Iranian Journal of Toxicology
ARAK UNIVERSITY OF MEDICAL SCIENCES

May 2026, Volume 20, Number 2

Research Paper

Activities of Metabolic Enzymes Linked with Dissipation of Toxic Pyrethroids in Malaria Vector Populations Breeding in Crude Oil Impacted Communities of Akwa Ibom State, Nigeria

Ndifreke Daniel Ekpo[1]*, Asanga Edet Effiong[2], Eteng Ofem Effiom[3], Emmanuel A Ating[4], Essien Ubong Benard[1], Udeme Udo Afia[1], Akon Umoekeyo Uno[5], Nworie Chika John[1], Akpan Bassey Effiong[1], Lawrence Usip[1]

[1] *Department of Animal and Environmental Biology, University of Uyo, Uyo, Nigeria.*
[2] *Department of Biochemistry, Arthur Jarvis University, Akpabuyo, Cross River State, Nigeria.*
[3] *Department of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria.*
[4] *School of Nautical Studies, Maritime Academy of Nigeria, Oron, Nigeria.*
[5] *Federal University of Technology, Minna, Nigeria.*

Scan to access website

How to cite this paper:
Ekpo ND, Effiong AE, Effiom EO, Ating EA, Benard EU, Afia UU, Uno AU, John NC, Effiong AB, Usip L. Activities of Metabolic Enzymes Linked with Dissipation of Toxic Pyrethroids in Malaria Vector Populations Breeding in Crude Oil Impacted Communities of Akwa Ibom State, Nigeria. Iranian Journal of Toxicology. 2026;20(2):77-86. doi: 10.22034/IJT.20.2.77
doi: 10.22034/IJT.20.2.77

Article info
Received: 05/10/2025
Accepted: 14/12/2025
Online Published: 15/05/2026

*** Corresponding author:**
Ndifreke Daniel Ekpo
Department of Animal and Environmental Biology, University of Uyo, Uyo, Nigeria

E- mail:
ndifrekeekpo@uniuyo.edu.ng

ABSTRACT

Background: Sustained insecticide toxicity against malaria vectors is crucial for effective vector control. Some mosquito populations have become resistant to commonly used insecticides for their control. Resistance, however, is often localized as it is shaped by local environmental factors that drive physiological and biochemical changes in local mosquito populations. The present study examined insecticide resistance and biochemical changes in adult female *Anopheles* mosquitoes from crude oil-polluted areas in Akwa Ibom State, Nigeria (March–November 2024).

Methods: Physico-chemical parameters of breeding sites were assessed using standard hydrobiological methods. Insecticide and piperonyl butoxide (PBO) bioassays, along with enzyme activity assays, were conducted according to standard protocols.

Results: Breeding sites were contaminated with hydrocarbons and other pollutants. Vectors showed substantial resistance to permethrin (16-71% mortality) but only marginal resistance to other pyrethroids (90-97% mortality). A strong negative correlation between permethrin mortality and hydrocarbon levels (r=-0.831, p=0.011) suggests pollution reduced insecticide efficacy. Pre-exposure to PBO remarkably raised permethrin mortality to 92-98%, implicating metabolic enzymes in the resistance dynamics. Local mosquito populations exhibited elevated detoxifying enzyme activities—especially mixed-function oxidase (MFO)—compared with Kisumu strains. These enzyme levels correlated significantly and positively with hydrocarbon content (r=0.766-0.985; p=0.000-0.027).

Conclusion: The findings suggest that crude oil pollution of anopheles breeding sites in Ibeno LGA, Akwa Ibom State, Nigeria, fosters enzyme-driven pyrethroid resistance in the local adult malaria vector populations. This presents a significant challenge for pyrethroid-based interventions, threatening malaria control efforts in the region.

Keywords: Anopheles, Insecticide resistance, Mixed function oxygenases, Petroleum pollution, Water quality

Introduction

Sustained insecticide toxicity against malaria vectors (female anopheles mosquitoes) is strongly influenced by local environments [1-4]. Aquatic stages of *Anopheles* mosquitoes usually develop in small, clean, scattered, sunlit, turbid, or temporary water bodies near dwellings [5]. However, many populations now adapt to polluted habitats, breeding in sites previously considered unsuitable [3]. Prolonged exposure to toxicants can trigger stress responses and metabolic adaptations that enhance insecticide tolerance, driving resistance evolution [6,7]. Organic pollutants, including crude oil, may upregulate detoxification enzymes such as cytochrome P450s, GSTs, and esterases, reducing the efficacy of control measures [8]. Adult mosquitoes in such environments may develop or inherit enhanced enzyme activity and gene expression, creating resistant populations with a high frequency of resistant phenotypes [2]. Resistance mechanisms involving mixed function oxidases, GSTs, and esterases can be identified using synergists like piperonyl butoxide (PBO), S.S.S.-tributylphoshorotrithiate (DEF), ethacrynic acid (EA), diethyl maleate (DM), and Chlorfenethol (CF) [9]. These compounds are not insecticides but inhibit detoxifying enzymes, revealing resistance pathways [9].

Pyrethroids remain the primary insecticides used in bed nets and sprays [10-13]. Nevertheless, their effectiveness is undermined by widespread resistance [5]. This is critical since pyrethroids are the main active ingredients for indoor sprays, fogs, and treated nets [10]. Vector

susceptibility is further shaped by environmental factors [14,15], making locally informed interventions essential, as recommended by WHO [1]. Pollution of breeding sites not only affects vector distribution but also enhances their resistance to insecticides [2,4,16,17].

The present study investigated resistance dynamics and metabolic enzyme activities in female *Anopheles* populations breeding in crude oil–impacted communities of Ibeno LGA, Akwa Ibom State, to inform malaria elimination strategies and prevent programmatic failure.

Materials and Methods

Study Area

The study was conducted in Ibeno Local Government Area (LGA), Akwa Ibom State, Nigeria (Figure 1), between March and November 2024. Ibeno, covering over 1,200 km² in the Niger Delta, lies south of the state along the Atlantic coast and consists of 31 gazetted communities [18]. Located in mangrove swamp ecozones, malaria transmission is highest between March and November [19]. A pre-survey, assisted by local guides, identified villages with high mosquito populations. Four sites—Okuritip, Iwo-opom, Upenekang, and Iwuonchang—were selected. These areas, with swamps, bushes, shallow wells, and waterlogging, provided ideal breeding habitats for mosquitoes.

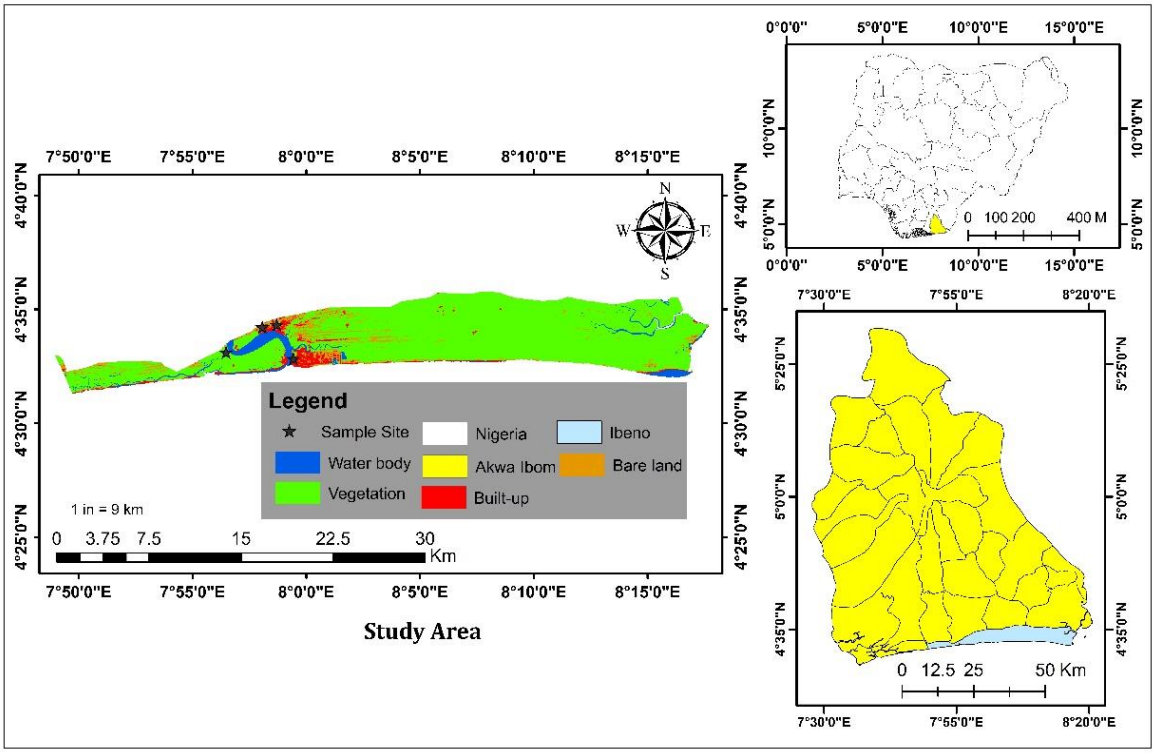

Figure 1. Map showing the location of the study sites

Larvae Collections and Laboratory Rearing of Mosquitoes

The study followed Kabula [20] with modifications. From May–November 2024, breeding sites (muddy water, swamps, wells, ponds, and stagnant pools) were surveyed on foot. Anopheles larvae, identified by their surface orientation, were collected with a 350 ml dipper, stored in 10 L jerrycans, and transported to the USAID/PMI/VECTORLINK Malaria Research Laboratory, University of Uyo. In the lab, larvae were reared in 20 L containers covered with fine mesh, fed a biscuit–yeast blend, and upon emergence, adults were aspirated into cages and maintained on 10% sugar solution.

Analysis of Physico-Chemical Parameters of Water Samples

Physico-chemical parameters of Anopheles breeding sites were assessed *in situ* and *ex situ* following Maiti [21,22]. The TDS, pH, temperature, DO, BOD, and EC were measured with calibrated portable probes (EXTECH models). Nutrients (NO_3^-, NO_2^-, PO_3^{2-}, SO_4^{2-}) were analyzed using standard colorimetric/turbidimetric methods, while total organic carbon was determined with the Lange TOC cuvette-test (Hatch Lange LCK 385). Analytical reagents were sourced from Sigma-Aldrich and BDH.

Insecticide Susceptibility Bioassay on Malaria Vector Populations

Insecticide susceptibility was assessed using WHO tube bioassays [9]. Non-blood-fed female Anopheles (2–5 days old, 25 per batch) were exposed for 1 h to papers impregnated with permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.75%), or lambda-cyhalothrin (0.05%); controls used untreated papers. Knockdown was recorded at intervals up to 60 min [9,23]. Mosquitoes were then held 24 h at 27±2°C and 75±10% RH with 10% sugar solution, after which mortality was recorded. Tests with >20% control mortality were discarded [24].

Synergist Assays with PBO

Resistance mechanisms involving metabolic enzymes were examined using WHO synergist assays [9]. Whatman papers impregnated with 5% PBO were used in four setups: control, PBO only, permethrin (0.75%) only, and PBO+permethrin. Each treatment had four replicates of 25 female Anopheles (2–5 days old). Mosquitoes were exposed for 1 hr, then transferred to holding tubes with sugar solution and maintained at 27±2°C, 75±10% RH. Mortality was recorded after 24 h.

Biochemical Assays

Enzyme activity was measured in the resistant adult population (with <90% mortality) using standard protocols [15,25]. A total of 40 non-blood-fed females (2–5 days old) that were not previously exposed to insecticides were randomly sampled in clusters and homogenized individually in 200 µl water on ice, centrifuged (14,000 g, 30 s), and supernatants were used as enzyme sources. Assays were run in 96-well plates, with OD values read using an ELISA microplate reader (Sunrise™, Tecan®, Switzerland). The same protocol was used to assay enzyme activities in reference strains (Kisumu mosquitoes) for comparison.

α- and β-esterases: A volume of 20 µl homogenate with 200 µl 30 mM α/β-naphthyl acetate; OD at 570 nm after staining. Activity expressed as nmole product/min/mg protein.

GST: A volume of 10 µl homogenate with GSH-CDNB mixture; OD at 340 nm. Activity calculated using Beer's Law, expressed as mmole CDNB/min/mg protein.

MFO: A volume of 2 µl homogenate with phosphate buffer, tetramethyl benzidine, and H_2O_2; OD at 650 nm after 2 h. The activity was expressed as cytochrome P450 equivalents/min/mg protein.

Statistical Analysis

Data were analyzed using Excel and SPSS (version 21) software. Descriptive statistics (mean, SD, range, %) summarized physico-chemical parameters, bioassay results, and enzyme activities. WHO bioassay outcomes were expressed as % mortality and classified as susceptible (98-100%), suspected resistance (90-97%), or resistant (<90%) [9]; Abbott's correction was unnecessary as control mortality was <5% [29]. Knockdown times (KDT_{50}, KDT_{95}) were estimated by log-time probit analysis. Analysis of variance (ANOVA) was employed to test differences among insecticide exposures, physicochemical parameters, and enzyme activities. Pearson's correlation assessed relationships between water parameters, resistance profiles, and enzyme activity, with significance at $p<0.05$.

Results

Physico-chemical Parameters of Breeding Sites

Water quality at mosquito breeding sites varied across study locations (Table 1). The pH value (7.30–7.85) was within the EPA range (6–9). Temperatures (33.9–34.2 °C) exceeded EPA limits. Electrical conductivity (257–384 µS/cm), TDS (85–123 mg/l), BOD (0.69–1.11 mg/l), alkalinity (122–199 mg/l), hardness (134–213 mg/l), and turbidity (0.37–0.44 NTU) were below EPA maximum permissible limits. Dissolved oxygen (2.07–3.09 mg/l) was far lower than the EPA standard. Nitrite nitrogen (1.00–1.98 mg/l) and hydrocarbons (4.22–8.90 mg/l) exceeded permissible levels at most sites, except hydrocarbons at Iwuonchang. Multivariate analysis showed significant site-specific differences ($p<0.05$), with Iwuonchang differing from other sites for most parameters.

Table 1. Physico-chemical parameters of larval breeding sites from Ibeno LGA

Parameter	Upenekang	Iwo Okpom	Iwuonchang	Okoroutip	EPA MPL
pH	7.54±0.52	7.85±0.23	7.30±0.09	7.82±0.19	6.5-9.0
Temperature (°C)	34.16±0.69	34.20±0.93	34.07±0.85	33.88±0.69	<3°C
Conductivity (µS/cm)	383.52±16.42	358.02±21.49	277.98±59.41^{a,b}	257.18±21.16^{a,b}	2500 µS/cm
TDS (ppm)	114.60±4.83	122.80±7.98	84.82±9.05^{a,b}	122.36±7.58^{c}	50 mg/l
DO (mg/l)	3.06±0.21	3.09±0.22	2.07±0.19^{a,b}	3.03±0.28^{c}	50 mg/l
BOD (mg/l)	1.09±0.11	1.11±0.14	0.69±0.15^{a,b}	1.08±0.12^{c}	5 mg/l
Total alkalinity (mg/l)	169.60 ±11.57	199.20±18.98^{a}	121.80±12.91^{a,b}	196.70 ±18.71^{c}	200 mg/l
Total hardness (mg/l)	181.20±8.70	212.60±19.20^{a}	133.60±12.95^{a,b}	212.32±18.74^{a,c}	<200 mg/l
Calcium (mg/l)	23.38±2.48	28.78±1.98^{a}	13.76±1.61^{a,b}	28.58±2.11^{a,c}	
Magnesium	20.46±2.65	25.58±1.98	25.07±2.13	20.46±2.65	30 mg/l
Turbidity (NTU)	0.40±0.04	0.44±0.11	0.37±0.42	0.42±0.08	5 NTU
Phosphate (mg/l)	1.65±0.17	1.56±0.21	0.73±0.13^{a,b}	1.50±0.19^{c}	
Nitrite (mg/l)	1.32±0.21	1.05±0.33	0.38±0.14^{a,b}	0.87±0.11^{a,c}	0.5 mg/l
Nitrate (mg/l)	1.98±0.12	1.81±0.13	1.00±0.23^{a,b}	1.71±0.11^{c}	50 mg/l
Sulphate (mg/l)	2.80±0.52	2.48±0.49	1.59±0.44^{a,b}	2.40±0.38	100 mg/l
Total hydrocarbon (mg/l)	8.34±1.31	8.90±1.33	4.22±0.26^{a,b}	8.77±1.29^{c}	5 mg/l

Values are expressed as Mean±S.D, n=3; EPA MPL: Environmental Protection Agency Maximum Permissible Limit
a=p≤0.05 (comparing values in other study sites to what was observed in Upenekang)
b=p≤0.05 (comparing values in other study sites to what was observed in Iwo Okpom)
c=p≤0.05 (comparing values in other study sites to what was observed in Iwuonchan)

Susceptibility to Pyrethroid Insecticides

Across the study sites, malaria vector populations showed strong resistance to permethrin but only suspected resistance to deltamethrin, lambda-cyhalothrin, and alpha-cypermethrin (Table 2-5). For the Upenekang population, Mortality was 19% with permethrin exposure, compared

with 96%, 97%, and 92% with deltamethrin, Lambda-cyhalothrin, and alpha-cypermethrin, respectively. Iwo Okpom population recorded 26% mortality following exposure to permethrin, compared with 96%, 97%, and 93% following exposure to deltamethrin, lambda-cyhalothrin, and alpha-cypermethrin, respectively**.** The Iwuonchang population showed the highest mortality with permethrin exposure (71%), and with deltamethrin, lambda-cyhalothrin, and alpha-cypermethrin exposure, 97%, 97%, and 95% mortalities, respectively, were recorded. Okoroutip vector populations had a mortality rate of 38% on exposure to permethrin, compared with 97%, 96%, and 95% mortalities on exposure to deltamethrin, lambda-cyhalothrin, and alpha-cypermethrin, respectively. In all sites, mortalities to deltamethrin, lambda-cyhalothrin, and alpha-cypermethrin were significantly higher ($p<0.05$) than to permethrin, but not different among themselves.

Table 2. Susceptibility of malaria vector population from Upenekang, Ibeno LGA, Akwa Ibom state, Nigeria to pyrethroid insecticides.

Insecticide Paper	No. exposed	No. of replicates	Mean mortality±SD	Total mortality (%)	Status
PY control	50	2	0.50±0.71	0	
Permethrin (0.75%)	100	4	4.75±0.50[a]	19	Resistant
Deltamethrin (0.05%)	100	4	24.25±0.50[a,b]	97	Suspected resistance
Alpha-cypermethrin (0.75%)	100	4	23.25±0.50[a,b]	93	Suspected resistance
Lambda-cyhalothrin (0.05%)	100	4	23.75±0.50[a,b]	95	Suspected resistance

Number of mosquitoes per replicate=25
a=p≤0.05 (comparing insecticide test groups with the PY control)
b=p≤0.05 (comparing other insecticide test groups with the permethrin)

Table 3. Susceptibility of malaria vector population from Iwo Okpom, Ibeno LGA, Akwa Ibom state, Nigeria to pyrethroid insecticides.

Insecticide Paper	No. exposed	No. of replicates	Mean mortality±SD	Total mortality (%)	Status
PY control	50	2	0.50±0.71	2	
Permethrin (0.75%)	100	4	6.50±1.00[a]	26	Resistant
Deltamethrin (0.05%)	100	4	24.00±0.82[a,b]	96	Suspected resistance
Alpha-cypermethrin (0.75%)	100	4	23.00±0.00[a,b]	92	Suspected resistance
Lambda-cyhalothrin (0.05%)	100	4	24.25±0.50[a,b]	97	Suspected resistance

Number of mosquitoes per replicate=25
a=p≤0.05 (comparing insecticide test groups with the PY control)
b=p≤0.05 (comparing other insecticide test groups with the permethrin)

Table 4. Susceptibility of malaria vector population from Iwuonchang, Ibeno LGA, Akwa Ibom state, Nigeria to pyrethroid insecticides.

Insecticide Paper	No. exposed	No. of replicates	Mean mortality ±SD	Total mortality (%)	Status
PY control	50	2	0.50±0.71	2	
Permethrin (0.75%)	100	4	17.75±0.50[a]	71	Resistant
Deltamethrin (0.05%)	100	4	24.25±0.58[a,b]	97	Suspected resistance
Alpha-cypermethrin (0.75%)	100	4	23.75±0.50[a,b]	95	Suspected resistance
Lambda-cyhalothrin (0.05%)	100	4	24.25±0.96[a,b]	97	Suspected resistance

Number of mosquitoes per replicate=25
a=p≤0.05 (comparing insecticide test groups with the PY control)
b=p≤0.05 (comparing other insecticide test groups with the permethrin)

Table 5. Susceptibility of malaria vector population from Okoroutip, Ibeno LGA, Akwa Ibom state, Nigeria to pyrethroid insecticides.

Insecticide Papers	Total No. exposed	No of replicates	Mean mortality ±SD	Total mortality (%)	Status
PY control	50	2	0.50±0.71	2	
Permethrin (0.75%)	100	4	9.50±1.00[a]	38	Resistant
Deltamethrin (0.05%)	100	4	24.25±0.50[a,b]	97	Suspected resistance
alpha-cypermethrin (0.75%)	100	4	23.75±0.50[a,b]	95	Suspected resistance
Lambda-cyhalothrin (0.05%)	100	4	24.00±1.15[a,b]	96	Suspected resistance

Number of mosquitoes per replicate=25
a=p≤0.05 (comparing insecticide test groups with the PY control)
b=p≤0.05 (comparing other insecticide test groups with the permethrin)

Knockdown Effect

Knockdown was consistently faster with deltamethrin, Lambda-cyhalothrin, and alphacypermethrin than with permethrin (Figures 2-5). All populations showed >90% knockdown within 1 h for deltamethrin and lambda-cyhalothrin. Exposure times resulting in 50% and 95% knockdown (KDT50 and KDT95) were estimated for each insecticide using a log-time probit model and are presented in Table 6. Permethrin indicated the slowest knockdown with a KDT_{50} range of 32.7–587.8 min and a KDT_{95} range of 201.6–2391.9 min. Deltamethrin had a KDT_{50} range of 21.4–32.3 min and a KDT_{95} range of 98.3–149.5 min. With lambda-cyhalothrin**,** the KDT_{50} range was 22.7–33.0 min, and the KDT_{95} range was 95.4–162.3 min. In addition, alpha-cypermethrin had a KDT_{50} range from 28.8–37.5 min and a KDT_{95} range from 142.1–177.7 min.

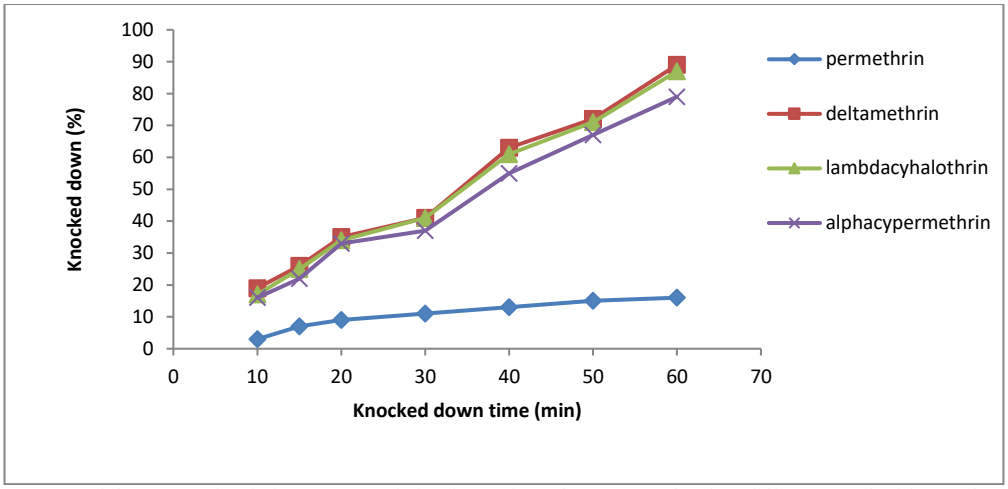

Figure 2. Knockdown rate of malaria vector population from Ukpenekang exposed to four different pyrethroid treated papers.

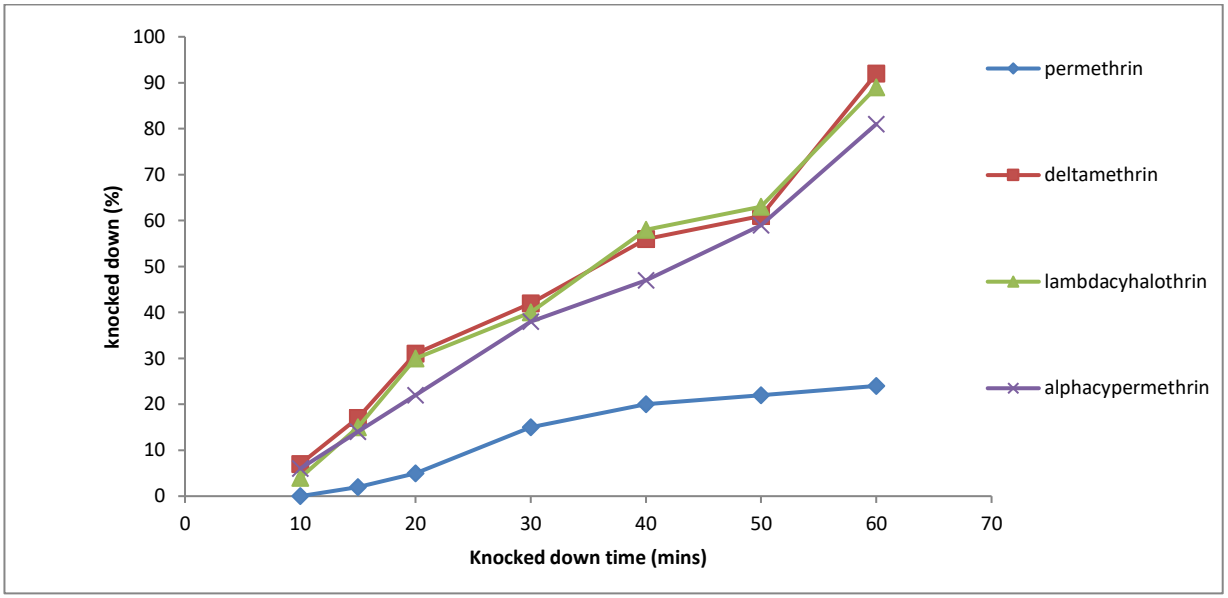

Figure 3. Knockdown rate of malaria vector population from Iwo-okpom exposed to four different pyrethroid treated papers.

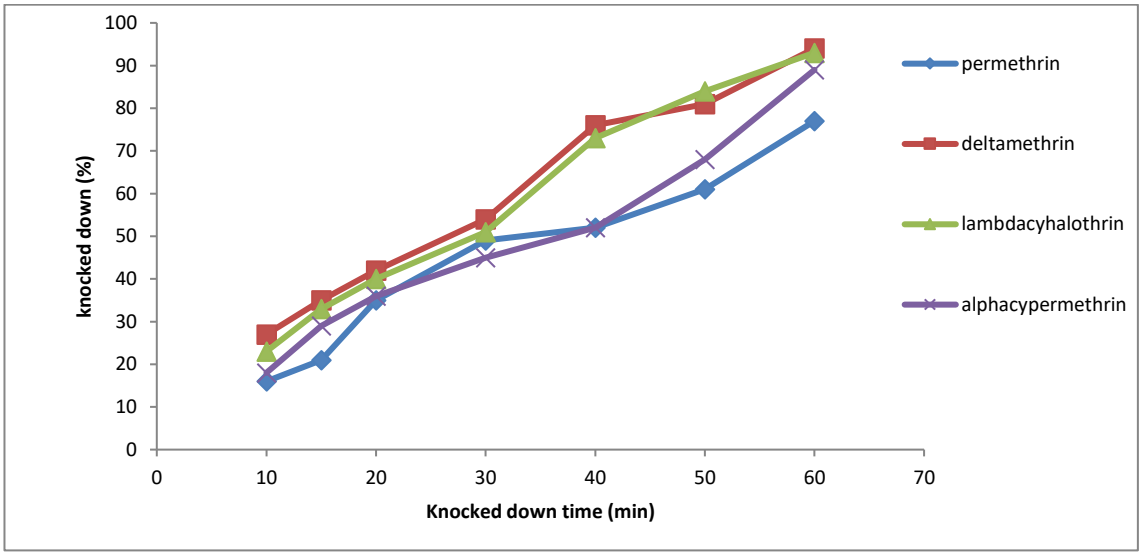

Figure 4. Knockdown rate of malaria vector population from Iwonchang exposed to four different pyrethroid treated papers.

Iranian Journal of Toxicology
ARAK UNIVERSITY OF MEDICAL SCIENCES

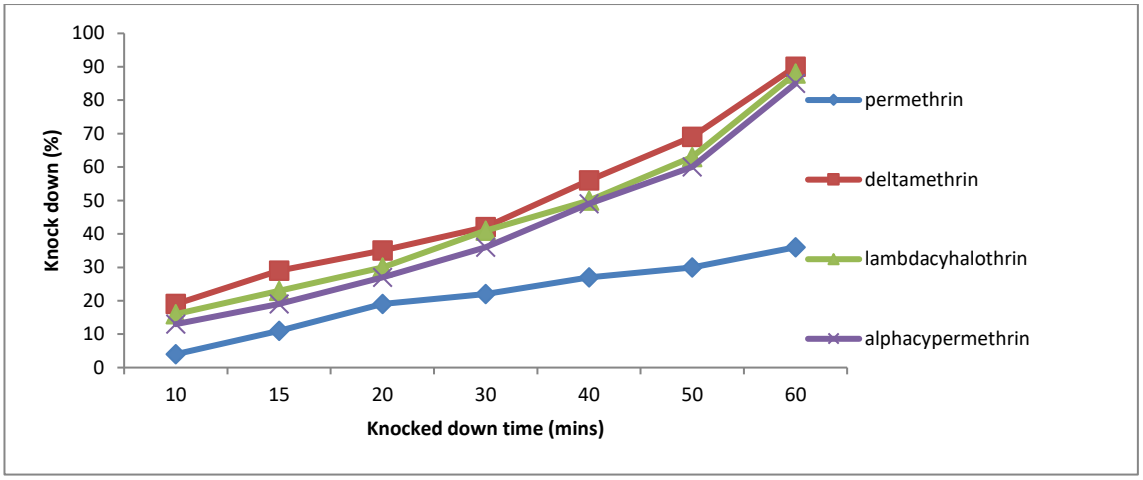

Figure 5. Knockdown rate of malaria vector population from Okoroutip exposed to four different pyrethroid treated papers.

Table 6. Knockdown times (KDTs) following exposure to different insecticides.

Study Site	No. exposed	Knocked Down Time (min)							
		Permethrin		Deltamethrin		Lambda-cyhalothrin		Alpha-cypermethrin	
		KDT_{50} (95 % CL)	KDT_{95} (95 % CL)	KDT_{50} (95 % CL)	KDT_{95} (95 % CL)	KDT_{50} (95 % CL)	KDT_{95}(95 % CL)	KDT_{50} (95 % CL)	KDT_{95} (95 % CL)
Upenekang	100	587.831 (206.311-17301.830)	29708.127 (2704.885 - 77347457.87)	27.818 (22.257-34.850)	131.552 (83.619-332.270)	28.887 (23.917-35.149)	135.764 (89.817-292.583)	32.607 (29.436-36.424)	177.694 (132.834-267.712)
Iwo-opom	100	107.252 (82.760-166.625)	580.479 (314.322-1718.662)	32.252 (26.212-40.749)	115.655 (76.927-269.214)	33.010 (28.167-39.296)	108.796 (70.787-192.034)	37.524 (34.444-41.265)	142.088 (114.013-191.144)
Iwuonchang	100	32.661 (29.292-36.774)	201.603 (145.660-322.044)	21.412 (16.785-26.081)	98.27 (67.090-204.167)	22.6770 (18.280-27.289)	95.354 (66.865-183.024)	28.827 (22.174-38.085)	157.669 (90.433- 585.482)
Okoroutip	100	102.707 (75.412-176.356)	1312.945 (549.692-6529.439)	28.415 (21.737-37.592)	149.510 (86.527-550.168)	32.116 (24.986-43.478)	162.278 (92.787-615.888)	35.056 (28.102-46.566)	165.408 (98.342- 517.738)

KDT_{50}: Knockdown time for 50% mosquitoes; KDT_{95}: Knockdown time for 95% mosquitoes; CL: Confidence limit

Correlation of Physico-chemical Parameters with Insecticide Resistance

Pearson's correlation (Table 7) indicated that permethrin resistance was strongly linked to high levels of several water parameters, including alkalinity (r=-0.796, p=0.018), pH (r=-0.725, p=0.042), total hydrocarbons (r=-0.831, p=0.011), dissolved oxygen (r= -0.947, p<0.001), BOD (r=-0.948, p<0.001), phosphate (r=-0.961, p<0.001), nitrite (r=-0.933, p=0.001), nitrate (r=-0.932, p=0.001), sulphate (r=-0.767, p=0.026), magnesium (r=-0.793, p=0.019), and calcium (r=-0.804, p=0.016). Higher values of these parameters consistently correlated with lower mortality (greater resistance). For alpha-cypermethrin, resistance was significantly correlated with hydrocarbons (r=-0.748, p=0.033), BOD (r=-0.750, p=0.032), phosphate (r=-0.773, p=0.025), nitrite (r=-0.831, p=0.011), and nitrate (r=-0.826, p=0.031). For lambda-cyhalothrin, only hydrocarbons were significantly associated (r=-0.738, p=0.037). For deltamethrin, correlations with water parameters were weak and not significant (e.g., hydrocarbons: r=-0.526, p=0.10).

Table 7. Pearson product-moment correlation analysis on the association between pyrethroid resistance and the physico-chemical parameters of the breeding site.

	Permethrin	Deltamethrin	Alpha-cypermethrin	Lambda-cyhalothrin
Alkalinity	- 0.796*	- 0.217	- 0.523	- 0.293
Temperature	0.117	- 0.418	- 0.167	0.021
pH	-0.725*	-0.686	-0.540	-0.555
THC (mg/L)	-0.831*	-0.526	-0.748*	-0.738*
EC (µs/cm)	-0.831	-0.526	-0.748	-0.738
DO	-0.947**	-0.356	-0.679	-0.512
BOD	-0.948**	-0.477	-0.750*	-0.614
PO_4^{2-} (mg/L)	-0.961**	-0.513	-0.773*	-0.636
Nitrite (mg/L)	-0.933**	-0.582	-0.831*	-0.702
Nitrate (mg/L)	-0.932**	-0.549	-0.826*	-0.655
SO_4^{2-} (mg/L)	-0.767*	-0.071	-0.327	-0.466
Magnesium (mg/L)	-0.793*	-0.229	-0.516	-0.303
Calcium (mg/L)	-0.804*	-0.239	-0.509	-0.291

Values represent the correlation coefficients (r-values)
**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed)

Synergist Bioassay

Table 8 indicates that across the study sites, pre-exposure of different malaria vector populations to PBO significantly increased the mortality rates on exposure to permethrin, which recorded high resistance (low mortality rates) without PBO pre-exposure. The mortality rate after 24 h exposure ranged from 19% to 81% for permethrin alone, but ranged from 92% to 97% with the pre-exposure to PBO. There was a significant difference ($p<0.05$) in the mean mortality of the malaria vector population from Iwonchang compared with those from other sites when exposure was conducted with only Permethrin. However, with pre-exposure to PBO, there were no significant differences ($p>0.05$) in the mean mortality rates among the different vector populations.

Table 8. Synergist bioassay

Study Site	Treatment									
	Control		Only PBO exposure		Permethrin only exposure			PBO +Permethrin exposure		
	Mean mortality±SD	Total mortality (%)	Mean mortality±SD	Total mortality (%)	Mean mortality±SD	Total mortality (%)	Status	Mean mortality±SD	Total mortality (%)	Remark
Upenekang	0.00±0.00	0	0.25±0.50	1	6.00±0.82	24	R	23.50±0.58	94	M R mechanism involved
Iwo Okpom	0.25±0.50	1	0.25±0.50	1	5.25±0.50	19	R	23.00±0.00	92	M R mechanism involved
Iwuonchang	0.00±0.00	0	0.25±0.50	1	20.25±0.50*	81	R	24.25±0.50	97	M R mechanism involved
Okoroutip	0.25±0.50	1	0.25±0.50	1	5.25±0.50	19	R	23.00±0.00	92	M R mechanism involved

Detoxifying Enzyme Activities

As shown in Table 9, mixed-function oxidase (MFO) enzyme activity was significantly ($p<0.05$) elevated in all vector populations across Ibeno LGA compared to the reference strain (Kisumu mosquitoes). No significant differences ($p>0.05$) were recorded for the MFO activities of the different malaria vector populations. Across the study sites in Ibeno LGA, alpha- and beta-esterase activity was significantly ($p<0.05$) lower than that observed in the reference strain. There were no significant differences ($p<0.05$) amongst the different malaria vector populations' esterase activities. Glutathione S-Transferase (GST) activity was slightly higher in vector populations across the study sites than in the reference strain; however, the difference was not significant ($p>0.05$).

Table 9. Detoxifying enzyme activities in Malaria vector populations across the sites in Ibeno LGA, Akwa Ibom State.

Populations	Mixed function oxidase (nmol P450/mg protein)	Alpha-esterase (µmol α-naphthol/min/mg protein)	Beta-esterase (µmol β-naphthol/min/mg protein)	GST (Nmol GSH conj/min/mg protein)
Kisumu strain (control)	0.0813±0.0025	0.0518±0.0013	0.0560±0.0014	0.1229±0.0027
Upenekang	0.1615±0.0219[a]	0.0180±0.0016[a]	0.0184±0.0019[a]	0.1265±0.0207
Iwo Okpom	0.1525±0.0171[a]	0.0171±0.0012[a]	0.0186±0.0026[a]	0.1278±0.0239
Iwuonchang	0.1472±0.0407[a,b]	0.0149±0.0016[a]	0.0157±0.0023[a]	0.1117±0.0144
Okoroutip	0.1500±0.0141[a]	0.0168±0.0014[a]	0.0176±0.0018[a]	0.1303±0.0265[d]

Values are expressed as Mean±S.D, n=40
a=$p<0.05$, comparing with the Kisumu strain
b=$p<0.05$, comparing with the Ukpekang population
d=$p<0.05$, comparing with the Iwuonchang population

Association between Physico-chemical Parameters and Detoxifying Enzyme Activities in the Malaria Vectors

Pearson's product-moment correlation (Table 10) revealed that high total hydrocarbon content was significantly and strongly associated with higher activities of all the detoxifying enzymes, including MFO ($r=0.766$, **p**=0.027), alpha esterase ($r=0.829$, **p**=0.011), beta esterase ($r=0.812$, **p**=0.014), and GST ($r=0.985$, **p**=0.000). High BOD content was significantly and strongly associated with higher activities of the detoxifying enzymes, for instance, MFO ($r=0.718$, **p**=0.045), alpha esterase ($r=0.712$, **p**=0.047), beta esterase ($r=0.760$, **p**=0.029), and GST ($r=0.926$, **p**=0.001). Furthermore, a significant and strong positive association was observed between Phosphate and MFO ($r=0.926$, $p=0.026$), Phosphate and alpha esterase ($r=0.926$, $p=0.046$), Phosphate and beta esterase ($r=0.926$, $p=0.024$), Phosphate and GST ($r=0.926$, $p=0.004$); Nitrite and MFO ($r=0.926$, $p=0.007$), Nitrite and alpha esterase ($r=0.926$, $p=0.030$), Nitrite and beta esterase ($r=0.926$, $p=0.017$), Nitrite and GST ($r=0.926$, $p=0.016$); Nitrate and MFO ($r=0.926$, $p=0.012$), Nitrate and alpha esterase ($r=0.926$, $p=0.021$), Nitrate and beta esterase ($r=0.926$, $p=0.010$), Nitrate and GST ($r=0.926$, $p=0.007$). Alkalinity, pH, DO, Magnesium, and Calcium were significantly and strongly associated with higher activities of GST only ($r=0.781$, $p=0.022$; $r=0.871$, $p=0.005$; $r=0.861$, $p=0.006$; $r=0.790$, $p=0.020$, and $r=0.768$, $p=0.026$, respectively).

Table 10. Association between physico-chemical environmental factors and detoxication enzyme activities

	MFO	Alpha esterase	Beta esterase	GST
Alkalinity	0.439	0.421	0.539	0.781*
Temperature	-0.091	0.306	0.154	-0.019
Ph	0.547	0.504	0.535	0.871**
THC (mg/L)	0.766*	0.829*	0.812*	0.985**
EC (µs/cm)	0.682	0.309	0.434	0.466
DO	0.618	0.604	0.670	0.861**
BOD	0.718*	0.712*	0.760*	0.926**
PO_4^{2-}(mg/L)	0.769*	0.716*	0.774*	0.878**
Nitrite (mg/L)	0.853**	0.755*	0.799*	0.806*
Nitrate (mg/L)	0.823*	0.786*	0.836**	0.852**
SO_4^{2-}(mg/L)	0.313	0.243	0.220	0.599
Magnesium (mg/L)	0.436	0.427	0.539	0.790*
Calcium (mg/L)	0.432	0.398	0.520	0.768*

Values represent the correlation coefficients (r-values)
**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Discussion

Pollution at mosquito breeding sites significantly affects vector distribution and resistance to insecticides [2,16]. In this study, Anopheles mosquitoes were found breeding in water bodies with visible oil films, indicating hydrocarbon contamination. Physico-chemical analysis confirmed that total hydrocarbons (THC), total dissolved solids (TDS), and nitrite levels exceeded EPA limits, consistent with high pollution [22]. Such contamination likely arises from crude oil spills and human activities, common in Ibeno LGA, a crude oil–rich region of Akwa Ibom State. Although oil can kill larvae by suffocation and reduce malaria transmission, dense mosquito populations persisted in these areas and tolerated indoor aerosol insecticides (field observation). This supports WHO [26], which links chronic crude oil pollution to high malaria endemicity. The likely explanation is that larvae adapted to polluted habitats, conferring resistance to pyrethroids in adults. Our findings contradict Gimnig *et al*. [5], who argued that Anopheles avoid polluted waters, but align with Awolola [3,26], who reported adaptation to environmental changes and pollution, including breeding in urban Lagos. Similarly, Imam and Yusuf [2] observed high Anopheles larval densities in contaminated waters in northwestern Nigeria and linked prior exposure to insecticide resistance in adults.

Our bioassays revealed high resistance to permethrin across study sites, while deltamethrin, lambda-cyhalothrin, and alpha-cypermethrin produced higher mortalities but still showed signs of emerging resistance. Overall, type II pyrethroids (deltamethrin, alphacypermethrin, lambda-cyhalothrin) had more sustained toxic effects than the type I pyrethroid (permethrin), consistent with earlier findings [27,28]. Bloomquist [27] attributed this to the cyano group in type II pyrethroids, which prolongs sodium channel depolarization and produces irreversible convulsions, unlike the shorter action of type I compounds. These results suggest differential selection pressures in the area and align with reports of widespread pyrethroid resistance in malaria vectors [16,29,30].

Pre-exposure of vectors to PBO markedly increased permethrin toxicity, raising mortality from as low as 19% to ≥87%, confirming metabolic enzyme involvement in resistance. PBO is known to inhibit MFOs, thereby restoring pyrethroid efficacy [4,16,31]. Biochemical assays corroborated this, showing elevated activities of CYP450s (MFOs), GSTs, and esterases in all field populations compared with Kisumu reference strains. Such enzyme overproduction is a well-documented mechanism of pyrethroid and DDT resistance in African malaria vectors [2–4,15,32,33]. Although esterase resistance is typically linked to organophosphates [16], elevated GST and esterase activities have also been associated with pyrethroid resistance in *Aedes* species [31]. Prior exposure to environmental xenobiotics (e.g., crude oil, herbicides, industrial pollutants) is known to induce detoxification enzymes and promote insecticide tolerance [2,34-35]. Our findings suggest that crude oil–polluted breeding sites in Ibeno LGA may have selected for enzyme-mediated resistance in local malaria vectors.

Conclusions

This study demonstrates that malaria vector populations from different communities in Ibeno LGA, Akwa Ibom State, Nigeria, successfully breed in crude oil–polluted waters, where hydrocarbon contamination may have influenced pyrethroid resistance. Susceptibility tests and synergist assays confirm that resistance is primarily metabolic, driven by elevated activities of MFOs and other detoxifying enzymes. Such enzyme-mediated resistance threatens the effectiveness of current pyrethroid-based control strategies and could undermine malaria elimination efforts. However, combining pyrethroids with PBO may restore efficacy and strengthen vector control in these polluted environments.

Data Access and Responsibility

As the principal investigator and corresponding author of the study, Ekpo, Ndifreke Daniel, has full access to all the study's data and is responsible for its accuracy and integrity.

Ethical Considerations

This study was performed in compliance with the

relevant laws and guidelines established by the University of Uyo's ethical committee, including the ARRIVE guidelines on animal experimentation.

Authors' Contributions

Research conceptualization was made by Ekpo ND. All authors were involved in Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Original Draft, and Review & Editing.

Acknowledgement

The authors are grateful to the PMI-VECTORLINK Project, Nigeria, for enabling the Laboratory environment and providing technical support for this research.

Conflict of Interests

There are no conflicts of interest to declare.

Funding

The study did not receive any external funding.

References

1. Chung HY, Cesari M, Anton S, Marzetti E, Giovannini S, Seo AY, et al. Molecular inflammation: Underpinnings of aging and age-related diseases. Ageing Res Rev. 2009;**8**(1):18-30. [**DOI**: 10.1016/j.arr.2008.07.002] [**PMID**: 18692159]
2. Odu P, Odu VK, Oyebanjo OT, Benneth BA, Onasanwo SA. Cognitive and neuroprotective effects of Vernonia amygdalina in scopolamine-induced memory impaired rats. Niger J Physiol Sci. 2024;**39**(2):233-40. [**DOI**: 10.54548/njps.v39i2.9] [**PMID**: 40156795]
3. Khansari N, Shakiba Y, Mahmoudi M. Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. Recent Pat Inflamm Allergy Drug Discov. 2009;**3**(1):73-80. [**DOI**: 10.2174/187221309787158371] [**PMID**: 19149749]
4. Çoban J, Betül-Kalaz E, Küçükgergin C, Aydın AF, Doğan-Ekici I, Doğru-Abbasoğlu S, et al. Blueberry treatment attenuates D-galactose-induced oxidative stress and tissue damage in rat liver. Geriatr Gerontol Int. 2014;**14**(2):490–7. [**DOI**: 10.1111/ggi.12096] [**PMID**: 23750655]
5. Omidkhoda SF, Mehria S, Heidaria S, Hosseinzadeh H. Protective effects of crocin against hepatic damages in D-galactose aging model in rats. Iran J Pharm Res. 2020;**19**(3):440–50. [**DOI**: 10.22037/ijpr.2019.15022.12825] [**PMID**: 33680043]
6. Rui L. Energy metabolism in the liver. Compr Physiol. 2014;**4**(1):177-97. [**DOI**: 10.1002/cphy.c130024] [**PMID**: 24692138]
7. Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, et al. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nat. 2010;**469**(7328):102–6. [**DOI**: 10.1038/nature09603] [**PMID**: 21113150]
8. Hunt NJ, Kang SW, Lockwood GP, Le Couteur DG, Cogger VC. Hallmarks of aging in the liver. Comput Struct Biotechnol J. 2019;**17**:1151-61. [**DOI**: 10.1016/j.csbj.2019.07.021] [**PMID**: 31462971]
9. Niccoli T, Partridge L. Ageing as a risk factor for disease. Curr Biol. 2012;**22**(17):741-52. [**DOI**: 10.1016/j.cub.2012.07.024] [**PMID**: 22975005]
10. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;**153**(6):1194–217. [**DOI**: 10.1016/j.cell.2013.05.039] [**PMID**: 23746838]
11. Chen P, Chen F, Zhou B. Antioxidative, anti-inflammatory and anti-apoptotic effects of ellagic acid in liver and brain of rats treated by D-galactose. Sci Rep. 2018;**8**(1):1465. [**DOI**: 10.1038/s41598-018-19732-0] [**PMID**: 29362375]
12. Zhang Q, Li X, Cui X, Zuo P. D-galactose injured neurogenesis in the hippocampus of adult mice. Neurol Res. 2005;**27**(5):552-6. [**DOI**: 10.1179/016164105x25126] [**PMID**: 15978184]
13. Ali T, Badshah H, Kim TH, Kim MO. Melatonin attenuates D-galactose-induced memory impairment, neuroinflammation and neurodegeneration via RAGE/NF-κB/JNK signaling pathway in aging mouse model. J Pineal Res. 2015;**58**(1):71–85. [**DOI**: 10.1111/jpi.12194] [**PMID**: 25401971]
14. Airoldi C, La Ferla B, D'Orazio G, Ciaramelli C, Palmioli A. Flavonoids in the treatment of Alzheimer's and other neurodegenerative diseases. Curr Med Chem. 2018;**25**(27):3228–46. [**DOI**: 10.2174/0929867324666170810142400] [**PMID**: 29424298]
15. Karak P. Biological activities of flavonoids: An overview. Int J Pharm Sci Res. 2019;**10**(4):1567–74. [**DOI**: 10.13040/IJPSR.0975-8232.10(4).1567-74]
16. Aziz N, Kim MY, Cho JY. Anti-inflammatory effects of luteolin: A review of in vitro, in vivo, and in silico studies. J Ethnopharmacol. 2018;**225**:342-58. [**DOI**: 10.1016/j.jep.2018.05.019] [**PMID**: 29801717]
17. Imran M, Rauf A, Abu-Izneid T, Nadeem M, Shariati MA, Khan IA, et al. Luteolin, a flavonoid, as an anticancer agent: A review. Biomed Pharmacother. 2019;**112**:108612. [**DOI**: 10.1016/j.biopha.2019.108612] [**PMID**: 30798142]
18. Mosa OF, Makkah KS. Protective impact of Luteolin on scopolamine induced-amnesia in wistar rats: An involvement of acetylcholinesterase, dopamine and lipid peroxidation. Life Sci J. 2021;**18**(3):4-11. [**DOI**: 10.7537/marslsj180321.02]
19. Mu J, Ma H, Chen H, Zhang X, Ye M. Luteolin prevents UVB-induced skin photoaging damage by modulating SIRT3/ROS/MAPK signaling: In vitro and in vivo studies. Front Pharmacol. 2021;**12**:728261. [**DOI**: 10.3389/fphar.2021.728261] [**PMID**: 34526903]
20. Yan Y, Jun C, Lu Y, Jiangmei S. Combination of metformin and luteolin synergistically protects against carbon tetrachloride-induced hepatotoxicity: Mechanism involves antioxidant, anti-inflammatory, antiapoptotic, and Nrf2/HO-1 signaling pathway. Biofactors. 2019;**45**(4):598–606. [**DOI**: 10.1002/biof.1511] [**PMID**: 31336028]
21. Zhang H, Tan X, Yang D, Lu J, Liu B, Baiyun R, et al. Dietary luteolin attenuates chronic liver injury induced by mercuric chloride via the Nrf2/NF-κB/p53 signaling pathway in rats. Oncotarget 2017;**8**(25):40982-93. [**DOI**: 10.18632/oncotarget.16684] [**PMID**: 28498799]
22. Cui X, Zuo P, Zhang Q, Li X, Hu Y, Long J, et al. Chronic D-galactose exposure induces memory loss, neurodegeneration, and oxidative damage in mice: Protective effects of R-alpha-lipoic acid. J Neurosci Res. 2006;**83**(8):1584–90. [**DOI**: 10.1002/jnr.20845] [**PMID**: 16555301]
23. Wang H, Wang H, Cheng H, Che Z. Ameliorating effect of luteolin on memory impairment in an Alzheimer's disease model. Mol Med Rep. 2016;**13**(5):4215–20. [**DOI**: 10.3892/mmr.2016.5044] [**PMID**: 27035793]
24. Rifai N, Warnick GR. Laboratory measurement of lipids, lipoproteins and apolipoproteins. Washington (DC): AACC Press. 1994:357. [**LINK**]
25. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974;**20**(4):470-5. [**PMID**: 4818200]
26. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem. 1973;**19**(5):476-82. [**DOI**: 10.1093/clinchem/19.5.476] [**PMID**: 4703655]
27. Crouse JR, Parks JS, Schey HM, Kahl FR. Studies of low-density lipoprotein molecular weight in human beings with coronary artery disease. J Lipid Res. 1985;**26**(5):566-74. [**DOI**: 10.1016/s0022-2275(20)34343-1] [**PMID**: 4020295]
28. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957;**28**(1):56-63. [**DOI**: 10.1093/ajcp/28.1.56] [**PMID**: 13458125]
29. Kind PR, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with Amino-antipyrine. J Clin Pathol. 1954;**7**(4):322-6. [**DOI**: 10.1136/jcp.7.4.322] [**PMID**: 13286357]
30. Du Z, Yang Y, Hu Y, Sun Y, Zhang S, Peng W, et al. A long-term high-fat diet increases oxidative stress, mitochondrial damage and apoptosis in the inner ear of D-galactose-induced aging rats. Hear Res. 2012;**287**(1–2):15-24. [**DOI**: 10.1016/j.heares.2012.04.012] [**PMID**: 22543089]
31. Gama AW, Akib R, Khaera U. The effect of giving D-galactose as an aging inducer on body weight, glucose levels and interleukin-6 levels in wistar rats. J Community Health Provis. 2025;**5**(1):1-3.

[DOI: 10.55885/jchp.v5i1.556]
32. Gill TM, Hardy SE, Williams CS. Underestimation of disability in community-living older persons. J Am Geriatr Soc. 2002;**50**(9):1492-7. [DOI: 10.1046/j.1532-5415.2002.50403] [PMID: 12383145]
33. Odu PO, Ujah GA, Uket JM, Odu VK, Inwang UA. Costus afer leaves extract ameliorates stress-induced alterations in hematological and lipid parameters in wistar rats. Trop J Nat Prod Res. 2025;**9**(6):2821-6. [DOI: 10.26538/tjnpr/v9i6.63]
34. Tarbiat S, Aydın B, Ergün K, Mohseni AR. Assessing the impact of oleuropein on dyslipidemia in male rats subjected to D-galactose-induced aging: A preliminary study. Eur Chem Biotechnol J. 2024;**25**(2):17-26. [DOI: 10.62063/ecb-21]
35. Lin S, Beck AN, Finch BK, Hummer RA, Master RK. Trends in US older adult disability: exploring age, period, and cohort effects. Am J Public Health. 2012;**102**(11):2157-63. [DOI: 10.2105/AJPH.2011.300602] [PMID: 22994192]
36. Zhou Y, Xu Q, Dong Y, Zhu S, Song S, Sun N. Supplementation of mussel peptides reduces aging phenotype, lipid deposition and oxidative stress in D-galactose-induced aging mice. J Nutr Health Aging. 2017;**21**(10):1314-20. [DOI: 10.1007/s12603-016-0862-3] [PMID: 29188895]
37. Shehnaz SI, Roy A, Vijayaraghavan R, Sivanesan S. Luteolin mitigates diabetic dyslipidemia in rats by modulating ACAT-2, PPARα, SREBP-2 proteins, and oxidative stress. Appl Biochem Biotechnol. 2023;**195**(8):4893–914. [DOI: 10.1007/s12010-023-04544-4] [PMID: 37103741]
38. Dai X, Liang B, Sun Y. Luteolin ameliorates rat model of metabolic syndrome-induced cardiac injury by apoptosis suppression and autophagy promotion via NR4A2/p53 regulation. BMC Complement Med Ther. 2025;**25**(1):14. [DOI: 10.1186/s12906-025-04749-6] [PMID: 39833877]
39. Galal RM, Zaki HF, Seif MM, Agha AM. Potential protective effect of honey against paracetamol-induced hepatotoxicity. Arch Iran Med. 2012;15(11):674–80. [PMID: 23102243]
40. Villegas R, Xiang YB, Elasy T, Cai Q, Xu W, Li H, et al. Liver enzymes, type 2 diabetes, and metabolic syndrome in middle-aged, urban Chinese men. Metab Syndr Relat Disord. 2011;**9**(4):305-11. [DOI: 10.1089/met.2011.0016] [PMID: 21495862]
41. Anadón A, Castellano V, Martínez-Larrañaga MR. Biomarkers in drug safety evaluation. Biomark Toxicol. 2014:923-45. [DOI: 10.1016/B978-0-12-404630-6.00055-5]
42. Lin Y, Shi R, Wang X, Shen HM. Luteolin, a flavonoid with potential for cancer prevention and therapy. Curr Cancer Drug Targets. 2008;**8**(7):634-46. [DOI: 10.2174/156800908786241050] [PMID: 18991571]
43. Zhang Q, Yang J, Wang J. Modulatory effect of luteolin on redox homeostasis and inflammatory cytokines in a mouse model of liver cancer. Oncol Lett. 2016;**12**(6):4767–72. [DOI: 10.3892/ol.2016.5291] [PMID: 28101223]
44. Alharbi OA, Almatroudi A, Alrumaihi F, Alghafis SF, Alwanian WM, Rahmani AH. The potential role of luteolin, a flavonoid in cancer prevention and treatment. CyTA J Food. 2024;**22**(1): 2381714. [DOI: 10.1080/19476337.2024.2381714]
45. Lakshmi BVS, Sudhakar M, Prakash KS. Protective effect of selenium against aluminum chloride-induced Alzheimer's disease: Behavioral and biochemical alterations in rats. Biol Trace Elem Res. 2015;**165**(1):67–74. [DOI: 10.1007/s12011-015-0229-3] [PMID: 25613582]
46. Yao C, Dai S, Wang C, Fu K, Wu R, Zhao X, et al. Luteolin as a potential hepatoprotective drug: Molecular mechanisms and treatment strategies. Biomed Pharm. 2023;**167**:115464. [DOI: 10.1016/j.biopha.2023.115464] [PMID: 37713990]