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Original Research Article

HEMOTOXIC AND GENOTOXIC EFFECTS OF LEAD ACETATE AND CHLORPYRIFOSE ON FRESHWATER CAT FISH *(Calarias gariepinus)*

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Abstract: This study was conducted to evaluate hemotoxicity and genotoxicity induced by lead acetate and chlorpyrifos in catfish (Clarias gariepinus) as a model for checking genotoxic pollutants in aquatic surroundings. Lead acetate was added by a dose of 24.4 mg/L (20% of the LC50) daily, chlorpyrifos was added by a dose of 1.65 mg/L (1/10 LC50) daily. Fish were kept in standard condition in which water temperature (25°C± 2); pH (7.6 ± 0.4) and dissolved oxygen (5.4±0.4 mg/L). Blood and liver were sampled after 4 weeks. The result revealed a significant reduction of RBCs count, Hb, hematocrit in fish exposed to lead and chlorpyrifos. Furthermore, MDA level and catalase activity showed significant increase and decrease, respectively in groups exposed to lead acetate and chlorpyrifos in comparison with the control fish. Toxic effect of lead acetate and chlorpyrifos confirmed by histopathological changes in liver sections which showed marked hepatic vacuolation and parenchymal hemorrhage. DNA damage detected by comet assay also revealed a significance increase in tail length, tail DNA% and tail moment at sublethal concentration of lead acetate and chlorpyrifos. This study concluded that lead acetate and chlorpyrifos have hemotoxic and genotoxic effect probably through, at least in part, induction of reactive oxygen species and chlorpyrifos has more hemotoxic and genotoxic effect than lead acetate.

Key words: catfish; lead acetate; chlorpyrifos; DNA damage

Introduction

Pollution of the aquatic surroundings is a serious and growing problem, caused by increasing variety and quantity of industrial, agricultural and business chemicals (1-3). Many water pollutants are capable of inducing oxidative stress on fish (4) which consequently led to cellular and tissues damage in fish (5). Heavy metals and pesticides are poisonous to animals just as individuals (6). Heavy metals are considered as the most important pollutants, because of their notable impacts on the aquatic system equilibrium, bioaccumulation in life forms, long term persistence and ability to aggregate in sediments and water (7). Heavy metals, like mercury, cadmium, copper, lead and zinc are the most vital poisons which influence aquatic environment and fish. They are extremely unsafe for the health of fish. These metals can ef-

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fectively affect the vital operations and reproduction of fish; weaken the immune system, and lead to pathological changes (8)

Lead (Pb) is a prevalent, cumulative and insidious environmental waste product that induces a broad range of physiological, biochemical and behavioral dysfunctions (9-11). With the increasing prevalence of lead pollution in the aquatic surroundings, the potential danger of lead to aquatic animals must be evaluated (12). Studies have demonstrated that lead can induce neurological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical effects in the animals and human (13, 14). One of the best known impacts of lead is its interference with synthesis of heme (15). High Pb concentration in soft tissues inactivate alpha-amino levulinic acid dehydratase (ALAD) enzyme which associated with rise of alpha -aminolevulinic acid (ALA) level (16). Increase of ALA level leads to production of reactive oxygen species (17)

Pesticides are used to control pests, insects, aquatic weeds, plant diseases, and Aquatic snails which transfer schistosomiasis. Pesticides have been observed to be very harmful not only to fish but also to the other organisms. (18). Widespread of pesticides and their improper use cause changes in the aquatic medium. Observing of different effects is wanted for good pesticides control (19). Two main types of pesticides; organophosphates and organochlorines, cause serious harmful impact on the aquatic system and their residues present in water are toxic to aquatic organisms (20, 21). One such organophosphate, is chlorpyrifos (CPF) [O, O-diethyl-O-(3,5,6 -trichloro-2-pyridyl) phosphorothionate] which is broadly utilized for the management of domestic and agricultural pests. CPF utilized additionally for over 10 years to control foliar insects (arthropoda) that influence farming yields, and subterranean termites (22). Oxidative stress which induced by pesticide has been a focal point of toxicological research for the last decade as a potential mechanism of toxicity (23, 24) . Toxicity of reactive oxygen species counteracted by antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR) (25). Antioxidants protect the cells and tissues from oxidative damage under normal conditions (26). The antioxidants in fish could be considered as biomarkers of exposure to aquatic contaminants (27). Exposure to pollutants causes several biochemical and physiological responses which may be adaptive or lead to toxicity. Thus, it is important that pollutant effects be detected and interpreted in biochemical terms, to show mechanisms of pollutant action, and possible ways to prevent there bad effects (28).

African catfish (*Clarias gariepinus*) is very important fish because of its high growth rate, high consumer acceptability and high resistance to bad water quality and low oxygen (29). It is omnivorous feeder and a general scavenger with a marked tendency to feed on benthic organisms and this make it in continuous exposure to different pollutants which concentrated in the sediments. Biomagnification of pollutants throuth eating fish which already accumulate chemicals make this fish accumulate pollutants in greater amounts than other herbivorous fish (30). Catfish considered as an excellent model for toxicological studies and has been used in fundamental research (31, 32). Since fish are important as proteins and lipids sources for humans and domestic animals, so health of them is very important for human beings (18). Therefore, this study was conducted to indicate the hemotoxic and genotoxic effect of both chlorpyrifose and lead acetate on African catfish

Materials and methods

Chemicals

Lead acetate trihydrate (Mol.Wt. 379.33 g/mol, product No. 316512, purity 95%)and other chemicals used in this study were purchased from (Sigma–Aldrich), chlorpyrifos was obtained as a commercial product (Pyrifos EL NASR 48% EC) from (El Nasr Pharmaceutical Company, Cairo-Egypt).

Experimental fish

Catfish (*Clarias gariepinus*) of both sexes, average weight 150 were purchased from fish

farm in Kafrelsheikh, Egypt. Fish was transported in large plastic water containers and maintained in the glass aquaria in the laboratory of faculty of veterinary medicine, Kafrelsheikh University. Fish left without any treatment for two week for acclimation before starting the experiment. Fish were kept in standard condition during the period of acclimation and the experiment in which water temperature is 25°C± 2, pH is 7.6 ± 0.4 and dissolved oxygen (5.4±0.4 mg/L). Water was supplied with oxygen by air pump, fish kept in 12/12hour light and dark, and were fed on commercial high protein diet (30 %) at ratio of 3% of body weight/day. The water was changed every 2 days to prevent the accumulation of fecal matter and to maintain the pollutants concentration.

Experimental design and treatment

A total of 120 fresh water catfish were divided into three groups, each group has two replicates (20 fish replicate), kept in (100×50×30 cm) glass aquaria contain 100L tap water (dechlorinated); the first group (G1) was considered as control group, the second group (G2) was exposed to lead acetate by a dose of 24.4 mg/L (20% of the LC_{50}) (33) and the third group(G3) was exposed to chlorpyrifos by a dose of 1.65 mg/L (1/10 LC_{50}) (34), for 4 weeks, the water was changed every 2 days to avoid the accumulation of fecal matter and to maintain the toxicant concentration.

Blood and tissue samples

By the end of the experimental period at 4th week, whole blood was collected from caudal blood vessels in clean and dry Eppendorf tubes containing EDTA as anticoagulant, other blood samples were collected without anticoagulant and left at room temperature to coagulate then centrifuged at 3000 rpm for 15 min and clear sera were separated and kept in -20 ^{o}C until biochemical analysis. Fish were then killed by spinal rupture; the liver was rapidly excised and divided into three parts: the first part was kept in sterile Eppendorf tube, was immediately immersed in liquid nitrogen and then kept in -20 ^{o}C for anti-oxidant determination, the second part about (1g) was kept in PBS in sterile plastic tubes then was stored in -20 ^{o}C for comet assay analysis and the third part was stored in 10% formalin for histopathological analysis.

Hematological analysis

Total red blood cell (RBCs $10^6/\mu l$**)** counts were determined by the Natt & Herrick (35) method (NH) using a Newbauer hemocytometer. The microhematocrit (PCV%) was estimated by the method of Hesser (36). Hemoglobin (Hb g/dl) values were assessed calorimetrically according to method of Wintrobe and Greer (37) by determining the formation of cyanomethemoglobin.

Biochemical analysis

Malondialdehyde was spectrophotometrically measured in serum based on (38) at wave length of 534 nm, catalase activity was determined in liver homogenates , briefly obtained as follow (homogenize 1 gm. of liver tissue in 5 ml of cold phosphate buffer saline PH:7.4 ,centrifuge at 4,000rpm/ 15 minute at 4^{o}C, then used supernatant for assay) according to Aebi (39).

Histopathological analysis

Fixed liver samples were dehydrated in ascending grades of ethanol, then embedded in paraffin wax, cut into sections of 5 µm thickness. Sections were stained using Mayer's hematoxylin and eosin (H&E) stains according to the method described by Bancroft (40) and were examined under a light microscope. Hepatic histological changes photographed by computer system with a digital camera (Nikon digital camera, Japan).

DNA damage detection

Comet assay (single cell gel electrophoresis, SCGE) was used to detect any prospective damage for DNA after treatments. DNA strand breaks and alkali labile sites detected by measuring the migration of damaged DNA from immobilized nuclear DNA. The comet assay was performed according to the protocol described by Singh et al., (41) and the calculations were done as previously described (42).

Statistical analysis

Data were analyzed by one way (ANOVA), followed by Bonferroni's Multiple Comparison Test to compare the significant differences between different groups. All the data were expressed as mean ±SE. A value of $P < 0.05$ was considered to be significant.

Results

Hematological parameters

The hematological parameters are listed in Table (1). Exposure to lead acetate and chlorpyrifos led to a significant reduction in RBCs count as compared to the control fish. Furthermore, lead acetate and chlorpyrifos exposed group illustrated a significant decrease in Hb concentration (in comparison with the control group. Also, there was a significant reduction in PCV% in group exposed to lead acetate and chlorpyrifos when compared with the control group.

Effect of lead acetate and chlorpyrifos on MDA level and catalase activity

Exposure to lead acetate and chlorpyrifos led to a significant increase in serum MDA level and a significant decrease in hepatic catalase activity as compared with the control fish a (Fig. 1). Furthermore, chlorpyrifos led to a higher increased MDA level than lead acetate.

Effect of lead acetate and chlorpyrifos on DNA (comet assay)

DNA damage in liver of lead acetate, chlorpyrifos-intoxicated fish were detected by comet assay. The results of comet assay were shown in Fig. (2) and Table (2). Fish exposed to lead acetate showed asignificant ($P<0.05$) increase in DNA damage as revealed by increase in tail length, tail DNA% and tail moment. Fish exposed to chlorpyrifos showed a significant ($P<0.05$) increase DNA damage with higher damage in chlorpyrifos than lead acetate groups as compared to the control group (G1).

Histopathological changes

Normal fish (Fig. 3A) showed normal hepatocytes separated with blood sinusoids, while liver of lead acetate -intoxicated fish (Fig. 3B) showed hepatic vacuolation, parenchymal haemorrahge and focal leukocytic infiltration. Furthermore, liver of chlorpyrifos - intoxicated fish (Fig.3C) showed hepatic vacuolation and hepatic necrosis.

Table 1: Effect of lead and chlorpyrifos on hematological parameters in cat fish

Groups	RBCs(10^6/µl)	PCV%	Hb(g/dl)
Control	3.90±0.06[a]	39.06±0.6 [a]	9.26±0.07[a]
Lead acetate	3.10±0.06 [b]	31.07±0.61[b]	7.76±0.08[b]
Chlorpyrifos	2.91±0.06[c]	29.10±0.07[c]	7.27±0.05[b]

Mean±SE (n=5/group) [a,b] Means in the same raw with different superscript are significantly different ($P<0.05$)

Table 2: Comet assay parameters obtained by image analysis in all groups

Group	Tailed %	Untailed %	Tails length (µm)	Tail DNA%	Tail moment
Normal	2	98	1.53±0.16 [c]	1.64	2.51
Lead acetate	19	81	6.13±0.49 [b]	5.48	33.59
Chlorpyrifos	29	71	9.34±0.70 [a]	8.02	74.91

Different superscript letters in the same column of tail length showed significance difference at $P< 0.05$

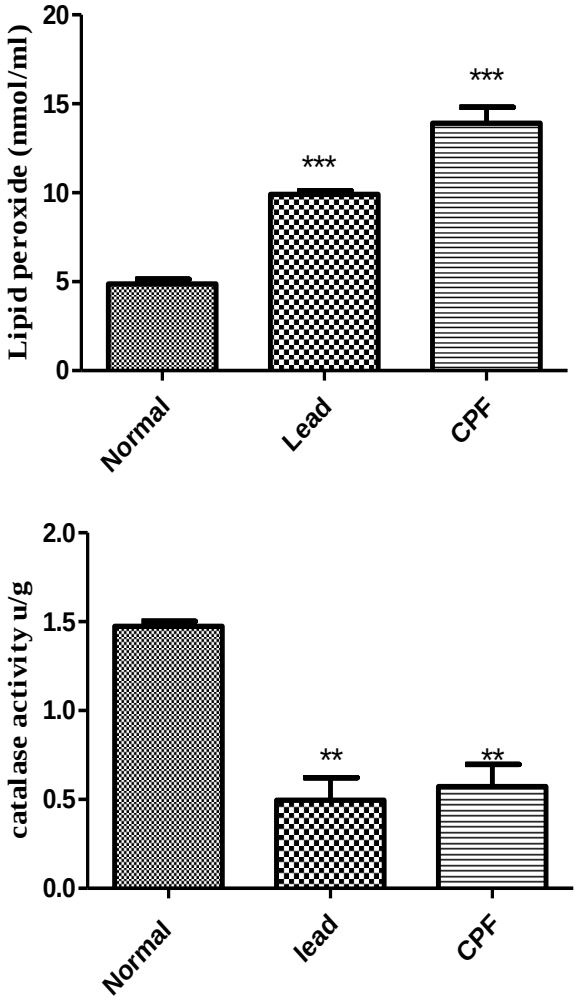

Figure 1: Showing effect of lead acetate and Chlorpyrifos on MDA and catalase activity. Data expressed Mean±SE. (n=10/group). *P <0.05 versus control group

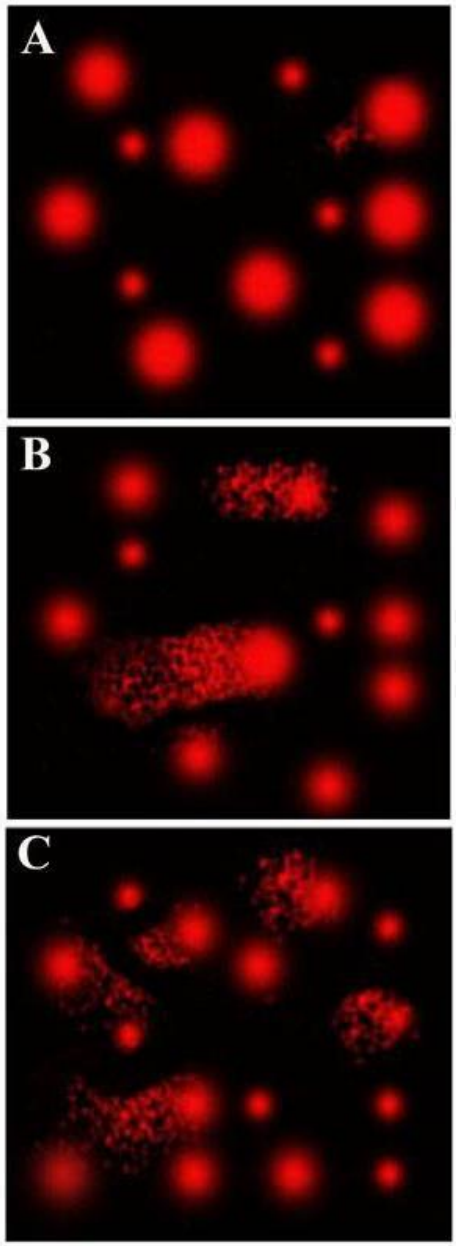

Figure 2: Showing DNA damage (comet assay) in normal fish (A), lead acetate -intoxicated fish (B) and Chlorpyrifos - intoxicated fish (C)

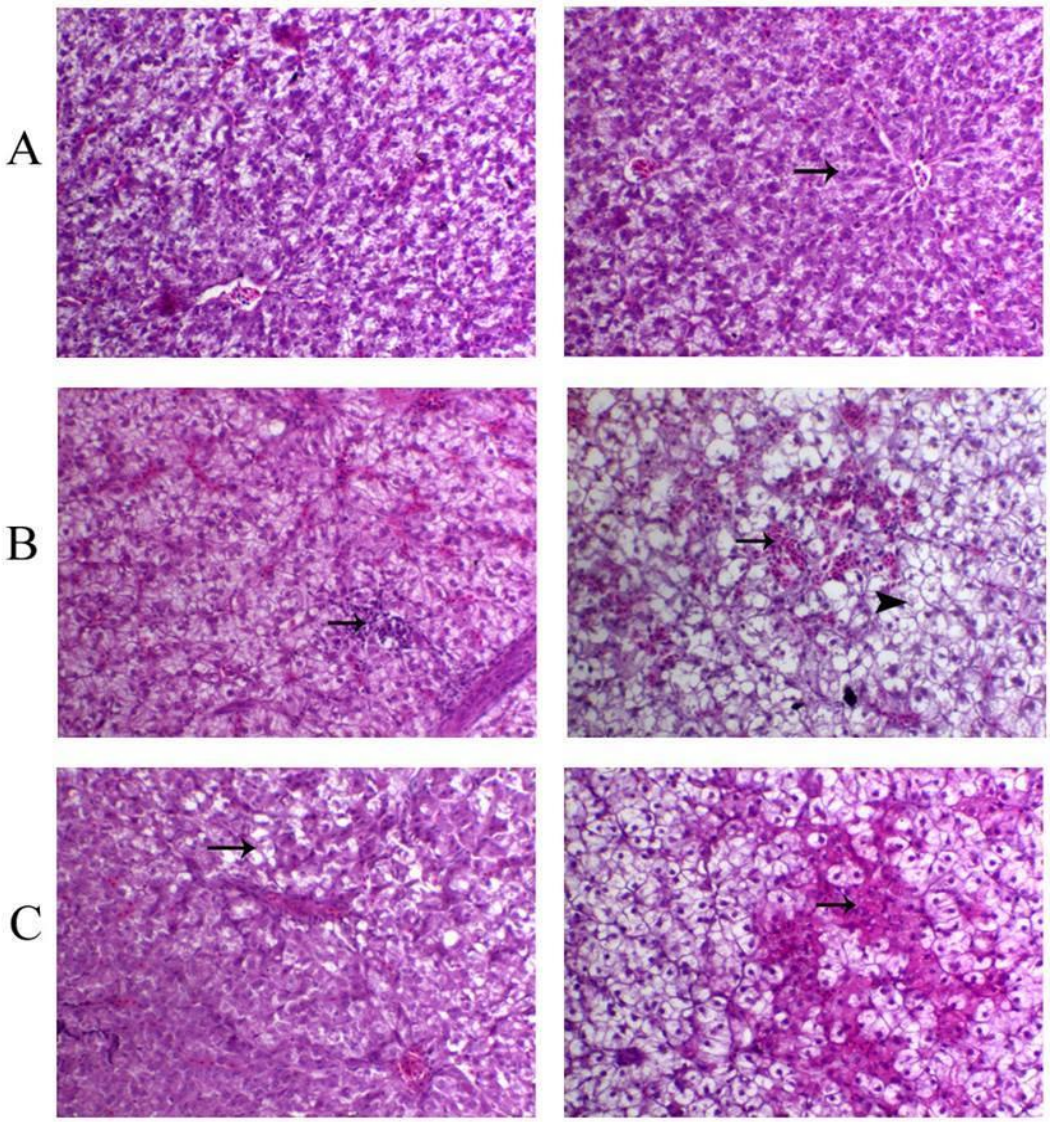

Figure 3: Showing Hematoxylin and eosin (H&E) stains of liver normal fish (A) ,lead acetate -intoxicated fish (B) and Chlorpyrifos - intoxicated fish (C)

Discussion

Because of over population and unintended civilization, many aquatic ecosystems are facing complicate problems of contamination (43). A few contaminates, for example detergents, household waste pesticides, insecticides, bacteria, parasites, metals, salts, acids, and other chemicals have been found in many aquatic surroundings (44). African catfish (*Clarias gariepinus)* is an excellent model for aquaculture, fundamental research, and environmental contamination studies (45, 46), So the present study try to illustrate, hemotoxicity and genotoxicity of lead and chlorpyrifos as pollutant on the *Clarias gariepinus* fish.

The result of present study revealed a significant decrease of RBCs count, Hb concentration and PCV values in lead acetate -intoxicated fish comparison with control fish. This was in harmony with (33) who reported that sub-chronic exposure of *C. gariepinus* to lead decreased RBCs count. Changes in Hb concentration and PCV values, were also compatible with

Adeyemo (47), who reported significant decreases in Hb, RBCs count and PCV values in *C. gariepinus* exposed to lead nitrate. Lead causes damage of hemopoietic organs , structural damage of RBC membranes resulting in hemolysis, impairment in hemoglobin synthesis, stress-related release of RBCs from the spleen and hypoxia (48). Lead causes early mortality of mature red blood cells and inhibition of hemoglobin synthesis through inactivation of RBC alpha-amino levulinic acid dehydratase (17). Also, our results revealed that RBCs count, hemoglobin concentration and hematocrit a significantly less in CPF -intoxicated fish in comparison with the control group. This result was compatible with (34) who reported that exposure of freshwater fish *Clarias batrachus* to chlorpyrifos led to significant decrease in RBCs and Hb indicating a condition of erythropenia and hemolysis. This may be attributed to inhibition of erythropoiesis, hemoformation, osmoregulatory dysfunction or due to increased rate of RBCs destruction in hematopoietic organ by chlorpyrifos (49). Indeed, chlorpyrifos more toxic than lead acetate.

Contaminants can possibly initiate oxidative stress in fish through creation of free radicals and reactive oxygen species (ROS) which lead to an imbalance between intracellular ROS levels and antioxidant defense (50, 51). ROS cause damage to proteins, lipids, carbohydrates and nucleic acids (52). This damage may alter cell functions, eventually leading to cell death (53). Our results showed that serum MDA was significantly increased and catalase activity was significantly decreased in fish exposed to lead as compared to the control fish. Similarly, other studies also reported that toxic action caused by lead might be due to its ability to produce ROS which cause oxidative damage in several tissues by increasing lipid peroxidation through Fenton reaction (54, 55). We also found a similar change following addition of CPF. Our study revealed that serum MDA was significantly increased and catalase activity was significantly decreased in tissue of CPF-exposed fish. Similar results obtained by Kaur and Jindal (56) who reported that CPF was very toxic to *Ctenopharyngodon idellus* even at very low concentration. Its administration increases MDA level and affected adversely the antioxidative defense system in different organs of the fish. This study indicated chlorpyrifos more toxic than lead acetate.

Histopathological examination of liver tissue is important marker for exposure to environmental stressors or pollutants, as the liver is important organ of detoxification (57). This study revealed that liver tissue in fish exposed to lead acetate showed hepatic vacuolation, parenchymal haemorrahge and focal leukocytic infiltration, which agreed with Rubio, et al., (58) who reported that lead cause inhibition of mono amino oxidase and acetylcholine esterase, to cause pathological changes in tissue and organs. Additionally, CPF also resulted in vacuolation and hepatic necrosis in hepatic tissue of cat fish, and this was in consistent with Deb and Das (59) who reported that common carp exposed to CPF revealed different degree of hydropic degeneration, vacuolization, pyknotic nuclei, and fatty infiltration in their liver tissue.. Although every metal have distinctive mechanism of toxicity however there are some common mechanism such as mimicry, adduct formation with DNA or protein and oxidative damage. Generation of ROS is caused by heavy metals in their ionic forms leading to oxidative changes in DNA, causing aberrant gene expression and carcinogenesis (60). The present study revealed induction of DNA damage by lead and CPF. In support, other studies also reported exposure to lead cause genotoxic effects, such as chromosome aberration, mutation, DNA breakage and DNA synthesis inhibition (61, 62). This result may due to lead can mimic the essential elements such as magnesium, iron, calcium and zinc, increased the production of inaccurate nucleotides in which it implicated as a co- carcinogen and effect on DNA repairing mechanisms (63). Similarly, Yin, et al., (64) reported that chlorpyrifos caused genotoxicity in RBCs and liver cells of Chinese toad (*Bufo gargarizans*) when the tadpoles were exposed to the sub lethal dose of chlorpyrifos. Indeed DNA damage was higher in chlorpyrifos than lead acetate.

Conclusion

This study illustrated that lead acetate and chlorpyrifos have hemotoxicity through decrease RBCs count, Hb concentration, and hematocrit, generate ROS and has genotoxicity through induction of DNA damage in cat fish after exposure to sub-lethal concentrations. Moreover chlorpyrifos is more toxic than Lead acetate

Conflict of interest

The authors declare that they have no conflict of interest.

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