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A Comparative Hepatotoxic and Genotoxic Effect of An Antifouling Agent on Three Catfish *Clarias gariepinus, Clarias batrachus* and *Heteropneustes fossilis*

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Abstract A single exposure to a less explored antifouling paint caused noticeable and detectable hepatopathologic and genotoxic effects in three species of catfish. Among the three catfish, one is exotic, *Clarias gariepinus*, and the other two are Indian species, *Clarias batrachus* and *Heteropneustes fossilis.* Antifouling paint (Power Excel Hi-Gloss Synthetic Enamel Paint, trade name Black Japan)-induced pathological changes were recorded in hepatic histology and histochemistry along with micronucleus tests in erythrocytes following 96 hours of a single exposure of 0.1% concentration. The detrimental changes included infiltrations of inflammatory cells, increased pyknotic nuclei, cytoplasmic vacuolation, dilation of blood vessels, melanomacrophage aggregation, hepatic necrosis, apoptotic cell, rupture of the cell wall of the central vein, haemorrhages, etc. in the hepatic tissue. A significant depletion in the hepatic PAS-positive components and DNA content in the treated groups was also noted. The adverse effects involved erythrocytic cellular and nuclear abnormalities. Results of the haematological assays indicated a significantly higher ($P<0.001$) level of micronucleus frequency in *H. fossilis* compared to its control counterpart, and also compared to the other two experimental catfish species. From our study, it could be commented that an almost unexplored antifouling paint contained potentially toxic components that caused a hazardous effect on the Indian catfish, especially on *H. fossilis* because the particular fish species found to be highly sensitive to our antifouling paint concerning the haematological and histopathological observations. In this context, we can state that *H. fossilis* could be used as a tool for screening the histopathological and genotoxic effects of antifouling paint.

Keywords: *Antifouling paint, Clarias gariepinus, Clarias batrachus, Heteropneutes fossilis, Hepatotoxicity, Micronucleus, PAS reaction, DNA content*

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1. Introduction

Various aquatic animals are referred to as biofouling agents, which cause a great economic loss to boat and ship owners. [1] Biofouling organisms including algae, bryozoans, barnacles, mussels, etc. attach to the boat and ship and cause increased friction that eventually leads to great fuel consumption. [2,3] Accumulating sessile organisms on living and non-living surfaces is common in aquatic environments and to overcome the problem the boat and ship owners have been using several antifouling agents (paints), which are potential sources of aquatic pollution. [4] Different biocides including lead, copper, zinc, tin etc. have been reported to be present in such antifouling agents. [5] It has also been reported that the potency of antifouling agents in producing oxidative stress in the form of ROS induces oxidative damage to DNA, protein and lipids. Copper and dibutyltin present in some paints have been reported to adversely affect the hepatic reduced glutathione, acetylcholinesterase, and glutathione-S-transferase activities. [6,7]

Most of the local people of the area of southern West Bengal are using synthetic enamel paint (trade name Black Japan), an antifouling agent and the pollution caused may have serious impacts on fish health and fish production. Decreased enzyme functions in shrimps exposed to PVC vessels painted with Flexguard vi-ii, an antifouling agent have been reported. [8] The heavy metal exposure is found to cause cell lesions, and tissue damage, that subsequently leads to organ dysfunction as noted in histological studies of gill and liver in fish. [9,10] Fish are widely used as indicators of environmental pollution to evaluate the health of the aquatic ecosystem and physiological changes. Hepatic histopathology can be an indicator to evaluate

such effects on aquatic animals. [11] The hepatic tissue architecture of teleost is highly sensitive to detrimental environmental changes and shows a severe decrease in cell membrane integrity and a decrease in metabolic activity. [12,13,14] There are cellular accumulations in fish hepatic tissue which have been identified as melanomacrophage centres (MMCs) as some immune cells contain pigments and are a prominent feature. [15] MMCs contain different types of cells such as phagocytes, fragments of erythrocytes, pigments etc. and the pigments include melanin, hemosiderin, lipofuchsin of reticuloendothelial cells of hepatic tissue the primary functions of those MMCs are the storage, destruction and detoxification of phagocytosed exogenous and endogenous materials and these are used as biomarkers of environmental pollution.[16] Among the variety of biomarkers for assessing aquatic environments, the micronucleus (MN) test is one of the most popular cytogenetic assays for determining the genotoxicity level *in vitro*. [17,18] MN is a small, chromatin-containing round-shaped body visible in the cytoplasm of the cells which is formed from acentric chromosome fragments or whole chromosomes that lag at anaphase during nuclear division. [19,20,21,22] The presence of erythrocytic primary nuclear abnormalities, and erythrocytic cellular abnormalities other than MN can also be considered to be indicators of genotoxic damage. [23,24,25].

According to the information received from the aforementioned reports, there was no comparative study done among the Indian and exotic catfish following the exposure of a particular antifouling paint, which has been very much in use in our local areas and is less explored in global respect. The present work thus suggested and aimed to observe the comparative hepatotoxicity and genotoxicity of Black Japan antifouling paint on *Clarias gariepinus* (Burchell, 1822) (African sharp tooth catfish), and two Indian catfish *Clarias batrachus* (Linnaeus, 1758) (walking catfish), and *Heteropneustes fossilis* (Bloch, 1794) (Indian stinging catfish) to determine whether a single dose of the antifouling paint could develop impact on hepatocellular activities and erythrocytic nucleus, MN in three catfish under investigation. In addition, a comparative account of the parameters under consideration of the three untreated control catfish was aimed to get information on whether there was any difference concerning the parameters.

2. Materials and Methods

2.1. Preparation of Test Compound

High gloss synthetic enamel paint (trade name Black Japan) was procured from the local market and used as an antifouling agent (paint)for the present experiment in the laboratory. The working concentration of the antifouling paint was prepared by adding 100 mg of the paint to each glass aquarium filled with 10 L of water. The mixture was vigorously shaken for proper mixing and a sublethal concentration (0.1%) of the paint compound was prepared. The lethal concentration (LC_{50}) of the antifouling paint for our catfish and the experimental dose was selected as described. [4]

2.2. Experimental Design

Three experimented fish species *C. gariepinus*, *C. batrachus*, and *H. fossils* were purchased from the local market weighing 100.12 ± 20.00 g and the length measuring 22.0 ± 2.0 cm. The fish was brought to the laboratory and kept in the aquarium filled with 10 L of plain water. They were given fish pellets once a day and kept for three days in plain water for acclimatization. The known volume of test compound 100 mg /10 L (0.1%) was added to the three different aquaria. A control set was also run with the same number of fish in the same volume of water without the test compound. During the period of the treatment the feeding was stopped.

2.3. Experimental Duration

Fish from experimental groups were exposed for 96 hours and subsequently, the fish were removed from the aquarium, killed and autopsied. The hepatic tissue from the fish of control and antifouling agent-exposed groups was carefully collected, washed in vertebrate normal saline and fixed in Carnoy's fixative solution.

2.4. Histological Examination

Following fixation, the tissue was processed for making paraffin tissue block and the tissue sectioning was performed in a microtome in the laboratory. Histological staining was done using the standard H&E counterstaining method, and histochemical PAS [26] and DNA Feulgen methods [27] were followed. The intensity of the colour of the stain both in the control and treated tissues for histology and histochemistry was observed. The effect of the toxicant was categorised as follows (a) no alteration (0–2% area of section (b) mild alteration, + (> 2–10 % area of section) (c) moderate alteration, ++ (> 10–40 % area of section and (d) severe alteration, +++ (> 40% area of section). The morphological changes of the hepatic tissue sections noted in the experimental fish were compared with those of the untreated control fish.

2.5. Preparation of Micronucleus (MN)

A blood sample was collected either from the caudal vein or directly from the heart. A thin film of blood was made on the grease-free glass slide, allowed to dry in the air, briefly fixed in methanol, and then stained for three mins in stock May-Grünwald stain followed by staining again with diluted May-Grünwald stain for five mins. The slides were rinsed in distilled water and finally stained in diluted Giemsa (1 part stock solution and 10 parts phosphate buffer [pH 6.8] about 15 mins). The slides were air-dried and mounted with DPX. At least fourteen fields were studied under the optical microscope and approximately 1670 cells were analyzed from each slide under the microscope (ZEISS Primoster 1) at 1000x magnification (oil immersion) for MN and other erythrocytic cellular and nuclear abnormalities. The MN was identified by the presence of small cell inclusion detached from the larger definitive nucleus.

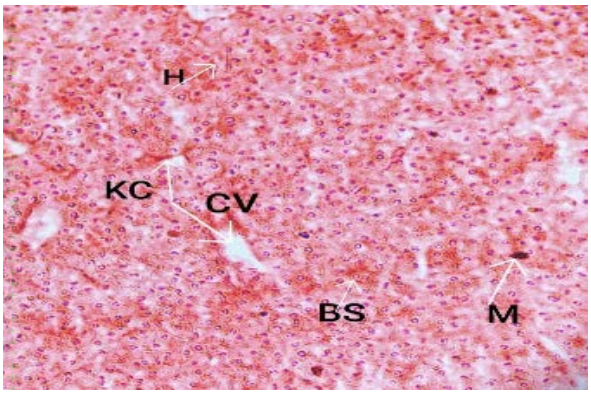
2.6. Statistical Methods

Assessment of the significant difference ($P<0.001$, $P<0.01$ and $P<0.05$) between control and treatment groups of three catfish species for the frequency of different types of cellular shape, nuclear shape, and frequency of MN was done with the execution of an unpaired t-test. A one-way ANOVA test was also performed to determine the significance among the aforementioned parameters in three control fish species. Whenever we found a significant F value, we then went for the next analysis- the Post hoc test (Tukey HSD beta) for any between-group comparison.

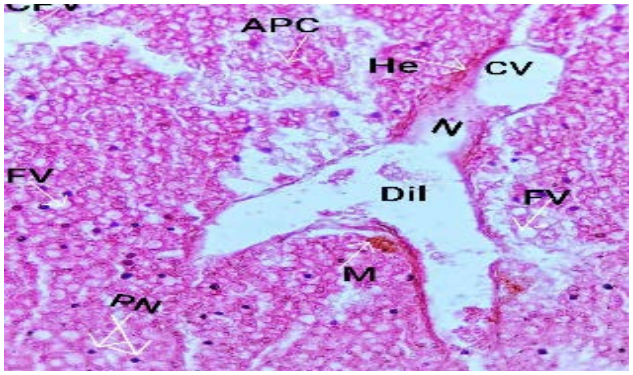
3. Results

3.1. Histopathological Studies

The microphotographs of the hepatic tissue of the unexposed and treated groups of three species of fish are shown in the figures (Figure 1A - Figure 9A) and (Figure 1B – Figure 9B) respectively. The control counterpart of all three catfish showed more or less a regular architecture with cords of hepatic cells, which appeared as a mass of hepatocytes. The hepatocytic cord was regularly interrupted by blood vessels and sinusoids. The cords of hepatocytes were in regular size, polygonal in shape with almost centrally located nuclei of *H. fossilis* as shown in the figure (Figure 1A). The treated hepatic tissue of *H. fossilis* (Figure 1B), on the other hand, revealed rupture of hepatocytes, vacuolations, lipid accumulation, and haemorrhage in the hepatic sinusoids along with a severe degree of cellular necrosis, increased Pyknotic nuclei, and aggregation of MMCs. A severe leucocytic infiltration and congestion of blood vessels were also observed, which were the indications of inflammation. An increased number of Kupffer cells was found in the antifouling paint-exposed hepatic tissue of *H. fossilis* compared to the control ones. In terms of the histochemical PAS (Figure 2A and Figure 2B) and nuclear DNA observations (Figure 3A and Figure 3B), the treated *H. fossilis* showed varied levels of damages. The PAS results showed a decreased level of hepatocytic glycogen content after the biocide exposure as compared with the control counterpart and the DNA content following the exposure was also found to decrease.



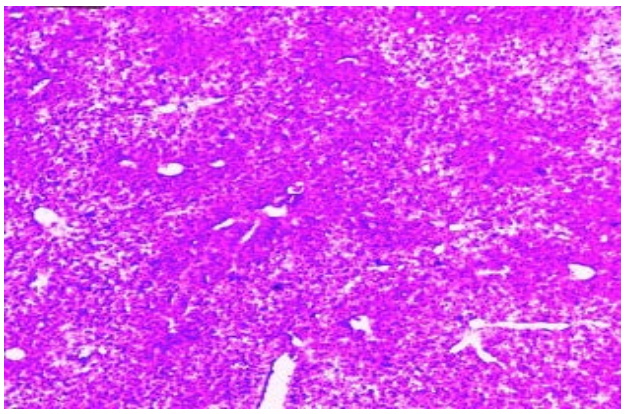
1A



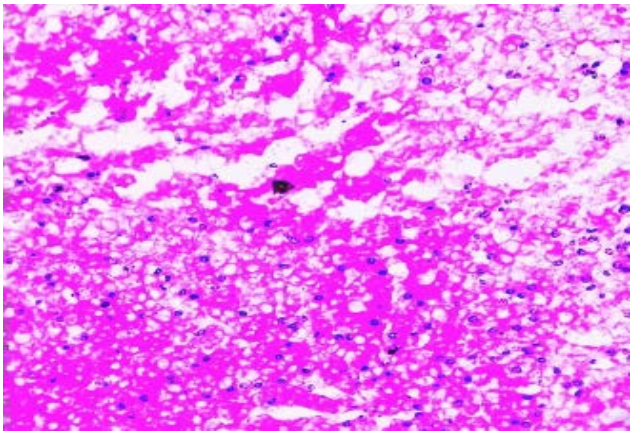
1B

Figure 1A. Hepatic tissue of untreated H. fossilis (H&E, 400x). H=Hepatocytes; KC=Kupffer cells; CV= Central vein; BS= Blood sinusoid, M= Macrophage

Figure 1B. Hepatic tissue of treated *H. fossilis* (H&E, 400x). APC= Apoptotic cell; He= Hepatocyte; Dil= Blood vessel dilation; M= Macrophage; N- Necrotic area; PN= Pyknotic nucleus; PV=Portal vein



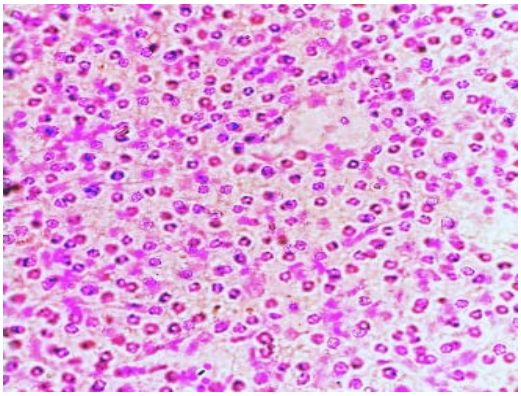
2A



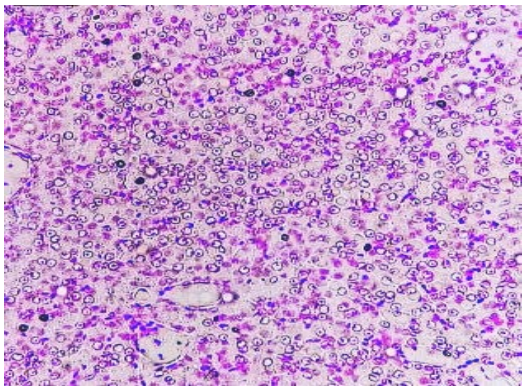
2B

Figure 2A. Hepatic tissue of untreated *H. fossilis* showing a great amount of glycogen content inside hepatocytes (PAS reaction, 400x).

Figure 2B. Hepatic tissue of treated *H. fossilis* showing a remarkable depletion in glycogen content inside hepatocytes (PAS reaction, 400x)



3A

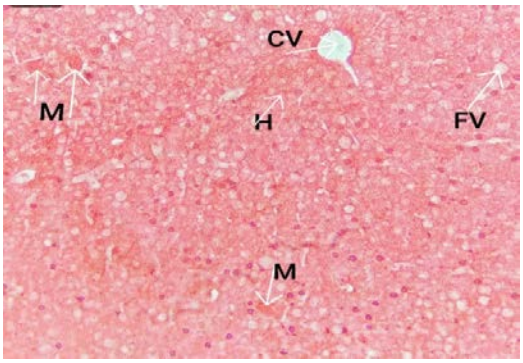


3B

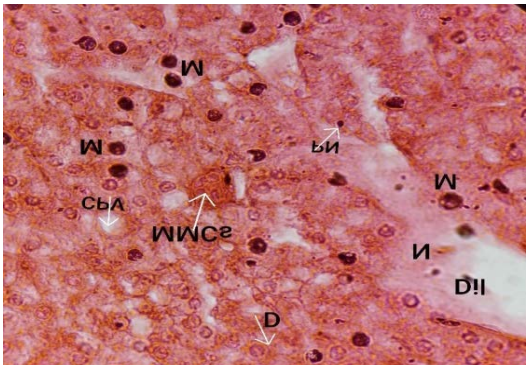
Figure 3A. Hepatic tissue of control *H. fossilis* showing a high amount of DNA content (Feulgen reaction, 400x)

Figure 3B. Hepatic tissue of treated *H. fossilis* showing a remarkable depletion in hepatocytic DNA content (Feulgen reaction, 400x)

The hepatic tissue of the unexposed control group of *C. batrachus* showed homogeneous distribution of polygonal-shaped hepatocytes but the antifouling paint led to an adverse effect on the hepatic tissue architecture including loss of regular cord-like architecture (Figure 4A and Figure 4B). The rupture of the cell membrane around the central vein was observed with the scattering of cellular content, pyknotic nuclei, cytoplasmic vacuolation, and lipid accumulation. The infiltration of inflammatory cells inside the central vein and between the hepatocytes was evident in the treated *C. batrachus*, and the aggregation of MMCs was found. The PAS reaction of treated *C. batrachus* showed a significant decrease in the PAS-positive components (Figure 5A and Figure 5B) along with the depletion in hepatocytic nuclear DNA content as compared to the control set (Figure 6A and Figure 6B).



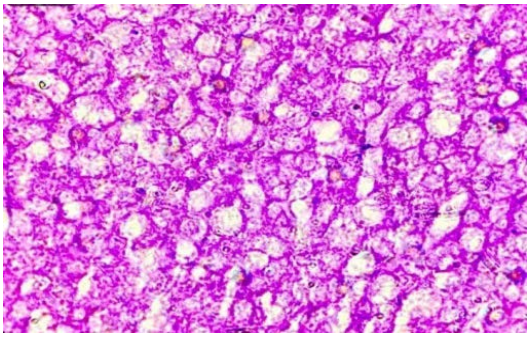
4A



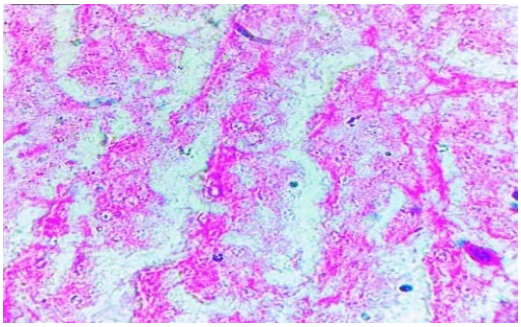
4B

Figure 4A. Hepatic tissue of untreated *C. batrachus* showing the normal architecture. H= Hepatocyte; CV=Central Vein; FV= Fat vacuole. (H & E, 400x)

Figure 4B. Hepatic tissue of treated *C. batrachus* showing the altered architecture (H & E, 400x). MMC= Melanomacrophag; Dil= Blood vessel dilation; PN= Pyknotic nucleus; M=Macrophage; N= Necrotic area



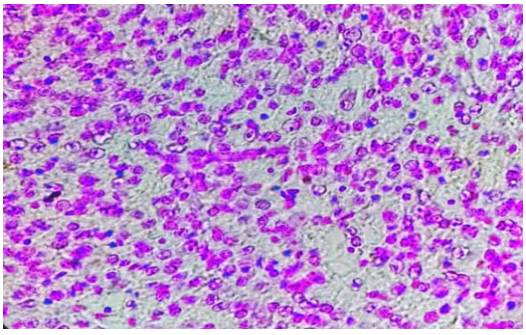
5A



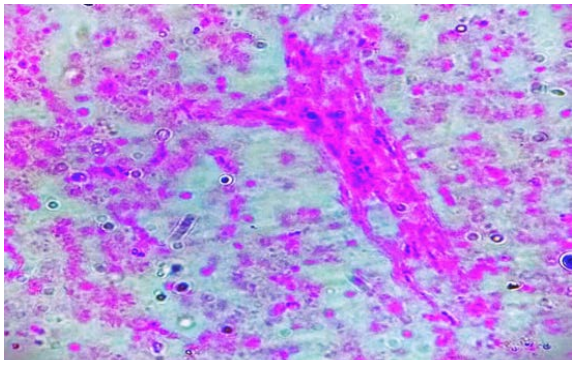
5B

Figure 5A. Hepatic tissue of untreated *C. batrachus* showing plenty of PAS- positive substances inside hepatocytes. (PAS reaction, x 400).

Figure 5B. Hepatic tissue of treated *C. batrachus* showing severe depletion in PAS positive substances (PAS reaction, x 400)



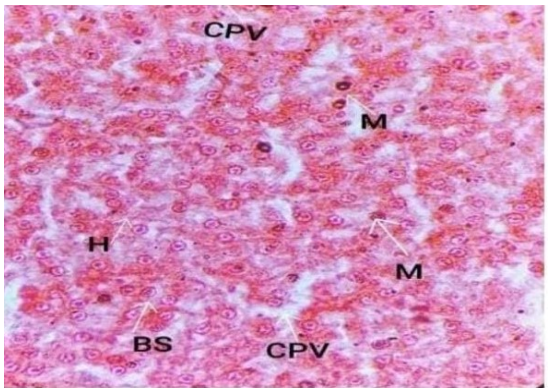
6A



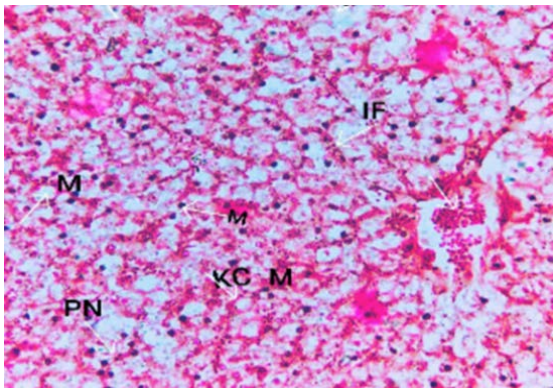
6B

Figure 6A. Hepatic tissue of untreated *C. batrachus* showing normal DNA content (Feulgen DNA reaction, 400x)

Figure 6B. Hepatic tissue of treated *C. batrachus* showing a lesser amount of DNA content (Feulgen DNA Reaction, 400x)



7A

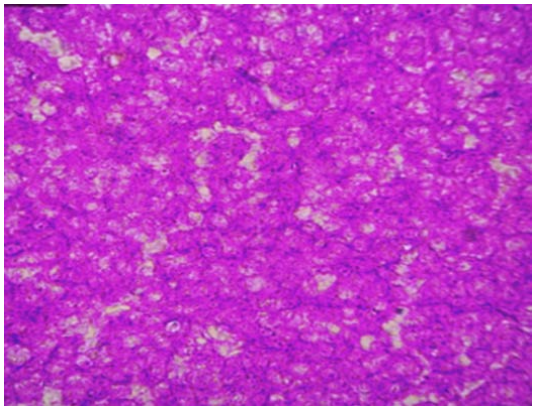


7B

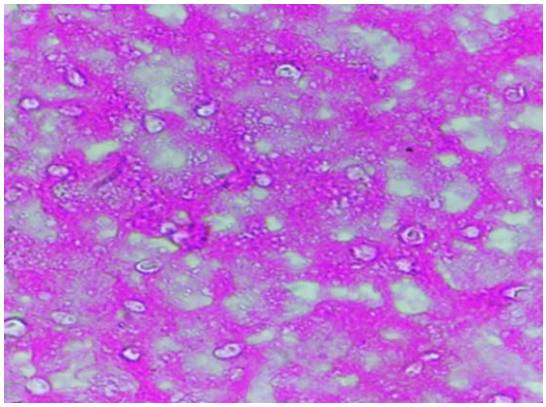
Figure 7A. Hepatic tissue of untreated *C. gariepinus* showing the normal architecture. H= Hepatocyte; CPV= Cytoplasmic vacuolation, M=Macrophage, BS= Blood sinusoid. (H & E, 400x)

Figure 7B. Hepatic tissue of treated *C. gariepinus* showing the altered architecture (H & E, 400x). MMC= Melanomacrophag; Dil= Blood vessel dilation; PN= Pyknotic nucleus; M=Macrophage; N= Necrotic area

We recorded the hepatic histological changes in *C. gariepinus* following exposure and noted several structural damages and abnormalities (Figure 7A and Figure 7B). The degree of hepatic architectural abnormalities was found very high. The figures show severe damages represented by the heterogeneous hepatic tissue structure. The hepatocytes of the treated *C. gariepinus* lost their usual polygonal shape, and the boundaries between cells became invisible correspondingly a high infiltration of leucocytes inside the blood vessels and between the hepatocytes was noted. An aggregation of MMCs inside the cytoplasm was observed. The individual MMCs were found to aggregate with each other forming a clamped shape in the antifouling paint-exposed *C. gariepinus*. The dilation of blood vessels and a large number of pyknotic nuclei with the accumulation of lipid vacuoles and proliferation of hepatocytes were noted. The PAS reaction in the case of the treated group revealed a decrease in the glycogen content (Figure 8A and Figure 8B) and the hepatocellular nuclear DNA content revealed a depletion as compared to the control set (Figure 9A and Figure 9B). Several levels of detrimental effects of the antifouling paint on hepatic architecture are summarized in Table 1. The table shows a comparative account of histopathological observations of the hepatic tissue of the experimental fish under investigation. Interestingly, the control untreated *C. gariepinus* showed some altered hepatic features for the endothelial dislocation, pyknotic nuclei and MMC aggregation as compared with the only MMC aggregation in the control untreated *C. batrachus.* The control untreated *H. fossilis* on the other hand showed no altered hepatic architecture. The antifouling paint exposure caused adverse changes in the three experimental fish but the degree of severity varied in them. The hepatic tissue architecture of treated *C. gariepinus* and *C. batrachus* altered similarly but the *H. fossilis* responded differently towards biocide exposure compared with the counterparts.



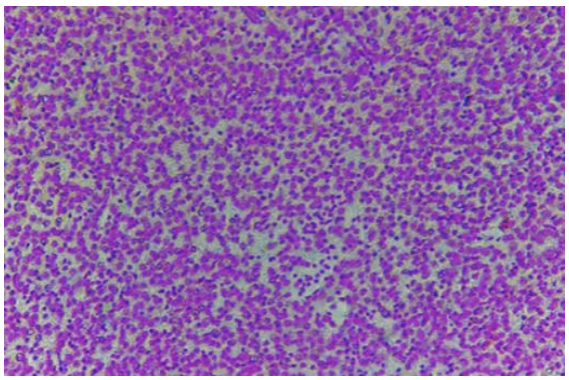
8A



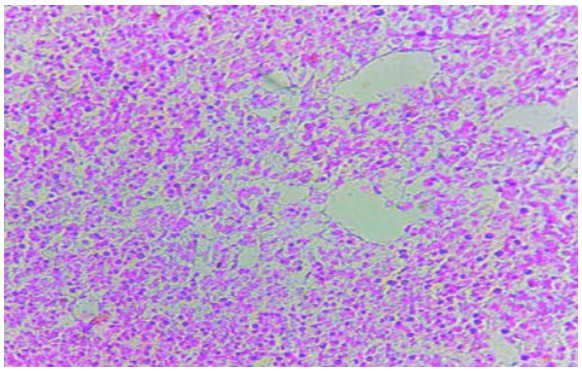
8B

Figure 8A. Hepatic tissue of untreated *C. gariepinu* showing compact and composed architecture. (H & E, 400x)

Figure 8B. Hepatic tissue of treated *C. gariepinus* showing depleted PAS positive substances inside hepatocytes (PAS reaction, 400x)



9A



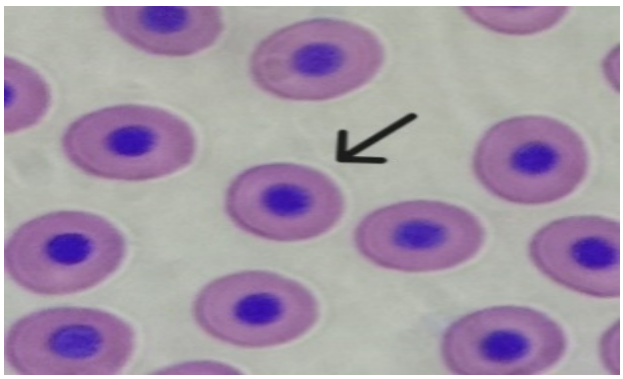
9B

Figure 9A. Hepatic tissue of untreated *C gariepinus* showing normal DNA content (Feulgen DNA reaction, 400x)

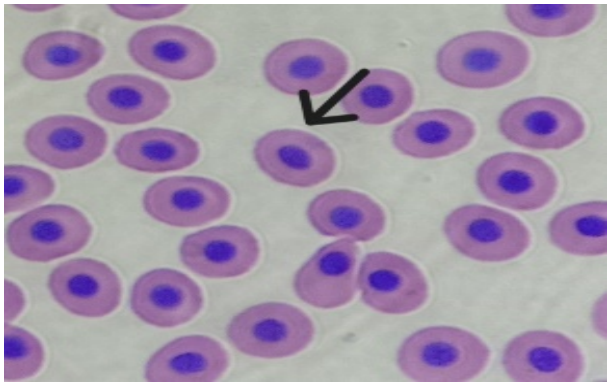
Figure 9B. Hepatic tissue of treated *C, gariepinus* showing depleted DNA content (Feulgen DNA reactio0n, 400x)

3.2. Haematological Studies

In the case of MN studies, the frequency of round cells (RC), elliptical cells (EC), irregular cells (IC) such as sickle-shaped cells, echinocytes, cells with deformed nuclei (DN), notched nuclei, nuclear degeneration and micronuclei (MN) (Figure 10A – Figure 10H) of antifouling paint-exposed the numerical values of are summarized in Table 2. Concerning the RC frequency, a significant decrease was observed in the treated *C. gariepinus* and *H. fossilis*, but *C. batrachus*, on the other hand, showed no change in the frequency. A highly significant decrease (~73%) was observed in *C. gariepinus* as compared with a ~34% significant ($P<0.01$) decrease in *H. fossilis*. Three catfish under investigation showed a highly significant ($P<0.001$) increase in the EC count in the treated groups. Similarly, the IC count indicated a significant increase in the post-exposed group and the value increased 600 - 800% in the antifouling agent-exposed two catfish except for the *C. gariepinus*, which indicated no change. Although the count of DN increased significantly ($P<0.001$) in the Indian catfish, the same was found to decrease significantly ($P<0.001$) in the exotic counterpart. Similar to that of DN, the MN showed a differential response towards the toxic exposure, while the MN value increased significantly both in *C. gariepinus* and *H. fossilis*, the same remained unchanged in *C. batrachus* as indicated by insignificant change. Following the treatment, the MN in *H. fossilis* increased ~805% from its control counterpart, which was found to be the highest increased value concerning that of the other two catfish under observation.



10A



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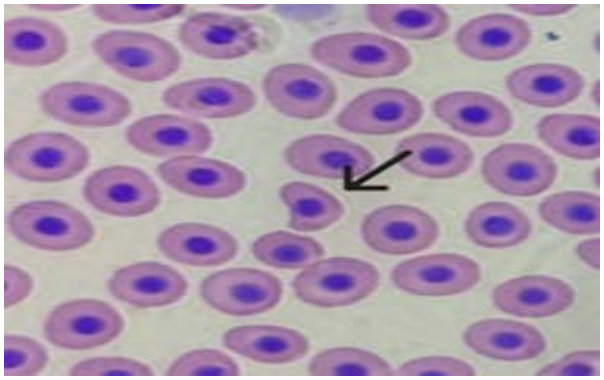

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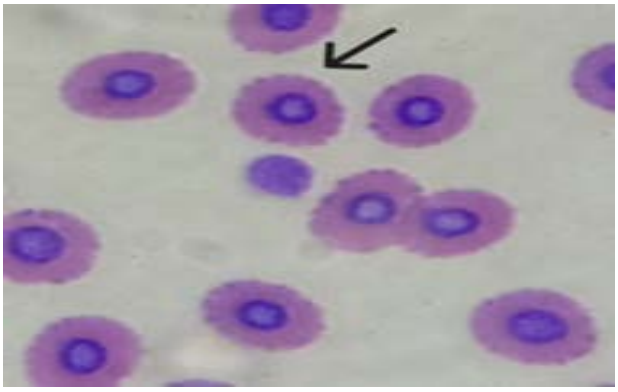

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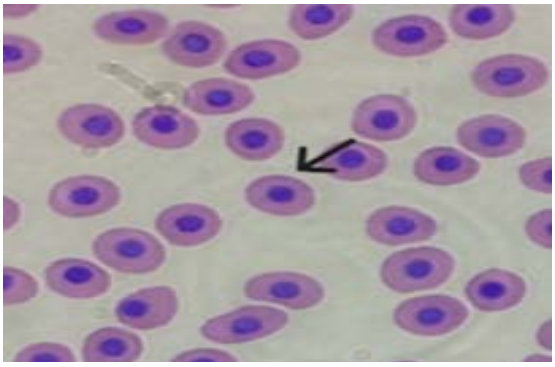

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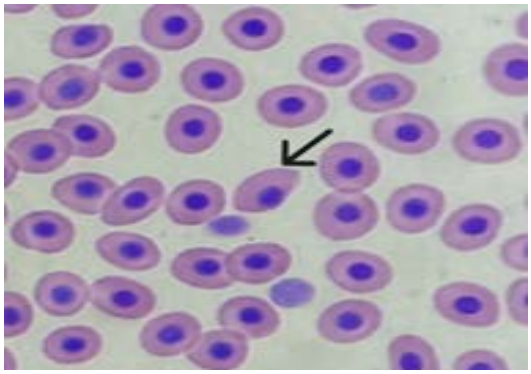
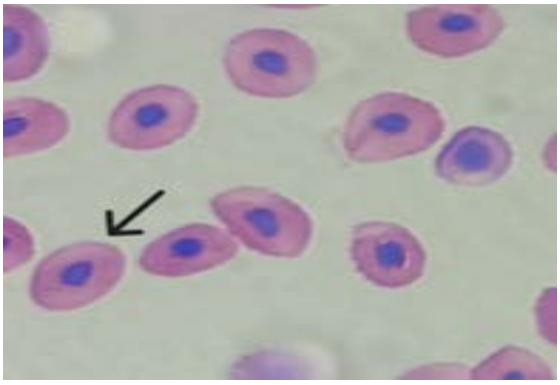
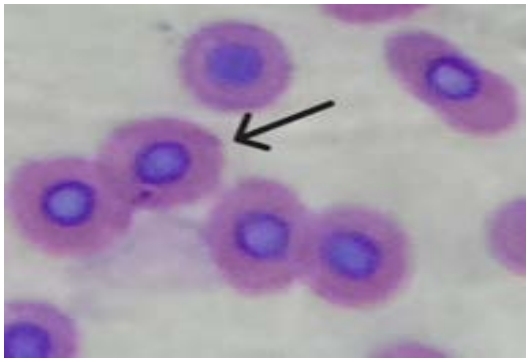


10G



10F



10H

Figure 10A-10H. Photomicrographs of erythrocytes from blood of experimental catfish species. Figure 10A) Round Cell; Figure 10B) Elliptical Cell; Figure 10C) Sickle-shaped Cell; Figure 10D) Echinocyte; Figure 10E) Normal Nucleus; Figure 10F) Notched Nucleus, Figure 10G) Nuclear Degeneration and Figure 10H) Micronucleus

Table 1. Histopathological observations in hepatic tissue of three experimental catfish species exposed to a single dose of antifouling paint for 96 hours

Features	Control			Treated		
	C. gariepinus	*C. batrachus*	*H. fossilis*	*C. gariepinus*	*C. batrachus*	*H. Fossilis*
Endothelial dislocation	+	-	-	+++	++	++
Blood vessel dilation	-	-	-	++	++	+++
Pyknotic nuclei	+	-	-	++	+++	++
Necrotic area	-	-	-	+++	+++	+++
Inflammatory cells in central vein	-	-	-	++	+++	+
MMC aggregation	+	+	-	+++	++	+
Kupffer Cells	+	+	+	++	++	+

Lesions were recorded on the basis of severity as '-' None; '+' Mild; '++' Moderate; '+++' Severe alterations following the antifouling agent exposure.

Table 2. Mean (±SD) number of different kinds of red blood cells with nuclear abnormalities and micronuclei in three species of control and treated catfish

Types of Erythrocytes	*C. gariepinus*		*C. batrachus*		*H. fossilis*	
	Control	Treated	Control	Treated	Control	Treated
Round Cell	76.17 ± 32.07	20.57[a] ± 6.14 (-73.00%)	16.36 ± 9.68	19.92[ns] ± 3.77 (+21.76%)	21.21 ± 7.26	13.93[b] ± 4.12 (-34.32%)
Elliptical Cell	55.33 ± 28.65	103.71[a] ± 21.61 (+87.44%)	13.58 ± 8.89	40.69[a] ± 4.68 (+199.63%)	45.57 ± 11.02	69.93[a] ± 8.24 (+53.46%)
Irregular Cell	12.33 ± 11.57	15.50[ns] ± 4.85 (+25.71%)	3.00 ± 2.25	23.23[a] ± 2.74 (+674.33%)	6.36 ± 3.05	56.07[a] ± 13.20 (+781.60%)
Deformed Nucleus	21.00 ± 4.82	11.14[a] ± 2.60 (-46.95%)	6.14 ± 3.16	13.46[a] ± 1.76 (+119.22%)	7.79 ± 2.69	56.71[a] ± 13.77 (+627.98%)
Micronucleus	0.67 ± 1.21	2.29[ns] ± 2.97 (+145.45%)	1.29 ± 1.54	0.77[ns] ± 1.01 (-40.31%)	0.71 ± 0.83	6.43[a] ± 3.63 (+805.63%)

[a](P<0.001); [b] (P<0.01); [ns] (not significant)

3.3. Statistical Analyses

The data in Table 3A indicates the value of the investigated parameters of the control catfish only. The results obtained from control catfish have been analyzed using the one-way ANOVA test and revealed that the values of all parameters have been significantly different. The F-values of RC (36.17), EC (24.14), IC (6.67), and DN (28.05) have shown significance at P<0.001, P<0.001, P<0.01, and P<0.001 respectively. On the other hand, the MN count of all three catfish has not shown any difference among themselves. The data with significant F values have been considered for the Post Hoc Tukey's HSD test to find out which two groups are significantly different from each other (Table 3B). Excepting the RC, IC, and DC counts between *C. batrachus* and *H. fossilis*, and the EC count between *C. gariepinus* and *H. fossilis*, all other parameters between comparing groups have shown significance.

Table 3A. Mean (± SD) values of different haematological parameters of three control catfish under study with the F value of ANOVA

Types of Erythrocytes	*C. gariepinus*	*C. batrachus*	*H. fossilis*	F-value
Round Cell (RC)	76.17 ±32.07	16.36 ±9.68	21.21 ±7.26	36.17^{a}
Elliptical Cell (EC)	55.33 ±28.65	13.58 ±8.89	45.57 ±11.02	24.14^{a}
Irregular Cell (IC)	12.33 ±11.57	3.00 ±2.25	6.36 ±3.05	6.67^{b}
Deformed Nucleus (DN)	21.00 ±4.82	6.14 ±3.16	7.79 ±2.69	45.23^{a}
Micronucleus	0.67 ±1.21	1.29 ±1.54	0.71 ±0.83	0.93^{ns}

[a] (P<0.001); [b] (P<0.01); [ns] Not Significant

Table 3B. Post Hoc Tukey HSD (beta) of the Table 3A

Comparison	RC	EC	IC	DN
CG vs CB	12.35^{a}	8.84^{a}	5.53^{b}	13.97^{a}
CG vs HF	11.35^{a}	2.07^{ns}	3.54^{c}	12.42^{a}
CB vs HF	1.00^{ns}	6.77^{a}	1.99^{ns}	1.54^{ns}

[a](P<0.001); [b](P<0.01); [c](P<0.05); [ns] Not Significant;
CG= *C. gariepinus*; CB= *C. batrachus*; HF= *H. fossilis*

4. Discussion

The findings of the present study indicate that antifouling paint frequently being used by some groups of people in the riverine West Benga for their fishing boats or public transport, has severe adverse effects on the non-target species (edible aquatic organisms), which potentially offers to the human food toxicity and scarcity. In the present study, we found the potential risk /threat of using antifouling paint in aquatic vessels because an agent like this caused detrimental effects on the hepatic tissue of the exposed non-target aquatic animal. The liver is an important organ in active metabolism, and detoxification and is extremely sensitive to pollutants and the organic solvents used in paint manufacturing and shoe-making industries can cause hepatotoxicity. [28] Hepatic histological damage has been used as an indicator of environmental pollution. [29,30] A foremost use of histopathological biomarkers in an organism such as fish is to specify and evaluate the toxic effect of biofouling agents when exposed to heavy metals.[31] Additionally, the histology of fish liver might be used as a pattern for research in the direct reaction between ecological factors and hepatic metabolic functions. [32]

Our study demonstrated several histopathological changes in the three experimental species of catfish exposed to a sub-lethal concentration of an antifouling paint and the treatment caused differential adverse effects on the three species because it might be the fact that the same toxicant was differentially handled by the hepatic tissues of the catfish under observation. So far as our knowledge goes the antifouling paint used in the present investigation was not reported to use in any previous toxicological studies. The observed hepatocytic vacuolations with pyknotic (condensed) nuclei were most likely due to the deposition of glycogen and lipids [33] as a result of hepatotoxicity induced in the presence of the toxicant. [34] The depletion of glycogen as observed in our present study may be due to direct intoxication or may also be due to a secondary diseased body condition following oxidative stress [35] because the antifouling paint exposure caused a higher number of hepatic lipid peroxidation with concomitant production of ROS. [4]

The hepatic tissue of control untreated *C. batrachus* and *H. fossilis* showed normal architecture but the same in the case of the control untreated *C. gariepinus* revealed some alterations and the indicated changes in them may be since the species were already under stressful conditions due to overcrowding and unhealthy farming condition and the exposure to the antifouling paint as per our investigation may lead to the observed and detectable changes including dilation of the hepatic central vein, heterogeneity of hepatocytes, rupturing of the blood vessels, and blood cellular congestion in the blood vessels. The toxicant-caused alterations of the liver parenchyma include vacuolization and necrosis, and those alterations were often associated with a degenerative-necrotic condition. [33] Besides *C. gariepinus*, the other two catfish could be considered efficient biomarkers as far as their response to the single exposure of the antifouling paint is concerned. Hepatic histology can be an indicator to evaluate the effect of water pollutants on aquatic animals because the architecture of liver histology of teleost is highly sensitive to environmental changes and shows a severe decrease in cell membrane integrity and a decrease in metabolic activity. [12,13,14] There are cellular accumulations in fish hepatic tissue which have been identified as MMCs as some immune cells that contain pigments and are a prominent feature in hematopoietic tissue. [15] The primary functions of the MMCs are the storage, destruction and detoxification of phagocytosed materials as functions of the reticuloendothelial system. [16] In our present investigation, we noted a significantly higher number of MMCs in the experimental fish.

Immature RBCs (erythroblasts) are usually rounded in shape [36,37,38] and they are known as round cells (RC). The decrease in the number of such RC in the present investigation indicated that the components of the antifouling paint may affect the production of RBC in bone marrow by influencing the process of erythropoiesis, which may cause anaemia. The RC value in *C. batrachus* remained unchanged and in this connection, it may be

commented that *C. batrachus* could successfully prevent the adverse effect of the particular toxicant used in the present study. During the process of development, the RCs become transformed into EC and mature. We found more ECs in the treated groups, which were the mature RBCs and this may be due to a sudden surge in the number to manage the toxicity-induced oxidative stress in them. The values of IC increased significantly in the treated groups of *C. batrachus* and *H. fossilis* but there was no difference in *C. gariepinus* between the control and treated groups. The erythrocytic abnormalities were reported to be caused by the modifications of the cell membrane of the cell itself following toxicity. [39,40] In recent years increased nuclear abnormalities were noted following toxicant exposure [24] but the mechanism of formation of such abnormal nuclei was not fully understood. [25] Increased deformities of erythrocytes could be due to the higher lipid peroxidation [41,42,43] which was one of the primary events associated with the cellular injuries that expressed in the form of tissue lesions due to DNA base oxidation. [44] This enhanced peroxidation corrupts the lipid membrane, thus, the increase in permeability and flexibility of the membrane results in an increased number of other cellular abnormalities [45] and eventually leads to cell death. The increased IC in the present study corroborated with the earlier observations and it could be said that our antifouling agent harmed the erythrocytic membrane, which could lead to the formation of more IC in the Indian catfish compared with the *C. gariepinus*, where the exotic variant was found resistant at least to the particular haematological parameter. We observed different nuclear abnormalities and they showed a significant difference within the three catfish. The frequency of DN increased in *C. batrachus* and *H. fossilis* but decreased in *C. gariepinus*. Earlier studies recorded erythrocytes with blebbed, notched and fragmented nuclei and that might be related to the failure of tubulin polymerization [40,46] and mitotic spindle fuses caused by the aneugenic actions of toxicants. [47] Toxicants were reported to produce excessive caspase-activated *DNAse*, which caused nuclear abnormalities [46] So, the present results of the DN in the exposed group may be due to the failure of tubulin polymerization, mitotic spindle fusions, excessive production of *DNAse*, etc. In this respect, it could be commented that the DN would be a biomarker for the Indian catfish but the exotic catfish was found resistant concerning this parameter. The increased frequency of the erythrocytic MN can be due to the mitotic disturbance through chromosomal aberration [23,40] and may also be due to mis-pairing or non-pairing of DNA fragments. [22] One of our experimental fish (*H. fossilis*) showed a significant increase in the MN frequency as compared with other experimental fish and concerning this, the fish species could be considered a biomarker for the MN frequency study.

5. Conclusion

In our present study, we recorded a high degree of histological, histochemical and genetic alterations in edible fish following a single dose of exposure for 96 hours to a very least-known antifouling paint which is being used to a large extent. In some parameters, we noted differential responses toward the toxic exposure as the two Indian catfish together responded differently from that of the exotic variant. Our study warrants further investigation with a series of doses to observe a similar effect on vulnerable tissues like gills, kidneys, spleen, intestine, etc. As we noted severe histopathological changes posttreatment in the hepatic tissue, the quantification of DNA, RNA and protein of the hepatic tissue can also be carried out along with the study of enzyme biomarkers like *catalase*, *glutathione-S-transferase*, *gamma-glutamyl transpeptidase* etc. As the RBCs were adversely affected by the treatment, the Hb content and total RBC, PCV etc. could help ensure the anaemic effect of the paint. The appearance of micronuclei following treatment in our investigation also warrants observations concerning chromosomal aberration studies. The mechanism of action of this particular paint pollutant has yet to be understood and thus there is ample scope to explore the potential toxic effect of the Black Japan antifouling paint on fish of economic importance. Finally, the bioaccumulation and biomagnification of the components of our antifouling paint can also be taken into consideration.

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Statement of Competing Interests

All the contributing authors declare that they have no conflict of interest.

List of Abbreviations

MMC= Melanomacrophage center; EC= Elliptical cell; IC= Irregular cell; RC= Round cell; DN= Deformed nucleus; MN= Micronucleus

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