

Malondialdehyde and Ascorbic Acid Activity of *Clarias Gariepinus* Cultivated in Crude Oil Contaminated Sediment

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Abstract

This study examined how crude oil from an oil exploration business in Delta State affected juvenile African catfish's blood, gills, kidney, heart, and liver. 25 juvenile African catfish were employed in the study, divided into 5 groups (5 per treatment) and used for the study. The juveniles were exposed to five concentrations of crude oil (0%, 0.1%, 0.3%, 0.5%, and 1%) for a period of 9 days. At the end of the test period, anti-oxidant activities were carried out on the blood, kidney, heart, and liver of the juveniles. The results of antioxidant parameters revealed a significant difference ($p < 0.05$) between the control and exposed groups, which according to previous studies indicates oxidative stress. Malondialdehyde (MDA) content increased in the serum of exposed groups except 0.5% crude oil contaminated group when compared with the control group. Ascorbic acid content decreased in the serum of all exposed groups when compared with the control group. In the control stock, none of the juveniles displayed any degeneration. In conclusion, this research has shown that exposing juvenile *Clarias gariepinus* to crude oil, even at low concentrations, may cause antioxidant alterations in the fish's blood, kidney, heart, liver, and gills.

Keywords: *Crude oil; Juvenile Clarias gariepinus, Malondialdehyde, Ascorbic acid, Anti-oxidant.*

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Background to the Study

Petroleum (predominantly hydrocarbons) discharges have adversely affected human health, and degraded communities in oil producing states, which has negatively impacted the regional economy, and caused socio-economic problems in Niger Delta (Edino *et al.*, 2010). Oil spill amongst other effect result in the interference with natural aeration of water due to the blanketing of water surface by oily film which could deplete the dissolved oxygen content of the water, aquatic lives are destroyed and the contamination of upland surface underground sources of water supply (Ogeleka *et al.*, 2017). Catfish not only provides food for the people, but it also allows for better protein nutrition because it has a high biological value in terms of high protein retention in the body, higher protein assimilation when compared to other protein sources, low cholesterol content, and is one of the safest animal protein sources (Daniel, 2018). The habitat of fishes is water bodies, hence physiological changes in fishes reflects physical and chemical changes in aquatic environment (Gebrekiros, 2016). Anti-oxidant changes in fishes exposed to contaminants have been proposed and used as biomarkers for pollutants such as petroleum products (Eseigbe *et al.*, 2013).

Lipid peroxidation is when free radicals "steal" electrons from the lipids in cell membranes, causing cell damage. Reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), are the end products of lipid peroxidation. The latter is also known as the "second messenger of free radicals" and a major bioactive marker of lipid peroxidation due to its numerous biological activities that are similar to those of reactive oxygen species. In addition, end-products of lipid peroxidation may be mutagens and carcinogenic (Anthonio *et al.*, 2014).

Vitamin C (ascorbic acid) is a powerful antioxidant and necessary nutrient for healthy growth and maintenance (Reang *et al.*, 2021). Another advantage of vitamin C, which has been demonstrated in a variety of animal species, including fish, is that it improves the non-specific immune response (Collins *et al.*, 2021). It also prevents oxidative renal and brain damage induced by stress and secures the body tissues against toxic effects of heavy metals by efficiently metabolizing these toxicants.

The liver's propensity to detoxify and store toxic substances makes it a popular environmental biomarker (Stori *et al.*, 2014). In fish, the kidneys are one of the bodily organs in charge of excretion and water balance regulation (Byron, 2014). The heart circulates blood (Graham and Dickson, 2004). Direct air oxygen absorption is possible through the gills (Byron, 2014). It is widely known that several research on the contact between pollutants and African Catfish (*Clarias gariepinus*) have been carried out. The effects of crude oil generated by Warri Refining and Petrochemical Company (WRPC) on the blood, liver, gills, kidney, and heart of juvenile African Catfish have not yet been investigated. The business is near a water body; it is vital to look at its consequences.

Materials and Methods

Collection and Accompaniment of Specimens

An open gallon half-filled with fresh tap water was used to transport 25 healthy *Clarias gariepinus* juveniles, each of which was 6 weeks old and weighed an average of 0.2 kilograms,

from a nearby fish farm in Delta State (AB farms, Agbarho). Each group of five juveniles were placed in five distinct bioreactors, each of which had a capacity of 25 liters and contained 5 kg of soil and 20 liters of water within Department of Petroleum Resources limit.

Ethical Disclaimer

With unlimited access to balanced pellet food and water, the animals were kept in typical laboratory settings. The experiment methodology was authorized by the ethics committee of the Federal University of Petroleum Resources, Effurun (FUPRE), Nigeria (FUPRE/ECC2019/SC/EMT001), and all moral rules for the use of animals in research were adhered to.

Clarias Gariepinus Experimental

Five groups of *Clarias gariepinus* were identified;

Group A: *Clarias gariepinus* grown on soil with no traces of crude oil

Group B: *Clarias gariepinus* grown in soil with a 0.1% crude oil contamination level.

Group C: *Clarias gariepinus* grown in soil with a 0.3% crude oil contamination level.

Group D: *Clarias gariepinus* grown on soil with 0.5% crude oil contamination.

Group E: *Clarias gariepinus* grown on soil with a 1.0% crude oil.

Tissue Homogenate and Serum

The fish were slaughtered, and their tissues (liver, kidney, gills, and hearts) were taken and placed in a beaker containing an ice cold 0.25M sucrose solution. The blood was collected using a heart puncture. Each blood sample was then centrifuged at 3,500rpm for roughly 15 minutes using chilled centrifuge RC650s, and the serum recovered was stored at -8°C until needed. The separated tissues were weighed, then a part of each tissue was taken out, diced into very small bits, and homogenized in an ice-filled dish using a pre-cooled pestle and mortar. The tissue homogenates were diluted 1:30 with 0.25M sucrose solution. The diluted homogenates were kept at -8°C.

Antioxidant Assay

The procedure outlined by Bird et al. (1982) was used to determine the MDA levels in the fish serum and tissues. MDA, which is produced when polyunsaturated fatty acids break down, functions as an easy way to gauge how much peroxidation has occurred. Thiobarbituric acid and MDA combine to form a red complex that absorbs light at a wavelength of 535 nm. Fish serum and tissues were tested for vitamin C using the Roe and Kuether (1943) technique. When ascorbic acid interacts with 2,4-dinitrophenylhydrazine, a colorful byproduct is produced that is absorbed at 520 nm. Ascorbic acid concentration is determined by comparing the color that standard solutions of ascorbic acid create in samples.

Statistical Examination

All data were evaluated using Analysis of Variance (ANOVA) utilizing Steel and Torrie's (1960) approach. Duncan's Multiple Range Test was used to examine whether there was a significant difference between the treatment means at the 5% confidence level (Duncan, 1955).

Results

In the present study, anti-oxidant activity such as Ascorbic Acid and Lipid peroxidation concentration (Malondialdehyde) were analyzed in Serum and tissues of the juveniles exposed to crude oil and control. The biomarkers of oxidative stress (Ascorbic Acid and MDA) analyzed are presented in Table 1 and 2 respectively. From table 2, it is observed that there is a significant difference in ascorbic acid activity of serum in the exposed groups of juveniles compared to the control. The ascorbic acid activity of in tissues of exposed juvenile groups are about the same with the control group. From table 1, it is observed that there is a significant difference in the activity of MDA in the serum and tissues of the juvenile exposed to crude oil when compared to control group.

Table 1: Malondialdehyde Activity of *Clarias Gariepinus* Cultivated in Crude Oil Contaminated Sediment

GROUP NAME	SERUM	HEART	LIVER	KIDNEY	GILLS
0%	92808±997 ^a	11615±8.8 ^a	11615±12.9 ^a	12308±9.4 ^a	11385±6.4 ^a
0.1%	99231±684 ^b	11346±7.7 ^b	11769±46.6 ^b	11346±12.2 ^b	11192±6.4 ^b
0.3%	96615±999 ^c	10923±14.8 ^c	10731±6.4 ^c	11000±10.0 ^c	10731±6.4 ^c
0.5%	83846±2166 ^d	11038±8.8 ^d	11731±18.8 ^d	10731±10.0 ^d	10731±8.4 ^d
1%	153346±3047 ^e	10846±4.2 ^e	10885±4.2 ^e	11231±10.3 ^e	10808±10.0 ^e

Results are means of five determinations ± SEM. Values on the same column carrying different superscripts are significantly different ($p < 0.05$)

Table 2: Ascorbic acid Activity of *Clarias Gariepinus* Cultivated in Crude Oil Contaminated Sediment

GROUP NAME	SERUM	HEART	LIVER	KIDNEY	GILLS
0%	4±0.002 ^a	3±0.002 ^a	3±0.001 ^a	3±0.001 ^a	3±0.0004 ^a
0.1%	3±0.001 ^b	3±0.005 ^a	3±0.005 ^a	3±0.002 ^a	3±0.002 ^a
0.3%	3±0.003 ^b	3±0.002 ^a	3±0.001 ^a	3±0.001 ^a	3±0.002 ^a
0.5%	3±0.002 ^b	3±0.002 ^a	3±0.001 ^a	3±0.004 ^a	3±0.003 ^a
1%	3±0.003 ^b	3±0.002 ^a	3±0.001 ^a	3±0.003 ^a	3±0.003 ^a

Results are means of five determinations ± SEM. Values on the same column carrying different superscripts are significantly different ($p < 0.05$)

Discussion

Since fish tissue absorbs contaminants, they can disrupt a variety of physiological and biochemical processes, making fish particularly vulnerable to water pollution (Durmaz et al. 2006). When xenobiotics' production of ROS surpasses the thresholds, biological components start to suffer. Oxidative stress is the name of this process (Oakes and Van der Kraak 2003). By measuring antioxidant activities, the toxicity brought on by exposure to crude oil was examined in the current study using the in vivo model *clarias gariepinus*.

The edible fish are seen as one of the primary sources of wholesome staple food for humanity as a whole. Changes in metabolism that appear as the inhibition of enzymes may be the cause of the biochemical alterations brought on by the stress from crude oil. Tissue antioxidant enzymes are necessary for healthy fish development and growth, and they also significantly strengthen the immune system (Droge, 2002).

In this study, Ascorbic acid was reduced in the serum of juveniles exposed to crude oil. According to (Luis et al., 2021) Ascorbic acid (AA) is a micronutrient essential for the mechanisms of reproduction, growth, and defense in fish. The reduction of ascorbic acid in the exposed juveniles demonstrates the decrease in ascorbic acid protective properties of the catfish caused by crude oil. Jigyasu and Paul (2020) also demonstrated the Protective effect of ascorbic acid against fenvalerate induced toxicity in air-breathing fish *Clarias batrachus*.

In the present study, malondialdehyde (MDA) levels increased in some serum and tissues of exposed group which is in agreement with Di Giulio et al. (1993) who reported a increase in lipid peroxidation with laboratory exposures of channel catfish to polluted harbour sediments. The results obtained in this study perhaps depends on the intensity and duration of stress applied, the physicochemical properties of the soil, water, and crude oil used as well as the susceptibility of the target species.

Conclusion

This study has shown the toxicity and effect of crude oil on serum and tissues of African Catfish. *Clarias gariepinus* is an important aquaculture in Nigeria and other parts of the world. The continuous discharge of crude oil into the environment either through oil spillage or tanker accidents will cause a deleterious effect to the environment in the long run. Refining companies should adhere to modern techniques of waste management and disposal to prevent adverse effects from crude oil on animals, human and environmental health.

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