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Research Article

Cytogenotoxicity of Abattoir Effluent in *Clarias gariepinus* (Burchell, 1822) Using Micronucleus Test

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The cytogenotoxic potential of abattoir effluent from Bodija, Nigeria, was investigated using micronucleus test in *Clarias gariepinus*. Fish was exposed to five different concentrations: 0.2, 0.4, 0.8, 1.6, and 3.1% of the effluent for 7, 14, and 28 days. Tap water and 0.02 mL/L of benzene were used as negative and positive controls, respectively. Physicochemical parameters and heavy metals were analyzed in the effluent in accordance with standard methods. After exposure, blood was collected from the treated and control fish and slides were prepared for micronuclei (MN) and nuclear abnormality evaluation in the peripheral erythrocytes. The effluent induced significant ($p < 0.05$) increase in the frequency of MN in a time dependent manner. Similarly, the frequency of total nuclear abnormalities (blebbing, notch, bud, binucleation, and vacuolation) was higher in the exposed fish than the negative control. Electrical conductivity, nitrate, biochemical oxygen demand, chemical oxygen demand, arsenic, and copper analyzed in the effluent may have provoked the observed cytogenetic damage. The findings herein suggest the presence of clastogens and cytotoxins in Bodija abattoir wastewater which are capable of increasing genomic instability in aquatic biota.

1. Introduction

Pollution of the aquatic ecosystems by incidental and deliberate discharge of xenobiotics is increasing at alarming rate worldwide. This is linked mainly to unprecedented human population growth which accounts for the increasing anthropogenic activities. For instance, increasing meat production to meet the protein needs of human population has contributed greatly to the pollution status of aquatic ecosystems [1]. Abattoirs, places where animals are slaughtered for meat collection, generate large amount of solid wastes and effluents containing rumen contents, blood, and large volume of wash water. Considering that animal slaughtering operations require large volume of water, most private and government owned abattoirs in Nigeria are sited close to rivers or water sources to enable proper washing of the meat after slaughtering [2]. This undoubtedly results in disposing of abattoir effluents and solid wastes directly into streams and rivers without treatment [1]. This act increases problems related to obnoxious odour of the water bodies and land, proliferations of flies (vectors of human and animal diseases), and surface and ground water contamination with pathogens and undesirable toxic metals and organic chemicals. There is increasing global concern due to possible health impacts on the biota and environmental degradation that may result from xenobiotics in abattoir solid wastes and effluent [2].

Studies have shown that abattoir solid wastes and effluents contaminated rivers contain high concentrations of hazardous trace metals such as Zn, Cu, Cr, Fe, Cd, and Pb [1, 3, 4] and high microbial loads [1, 2]. However, studies assessing the genotoxicity and cytotoxicity of abattoir effluents using micronucleus test in aquatic vertebrates are relatively scarce. There is need to investigate the possible cytogenotoxic effects of abattoir effluents in fish, in order to protect aquatic biota from predisposition to genetic related abnormalities and biodiversity loss. The use of genetic biomarkers in the

routine monitoring of industrial and agricultural effluents for the presence of xenobiotics that are capable of eliciting DNA damage in aquatic biota will enhance the survival through prompt reproduction of the aquatic forms and reduce pollutant-induced stress syndromes [5, 6].

Clarias gariepinus (African catfish) is a benthopelagic fresh water fish, with feeding habits and ecological distribution that increases its exposure to xenobiotics [7]. Its use in previous studies to assess the cytogenotoxic effects of municipal landfill leachate [8], e-waste leachates, and e-waste contaminated underground water [9] and textile effluent [10] using micronucleus test suggests its sensitivity to detecting the DNA damaging effects induced by mixture of xenobiotics in the effluents. The ease to culture *C. gariepinus* due to rapid turnover rate and high adaptation to varying laboratory conditions accounts for its use in scientific studies and as the most locally consumed African clarrid in Nigeria. In this study, we investigated the genotoxic and cytotoxic potentials of abattoir effluent in *C. gariepinus* using micronucleus test. Some physicochemical parameters and heavy metals were also analyzed in the effluent.

2. Materials and Methods

2.1. Sampling Site. Bodija abattoir (longitude $7^{\circ}25'35''$ N and latitude $3^{\circ}54'39''$ E), located in Ibadan, Oyo State in Nigeria, is the major recipient of bovine and swine from different parts of the country. The abattoir is an open slaughtering slab for daily killing of 300–350 cattle heads, 150–200 sheep and goats, and 50–100 pigs by dehairing with hot water and/or burning using fire [3]. The butchering of the slaughtered animals and washing of the guts and meat release large volume of wastewater into Alamuyo River (about 500 m from the slaughter house) [11].

2.2. Wastewater Sampling, Heavy Metals, and Physicochemical Analysis. Abattoir effluent collected from Bodija abattoir at different discharge points was filtered to remove debris and mixed to obtain a composite sample. The physical and chemical parameters of the effluent, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), chloride, pH, acidity, alkalinity, electrical conductivity, phosphate, and nitrate, were measured in accordance with APHA [12]. The concentrations of arsenic (As), lead (Pb), copper (Cu), cadmium (Cd), nickel (Ni), and chromium (Cr) were determined according to APHA [12] and USEPA [13] using PerkinElmer A3100 atomic absorption spectrophotometer.

2.3. Animal and Experimental Design. Juvenile *C. gariepinus* (mean ± SD body weight 6.60 ± 2.40 g and length 8.30 ± 1.00 cm) obtained from the Fisheries Department, Oyo State Ministry of Agriculture and Natural Resources, Ibadan, were used for the study. They were acclimated to laboratory conditions of 26°C and 12/12 h dark/light modes for not less than 14 days prior to the experimental set-up. They were stocked at a population density of 15 fish per 25 L transparent plastic aquarium containing dechlorinated water and fed 5% of their body weight with standard fish feed which contains 35% crude protein twice daily.

0.20, 0.40, 0.80, 1.60, and 3.14% (v/v; effluent/dechlorinated tap water) concentrations of the effluent corresponding to 1/32, 1/16, 1/8, 1/4, and 1/2, respectively, of the 96 hr LC_{50} of 6.28% [14] were selected for the sublethal toxicity study. *C. gariepinus* were exposed to the different effluent concentrations by immersion for 7, 14, and 28 days, and five fish were analyzed at each sampling time from each concentration of the effluent sample. Similar treatments were given to fish exposed to dechlorinated tap water and 0.02 mL/L of benzene (Sigma, St. Louis, MO, Australia) as negative and positive controls, respectively. The test effluents and control substances were replaced every 48 h to reduce accumulation of metabolic wastes, remains of food particles, and volatilization of less stable substances in the effluent and benzene.

2.4. Micronucleus Analysis. At the end of each exposure time, blood was collected from the caudal vein of each fish in a test group and control groups for MN analysis. Thin smear of the peripheral blood was made on three precleaned slides per fish. The slides were air dried, fixed in absolute methanol for 30 minutes, and counterstained with 10% May-Grunwald and 5% Giemsa [8, 15]. 3000 erythrocytes per fish were scored for micronucleus induction at ×1000. Nuclear abnormalities (NAs) were also scored as cytotoxic parameters [9, 16] at the same magnification. Cells with two nuclei were considered as binucleated (BN). Blebbed nucleus (BL) presents a relatively small evagination of the nuclear membrane, which contains euchromatin. When the evagination is larger than the blebbed nuclei and containing several lobes, it was considered as lobe nucleus (LB), while notched nucleus (NT) contains vacuoles and appreciable depth into the nucleus that does not contain nuclear materials. Other scored NAs are budding nucleus. Only cells with intact cell and nuclear membranes were scored.

2.5. Statistical Analysis. All statistical analyses were conducted using Graphpad prism 5.0 computer programs. Data are presented as mean ± standard error (SE). One-way analysis of variance (ANOVA) was used to determine the differences among various groups, while Dunnett multiple post hoc test was used to compare the level of significance ($p < 0.05$) of each treated group with the negative control.

3. Results

The physicochemical parameters and heavy metals analyzed in the abattoir effluent are presented in Table 1. pH, alkalinity, electrical conductivity, nitrate, TS, BOD, COD, and As and Cu concentrations were higher than respective values in the negative control and national and international allowable limits for effluent quality criteria standards. Pb, Cd, Ni, and Cr were below detectable limit, while chloride, DO, and phosphate were below permissible limits by NESREA (Nigeria) and USEPA (USA) standards.

Table 1: Physicochemical parameters and heavy metals analyzed in the abattoir effluent and tap water.

Parameters	Effluent	Tap water	NESREA[a]	USEPA[b]
pH	8.93	8.20	6.5–8.5	6.5–8.5
Acidity	7500.02	1.51	—	—
Alkalinity	250.09	3.06	—	20
EC[c]	850	44	—	—
Phosphate	2.12	0.20	3.5	5
Nitrate	586.2	0.18	9.1	10
TS[d]	8876	98.4	—	—
DO[e]	2.1	7.7	—	—
BOD[f]	582.6	0.78	3	250
COD[g]	728.3	1.69	30	410
Chloride	13.5	56.7	300	250
Arsenic	0.68	0.004	0.05	—
Lead	BDL	BDL	0.01	0.015
Copper	0.10	0.05	0.001	1.3
Cadmium	BDL	BDL	0.005	0.005
Nickel	BDL	BDL	0.01	—
Chromium	BDL	BDL	0.01	0.1

All values are in mg/L except pH, alkalinity, acidity, and EC (μS/cm). BDL, below detectable limit.

[a]NESREA: National Environmental Standards and Regulations Enforcement Agency (2011) (Nigeria) maximum permissible limits for effluent for fisheries and recreational quality criteria standards.

[b]USEPA: United States Environmental Protection Agency (2006) (http://www.epa.gov/safewater/mcl.html).

[c]Electrical conductivity; [d]total solid; [e]DO: dissolved solid; [f]biochemical oxygen demand.

[g]Chemical oxygen demand.

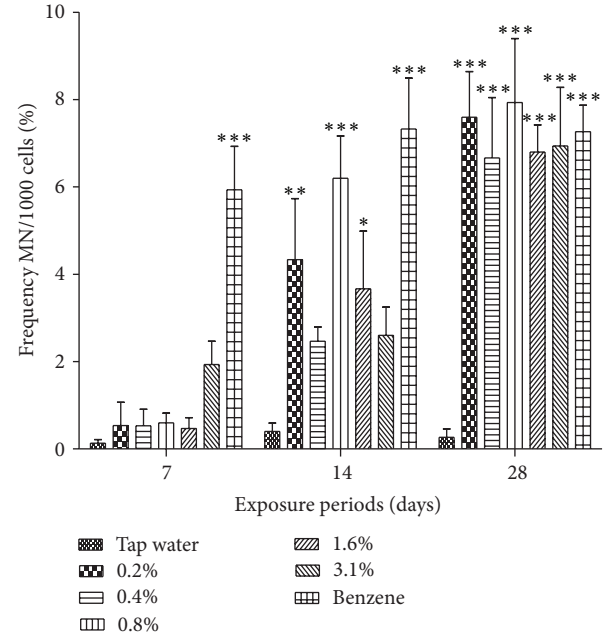

Figure 1: Percentage frequency of micronucleus formation in peripheral erythrocytes of *C. gariepinus* exposed to abattoir effluent. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ which are significantly different from corresponding negative controls (tap water) using Dunnett's multiple post hoc comparison test. Benzene (0.02 mL/L) positive control.

The abattoir effluent induced significant increase in percentage frequency of micronucleated erythrocytes in peripheral blood of *C. gariepinus* at the different exposure durations (Figure 1). The induced MN in the effluent treated *C. gariepinus* was higher than the tap water cultured fish by 4.05-, 4.03-, 4.55-, 3.53-, and 14.65-fold for 7 days' exposure; 10.84-, 6.17-, 15.50-, 9.17-, and 6.50-fold for 14 days' exposure; and 28.55-, 25.06-, 29.83-, 25.56-, and 26.08-fold for the 28 days' exposure according to 0.20, 0.40, 0.80, 1.60, and 3.14% effluent concentrations, respectively. MN induction did not show concentration dependent increase according to the effluent concentrations but increased according to exposure duration. Table 2 showed concentration independent significant increase in percentage frequency of total nuclear abnormalities in peripheral erythrocytes of *C. gariepinus*. The nuclear abnormalities decreased with increasing exposure time. Figure 2 presents the photomicrographs of the various micronucleated and nuclear abnormalities scored in the peripheral erythrocytes of *C. gariepinus*.

4. Discussion

Pollution of most aquatic and terrestrial ecosystems due to abattoir effluent discharge is eliciting national and international response due to the release of emergent xenobiotics into the environment [17, 18]. Chemical and microbial constituents of most abattoir effluents are known mutagens and carcinogens that are capable of increasing DNA damage in most biota [19]. The cytogenotoxicity induced by the abattoir effluent in *C. gariepinus*, *vis-à-vis* increased micronucleus and nuclear abnormalities in peripheral erythrocytes, suggests that xenobiotics, mostly toxic metals and organic compounds, in the tested effluent are clastogens and/or aneugens. The observed micronucleus and nuclear abnormalities indicate increased genetic alterations which may enhance somatic mutation and cancer formation [20]. This may lead to decreased embryonic viability, genetic disorders, reduced fitness, and biodiversity loss in aquatic biota [21].

Studies are scarce on the cytotoxicity and genotoxicity of abattoir effluents in both terrestrial and aquatic organisms, but there are similar observations as obtained herein with other wastewater/effluents from Nigeria [8–10]. Arsenate observed at higher concentrations in the tested abattoir effluent has been reported to possess clastogenic and aneugenic properties which may increase genomic instability enhancing mutagenesis and carcinogenesis in biological systems [22, 23]. Its genotoxic effect was linked to oxidative DNA adducts and DNA-protein cross-links [24]. Also Cu observed in the effluent though is considered an essential element that plays vital roles in normal enzyme activities but may induce DNA damage at higher concentration by binding to biologically sensitive molecules and forming dangerous free radicals [25–27]. Higher concentration of nitrate in the effluent is attributed to the evisceration and slaughter processes which release undigested stomach content into the wastewater [3,

Table 2: Mean ± SE/1000 cells of total nuclear abnormalities (NA) in peripheral erythrocytes of *C. gariepinus* exposed to abattoir effluent.

Treatment	Conc. (v/v; %)	7 days	14 days	28 days
Tap water	0	0.01 ± 0.08	0.01 ± 0.01	0.00 ± 0.00
Benzene	0.02 (mL/L)	0.19 ± 0.0[a]	0.32 ± 0.10[a]	0.22 ± 0.16[a]
Abattoir effluent	0.2	0.19 ± 0.11[a]	0.03 ± 0.12	0.10 ± 0.04[a]
	0.4	0.61 ± 0.29[c]	0.21 ± 0.10[a]	0.28 ± 0.12[a]
	0.8	0.91 ± 0.25[c]	0.14 ± 0.04[a]	0.29 ± 0.16[a]
	1.6	0.64 ± 0.27[c]	0.08 ± 0.03	0.28 ± 0.09[a]
	3.1	0.25 ± 0.08[a]	0.08 ± 0.05	0.22 ± 0.12[a]

Superscripts are significantly ([a] $p < 0.05$; [c] $p < 0.001$) different from negative control using Dunnett's multiple post hoc comparison test.

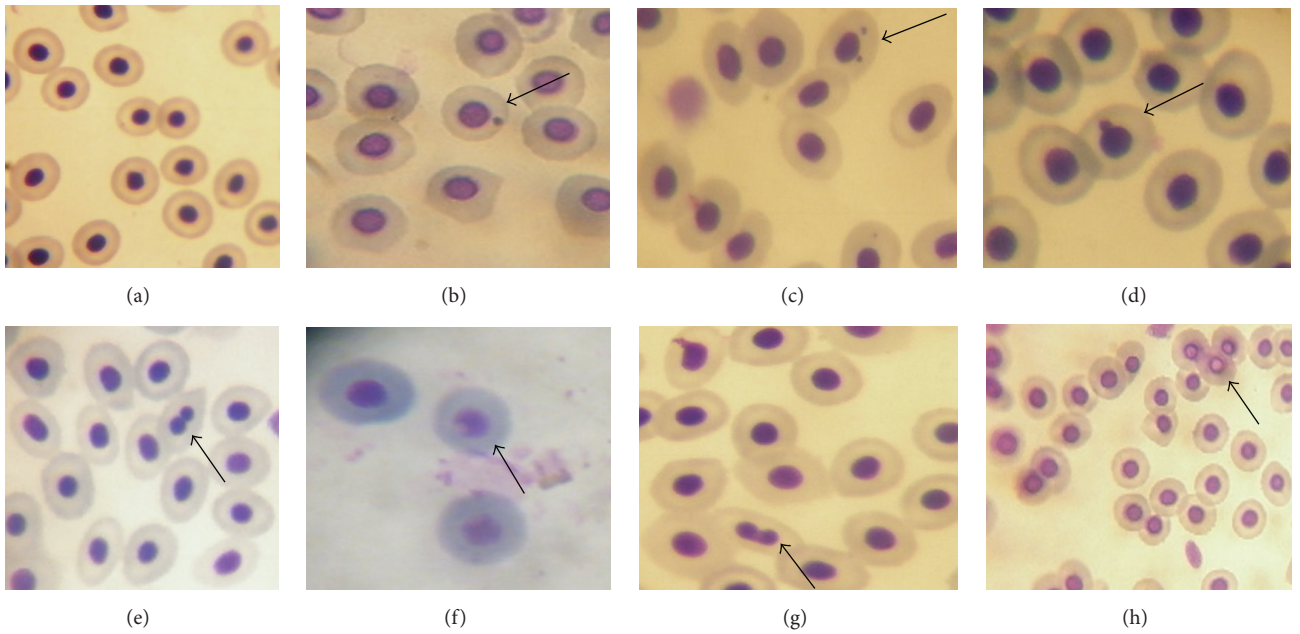

Figure 2: Micronucleated (MN) and nuclear abnormalities observed in abattoir effluent treated *C. gariepinus* are shown by the arrows: (a) normal peripheral erythrocyte. (b) MN peripheral erythrocyte. (c) Bi-MN peripheral erythrocyte. (d) Budded nucleus. (e) Binucleated peripheral erythrocyte. (f) Peripheral erythrocytes with notched nucleus. (g) Binucleated peripheral erythrocyte. (h) Vacuolated nucleus in peripheral erythrocytes.

28]. It is plausible that high nitrogenous compounds in the effluent formed reactive free nitric acid (NO), a cytotoxin, with mutagenic and carcinogenic properties, which increased the induction of micronucleated erythrocytes in the treated *C. gariepinus* [29]. The dose and time dependent variations in MN and NAs formation are in accordance with Hooftman and de Raat [30], who reported similar trend in eastern mud minnow, *Umbra pygmaea*, when exposed to ethyl methanesulfonate.

Nuclear abnormality assessment along with MN during cytotoxicity and genotoxicity monitoring of xenobiotics is efficient in fresh water fishes and under laboratory controlled conditions. Significant increase in total nuclear abnormalities observed in the treated *C. gariepinus* compared to the control suggests the presence of cytotoxins in the effluent. Their formations are linked to xenobiotic interference with the cell cycle and DNA synthesis via various mechanisms [31]. The formation of binucleated cells suggests that constituents of the effluent are capable of blocking cytokinesis of a normal dividing cell at M phase of the cell cycle [32] and may increase mutational frequency and carcinogenesis [33]. The presence of notch and bleb nuclei is associated with chromosome aneuploidy due to disturbance of the chromatin materials by xenobiotics in the abattoir effluent [32]. Vacuolated cell and nuclear bud formation (Figure 2) are associated with cell injury, cell death, and apoptosis induced by xenobiotics in the effluent [34].

High concentrations of electrical conductivity, TS, phosphates, nitrates, BOD, and COD and low concentration of DO in the abattoir effluent are in concert with those reported from Ogun River, Nigeria, which also received untreated abattoir effluent from slaughter houses [35]. These parameters along with heavy metals and unanalyzed organic compounds contributed to the induction of MN and NAs in peripheral erythrocytes and gill erythrocytes of *Synodontis clarias*, in the river [36]. This suggests threats to the functioning of the

aquatic ecosystems and the survival of most aquatic biota in abattoir wastewater polluted water bodies.

5. Conclusion

Clarias gariepinus were exposed to different concentrations of abattoir effluents from Bodija slaughtered house in Ibadan, Nigeria. The high concentration of the physicochemical parameters and some toxic heavy metals in the abattoir effluent provoked DNA damage via elevated micronucleated erythrocytes and erythrocytes with nuclei abnormalities in *C. gariepinus*. Xenobiotics in abattoir effluents are emerging clastogens and/or aneugens that are capable of inducing cytotoxicity and genotoxicity in aquatic biota.

Conflict of Interests

The authors declare no conflict of interests.

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