Journal of Advances in Biology & Biotechnology

Volume 27, Issue 5, Page 352-364, 2024; Article no.JABB.115656
ISSN: 2394-1081

Genotoxic Effects of Cassava Effluent on the Expression of Selected Genes in the African Catfish, *Clarias gariepinus*

Daniel I. Olorunfemi [a*], Oghenekparobo Oteri [a], Osuvwe C. Orororo [b], Tina-Bayoko B. Enize [c], Charles Pere [d] and Ejovi Osioma [e*]

[a] Department of Environmental Management and Toxicology, University of Benin, Benin, Nigeria.
[b] Department of Medical Biochemistry, Delta State University, Abraka, Nigeria.
[c] Department of Fisheries and Aquaculture, University of Africa, Toru-Orua, Nigeria.
[d] Department of Chemistry, Federal University of Petroleum Resources, Effurun, Nigeria.
[e] Department of Biochemistry, Federal University, Otuoke, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author DIO conceptualized the study; Author OO and OCO did Data curation; Author OO, OCO, CP and TBBE did Formal analysis and Investigation. Author EO, OCO, TBBE and CP performed Methodology; Authors OO did Project administration; Author CP and EO searched for Resources; Author DIO supervised the study; Author OO and DIO did data visualization; Authors OO, OCO and DIO wrote original draft. Authors DIO and OO wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2024/v27i5794

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/115656

Original Research Article

Received: 06/02/2024
Accepted: 09/04/2024
Published: 12/04/2024

ABSTRACT

Aim: A cause for worry is the wastewater that is released into the environment or public sewers without any sort of purpose during the processing of *Manihot esculenta* Crantz. Fish and other aquatic species may suffer negative consequences if wastewater is discharged into streams and

**Corresponding author: E-mail: danfem@uniben.edu;*

rivers, either directly or indirectly. This study was conducted to examine the effects of cassava effluents on gene expression in the African catfish (*Clarias gariepinus).*

Methodology: *C. gariepinus* juveniles were exposed to 0.1, 0.5, 1.0 and 1.5 % concentrations of cassava effluent, respectively. The subsequent tissue extraction, RNA isolation, cDNA synthesis and electrophoresis analysis were all done following standard procedures.

Results: From the results obtained, there were varying levels of upregulation in the expression levels of all the nine genes assessed: IL-1β, CYPIIA, HSP70, DMRTI, HSD17B, FOX12, MEL1C, CAMKIIg and GH genes. Statistical analysis to compare the expression levels of the genes at the different concentrations with their corresponding control experiments showed that the upregulation of only two genes, (HSD17B and GH), upon exposure to cassava effluents were not significant (p >0.05) at any concentration. The upregulation of the seven other genes is an indication that the cassava effluents exerted adverse impacts on the physiology of *C. gariepinus.*

Conclusion: It is therefore recommended that cassava effluents be properly treated before being discharged into the aquatic environment.

Keywords: Cassava processing effluents; toxicity; water quality; gene expression.

1. INTRODUCTION

One of the earth's most plentiful natural resources is water. Water is a necessary element for all biological processes carried out by living things, such as plant development, chemical reactions, the transportation of nutrients, and body temperature regulation [1]. Water is used by many living things for a range of purposes in the environment, including transportation, habitat provision, and soil formation [2]. Water covers over 70% of the surface of the Earth. This water is found in a variety of bodies of water, including lakes, rivers, ponds, streams, and seas. It can be either freshwater or saltwater [3]. Since water can dissolve a wide range of chemicals, it is a universal solvent, which contributes significantly to its susceptibility to contamination [4].

Recent years have seen an increase in pollution from both point and non-point sources, raising questions about the cleanliness of water bodies [5]. Trash generation has increased due to urbanization and growth, and a large portion of this trash ends up in water bodies [3]. Agricultural wastes, such as food processing, animal faeces and runoff from farmlands, industrial and sewage discharges, seepage of waste components from waste dumps and landfills, and urban runoff are among the wastes that contaminate water [6]. According to Backhaus et al. [7], water pollution results in alterations to the water's quality, such as a decrease in the amount of dissolved oxygen, eutrophication, toxicity, elevation of temperature, and addition of colour, odour, and taste. The introduction of garbage may also encourage the growth of dangerous bacteria, viruses, protozoa, and coliforms—microbes that cause disease. The quality and survival of aquatic species, as well as the usability of water, are negatively impacted by these changes [8].

In many places of the world, cassava and its derivatives are staple foods. According to Eze and Azubuike [9] and [10], cassava processing mills are frequently located close to water bodies into which the wastewater produced during the conversion of cassava into final products is dumped. These effluents have different concentrations of dangerous materials, such as cyanides, organic compounds, and other heavy metals, which alter the water's colour, chemical and biological oxygen demands, and other characteristics [11]. The components of cassava effluents have a negative impact on the different aquatic creatures that live in the affected water body in addition to the worsening of the water quality [12]. According to Jegede et al. [13], one of the impacts of cassava effluents in the aquatic environment is alterations to the medium's chemical composition, which have an impact on the aquatic organisms' physiology, behaviour, haematology, and biochemistry.

Given that fish are harmed in terms of number, location, and size, they are among the creatures most negatively impacted by hazardous chemicals found in cassava effluents, particularly hydrogen cyanide. Additionally, they have the capacity to accumulate these substances, which eventually make their way into human bodies through the food chain [14,15]. Cassava peel-containing wastewaters serve as breeding grounds for mosquitoes and flies, which are disease vectors, and they also contribute to the production of hydrogen cyanide in water [16]. Humans may come into touch with contaminated water or fish or through skin contact when exposed to hydrogen cyanide derived from

cassava. In addition to its numerous carcinogenic and mutagenic effects, hydrogen cyanide causes partial blindness in humans [17]. One reason for the reduction in the advantages associated with fisheries resources is the contamination of water bodies caused by cassava effluents. Alternative methods of treating these effluents prior to their discharge into water bodies should be put into place in order to safeguard water bodies and increase fisheries yield [13].

The impacts of cassava effluents on various aquatic creatures, particularly fish, have been the subject of several research [11,18,13,14]. It is a known fact that fish, because of their sensitivity to pollution, are useful as markers of the health of aquatic ecosystems [14]. We are not aware of any report on the gene expression of effluents obtained from cassava processing effluents on the African catfish. Thus, the purpose of this study was to evaluate how cassava effluents affect the African catfish's (*C. gariepinus*) gene expression.

2. MATERIALS AND METHODS

2.1 Collection of Cassava Effluent

The cassava effluent used in the study was obtained from a local cassava processing plant located in Agbarha-Otor, in Agbara of Delta State (Fig. 1). Agbarha-Otor is geographically located at 5°31’15” N, 6°2’49” E and has an elevation of 14 m above sea level. It is one of the towns in the Urhobo Kingdoms of Ughelli in Delta State, Nigeria.

During the collection, cassava effluent was homogenised and then transferred from plastic basins into 5-litre and 10litre kegs. After collection of the samples, they were then stored in a refrigerator at 10°C until the introduction of the test organisms into the effluents.

2.2 Collection of Fish Samples

Clarias gariepinus, the African catfish, served as the study's test organism. Juvenile *C. gariepinus* were purchased from a commercial fish farm in Benin City, Edo State, Nigeria. The fish were allowed to acclimate for seven days in a plastic aquaculture tank filled with dechlorinated water. Due to waste accumulation in the water and to lower stress levels, this water was changed daily. Fish feed pellets with a diameter of 2 mm were used to feed the fish twice a day. The feeding schedule was terminated 24 hours before the fish were exposed to the wastewater.

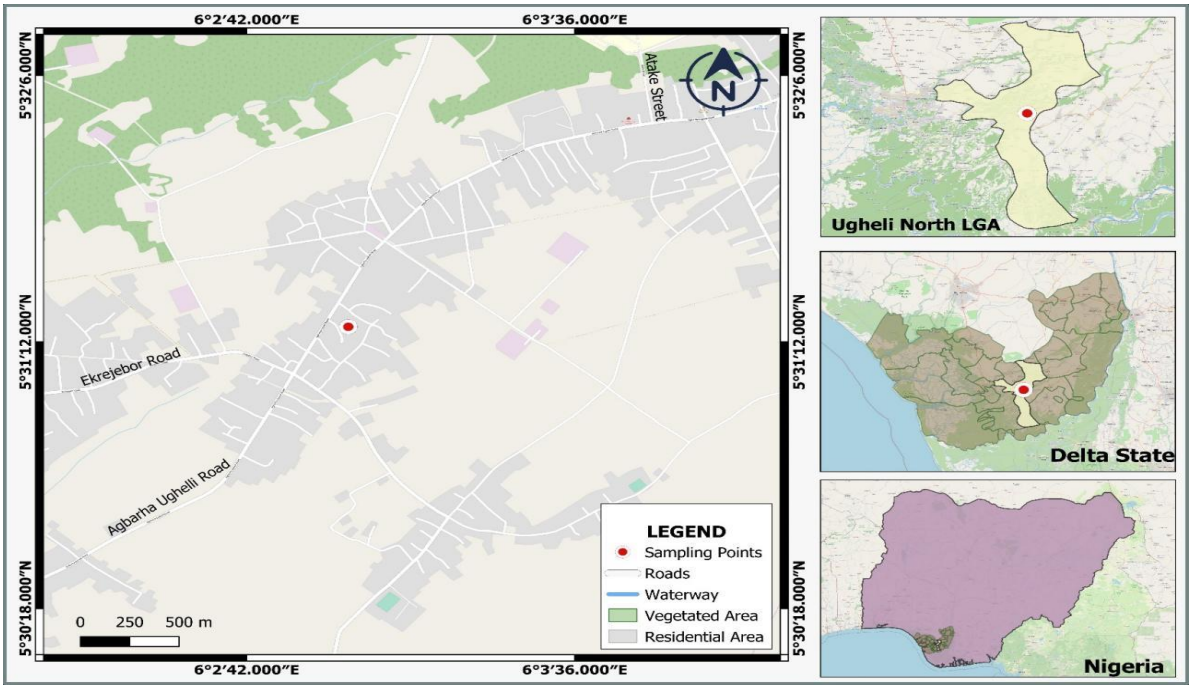

Fig. 1. A map of the study area showing the sampling location.

Plate 1. Generation of cassava effluent

Plate 2. Cassava effluent stored in plastic basins awaiting disposal

Plate 3. Samples of *Clarias gariepinus* juveniles

2.3 Analytical Procedures

2.3.1 Exposure to effluent

Exposure of the test organisms to the cassava effluent was done in accordance with guidelines recommended by UNEP [19]. Ten fishes each were transferred to five 16-litre aquariums for a total of 50. The concentration of cassava effluent in the aquariums were as follows 0.1%, 0.5%, 1.0%, 1.5% and 0% in the control which only contained dechlorinated water. One fish each from the aquariums were removed and observed for abnormalities and mortality after 6, 12, 48, 72 and 96 hours. After observation, the fish were properly labelled and stored in a freezer.

2.3.2 Extraction of tissue sample

The extraction of tissue samples followed the modified methods of [20]. For this procedure, the fishes were taken out from the freezer and

allowed to thaw. They were then dissected using appropriate equipment. An incision was made in the pectoral region of each fish and the liver was removed, placed in an EDTA tube and stored in a freezer to prevent denaturing. The liver was selected for this study and is appropriate because it is a large organ which is easy to locate and remove; it is the main organ for detoxification; and the extraction of DNA from the liver is not cumbersome [20].

2.3.3 RNA isolation

The isolation of RNA followed the modified methods of [20]. The liver tissues extracted from the fish were homogenised by mechanical membrane destruction in an Eppendorf tube using a plastic pestle to break the nuclei and release the DNA and RNA contents. Thirty (30) ml of RNA Lyase buffer (SOL A) was then pipetted into the homogenised sample to facilitate the breakage of plasma membranes in the event of improper homogenisation. After that, the samples were then placed in a water bath and incubated for 10 minutes at 30°C. When this was completed, the samples were centrifuged for 15 minutes at an oscillation rate of 10000 rpm. The centrifuged samples were then divided into three parts i.e. DNA, RNA and proteins. The RNA was pipetted into a new tube and ten (10) ml of the precipitating buffer (SOL B) which contained DNase enzymes which served the purpose of breaking down the residual DNA impurities in the RNA sample. The resulting mixture was incubated again in the water bath for 10 minutes at 30°C, followed by centrifuging for 15 minutes at 10000 rpm. When centrifugation was complete, the supernatant was decanted and there was a tiny pellet left at the bottom of the test tube. RNA buffer (SOL C) was added to the test tube. This solution serves to wash out RNA from all the solvents which have been used. The resulting solution was then decanted and allowed to dry.

2.3.4 Quantification and normalisation

The quantification and normalisation of RNA followed the modified methods of [20]. Fifty (50) µl of nuclease-free water which contained inhibitors of DNA and RNA was added to the RNA after it had dried. Nuclease-free water is added to prevent the breaking down of the RNA sample. Purification of the sample was carried out with the aid of the UV spectrophotometer set at 260 nm. The amount of RNA isolated was determined by quantification and then to ensure that the concentrations of RNA present in all the samples were the same, normalisation was carried out.

2.3.5 cDNA synthesis

The synthesis of cDNA followed the modified methods of [20]. The reverse transcription procedure facilitated by reverse transcriptase was used in the conversion of the normalised RNA to cDNA. Aside from the reverse transcriptase, other reagent used in this process included the reverse transcriptase buffer, a random primer, Oligo DT primer and dNTPs. These reagents were mixed into a ‘cocktail’ mixture and then added to the sample. The samples were then placed in a PCR machine at 42°C for transcription. The operating temperature was increased to 72°C to denature the transcriptase. When the synthesis of cDNA was complete, gene amplification involving denaturation, annealing and elongation was carried out through the polymerase chain reaction technique. The reagents in this process were the cDNA itself, TAG polymerase, a buffer solution, primers and magnesium. The solution was first denatured in the PCR machine at 94°C which resulted in the physical separation of the two strands which make up the DNA double helix. A gradual reduction in the operating temperature of the system was then effected to 55°C for annealing which resulted in the binding of the primers to complementary DNA sequences. An exponential amplification of the original DNA template occurred at 72°C occasioned by a chain reaction which resulted from the addition of nucleotides to the primers by TAG polymerase.

2.3.6 Agarose gel electrophoresis

Electrophoresis with agarose gel followed the modified methods of [20]. After amplification of a solution is complete, it is referred to as an amplicon. To the amplicons, gel loading dye was added to monitor the migration of DNA within the electrophoretic tank while ensuring that the amplicons maintain a high density due to the presence of glycerol in the gel. The amplicons which were negatively charged migrated through the agarose gel pores in the direction of the positively charged gel and vice versa. Higher amplicon density was accompanied by slower movement which made it easier to acquire sharp and clear bands for the results. Image J software was used in the capturing the various bands produced which were then used in the determination of the level of intensity for each band.

Table 1. Primer sequences of the genes

GENE	FORWARD PRIMERS	REVERSE PRIMERS
BETA ACTIN	CATCGGCAATGAGCGTTTC	GATGGAGTTGAAGGTGGTCTC
1L-1BETA	CAGTGAATCCAAGCGCTACA	AAGCGAGCAGAAGAGGAAA
CYP11A1	GCAGGGACTATCGCATCTTT	CAAAGCCAAAGCCCAAACTC
HSP70	TGGCCTTTCAAGGTCATCAG	CAGCACCATGGAGGAGATTT
DMRT1	CTCTGACTCTGGAGCGTTTAC	CACAGTGCCATGAGGTAAGT
HSD17B2	AGGTGATCCTGTGCTGTTG	GCCTTCCTGAAGTGAAGTAGAG
FOXL2	ACTCCTCGTACAACCCTTACT	CTGCTGATGGTGCTGATGT
MEL1C	GATTTGGGCGACAGCAATTC	CCAGCATACAGCAAACAACAC
CAMKIIG	GACTTTGGACTGGCGATTGA	AGGGTCCTTTCTCAGGACTT
GH	CCCTGTCATTCTGCAACTCT	CCATGACTCGATCAGACGATAAG

2.4 Data Analysis

SPSS 20.0 statistical package was used for all data analysis. The results of the root inhibition at each concentration of the effluents were expressed as mean ± standard deviation. The differences between the control and different concentrations of the cassava effluents were compared using one-way analysis of variance (ANOVA). To assess whether the means of groups were statistically different from each other, the least significant difference (LSD) test was adopted. In all cases, a value of $p<0.05$ was considered significant.

3. RESULTS AND DISCUSSION

Cassava effluents have been shown by previous studies to be toxic due to their physicochemical properties and the fact that they often contain certain amounts of some heavy metals [14]. Upon the release of these effluents into water bodies, they alter the physicochemical properties of the aquatic system and are able to induce adverse effects in fish [21]. Organs such as kidneys, brains, livers, gills and gonads are often affected. Among the effects reported in these studies are congestion in central veins, formation of vacuoles in hepatocytes and necrosis of the cells [22]. Some studies have reported that exposure to extreme concentrations of these effluents result to mortality [14]. The present study was conducted with the aim of determining the effects of exposure to cassava effluents on gene expression in *Clarias gariepinus.* These effects were assessed at various concentrations i.e. 0.1, 0.5, 1.0 and 1.5% for the expression levels of the following genes: IL-1β, CYPIIA, HSP70, DMRTI, HSD17B, FOX12, MEL1C, CAMKIIg and GH genes.

3.1 Expression of IL-1β Gene

The changes in the expression levels of the IL-1β gene are shown in Fig. 2. The results show that with increasing concentrations of cassava effluent, there was a corresponding increase in the expression of the gene. The largest increase in the expression levels was recorded when the effluent concentration was at 1.5%.

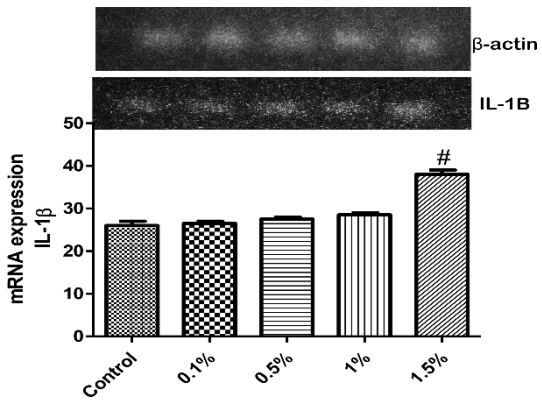

Fig. 2. Expression of IL-1β gene at various concentrations

The IL-1β gene, known as Interleukin-1 beta, is a gene of the cytokine family which is pro-inflammatory and is an important mediator of the response of an organism to infections by microorganisms, injuries to tissue and immunological reactions [23]. The changes in expression levels of IL-1β upon exposure to different concentrations of cassava effluent are shown in Fig. 2 and it clearly shows that minute increments in expression were recorded at 0.1, 0.5 and 1.0% when compared to the control sample. However, there was a spike in expression levels when the concentration was 1.5%. This suggests that a concentration of 1.5% cassava effluent is potentially injurious to *Clarias gariepinus* [24], hence, the increased expression of the gene. And also. the results from the statistical analysis shows there is a significant difference (p<0.05) in the genes expressed.

3.2 Expression of CYPIIA Gene

The variations in the expression of the CYPIIA gene as the concentration of the cassava effluent changed is shown in Fig. 3. The greatest increase in the expression levels was recorded when effluent concentration was 1.5%, and the least was recorded when effluent concentration was 1%.

For the cytochrome P4501A gene, also known as the CYP1A gene, it serves a variety of functions within the bodies of living organisms including fish especially biotransformation of xenobiotic compounds which may result to these compounds being detoxified or their reactive intermediates being formed. The gene is often expressed upon exposure to such toxic substances and is known to be active in organs including the liver, gills, heart and kidneys, among others [25]. In this study, the expression levels of CYP1A was greater at all concentrations of cassava effluent than in the control sample and also a statistical significance (p<0.05) in the result obtained. The highest increase in expression levels was recorded at 1.5%. This shows that at all concentrations, cassava effluent is viewed as a xenobiotic in the physiology of the fish and as such the gene is expressed to detoxify its components [26].

3.3 Expression of HSP70 Gene

Fig. 4 presents the expression levels of the HSP70 gene at the various concentrations of cassava effluent. At 0.5% and 1.5% concentrations of cassava effluents, the highest increases in the expression of HSP70 gene were recorded. The least increment was recorded at 0.1%.

The heat shock protein 70 (HSP70) gene is produced in fish as a response when exposed to cold or heat shock, under pathological conditions, exposure to heavy metals and ultraviolet irradiation [27]. In this study, there was an upregulation of the expression of the HSP70 gene at all concentration in comparison with the control sample, suggesting that the presence of cassava effluent in the aquatic environment is a stress factor for *Clarias gariepinus* [28]. The result however did not indicate a statistical significance (p>0.05) when the gene was expressed.

3.4 Expression of DMRTI Gene

The variations in the expression levels of the DMRTI gene in the presence of various concentrations of cassava effluents are shown in Fig. 5. The least increase in expression of the DMRTI gene was recorded at 0.5%. The increases at 0.1% and 1% were identical, and the highest increases was recorded at 1.5%.

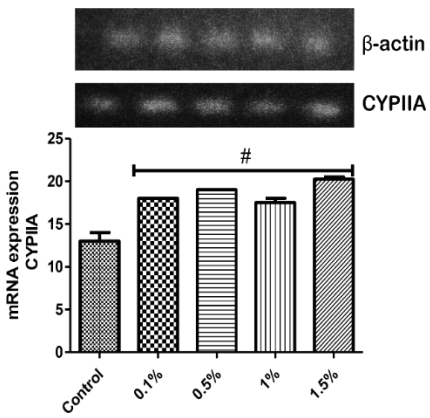

Fig. 3. Expression of CYPIIA gene at various concentrations

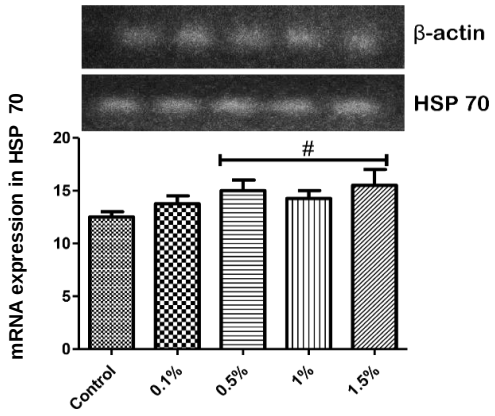

Fig. 4. Expression of HSP70 gene at various concentrations

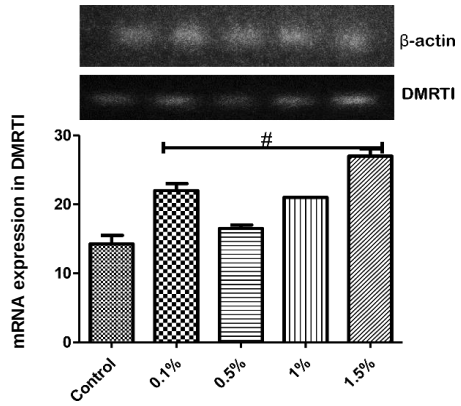

Fig. 5. Expression of DMRTI gene at various concentrations

The double-sex and mab-3 related transcription factor 1 (DMTR1) gene is a determinant of the differentiation of gonadal sex in metazoan animals [29]. At 0.1, 1.0 and 1.5% concentrations of cassava effluent in this study, there were large upregulation differences between the samples and the control experiment for the DMRT1 gene and the analysis indicated a difference statistically (p<0.05). Given the function of the DMRT1 gene, it is inferred that the increase in the expression of the gene upon exposure to cassava effluent is an immune response to prevent the mutation of *Clarias gariepinus* samples as that would result to the development of only female individuals or complete reversal of all male individuals to females, while any males that remain would be characterised with sterility and dysgenesis of the testis as explained in [30].

3.5 Expression of HSD17B Gene

The various levels of the expression of the HSD17B gene at corresponding concentrations of cassava effluents is displayed in Fig. 6. The smallest increases in expression levels for the HSD17B gene were recorded at 0.1% and 0.5%. In contrast, the largest increase was recorded at 1% concentration.

The 17β-hydroxysteroid dehydrogenase (HSD17B) is one of a group of enzymes of a steroidogenic nature which are important factors in metabolism, biosynthesis and sex differentiation and determination in many vertebrates [31]. When compared to the control sample, there were varied increments in upregulation of HSD17B expression at all concentrations with the highest being at 1.0% suggesting there was a significant difference (p<0.05) in the expressed gene. In the same vein as the DMRT1 gene, the upregulation of HSD17B is a mechanism to prevent mutations that cause the development of only one sex in *Clarias gariepinus* populations as they encode enzymes which catalyse the synthesis of sex hormone in fish and control the direction of gonadal development [32].

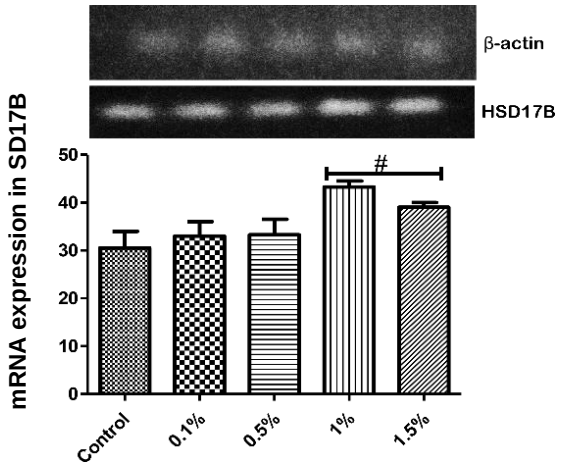

Fig. 6. Expression of HSD17B gene at various concentrations

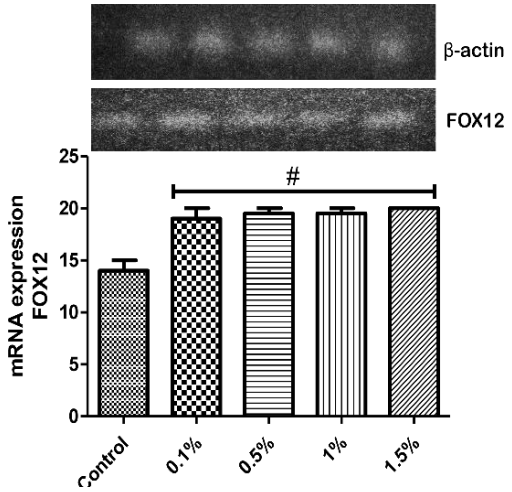

Fig. 7. Expression of FOX12 gene at various concentrations

3.7 Expression of FOX12 Gene

The expression levels of the FOX12 gene at the various concentrations of cassava effluents used during this study are shown in Fig. 7. The expression levels at 1.5% was the highest, followed by those at 0.5% and 1%, and the least was recorded at 0.1%. The differences in the expression levels were minute.

The forkhead transcriptional factor 2 (FOXL2) gene is one of the proteins which possess a winged helix. The gene has been shown to be one of the initial determinants of the differentiation of the ovaries in vertebrates [33]. There was a spiked upregulation of the FOXL2 gene at 0.1% and the upregulation at other concentrations in contrast with that of the control were not much different from that at 0.1%. The upregulation of the expression of FOXL2 gene shows that the presence of cassava effluent in the aquatic environment can cause changes in the sex of *Clarias glariepinus* individuals [34] and the statistical analysis indicated a difference (p<0.05).

3.8 Expression of MEL1C Gene

The variations in the expression of the MEL1C gene with changes in the concentration of the cassava effluent are shown in Fig. 8. The least variation in comparison with the control was recorded at 1% of cassava effluent, and the highest increase in the expression levels was recorded at 1.5% followed by 0.5%.

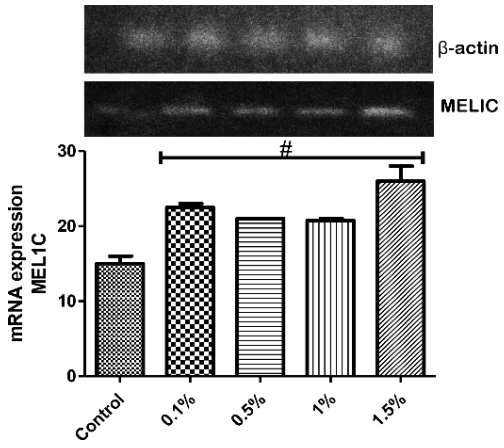

Fig. 8. Expression of MEL1C gene at various concentrations

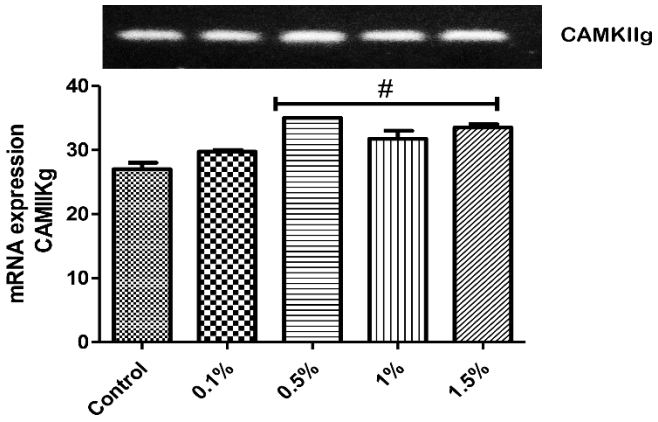

Fig. 9. Expression of CAMIIKg gene at various concentrations

MEL1C is a gene which codes for a protein that binds melatonin in fish, birds, and amphibians. Melatonin is a neurohormone which is responsible for maintaining circadian information in an organism by way of the diffusion of blood circulation to the peripheral organs [35]. In the case of the MEL1C gene in this study, the upregulation of MEL1C expression showed a spike at 0.1% compared to the control sample. There was a slight decrease in the upregulation from 0.1% at 0.5% and 1%. However, at 1.5% MEL1C expression spiked again and statistical analysis indicated a difference (p<0.05). Upregulation of MEL1C suggests that blood circulation in *Clarias gariepinus* is adversely affected by exposure to cassava effluent.

3.9 Expression of CAMIIKg Gene

The various levels of the expression of the CAMKIIg gene at corresponding concentrations of cassava effluents is displayed in Fig. 9. There were slight increases above the expression levels in the control experiment, with the smallest increment recorded at 0.1%. The largest increase in the expression levels of the gene was recorded at 0.5% followed by 1.5%.

The CaMK2g gene is one of those which encodes the calcium-dependent protein kinase CaMKII. The gene's importance is evident in the early development of most teleost fish species as its role in calcium signalling is key in many aspects of plasticity at glutamatergic synapses [36]. The expression of the CaMK2g gene in this present study steadily increased from the control sample to 0.1% and 0.5%. A small reduction in expression levels was recorded at 1.0% with another increase at 1.5% and that suggested a statistical difference when analysis was conducted (p<0.05).

3.10 Expression of GH Gene

The variations in the expression levels of the GH gene in the presence of various concentrations of cassava effluents are shown in Fig. 10.

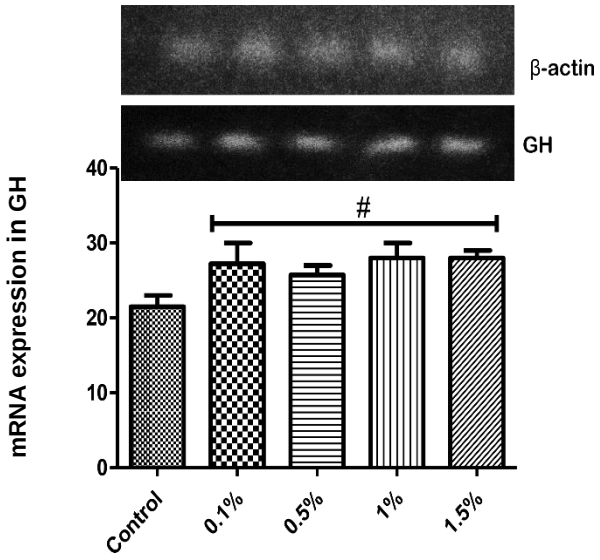

Fig. 10. Expression of GH gene at various concentrations

There were increases in the expression levels of the GH gene compared to when cassava effluent was absent. The least increment was recorded at 0.5% and the greatest was recorded at 1.5% and 1%.

The main function of the GH gene in living organisms is the promotion of growth while it also has an influence on metabolism. Expression of the GH gene in fish causes an increase in aggression, swimming activity and appetite [37]. Concerning the expression of the GH gene, it was upregulated at all concentrations and was found to be greater than the expression in the control sample. This indicates that upon exposure to cassava effluent, *Clarias gariepinus* may show increase in size. And the statistical analysis suggests there was no statistical difference (P>0.05).

Statistical analysis of the results obtained showed that at a 5% confidence level there was significant difference (p<0.05) in the expression of the following genes: 1L-1β, CYPHA1, DMRT1, HSD17B, FOX12, MEL1C, CAMIIKG**;** except two (2) genes: HSP70 and GH.

4. CONCLUSION

Gene expression is a key cellular operation in every living organism as every activity that takes place in the body is regulated in one way or another by the genetic material. This study was conducted to examine the effects of cassava effluents on gene expression in *Clarias gariepinus.* The expression levels of a number of genes were assessed during the study and the results showed that exposure to cassava effluents caused varying levels of upregulation in the expression of the various genes. Although, there were increases in expression, these increments were found to be significant in seven (7) out of the nine (9) genes expressed. It is concluded that cassava effluents are environmental pollutants which exert adverse impacts on *Clarias gariepinus.* It is therefore recommended that the appropriate regulatory bodies should ensure that cassava effluent should be properly treated with improved treatment methods before being indiscriminately discharged into arable land and aquatic environments as the effluent contain toxic substances which may constitute a risk to the environment and human health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jequier E, Constant F. Water as an essential nutrient: the physiological basis of hydration, Eur. J. Clin. Nutr, 2010; 64:115-123.
2. Son CT, Giang NTH, Thao TP, Nui NH, Lam NT, Cong VH. Assessment of Cau River water quality assessment using a combination of water quality and pollution

indices, J Water Supply Res. Technol. 2020;69(2):160 - 172.
3. WHO (World health organization). Guidelines for Drinking Water Quality. 3rd edition. WHO: Geneva, Switzerland. 2002;668.
4. Ali H, Khan E, Ilahi I. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity and bioaccumulation; 2019. Available:https://doi.org/10.1155/2019/6730305. Accessed May 20th, 2022.
5. Irfan S, Alatawi A. Aquatic ecosystem and biodiversity: a review, Open J Ecol. 2019;9:1-13.
6. Madilonga RT, Edokpayi JN, Volenzo ET, Durowoju OS, Odiyo JO. Water quality assessment and evaluation of health risk in Mutangwi River, Limpopo Province, South Africa, Int. J. Environ. Res. Public Health. 2021;18:67-83.
7. Backhaus T, Brack W, van den Brink P, Deutschmann B, Hollert H, Posthuma L, Segner H, Seiler TB, Teodorovic I, Focks A. Assessing the ecological impact of chemical pollution on aquatic ecosystems requires the systematic exploration and evaluation of four lines of evidence. Environ. Sci. Eur. 2019;1:49 - 62.
8. Bashir I, Lone FA, Bhat RA, Nir AS, Dar ZA, Dar SA. Concerns and threats of contamination on aquatic ecosystems. Bioremediat. Biotechnol. 2020;27:1-26.
9. Eze VC, Onyilide DM. Microbiological and physiochemical characteristics of soil receiving cassava effluents in Elele, Rivers State, Nigeria. J. Appl. Environ. Microbiol. 2015;3(1):20–24.
10. Okunade DA, Adakalu KO. Physico-chemical analysis of contaminated water resources due to cassava wastewater effluent disposal. Eur. J. Sci. Technol. 2013;2(6):75-78.
11. Adewoye SO. Assessment of toxic potentials of cassava effluent on *Clarias gariepinus,* Int. J. Agric. Sci. Res. 2013; 3(3):157 – 160.
12. Omotioma M, Mbah GO, Akpan IJ, Ibezim OB. Impact assessment of cassava effluents on Barika Stream in Ibadan, Nigeria. Int. J. Environ. Sci. Manage Eng. Res. 2013;2(2):50-52.
13. Jegede T, Ariyomo TO, Adeniran AF, Omobepade O. Toxicity of cassava effluents to catfish, *Clarias gariepinus,* and the effects on some target organs, Ann. Aquac. Res. 2017;4(2):1034 - 1041.
14. Olorunfemi DI, Ehighiator FA, Ofomata RC. Evaluation of acute toxicity of cassava effluent on the African catfish (*Clarias gariepinus*) and Freshwater clam (*Egera radiata*), Afr Sci. 2019; 20(20):81- 86.
15. Egbune OU, Egbune EO, Orororo OC, Ezedom T, Onojakpor O, Sabo AM, Amadi K. Chronic cassava meal modulates body weight, histology and weight of reproductive organs in male albino rats. Toxicol. Environ. Health Sci. 2023;15:257–266. Abailable: https://doi.org/10.1007/s13530-023-00179-4.
16. Oghenejoboh KM. Effects of cassava wastewater in the quality of receiving water body intended for fish farming, Br. J. Appl. Sci. Technol. 2015;6(2):164 - 171.
17. Ehiagbonare JE, Enabulele SA, Babatunde BB, Adjarhore R. Effect of cassava effluents on Okada denizens. Sci. Res. Essay. 2009;4(4):310-313.
18. Olaniyi CO, Adewoye SO, Ismail MO, Adeseun FM. Effect of cassava mill effluents on haematological and biochemical characteristics of adult African catfish (*Clarias gariepinus*), J. Biol. Agric. Healthcare. 2013;3(3):191 – 197.
19. United Nations Environmental Programme (UNEP): Estimation of the lethal toxicity of pollutants in marine fish and invertebrates. Reference Methods for Marine Pollution Studies No. 198;943:27.
20. Elekofehinti OO, Lawal AO, Ejelonu OC, Molehin OR, Famusiwa CO. Involvement of fat mass and obesity gene (FTO) in the anti-obesity action of *Annona muricata* Annonaceae: *In silico* and in vivo studies. Diabetes Metab. Disord**.** 2020; 2(4):302-310.
21. Okayi RG, Annune PA, Tachia MU, Oshoke OJ. Acute toxicity of glyphosate on *Clarias gariepinus* fingerlings. J. Res. For. Wildl. Environ. 2010;2(2):150 - 155.
22. Agboola OA, Fawole OO. Chronic toxicity of pharmaceutical effluent to *Clarias gariepinus* (Burchell, 1822). Covenant J. Phy. Life Sci. (CJPL). 2014;1(2):27- 42.
23. Husain M, Bird S, van Zwieten R, Secombes CJ, Wang T. Cloning of the IL-1β3 gene and the IL-1β4 pseudogene in salmonids uncovers a second type of IL-1β gene in teleost fish, Devel. Complex Immunol. 2012;38:431-446.
24. Liang Q, Li W, Guo N, Tong C, Zhou Y, Fang W, Li X. Identification and functional analysis of Interleukin-1β in the Chinese

soft-shelled turtle *Pelodiscus sinensis*, Genes. 2016;7(5):18-22.

25. Sarasquete C, Segner, H. Cytochrome P4501A (CYP1A) in teleostean fishes: a review of immunohistochemical studies, Sci Total Environ. 2000;247(2-3):313 - 332.
26. Olsvik PA, Lie KK, Saele O, Sanden M. Spatial transcription of CYP1A in fish liver, BMC Physiol. 2007;7(12):74 - 80.
27. Ma F, Luo L. Genome-wide identification of HSP70/110 hgenes in rainbow trout and their regulated expression in response to heat stress. Peer J. 2020;8:e10022 DOI:10.7717/peerj.10022.
28. Cho Y, Jeong T, Choi M, Kim J, Lim H. Heat shock protein 70 gene expression and stress response of red-spotted (*Ephinephilus akaara*) and hybrid (*E. akaara* female x *E. lanceolatus* male) groupers to heat and cold shock exposure, Fish Physiol. Biochem. 2021;47:2067-2080.
29. Bradley KM, Breyer JP, Melville DB, Broman KW, Knapik EW, Smith JR. An SNP-based linkage map for zebrafish reveals sex determination loci, G3-Genes Genom Genet. 2011; 1: 3 - 9.
30. Webster KA, Schach U, Ordaz A, Steinfeld JS, Draper BW, Siegfried KR. DMRT1 is necessary for male sexual development in zebrafish, Dev. Biol. 2018;422(1):33 - 46.
31. Xiao L, Guo Y, Wang D, Zhao M, Hou X, Li S, Lin H, Zhang Y. Beta-hydroxysteroid dehydrogenase genes in orange-spotted grouper (*Epinephelus coioides*): genome-wide identification and expression analysis during sex reversal. Front. Genet. 2020;11:161-170.
32. Zou C, Wang L, Zou Y, Wu Z, Wang W, Liang S, Wang L, You F. Characteristics and sex dimorphism of 17β-hydroxysteroid dehydrogenase family genes in the olive flounder *Paralichthys olivaceus.* J. Steroid Biochem. Mol. Biol. 2020;199(3):105597.
33. Kobayashi Y, Hiroguchi R, Nozu R, Makamura M. Expression and localisation of forkhead transcriptional factor 2 (foxl2) in the gonads of protogynous wrasse, *Halichoeres trimaculatus,* Biol. Sex Differ, 2010;1:3-9.
34. Govoroun MS, Pannetier M, Pailhoux E, Cocquet J, Brillard JP, Couty I, Batellier F, Cotinot C. Isolation of chicken homolog of FOXL2 gene and comparison of its expression patterns with those of aromatose during ovarian development, Dev. Dyn. 2004;231:859-870.
35. Gautier C, Guenin S, Riest-Fery I, Perry J, Legros C, Nosjean O, Simonneaux V, Grutzner F, Boutin J. Characterisation of the MEL1C melatoninergic receptor in platypus (*Ornithorhynchus anatiinus*), PLoS One, 2018;13(3)**:**191 - 202.
36. Onori MP, Koopal B, Everman DB, Kury S, van Woerden G. The intellectual disability-associated CAMK2G p.Arg292Pro mutation acts as a pathogenic gain-of-function, Hum. Mutat. 2018;39(12):2008-2024.
37. Jonsson E Bjornsson BT. Physiological functions of growth hormone in fish with special reference to its influence on behaviour, Fish Sci, 2002;68(1):742-748.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/115656