Trop J Nat Prod Res, July 2022; 6(7):1159-1164 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

# Cytogenotoxicity of Aqueous *Azadirachta indica A. Juss* Extracts on Nile Tilapia – O*reochromis niloticus (*Linnaeus, 1758) Under Static Exposure

Akinjogunla V. Folakemi[1]*, Muazu T. Abubakar[2], Ajeigbe S. Olawale[3], Usman M. Dahiru[4], Ibrahim Halima-Sadiya[5]

[1]*Department of Fisheries and Aquaculture, Faculty of Agriculture, Bayero University, Kano, Nigeria*
[2]*Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Bayero University, Kano, Nigeria*
[3]*Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Federal University of Agriculture, Zuru Kebbi State, Nigeria*
[4]*Department of Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Bayero University, Kano, Nigeria*
[5]*Department of Plant Biology, Faculty of Life Sciences, Bayero University, Kano, Nigeria*

ARTICLE INFO

*Article history:*
Received 30May 2020
Revised 08July 2022
Accepted 17July 2022
Published online 03 August 2022



ABSTRACT

The indiscriminate use of plant extracts as anti-microbial agent on culturable fish (catfishes and the Cichlids) in confinement is alarming, especially the use of neem leave extracts. Neem (*Azadirachta indica*) is a medicinal plant containing active biological and chemical properties. The effects of aqueous *Azadirachta indica* (neem) extract on the cytology and genome composition of hundred post juvenile *Oreochromisniloticus* with mean length and weight of 9.37±0.67cm and 8.99±1.84 g respectively was investigated in a tank culture system to determine the median lethal concentration ($LC_{50}$) at 96 h of exposure. Five graded concentrations of 1.2, 1.4, 1.6, 1.8 and 2.0 L of the aqueous extract and control were diluted in 15 L of water in triplicate. The control group and group exposed to 1.2 L concentration of neem extract showed normal behaviour while group exposed to 1.6 and 1.8 L concentrations showed abnormal behavioural changes such as rolling movement, gulping of air, weakness, swimming on the back and eventually death was observed. There was significant difference ($p<0.05$) in the toxicity effects of neem extract at 14$^{th}$ and 28$^{th}$ day of exposure. Cytotoxic effect was evident by cellular apoptosis which increases with increase concentration of the extract while genotoxic effect was manifested by formation of nuclear aberrations within the erythrocytes. The results obtained in this study revealed that neem extract has clastogenic and apoptotic effects on the erythrocytes of *Oreochromis niloticus*.

***Keywords***: *Azadirachta indica*, Cytogenotoxicity, $LC_{50}$, *Oreochromis niloticus*.

## Introduction

According to Food and Agricultural Organization (FAO),[1]artificial rearing of fishes in ponds and tanks is one of the rising sources of food production sectors in the world, worldwide production is expected to increase from 179 million tonnes in the year 2018 to 204 million tonnes in the year 2030. In Nigeria, aquaculture helps to provide an appropriate and affordable replacement for nutritional and digestible protein. With this production rise, aquaculture services are progressively depending on the input of formulated feeds and disease- resistant drugs for increased production.[2]Fish is a principal supply for humans, especially as source of animal protein, digestible minerals, commercial (profit-making) or subsistence (survival) farming.[3,4] Fin (Tilapia, Catfishes) and Shell (Oysters, Clams, Mussels) fishes serve as effective genetic replica for the evaluation of effluence in aquatic ecosystems [5] and can play major roles in assessing imminent vulnerability allied with contagion in aquatic ecosystem since they are directly exposed to chemicals (trace or heavy) resulting from agricultural production via surface overspill or tactfully, through the food chain of the ecosystem.[6]

*Corresponding author. E mail: vfakinjogunla.faq@buk.edu.ng
Tel: +2348068036484

**Citation**: Akinjogunla VF, Muazu TA, Ajeigbe SO, Usman MD, Ibrahim, H Cytogenotoxicity of Aqueous *Azadirachta indica A. Juss* Extracts on Nile Tilapia – O*reochromis niloticus (*Linnaeus, 1758) Under Static Exposure.Trop J Nat Prod Res. 2022; 6(7):1159-1164. doi.org/10.26538/tjnpr/v6i7.20

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Neem plants with the botanical name *Azadirachta indica A. Juss* is grown worldwide in various continents like Africa, Asia and Austrialia.[7] Neem has been extensively used by humans since primeval times to treat diverse illnesses.[8] The Neem plants have various names amongst which are "Twenty-first-century trees" and "a-tree-for-solving-global-problems" because of its extraordinarily natural properties (anti-bacterial, anti-fungal, anti-inflammatory, immune-modulatory, anti-hyperglycemic, anti-ulcer, anti-mutagenic, anti-cancer, anti-malarial, anti-viral, anti-oxidant, [9] anti-fertility, [10,11] in treating various diseases and has many benefits to humans and animals. [12]

In Aquaculture, it is common management practice to get rid of fish diseases which cause enormous economic losses in productivity and are mainly controlled with noxious chemicals which are usually applied erratically. [13] Thus, the use of pesticides to eradicate fish diseases and parasites leads to elevated levels of residues in the animals and thus affect their performance. [14] In view of the ecological harms arising from the use of synthetic chemicals and the urgent need for other methods that will help minimize this damage, there has been massive researches on controlling fish diseases using plants extracts.[15] The organic and chemical activities of neem extracts have been investigated in various countries.[16,17]stated that varieties of neem leave extracts have been broadly used in fish-farms as substitutes to monitor fish parasites and predators such as dragon-fly larvae. Aqueous extracts of the bark of the neem plant caused respiratory problems in *Coptodonzillii*[18] even though its toxicity is low to non-target aquatic biota while prolonged exposure to minimal concentrations of the crude extract of *A. indica* can prolong the development of this Cichlid fish.[19] Nile Tilapia is one of the principal fish species that is widely cultivated in Nigeria because of its profitability and to meet up with the increasing demand for protein intake. Nile tilapia is commonly

found in lakes and rivers with ample flora across Africa, Middle East, Coastal India, Central and South America.[20]
Several studies have been done on feed intake and body mass gain activity of Neem in Poultry birds; [21] ruminants; [22-24] Fish; [25, 26] rats [27, 28] and on Human. [29, 12]
Meanwhile, majority of species of fishes available and consumed in the Northern region of Nigeria are from dams, rivers and aquaculture (nursery, ponds and culture tanks) because of the arid nature of the region. Yet, there is dearth of information on control or prevention of fish diseases on freshwater fish species from aquaculture sector in the Northern region of Nigeria.
This present study is therefore undertaken to investigate the effects of Neem leaves (*Azadirachta indicaA. Juss*) extract on the Nile Tilapia, *Oreochromis niloticus* also known as *"Karfasa"* in Hausa language and to also examine the cytology and genome composition of the extracts on the erythrocytes. The fish species was selected for this research because of the availability all year round [30, 31]and its consumption by Northern inhabitants.

## Materials and Methods

*Experimental animals*
One hundred (100) Nile Tilapia (*Oreochromis niloticus*) were identified by their vertical stripes on the caudal fin with brownish to grayish overall coloration and indistinct banding on their body from a fish farm in Kano State, Nigeria and were conveyed in open troughs containing water from the pond to the Department of Fisheries and Aquaculture, Bayero University (BUK), Kano State, Nigeria. They were allowed to acclimate for 14 days in 400L tanks filled with de-chlorinated water (temperature 20–21°C, pH 7.5–7.7 and hardness 80–90 mg/L, $CaCO_3$). They had 12 h of dark and 12 h light phase with stable ventilation and were fed with extruded feed (1.5 mm in size) twice daily except the day preceding the experiments.

*Preparation of Aqueous Neem Leaf Extract*
Dry Neem leaves with voucher number BUK0211 were obtained from the Department of Plant Biology herbarium, Faculty of Life Sciences, Bayero University Kano, Nigeria on Wednesday, 24th November, 2021. Fifty grams (50 g) of the Neem powder was dissolved in 1litre of dechlorinated water for a whole day (24 h) at 32±2°C (Standard room temperature).[32] The blend was sieved by 25-mesh diameter sieve and the extract (50g/L) was used at various concentrations.

*Acute toxicity test*
The definitive test was conducted for 96 h to ascertain the acute toxicity effect ($LC_{50}$) after the range finding test. The following concentrations of neem extract (1.2, 1.4, 1.6, 1.8 and 2.0L) were used in 15 L of water in triplicate and control. 95% confidence limits were derived for the toxicity indices ($LC_{95,}$ $LC_{50}$ and$LC_{5)}$ using this formula: TF = $LC_{50}$ at 24hours / $LC_{50}$ at any other exposure time. [33]

*Sublethal Analysis for the Determination of Genotoxicity and Cytotoxicity Effects*
The fish were exposed to sublethal concentrations of Neem extract at $1/10^{th}$, $1/20^{th}$ and $1/50^{th}$ of the $LC_{50}$ (Lethal concentration) for 28 days. *Oreochromis niloticus* were selected at random from each concentration of the triplicate including control.

*Slide preparation*
Blood sample was collected from each of the selected fish via the tail vein and were smeared on a glass slide at Fishery Department Culture Laboratory Bayero University Kano-Nigeria. The glass slides were air dried and fixed in 10% methanol for 40 minutes. They were removed and air dried and then stained with 3% Giemsa (sigma) stain, rinsed with water and allowed to dry.
The slides were examined using light microscope at magnification of x1000 (oil immersion). The method of [34] was adopted for scoring the micronuclei.

*Statistical analysis*
One-way parametric ANOVA (Kruskal-Wallis) test was used for significance differences between groups exposed to different concentrations of Neem extract while the differences were identified using the Student–Newman–Keuls (SNK) multiple-range test. Values of $p \leq 0.05$ were considered significant.

*Ethical statement*
The experimental procedures were approved by the Institutional Animal Ethics Committee of Bayero University Kano-Nigeria

## Results and Discussion

*Range Finding Tests and Behavioral Observation at 1.2L concentration of Neem Extract*
Table 1 shows the total mortality rate observed when *Oreochromis niloticus* was exposed to neem extract concentration above 1.2 L in 12.5 L of water. After the introduction of the above concentration, an erratic movement was observed among the exposed fish and total mortality was recorded in less than 24 hours. Aggressive behaviors were observed with the Nile tilapia juveniles on contact with *Azadirachta indica A. Juss* extract. Fish from the control group and the group exposed to 1.2 L of neem extract showed normal body colour with a change in fin colour. Their behavior ranges from dynamically active, motionless symmetry, energetic swimming, normal gill movement and lying horizontally in water. Those from the group exposed to 1.6L and 1.8 L of neem extract showed abnormal behavior characterized by agitation, sudden and quick movement, air gulping, rolling movements and swimming on the back. There was an increase in the amount of mucus with widespread pigmentation mainly on the dorsum in the group exposed to 1.8 L neem extract. Weakness, swift movement and eventually death are reactions observed during excitation. There was an increment in the mortality rate at 24 hours intervals between the groups treated with different concentrations of neem extract.
These erratic and aggressive behaviors of the Nile Tilapia observed in this work agrees with the report of Ajani and Ayoola (2010)[35] who worked on *Sarotherodon gallilaeus* using *Adenia cissampeloides* extract and also Akinbulumo *et al* (2004)[36] who worked on *Clarias gariepinus* using *Derris elliptica* roots. The observed behaviors (abnormal movement, high respiration rate, restlessness) in the fish samples were due to the effect of the toxic substances from the neem extract. These rapid, aggressive and erratic behavioral changes as well as mucus secretion on gills prior to death were also reported by.[37]

*Definitive Test with Neem Extract*
The definitive test was carried out using 12.5L of dechlorinated water with the following concentrations of neem extract: 1.2L, 1.4L, 1.6L, 1.8L, 2.0L and a control. It was observed that mortality increases with increase in concentration and longer duration (Table 2).

**Table 1:** 96 h Mortality rate of *Oreochromis niloticus* for the range test

| **Concentrations (Litres)** | **MORTALITY (HRS)** | | | | |
|---|---|---|---|---|---|
| | **Test fishes** | **24hrs** | **48hrs** | **72hrs** | **96hrs** |
| 1.2 | 5 | 0 | 0 | 0 | 2 |
| 1.4 | 5 | 0 | 2 | 2 | 2 |
| 1.6 | 5 | 1 | 2 | 4 | 4 |
| 1.8 | 5 | 1 | 1 | 1 | 2 |
| 2.0 | 5 | 1 | 1 | 3 | 3 |

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

**Table 2:** 96 h of Mortality rate of *Oreochromis niloticus* during definitive test

| Concentrations (Litres) | MORTALITY (HRS) | | | | |
|---|---|---|---|---|---|
| | Test fishes | 24hrs | 48hrs | 72hrs | 96hrs |
| 0.0 | 30 | 0 | 0 | 0 | 0 |
| 1.2 | 30 | 2 | 4 | 5 | 6 |
| 1.4 | 30 | 2 | 4 | 8 | 9 |
| 1.6 | 30 | 3 | 9 | 12 | 13 |
| 1.8 | 30 | 13 | 16 | 23 | 26 |
| 2.0 | 30 | 14 | 23 | 27 | 30 |

*Bioaccumulation of Neem Extract*

The result showed that the concentration of the exposure dose had effect on the bioaccumulation of neem extract at day 14 of the experiment. The mean neem extract point in the serum of fish and water media exposed to neem extract was found to be concentration-dependent at day 14$^{th}$ of the experiment.

*Probit analysis at 96 h $LC_{50}$*

Table 3 shows the percentage mortality from the definitive test which was subjected to probit analysis to generate the probit value of the response and to determine the $LC_5$, $LC_{50}$ and $LC_{95}$ respectively. The log concentration and probit values observed in the definitive test at 96 hours was used to generate the lethal concentration at 96hours $LC_{50}$ (Figure 1). The fish samples became inactive with longer exposure to the extract and at higher concentration. The lethal toxicity values of the neem extracts obtained in this study indicates its potent toxic effects on *Oreochromis niloticus*.

**Table 3:** Probit analysis data

| Concentrations (Litres) | Log Concentrations | Number of fishes | Observed responses | Percentage mortality | Probit |
|---|---|---|---|---|---|
| 0.0 | 30 | 0 | 0 | 0 | 0 |
| 1.2 | 30 | 2 | 4 | 5 | 6 |
| 1.4 | 30 | 2 | 4 | 8 | 9 |
| 1.6 | 30 | 3 | 9 | 12 | 13 |
| 1.8 | 30 | 13 | 16 | 23 | 26 |
| 2.0 | 30 | 14 | 23 | 27 | 30 |

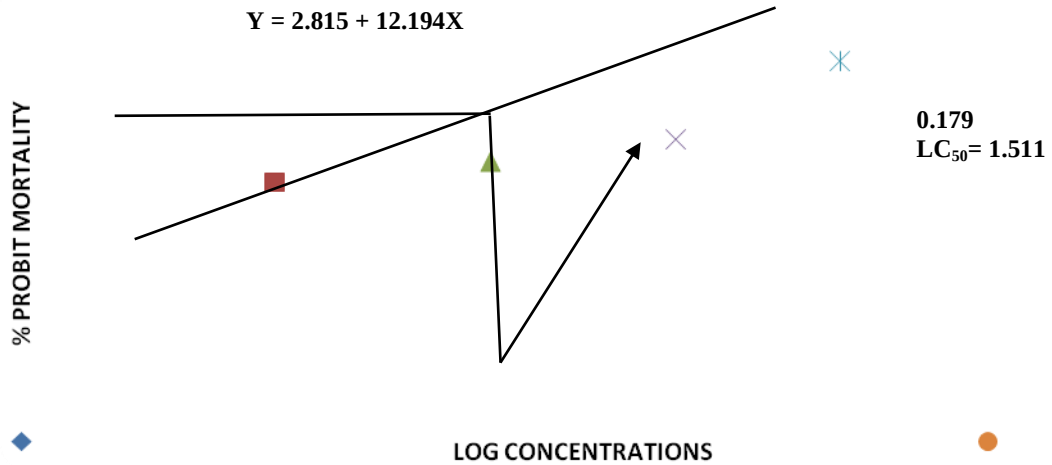



**Figure 1:** Lethal Concentrations ($LC_{50}$) at 96hours

**Table 4:** Mean and standard deviation of nuclear abnormalities

| Conc. (Litres) | MN | BN | LB | BL |
|---|---|---|---|---|
| 0.0 | $1.00 \pm 1.0^{a}$ | $0.00 \pm 0.5^{a}$ | $0.0 \pm 0.0^{a}$ | $0.00 \pm 0.0^{a}$ |
| 0.60 | $2.0 \pm 1.0^{ab}$ | $0.33 \pm 0.6^{a}$ | $1.0 \pm 1.0^{a}$ | $0.00 \pm 0.0^{a}$ |
| 0.80 | $2.3 \pm 1.5^{ab}$ | $0.67 \pm 1.2^{a}$ | $3.0 \pm 1.0^{b}$ | $3.00 \pm 1.7^{ab}$ |
| 1.00 | $3.7 \pm 2.1^{ab}$ | $1.67 \pm 1.2^{a}$ | $2.7 \pm 0.6^{b}$ | $5.00 \pm 1.7^{b}$ |
| 1.20 | $5.0 \pm 1.0^{b}$ | $1.67 \pm 0.6^{a}$ | $5.0 \pm 1.0^{c}$ | $5.67 \pm 2.5^{b}$ |

*MN – micro-nucleated cell; BN – binucleated cell; LB – lobbed cell; BL – blebbed

**Mean with the same superscript in the same vertical column are not significantly different at ($p>0.05$) from each other.

This is in line with the findings of [38] who reported that neem plants contain active compounds that are potentially lethal to fish. The $LC_{50}$ ($1.511g^{-L}$) of neem extract expressed at 96 hours was higher than that reported by [13] in *Cirrhinusm rigala* (1.035 g/L) and lower than that reported by [24] in *Prochilodus lineatus* (4.80 g/L). However, the result obtained is in conformity with the findings of [38] who reported that differences in lethal toxicity could be as a result of differences in fish species, age, sex, size, water quality and even methodology or the origin and part (s) of the neem tree used. [20]

*Cytotoxicity and Nuclear abnormality on Blood Serum of Oreochromis niloticus Exposed to Neem extract at Day 14 and 28*

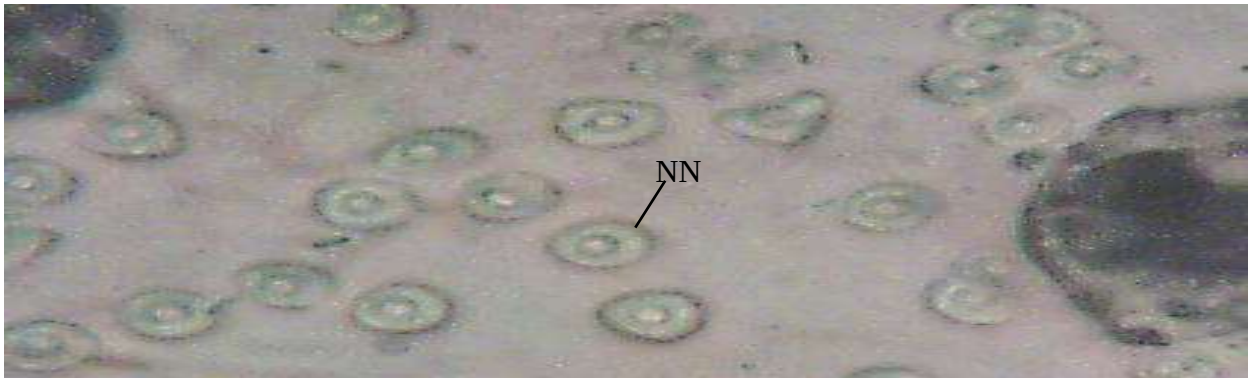
The Neem extract induced significant increase in percentage frequency of micro-nucleated erythrocytes in peripheral blood of *Oreochromis niloticus* at the different exposure durations while at 0.00ml, the nucleus cells are normal (Plate 1).

**Table 5:** Mean and standard deviation of nuclear abnormalities

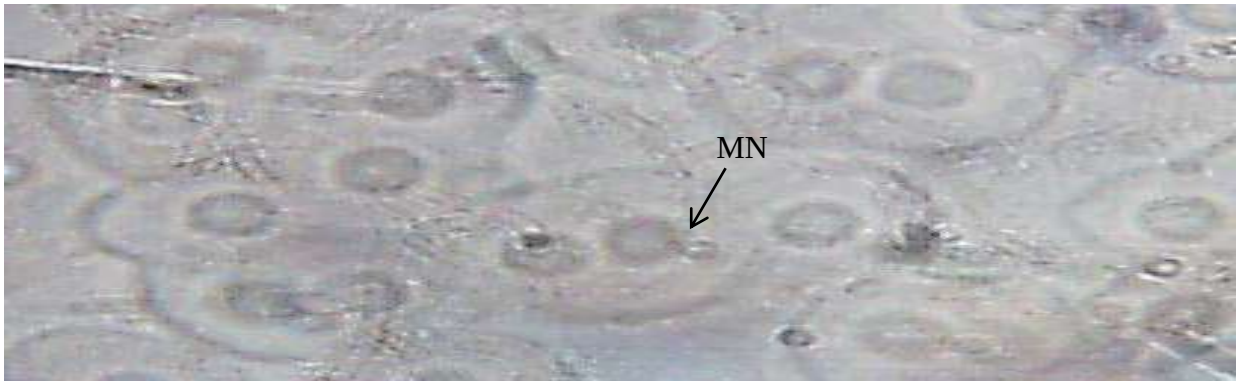
| Conc. (Litres) | MN | BN | LB | BL |
|---|---|---|---|---|
| 0.0 | $1.67 \pm 1.2^{a}$ | $0.00 \pm 0.0^{a}$ | $0.00 \pm 0.0^{a}$ | $0.00 \pm 0.0^{a}$ |
| 0.60 | $3.00 \pm 1.0^{ab}$ | $2.67 \pm 1.5^{ab}$ | $3.67 \pm 1.2^{b}$ | $2.00 \pm 1.0^{a}$ |
| 0.80 | $4.00 \pm 1.0^{ab}$ | $5.00 \pm 2.7^{bc}$ | $5.33 \pm 1.2^{b}$ | $3.67 \pm 1.2^{a}$ |
| 1.00 | $6.67 \pm 2.1^{ab}$ | $5.33 \pm 1.2^{bc}$ | $7.0 \pm 1.0^{b}$ | $6.00 \pm 3.0^{a}$ |
| 1.20 | $11.0 \pm 6.9^{b}$ | $7.67 \pm 2.5^{c}$ | $7.0 \pm 2.7^{b}$ | $10.67 \pm 4.5^{b}$ |

*MN – micro-nucleated cell; BN – binucleated cell; LB – lobbed cell;BL – blebbed
**Mean with the same superscript in the same vertical column are not significantly different at ($p>0.05$) from each other.

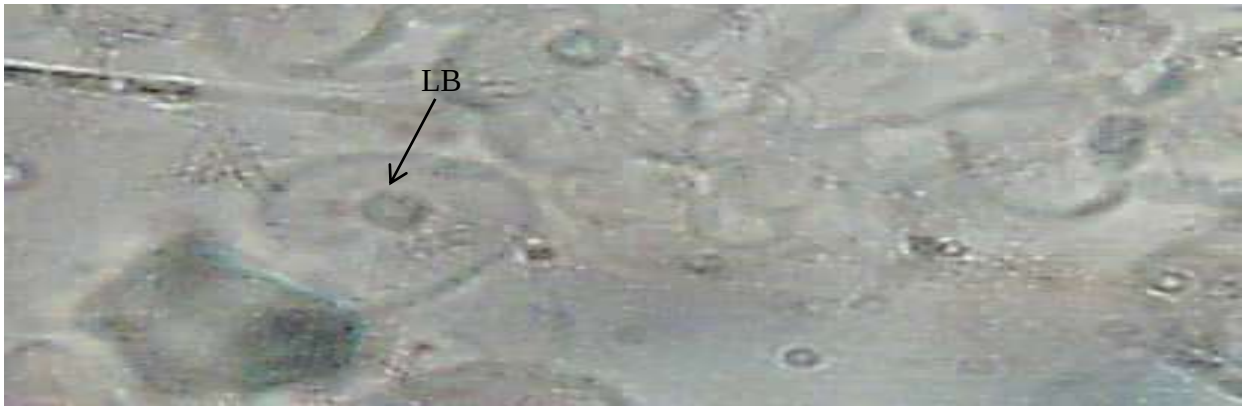
The micronucleus aberration (Plate 2) and lobbed aberration (Plate 3) recorded the highest range at concentration of 1.2L and the lowest range at concentration of 0.00ml (control). The analysis of variance indicated a significant difference (day 14 = 0.041; day 28 = 0.048) in the toxic effect of Neem extract on *Oreochromis niloticus* (Table 4). While the Student–Newman–Keuls analysis showed significant difference between the control and the test media. The clastogenic effect of neem extract observed in this study is in line with the report of [25] in the root tip meristems of *Alium cepa* and also agrees with the findings of [33] who reported the effects of *Pendimethalin* on maize and onion. The aberrations produced by neem extract on the erythrocyte nucleus of *Oreochromis niloticus* could be used as a biomarker in water pollution. The increased cellular apoptosis observed in this study is in support with the findings of [25] who reported that silver nano-particles induce endoplasmatic reticulum stress response in Zebrafish.




Mg: x1000 (Oil Immersion)
**Plate 1:** Erythrocytic of *O. niloticus* (normal nucleus - NN) cells at 0.00ml concentration of *Azadirachta indica A. Juss* extract.




Mg: x1000 (Oil Immersion)
**Plate 2:** Erythrocytic of *O. niloticus* cell (micro-nucleated - MN) at 1.2L concentration of *Azadirachta indica A. Juss* extract




Mg: x1000 (Oil Immersion)
**Plate 3:** Erythrocytic cell of *O. niloticus* (lobbed cell- LB) at 1.2L concentration of *Azadirachta indica A. Juss* extract.


Mg: x1000 (Oil Immersion)

**Plate 4:** Erythrocytic cell of *O. niloticus* (binucleated cell - BN) at 1.2L concentration of *Azadirachta indica A. Juss* extract

The highest mortality rate was recorded at 2.0L concentration of neem extract; this is in line with that reportedin Nile Tilapia by [37] and [39] in juvenile of Atlantic salmon exposed to neem leave extracts. The nuclear aberrations observed in this study signifies that neem leave extract is a potential mutagen, this is in agreement with the findings of [11, 28,] who reported that electro-negativity of neem leave extract is of the same magnitude with DNA-reactive molecules, which implies that neem extract is a potential mutagen. The highest range of bi-nucleated aberration (Plate 4) was recorded at a concentration of 1.2L and the lowest range at concentration of 0.00ml (control). The analysis of variance indicated that there was no significant difference at day 14 (0.096); while day 28 (0.005) showed significant difference in the toxicity effect of Neem extract on *Oreochromis niloticus.* Student–Newman–Keuls analysis showed no significant difference between the control and the test media.The highest range of blebbed aberration was recorded at a concentration of 1.2L and the lowest range at concentration of 0.00ml (control). The analysis of variance indicated that there was a significant difference (day 14=0.003; day 28= 0.004) in the toxicity effect of Neem extract on *Oreochromis niloticus* and the Student–Newman–Keuls analysis showed significant difference between the control and the test media.

The results reported above are imperative to evaluate the functional and morphological responses in fishes exposed to sub-lethal concentrations of plant extracts used in aquaculture.

## Conclusion

The results obtained in this study revealed that *Azadirachta indica A. Juss* (neem leave) extract has clastogenic effects on the erythrocytes of *Oreochromis niloticus* (*O. niloticus*) post juveniles. Neem extract equally has cellular apoptotic effect on the erythrocytes of *Oreochromis niloticus* and increases mortality rate of *Oreochromis niloticus* within 96 hours of exposure. Neem extract causes morphological, functional and histopathological changes in the fish. Therefore, the use of *Azadirachta indica A. Juss* extracts at the experimented concentrations for the treatment of fish diseases should be discouraged. However, Further works are suggested to be carried out in order to provide information for appropriate methods of application and to determine patent criteria for its cautious use in fish-farming for future use particularly in aquaculture facilities.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

## References

1. Food and Agricultural Organization (FAO). The State of World Fisheries and Aquaculture. Rome. 2014;223p.
2. Ubiogoro OE, Alarape SA, Saka AB, Adeyemo OK. Growth performance and sensory parameters of African catfish (*Clarias gariepinus*) fed with sublethal dose of neem leaf extracts and its antibacterial effects. Vet Arch. 2019; 89:709-721.
3. Ineyougha ER, Orutugu LA, Izah SC. Assessment of microbial quality of smoked *Trachurustrachurus* sold in some markets of three South-south States, Nigeria. Int J Food Res. 2015; 2:16–23.
4. Akinjogunla VF, Lawal-Are AO, Soyinka OO. Proximate Composition and Mineral Contents of Mangrove Oyster (*Crassostreagasar*) from Lagos Lagoon, Lagos, Nigeria. Nig J Fisheries Aquac. 2017; 5:36–49.
5. Moruf RO and Akinjogunla VF. Concentration of Heavy Metals in Sediment of Two Interconnecting Brackish/Freshwater Lagoons and the Bioaccumulation in the Crustacean, Farfantepenaeus notialis (Pérez-Farfante, 1967). J Fisheries Environ. 2019; 43:55-62.
6. Akinjogunla VF and Lawal-Are AO. Seasonal Assessment of the Impacts of Heavy Metal Deposits In (Adanson, 1757) from the Mangrove Swamp of The Lagos Lagoon Lagos. NigJ Exp Res. 2020; 8:21–31.
7. Ghosh V, Sugumar S, Mukherjee A, Chandrasekaran N. Neem (*Azadirachtaindica*) oils Essent Oils Food Preserv Flavor Safety. 2016; 67:593-599.
8. Seriana I, Akmal M, Wahyuni S. Neem leaves extract (*Azadirachtaindica* A. Juss) on male reproductive system: a mini-review. Earth Environ Sci. 2019;399 (1): 012106.
9. Gupta SC, Prasad S, Tyagib AK, Kunnumakkarac AB, Aggarwal BB. Neem an Indian traditional panacea with modern molecular basis Phytomedicine, 2017; 34:14-20.
10. Daniyal M and Akram M. Antifertility activity of medicinal plants. J Chinese Med Assoc. 2015; 78:382-388.
11. Saleem S, Muhammad G, Bukhari YNA, Hussain MAA. Comprehensive review of phytochemical profile, bioactives for pharmaceuticals and pharmacological attributes of *Azadirachta indica*. Phytother Res. 2018; 32 (7): 1241 -1272.
12. Kumar VS and Navaratnam V. Neem (*Azadirachtaindica*) prehistory to contemporary medicinal uses to human kind. Asian Pac J Trop Biomed. 2013; 3: 505-514.
13. Saravanan M, Ramesh M, Malarvizhi A, Petkam R. Toxicity of neem leaf extracts (*Azadirachtaindica*A. Juss) on some haematological, ionoregulatory, biochemical and enzymological parameters of Indian Major Carp, *Cirrhinusmrigala*. J Trop Forest Environ. 2011; 1:14-26.
14. Wendelaar SE. Bonga - The stress response in fish. Physiol Rev. 1997; 7:591-625.
15. Wan MT, Watts RG, Isman MB, Strub R. Evaluation of the acute toxicity to juvenile Pacific North west salmon of

*azadirachtin*, neem extract, and neem-based products. Bull Environ ContamToxicol. 1996; 56:432–439.
16. Biswas K, Chattopadhyay I, Banerjee R, Bandyopadhyay U. Biological activities and medicinal properties of neem*(Azadirachtaindica*). Curr Sci. 2002; 82:1336–1345.
17. MartinezSO. NIM *Azadirachta indica*: natureza, ususmúltipl oseprodução. InstitutoAgronômico do Paraná (IAPAR), Londrina,PR. 2002; 134p.
18. Omoregie E and Okpanachi MA. Acute toxicity of water extracts of bark of the Neem plant, *Azadirachtaindica* (Lodd) to the cichlid *Tilapia zillii* (Gervais), ActaHydrobiol. 1997; 39:47–51.
19. Omoregie E and Okpanachi MA. Growth of *Tilapia zilli* exposed to sublethal concentrations of crude extracts of*Azadirachtaindica*,ActaHydrobiol. 1992; 34:281-286.
20. Picker MD and Griffiths CL. Alien and Invasive Animals – A South African Perspective. Randomhouse/Struik, Cape Town, South Africa. 2011; 24p.
21. Nnenna OP and Aniebo AO. Toxicity and nutritional assessment of aqueous *Azadirachtaindica*(neem) leaf extract in broiler chicks, Int J Biosci. 2013; 3:172-180.
22. Cruz C, Machado-Neto JG, Menezes ML. Toxicidadeaguda do inseticidaPrationmetilico e do biopesticidaazadiractina de folhas de neem (*Azadirachtaindica*) paraalevino e juvenile de pacu (*Piaractusmesopotamicus*). Pesticidas: R. Ecotoxicol. eMeioAmbiente. 2004; 14:92-102.
23. Aruwayo A and Maigandi SA. Neem (*Azadirachta indica*) seed cake/kernel as protein source in ruminants' feed. Am J Exp Agric. 2013;3 (3): 482-494.
24. Tiwary MK and Pandey A. Feeding neem (*Azadirachtaindica*) products to small ruminants as anthelmentics. Food SciTechnolLett. 2010; 1:10 -17.
25. Shailender M, Suresh-Babu CH, Rajagopal RS. Toxic effect of Neem, *Azadirachtaindica*plant bark on growth and survival of freshwater catfish,*Pangasiushypophthalmus*. Int J ToxicolApplPharmacol. 2013; 3:39-43.
26. Oniovosa UE, Aina OO, Alarape SA, Babalola OE, Adeyemo OK. Effects of Neem Leaf Aqueous Extract on Organ Histology, Haematological Parameters and Biochemical Indices in Catfish.Alex J Vet Sci. 2017; 54:17-24.
27. Trivedi SP. Phytopiscicides: A hidden threat for fish biodiversity. Proceedings of 22nd All India Congress of Zoology and national Seminar on Recent Advances in Biological Sciences: Biodiversity and human Welfare, (2011) 29-31,Department of Zoology, University of Lucknow, Lucknow and Zoological Society of India, Bodhgaya. (2011), 29 – 31
28. Ekaluo UB, Ikpeme EV, Udensi O, Markson AA, Umana EJ, Madunagu BE, Omosun G. Effect of aqueous leaf extract of neem (*Azadirachtaindica*) on the hormonal milieu of male rats. Int J Curr Res. 2011; 4:001-003.
29. Tiwari S, Verna AK, Chakraborty S, Dharma K, Singh VC. Neem(*Azadirachtaindica*) and its potential for safe guarding health of animals and human.J Biol Sci. 2014; 14:110-123.
30. Lue X, Ma Y, Wu S, Wu D. Two novel azadirachtinderivates from *Azadirachtaindica*. J Nat Prod. 1999; 62:1022-1024.
31. Abdullahi UA and Mukhtar UY. Appraisal of the current fish composition, abundance and operative fishing gears in Thomas dam, Danbatta, Kano state and Daberam Dam Daura, Katsina state. Bayero J Pure Appl Sci. 2015; 8:6-9.
32. Abu N and Mba K. Mutagenicity testing of pharmaceutical effluents on Allium cepa root tip meristems. J Toxicol Environ Health Sci. 2011; 3:44-51.
33. Promkaew N, Soontornchainaksaeng P, Jampatong S, Rojanavipart P. Toxicity and Genotoxicity of Pendimethalin in maize and onion. Kasetsart J Nat Sci. 2010; 44:1010–1015.
34. Christen V, CapelleM,Fent K. Silver nanoparticles induce endoplasmatic reticulum stress response in zebrafish. ToxicolApplPharmacol. 2013; 272:519–528.
35. Ajani EK and Ayoola SO. Acute toxicity of Piscicidal Plant Extracts (*Adeniacissampeloides*) on Tilapia (*Sarotherodongalilaeus*) Juveniles. Iranica J Energ Environ. 2010; 1:246–254.
36. Akinbulumo MO, Fagbenro OA, Fasakin EA. Acute toxicity of ethanolic extract of Derris Elliptica roots to African catfish (*Clariasgariepinus*,Burchell, 1822) fingerlings.Biol Environ Sci J Trop. 2004; 2:126–131.
37. Ayoola SO, Kuton MP, Idowu AA, Adelekun AB. Acute toxicity of Nile tilapia (*Oreochromisniloticus*) juveniles exposed to aqueous and ethanolic extracts of Ipomoea aquatic leaf. Nat Sci. 2011; 9:91-99.
38. Ayoola SO and Akaeze CO. Genotoxic Evaluation and Toxicity of Spent Engine Oil on *Clariasgariepinus*. Res J Environ Toxicol. 2012; 6:133-141.
39. Farmen E, Mikkelsena HN, Evensenc Ø, Einset J, Heier LS, Rosselanda BO, Salbua B, Tollefsena KE, Oughtona DH. 'Acute and sub-lethal effects in juvenile Atlantic salmon exposed to low µg/L concentrations of Ag nanoparticles.Aqua Toxicol. 2012; 108:78-84.