# BIOCHEMICAL EFFECTS OF AQUEOUS EXTRACT OF *Azadirachta indica* ON *Clarias gariepinus*

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**ABSTRACT**

The study investigates the impact of sublethal toxicity in *Clarias gariepinus* following the administration of aqueous extracts of *Azadirachta indica* leaves via intramuscular injection, with a specific focus on biochemical factors like protein and glucose. For the experiment, juvenile *C. gariepinus* raised in tanks were used. They were placed in a large 140L plastic container containing clean borehole water for a 14-day acclimatization period and were fed commercial fish feed pellets twice daily until they were full. The research utilized various test concentrations (0.5, 1.0, 2.0, 4.0, and 0.00g/l) over a 7-day period. Following the experimental period, the fish were euthanized to analyze biochemical parameters such as total protein and glucose levels in the fish. The levels of total protein in the serum with percentage change of 60.18%, 63.55%, 65.38% and 75,97%, muscles with percentage change of 3.92%, 9.80%, 69.28% and 116.01%, and kidney with percentage change of 44.18%, 26.55%, 45.38% and 42.97% increased with higher concentrations of the plant, while there was a significant decrease ($p<0.05$) in glucose levels in the muscles with percentage change of 15.95%, 44.87%, 51.71 and 51.14% and a significant increase ($p<0.05$) in the serum with percentage change of 3.56%, 70.56%, 73.78% and 76.67% and kidney with percentage change of 9.15%, 23.56%, 26.78% and 34.75%. Based on the study results, it can be inferred that exposing *A. indica* to *C. gariepinus* may pose a threat to the survival of the fish species. The observed variations suggest that the toxicity of *A. indica* to *C. gariepinus* primarily impacts the fish's biochemical parameters, which in turn negatively affects their physiological well-being. These alterations can be used as indicators of the stress experienced by the fish when they are exposed to *A. indica*.

**Keywords:** *Azadirachta indica*, *Clarias gariepinus*, crude extract, biochemical response.

## Introduction

In Northern Nigeria, the neem (*Azadirachta indica*) plant known as "dogonyaro" is a member of the Milliaceae family. According to Adamu et al. (2017), the plant thrives in sub-Sahelian Nigeria, which has poor, shallow, and even salty soils and extreme dryness. Azadirachtin, meliacin, gedunin, salanin, nimbin, valassin, margosine, eriterperenoid, azatin, rotinine, and quinine are among the biologically active chemicals that have been identified from various plant sections (Adamu et al., 2017). Ancient medicinal writings have suggested the plant as a remedy for intestinal infections, diarrhea, skin sores, malaria, and infectious diseases. Numerous pharmacological effects have been attributed to every component of the neem tree, particularly its antibacterial, antifugal, antiulcer, antifeedant, repellant, pesticidal, molluscidal, ecdysone inhibitor, and sterilant qualities. It contains tetranitroterpenoid chemicals called meliatoxins, which are extremely harmful to both insects and mammals, making it a natural pesticide (Adamu et al., 2017).

In order to provide the much-needed animal protein at a cheap cost, the use of aquatic plants and weeds with high nutritional value as feed components has expanded in recent years. Another benefit of growing aquatic weeds with farmed fish species is that they share water resources and/or farm effluent (Patel et al., 2021). In semi-intensive and widespread aquaculture production, they provide as food for both herbivorous and omnivorous fish species (Patel et al., 2021). Perhaps the most worried tree in the world, *A. indica* is one of the most promising plants with chemicals recognized for their insecticidal qualities.

Water-soluble extract of *A. indica* leaves exhibited significant hypoglycemic, hypolipidemic, hepatoprotective, anti-fertility, and hypotensive properties (Patel *et al*., 2021). Fish parasites and predators cause significant production losses in freshwater fish farming, which are frequently managed by indiscriminately using hazardous pesticides. In addition to causing fish to have elevated amounts of harmful substances, this might also impair their functionality. Research on using plant-based compounds for pest management has been extensive in an effort to lessen the harm caused by the usage of synthetic poisons. Patel et al. (2021) claim that neem and its derivatives are useful in aquaculture systems for managing parasites and fish predators. Neem extract has been the subject of several studies and applications in Nigeria for a variety of reasons.

Similar to this, fish farms have made considerable use of neem leaf aqueous extract and other neem-based products as an alternate method of controlling fish parasites and fish fry predators like dragonfly larvae. Water bodies (lakes, rivers, and streams) may be impacted by the usage of this plant or its products, either directly or indirectly (Adamu et al, 2017).

African catfish are widely cultivated in Africa, Europe, and certain parts of Asia due to their hard character. It has shown to be a suitable match for aquaculture because of its high prolificacy, ease of culture, arborescent air-breathing organ, omnivorous feeding behavior, quick development rate, and high feed conversion rate (Okey *et al*., 2022). *Clarias graiepinus* is quite popular among Nigerian customers because of its amazing attributes and delectability (Okey *et al*., 2022). This fish species is most often raised in Nigerian aquaculture, and researchers have been using it as a model for studying aquatic vertebrates for about 20 years (Okey et al., 2022). These days, biomarkers are frequently used as early diagnostic indicators to assess the environmental quality of contaminated water bodies (Jessa et al., 2022).

The African Catfish (*C. gariepinus*, Family Clariidae), which is extensively cultivated in many tropical and subtropical parts of the world, is one of the fishes of interest for this study. This fish, which grows well in a variety of culture techniques, makes up the biggest category of cultivated species. There are biomarkers that show the impacts of neem (*A. indica*) on non-target organisms, such fish, long before the creature dies (Adamu, 2009; Adamu et al 2017). According to Adamu and Idris (2014), these indicators include, among other things, total protein, glucose, cholesterol, uric acid, creatinine, and triglyceride levels.

Therefore, the study was aimed at determining the effect of neem leaf powder on the selected biochemical parameters of African catfish after the seven (7) days exposure period.

## Materials and methods

### Experimental Animals

One hundred Juvenile *C. gariepinus* (mean total length 28.39 ± 0.33 cm, SE; mean weight, 99.34 ± 2.48g SE) grown in tanks were acquired locally from a commercial fish farm. They were then sent to Delta State University of Science and Technology, Ozoro, to be housed at the Marine Science Research Laboratory. The fish in the experiment were housed in 140-liter plastic aquariums and fed fresh water directly from the borehole. The fish were acclimated and given commercial fish feed pellets (Coppens feed) twice a day for a total of fourteen days, until they were satisfied. During the testing and acclimation phase, waste and uneaten food were removed daily with a hose. Any deceased fish were also immediately removed in order to prevent infection.

**Plant Material**

On the premises of Delta State University Abraka, leaves from the *A. indica* tree were collected. After then, they were recognized in the Department of Plant Science and Biotechnology's herbarium division. After being allowed to air dry for two weeks, the leaves were oven-dried for three hours at 60°C until they reached a consistent weight. Following drying, the leaves were ground into a powder using an electric blender (MX-2071, Nakai, Japan), sieved, and the fine powder kept dry and sealed in a container until needed.

**Toxicant Preparation**

The extract that had been kept was combined with distilled water to create stock solutions that included 4 grams per liter of *A. indica* aqueous solution. Subsequent dilution from this stock solution produced four distinct test concentrations (0.5, 1.0, 2.0, and 4.0 g/L) after preliminary study.

**Experimental Procedure**

After a period of acclimation, the experimental fish were divided into five (5) groups, each containing ten juvenile individuals of fish, to assess *A. indica's* sublethal effects on the biochemical parameters. The upper part of each container was sealed with a cover made of fine, 1 mm-mesh polyethylene gauze screen. Before the fish were placed in the water, 2 milliliters of the extract were injected intramuscularly above the lateral line of each fish specimen. The fish in the control group were injected with the same amount of distilled water. The fish were housed in 140-liter plastic containers for the duration of the seven-day exposure period. For both exposure and acclimatization, borehole water was used. Both the test and control groups' water quality characteristics were evaluated using industry-standard techniques (APHA, 1998).

**Sampling Procedure**

Following the seven days of exposure, the fish from the test and control tanks were taken out, sacrificed, and then the analysis described below was performed on them. Six fish were removed from each aquarium separately using a small hand net. Initial weight and length measurements were taken before the fish were gently placed on their backs to draw blood samples from the caudal circulation. The instruments were a disposable plastic syringe with heparinized 2 cm3 and a disposable hypodermic needle with gauge 21. Glass contact can reduce the coagulation period, hence it's important to use a plastic syringe while handling fish blood (Smith et al., 1952).

To avoid mucus contamination, the puncture site (which was selected to be around 3–4 cm from the vaginal entrance) was dried using tissue paper. The penetration of the needle was performed with gentle aspiration while it was introduced perpendicular to the fish's spinal column. After that, it was gradually pushed down until blood started to flow, signifying that a caudal blood artery had been ruptured by the needle.

**Determination of Biochemical Parameters**

Using a spectrophotometer (AJ-1C03 Spectrophotometer Anqing Anjue Export Ltd., China) and a commercial diagnostic kit (Randox Ltd., UK), the levels of glucose and protein in serum and tissue were determined in compliance with the manufacturer's instructions.

**Data Analysis**

The treatment mean data were statistically analyzed using one-way analysis of variance (ANOVA) to determine the significance levels of several sublethal dosages of *A. indica*. The mean comparisons were then separated using Dunnet's Multiple Comparison Test. Graphical representation of results were performed using Graph pad Prism (Version 8).

**Results**

Following a seven-day intramuscular injection of two milliliters of different quantities of the crude extract of *A. indica* leaves, the biochemical parameters of *C. gariepinus* are listed below.

**Effect on Total Protein Level**

The average total protein (TP) levels in *C. gariepinus*, which were intramuscularly injected with 2 ml of various concentrations of the crude extract of *A. indica* leaves over a period of seven (7) days, are displayed in Figure 1 and Table 1. Serum and kidney total protein levels showed a substantial increase ($p<0.05$) at all test doses. Muscle total protein levels increased significantly ($p<0.05$) at the last two test concentrations. After the fish were exposed for seven days, the total protein activity was statistically most noticeable in the serum, less noticeable in the muscle, and least noticeable in the kidney.

## Effect on Glucose Levels

Table 1 displays the average glucose level of C. gariepinus after seven (7) days of intramuscular injection of 2 ml of different concentrations of the crude extract of A. indica leaves. Serum and kidney considerably rose ($p<0.05$) at the second to fourth test concentrations, but muscle significantly reduced ($p<0.05$) in all test doses. After the fish were exposed for seven days, the muscle showed the greatest statistical activity of glucose, followed by the kidney and the serum.

**Table 1:** Effect of Crude Extract of *A. indica* leaves on Total Protein and Glucose Levels in Serum, Muscle and Kidney of *C. gariepinus*

| Parameter | Control 0.0 g/L | Experimental Regimes | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0.50 g/L | % Change | 1.00 g/L | % Change | 2.0 g/L | % Change | 4.0 g/L | % Change |
| **Serum** | | | | | | | | | |
| **Total Protein (g/L)** | 3.49 | 5.59 | -60.18 | 5.69 | -63.55 | 5.72 | -65.38 | 6.56 | -75.97 |
| **Glucose (mM/L)** | 4.50 | 4.66 | -3.56 | 7.29 | -70.56 | 7.48 | -73.78 | 7.95 | -76.67 |
| **Muscle** | | | | | | | | | |
| **Total Protein (g/L)** | 3.06 | 3.18 | -3.92 | 3.36 | -9.80 | 5.18 | -69.28 | 6.61 | -116.01 |
| **Glucose (mM/L)** | 8.78 | 7.38 | 15.95 | 4.84 | 44.87 | 4.24 | 51.71 | 4.29 | 51.14 |
| **Kidney** | | | | | | | | | |
| **Total Protein (g/L)** | 2.49 | 3.59 | -44.18 | 3.40 | -36.55 | 3.62 | 45.38 | 3.56 | -42.97 |
| **Glucose (mM/L)** | 5.90 | 5.36 | 9.15 | 7.29 | -23.56 | 7.48 | -26.78 | 7.95 | -34.75 |

**Discussion**

Biochemical indicators like proteins and glucose are used to analyze fish and their surroundings in order to determine the health of the fish (Sayed *et al.*, 2022). Fish under stress need more protein to meet their higher energy needs, which are caused by increased physical activity, biotransformation, and xenobiotic excretion (Adamu et al., 2017). The biochemical reaction of *C. gariepinus* to sublethal dosages of *A. indica* aqueous extracts over a seven-day period showed an intriguing pattern that led to metabolic changes.

Protein is necessary for all living things to operate because hormones and enzymes, which are also types of protein, control every biological activity. Therefore, assessing the protein concentration might be a diagnostic method to ascertain the cell's physiological state. Due to abnormalities in protein metabolism brought on by exposure to dangerous substances, experimental fish's kidney and muscle may show a decrease in total protein content.

The primary function of proteins is in cell architecture. The primary source of nitrogenous metabolism is proteins (Adamu and Kori-Siakpere, 2011; Adamu *et al.*, 2017). Accordingly, the change in total protein levels observed in this study was corroborated by previous research (Adamu and Kori-Siakpere, 2011; Adamu *et al*., 2017), which hypothesized that the change value might be caused by protein hydrolytic activity as a result of increased protease production. Thus, the test fish's method of action was comparable to that of tobacco leaf powder, as described by Adamu and Kori-Siakpere (2011). This plant powder caused a stress-induced effect on protein synthesis, resulting in changes in blood total protein, kidney, and muscle total protein.

This result is comparable to previous reports by Saravanan *et al.*, (2010) on *Labeo rohita* and Mousa *et al*., (2008) on African Catfish. Adults of *C. gariepinus* exposed to aqueous and ethanolic extracts of *Parkia biglobosa* pods demonstrated increased protein content in both blood and tissue levels due to the higher concentration of plant extract (Samson *et al*., 2011). Additionally, Adamu *et al*., (2017) speculate that increased de novo synthesis, lower plasma water content, or relative changes in blood protein mobilization might account for the increase in protein concentration seen in fish exposed to the ethanolic extract.

This might possibly be the exposed fish's response to the growing demand for detoxification from hazardous chemicals. According to Omitoyin (2007), fish with hyperproteinemia may also be displaying physiological sensitivity to these toxic substances and indications of a strong immunological response. According to Kamal and Omar (2011) and Yaji *et al.* (2011), protein levels rose and were similar to the serum protein of the experimental fish. This implies that the fish could have adjusted to their new surroundings by increasing their metabolic activity, changing the kind and quantity of certain enzymes involved in energy metabolism, and altering the pace at which proteins are synthesized in response.

The study's findings of low serum glucose are consistent with those of Mousa *et al*., (2008) and Adamu *et al*., (2017). Fish exposed to plant leaf powder may have high kidney glucose, which might be a sign that the liver is unable to perform its role in glycolysis efficiently, decreasing the amount of glucose in the muscle and serum. Therefore, it can be concluded that the plant has an impact on glucose metabolism because as the concentrations of the plant powder increased, the liver was unable to efficiently use the glucose produced, which raised its liver concentration and caused values in the serum and muscle to decrease (Adamu *et al*., 2017). The primary energy source under stressful situations is carbohydrate. As a result, the pollutants caused the fish in the study to have significantly lower muscle glucose levels. When juvenile *Oreochromis aureus* fish were exposed to phenol, Mohamed and Gad (2008) and Abdel-Hameid (2007) saw comparable drops in glucose levels. A drop in glucose levels might be a sign of a problem with the way carbohydrates are metabolized, which would result in the small intestine not absorbing enough soluble glucose. The observed decrease may indicate higher energy use as a result of stress caused by the pollution. As demonstrated in adult *C. gariepinus* fish exposed to ethanolic and aqueous extracts of *Parkia biglobosa* pods, the increase in plant concentration is accompanied with a corresponding rise in blood and tissue glucose levels (Samson *et al.*, 2011). According to Adamu *et al.* (2017), increased energy requirements may result in a considerable increase in plasma glucose levels, which may then raise the synthesis of corticosteroids and catecholamines in the plasma. It has been demonstrated that these substances result in the overproduction of adrenaline, which inhibits the adrenal medulla's neuroeffector sites and promotes the conversion of glycogen to glucose. According to Adamu *et al.*, (2017), these increases might possibly be the result of the fish's enhanced gluconeogenesis in response to stress.

When under stress, the primary energy source is carbohydrates. Consequently, the presence of pollutants led to a considerable drop in the muscle glucose levels of the fish subjects in the research. Abdel-Hameid (2007) and Mohamed and Gad (2008) observed similar decreases in glucose levels in juvenile Oreochromis aureus fish exposed to phenol. Reduced blood glucose levels might be a sign of a carbohydrate metabolism issue, which would prevent the small intestine from absorbing enough soluble glucose. This decrease might also indicate higher energy use as a result of stress from the pollution. As demonstrated in adult *C. gariepinus* fish exposed to ethanolic and aqueous extracts of *Parkia biglobosa* pods, the increase in plant concentration is accompanied with a corresponding rise in blood and tissue glucose levels (Samson *et al.*, 2011). Increased energy requirements may result in a substantial increase in plasma glucose levels, which may raise the synthesis of corticosteroids and catecholamine in the plasma. It has been demonstrated that these substances produce too much adrenaline, which blocks the adrenal medulla's neuroeffector sites and promotes the conversion of glycogen to glucose. According to Adamu *et al*., (2017), these increases might possibly be the result of the fish's enhanced gluconeogenesis in response to stress.

## Conclusion

The study highlights how the test fish, *C. gariepinus*, are negatively affected biochemically and in terms of health when low concentrations of water-based extracts of *A. indica* are administered. As the *A. indica* extract concentrations rise, these adverse effects become more noticeable. Fish ponds must carefully regulate this toxin to protect fish and other aquatic life. The results of this study indicate that the fish underwent stress after receiving an intramuscular injection of *A. indica* crude extract.

## References

Abdel-Hameid, N. A. H. (2007). Physiological and histopathological alterations induced by phenol exposure in *Oreochromis aureus* juveniles. *Turkish J. Fisheries & Aqua. Sci.* **7**: 131-138.

Abdel-Hameid, N. A. H. (2009). A protective effect of calcium carbonate against arsenic toxicity of the Nile catfish, *Clarias gariepinus. Turkish J. Fisheries & Aqua. Sci.* **9**: 191–200.

Adamu, K. M. and Kori-Siakpere, O. (2011). Effects of sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust on some biochemical parameters of hybrid catfish (*Clarias gariepinus* and *Heterobranchus bidorsalis*). *Brazilian Arch. Biol. & Tech.* **54**(1): 183-196

Al-Attar, A. M. (2005). Biochemical effects of short-term cadmium exposure on the freshwater fish, *Oreochromis niloticus*. *J. Biol. Sci.* **5(3):** 260-265.

American Public Health Association (APHA) (1998). Standard Methods for the Evaluation of Water and Waste Water. 20th Edn., *American Public Health, Washington D.C.* 270-274.

Bhure, D. D., Nanware, S. S. and Mali, R. P. (2011). Effect of CuSO4 on protein content of *Channa punctatus. J. of Exp. Sci.* **2(7):** 36-37.

Das, B. K., Mukherjee, S. C., and Murjani, O. (2002). Acute toxicity of neem *(Azadirachta indica)* in India major carps. *J. Aqua. Trop.*, **17**: 23-33

Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture* **500**: 237–242.

Gill, T. S., Tewari, H. and Pande, J. (1990). Sub-lethal effects of an organophosphorus insecticide on certain metabolic levels in a fresh-water fish, *Puntius conchonius Hamilton. Pest. Biochem. Physiol*. **36**: 290-299.

ICAR, World Neem Conference Souvenir, (1993). ICAR, Bangalore, India.

Jessa. O. E., Okagabre. A., David I., Ojotule, P. E. and Fejor E.I. (2022). Haematological response of *Clarias gariepinus* to crude extract of *Azadirachta indica. African Scientist*, **23(4):**274-282.

Kamal, S. M. and Omar, W. A. (2011). Effect of different stocking densities on hematological and biochemical parameters of silver carp, *Hypophthalmichthys molitrix* fingerlings. *Life Sci. J.*, **8(4):** 580 – 586.

Lavanya, S., Ramesh, M., Kavitha, C., and Malarvizhi, A. (2011). Hematological, biochemical and ionoregulatory responses of Indian major carp Catla catla during chronic sublethal exposure to inorganic arsenic. *Chemosphere* **82**: 977–985.

Mohamed, F. A. S. and Gad, N. S. (2008). Environmental pollution-induced biochemical changes in tissues of *Tilapia zillii*, *Solea vulgaris and Mugil capito* from Lake Qarun, Egypt. *Global Vet.* **2 (6):** 327 – 336.

Omitoyin B. O., (2007). Plasma biochemistry changes in *Clarias gariepinus* (Burchell 1822) fed poultry litter. *Asian J. Anim. Sci*. **1(1):** 48-52.

Ramesh, M., Thilagavathi, T., Rathika, R., and Poopal, R. K. (2018). Antioxidant status, biochemical, and hematological responses in a cultivable fish Cirrhinus mrigala exposed to an aquaculture antibiotic Sulfamethazine. *Aquaculture*, **491**: 10–19.

Samson E. A., King A. N. E. and Shodeinde V. O. S. (2011). Evaluation of biochemical changes in *Clarias gariepinus* adults exposed to aqueous and ethanolic extracts of *Parkia biglobosa* pods. *African J. Biotech.* **10 (2):** 234-240

Sayed AE-DH, Taher H, Soliman HAM and Salah El-Din AE-D (2022), Immunological and hematobiochemical effects on catfish (*Clarias gariepinus*) exposed to dexamethasone. *Front. Physiol.* **13**:1018795. doi: 10.3389/fphys.2022.1018795

Winkaler, E. U., Santos, T. R. M., Machdo-Neto, J. G and Martinez, C. B. R, (2007).Acute lethal sublethal effect of neem leaf extract on the Neotropical freshwater fish *Prochilodus lineatus*. *Comp. Biochem. and Physiol.*, **145**: 236-244.

Yaji, A. J., Auta, J., Oniye, S. J., Adakole, J. A. and Usman, J. I. (2011*)*. Effects of cypermethrin on behaviour and biochemical indices of fresh water fish *Oreochromis niloticus*. *Elect. J. of Environ., Agric. and Food Chem.* **10 (2):** 1927-1934.

Sogbesan, A. O. and Ugwumba, A. A. A. (2008) Nutritional values of some non-conventional animal protein feed stuffs used as fishmeal supplement in aquaculture practices in Nigeria *Turkish Journal of Fisheries and Aquatic Sciences,* **8**: 159-64.

Okey, I. B., Igiri, M. R. Ekpenyong, J. J. and Inya, F. U. (2022) Haematological and Biochemical responses in African catfish, (*Clarias gariepinus*) juveniles immobilized with clove basil, (*Ocimum gratissimum*) powder Anaesthetic. *J Aquat Sci Oceanogr* **3**: 1022.

Adamu, K.M. , Aliyu-Paiko, M., Abdullahi, F. and Mustapha, A.Y. (2017). Effects of *Azadirachta indica* Leaf Powder on some Biochemical Parameters of the African Catfish (*Clarias gariepinus*). *Nigerian Journal of Basic and Applied Science*, **25**(2): 41-50.

Patel, B., Bhargava, D. and Vyas, V. (2021). Effects of *Azadirachta indica* leaf powder supplementation on growth and survival of fish *Ctenopharyngodon idella* fingerlings. *International Journal of Fisheries and Aquatic Studies,* **9**(2): 206-209.

Adamu, K. M. and Ademuyiwa, S. A. (2014). The effect of Moringa oleifera leaf dust on selected biochemical parameters of hybrid catfish. *Nigeria Journal of Technological Research*, **9**(2): 69-77.

Adamu, K. M. (2009). Sublethal effects of tobacco (Nicotiana tobaccum) leaf dust on enzymatic activities of Heteroclarias (a hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*). *Jordan Journal of Biological Sciences*, **2**(4): 151-158