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Lipid Peroxidation and Some Antioxidant Enzymes of *C. gariepinus* Fingerlings Exposed to Diethyl Phthalate

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Authors' contributions

This work was carried out in collaboration between all authors. Author CBI, provided the chemical diethyl phthalate which was procured from Sigma Aldrich Chemical, Ohio, USA and prepared the stock solutions including procurement of plastic aquaria, the experimental design and first draft of the manuscript. Author RNNO analysed the research work and edited the work while author ICO took care of the laboratory analysis and reviewed the manuscript before submission. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Aims: Diethyl phthalates an example of phthalates which are a group of multifunctional chemicals is one of the most frequently used phthalates for manufacturing numerous products. Its persistence in the waterways could cause metabolic changes in fishes. The present investigation was undertaken to evaluate the changes induced by DEP intoxication in fish antioxidant in fish system.
Study Design: Complete randomized design was used.
Place and Duration of Study: Department of Zoology and Environmental Biology, wet laboratory and the experiment lasted for 21 days.
Methodology: One hundred and twenty fish were randomly divided into four treatment groups (A-D) in 25litre glass aquarium filled to 20 litres mark with aerated deep well water. The fish were subjected to sub lethal concentrations of DEP (0.01 ug/L, 0.03 ug/L and 0.05 ug/L) in a renewal bioassay system. Superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GPx),

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Lipid peroxidation (LPO) and total protein activity of liver and kidney was assayed in DEP exposed *C. gariepinus*.
Results: It was observed that DEP significantly lowered (P<0.05) CAT, SOD and GPx activity in the entire organ except LPO and total protein that had significantly (P<0.05) increased activity in all the organs at different concentrations of DEP. It can be elucidated that at different concentration of DEP, oxidative stress, total protein and antioxidant enzyme system on *C. gariepinus* was significantly disturbed.
Conclusion: DEP has being seen to cause alterations in antioxidant, lipid peroxidation and total protein of *C. gariepinus*. Therefore, there is need for more studies in the oxidative stress, antioxidant status, and biochemical alterations by DEP to fish species.

Keywords: Sublethal toxicity; diethylphthalate; C. gariepinus; antioxidants; lipid peroxidation and total protein.

1. INTRODUCTION

Diethyl phthalate (C_{12} H_{14} O_4) is produced industrially and manufactured for many uses such as mosquito repellent, cosmetics, after shave lotion etc. it may enter into the environment in industrial waste water by evaporation into the air from disposal sites, directly from consumer products, burning of plastic products and leaking from landfills into soil and water including ground water (ATSDR, 1995).

Within the last two decades, there has been increasing awareness and concerns among environmentalists regarding the effects of agrochemicals on the status of aquatic health, particularly living resources like fish (Mgbenka et al, 2003). The public health implication of eating fish contaminated with poisonous chemicals is worrisome especially after the well-known minimata bay mercury pollution incident (Mgbenka et al, 2003).

DEP as a raw material for producing some products mentioned earlier interfere with intracellular electron transfer systems with reactive oxygen species (ROS) including the superoxide anion and hydroxyl radicals, which interact with the unsaturated membrane lipids and results in the destruction of organelles and ultimately lead to death [1]. Toxic substances in aquatic environment can affect fish growth indirectly by reducing food availability, or directly by changing their metabolism. Thus, toxins cause an increase in energy requirements for maintaining homeostasis previous studies have investigated the toxicological effect of DEP in fish.

However, the effects of DEP on tropical fish species have not been completely shown. Due to its inexpensive cost, *Clarias garieprius* is the main source of animal protein in most commercial food fish [1]. This fish is usually obtained in most natural fresh water bodies and is also a good aquaculture candidate. It appears as an excellent model for ecotoxicological studies. Impact of DEP on total protein, lipid peroxidation and antioxidant status of *Clarias gariepinus* juveniles were studied.

2. MATERIALS AND METHODS

2.1 Procurment of the Experimental Fish and Chemical

One hundred and twenty (120) fingerlings of mean weight (13.13±2.27 g) and length (10.45±0.1 cm) used in this study were obtained from Aqua fish limited Awka, Anambra State, Nigeria. They were identified using taxanomic key of Reed et al. (1967) and were disinfected with 0.05% $KMnO_4$ solution for 2 mins to avoid dermal infections. The fish were transported to the University of Nigeria, Nsukka wet lab and acclimated for three (3) weeks. They were fed 35% crude protein which was 3% of their body weight at 8h intervals. The diethyl phthalate (99.9% purity) was obtained from Ohio, USA.

2.2 Range Finding Test

The exploratory range of concentration of test chemicals was initially conducted according to American Public Health Association (1996) using geometric series of concentrations values to identify the highest concentration that will kill 100% of the test organism and the least concentration that will have no effect on them, thereafter, 0.01 ug/l, 0.03 ug/l, 0.05 ug/l were selected for the sublethal treatments.

2.3 Treatment of Fish and Collection of Tissue Samples

One hundred and twenty fish were randomly divided into four treatment groups (A-D) in 25 litre glass aquarium filled to 20 litres mark with aerated deep well water. They were further replicated thrice with each replicate having ten fish. The fish were subjected to sub lethal concentrations of DEP (0.01 µg/L, 0.03 µg/L and 0.05 µg/L) in a renewal bioassay system. Both the water and DEP were changed daily to maintain the toxicant concentration and at the same time minimize the accumulation of waste products that may induce stress in fish. Water quality was determined according to the standard protocol of APHA (1990). The experiment lasted for 21 days. The fish were sacrificed and the tissues (Liver and Kidney) collected at the end of each week were homogenized immediately in ice cold distilled water and centrifuged at 3000 rpm for 15 minutes. The homogenized tissues were then removed and stored in plan bottles at –20℃ for further analysis.

2.4 Antioxidant Enzymes Determination

The catalase activity was assayed using the method of Luck [2] and results expressed as µmol of H_20_2 decomposed/min/mg protein, superoxide dismutase [3] and result expressed as units/mg protein while glutathione peroxidase using [4] protocols were expressed as nmol of NADPH oxidized/min/mg protein.

2.5 Total Protein

This was determined using the method [5].

2.6 Lipid Peroxidation

This was determined using the methods of Will [6] and LPO expressed as in nmol of MDA formed/mg protein as $1.56x10^{5.}$

2.7 Statistical Analysis

This was done using the one way analysis of variance (ANOVA) with significance level fixed at $P<0.05$ while the means where compared for significant difference using Duncan multiple range test. The statistical package for social science (SPSS) version 16 was used.

3. RESULTS

Table 1 and 2 shows changes in the total protein, lipid peroxidation, superoxide dismutase catalase and glutathione peroxidase of in the liver and kidney of *C. gariepinus* exposed to DEP. The catalase enzyme activity in the liver and kidney of *C. gariepinus* exposed to varied sublethal concentration of DEP had a significantly (P=.05) low activity when compared to the control throughout the exposure period, there was increased SOD and GPx activity in the exposed fish throughout the duration of the experiment. The decrease in SOD and GPx activity on the liver and kidney of *C*. *gariepinus* exposed to DEP differed significantly (P=.05) when compared to the control, although in Day 14, the GPx activity in the kidney of the exposed fish did not differ significantly ($P\neq.05$). However, the LPO activity in the liver and kidney of the exposed fish significantly decreased (P=.05) compared to the control group. The plasma protein level significantly increased (P=.05) in the treated group when compared (P=.05) in the treated group when compared to the control group throughout the experiment.

4. DISCUSSION

Enzymes analysis of organs such as kidney and liver can provide important information about the internal environment of the organism (Gabriel et al, 2012). In the present study the antioxidant, lipid peroxidation and total protein of three 0.01 ug/l, 0.3 ug/l and 0.05 ug/l sublethal concentration of DEP on *C. gariepinus* juvenile was investigated. From this study, it can be deduced that different concentration of DEP lead to dose dependent significant increase in the lipid, peroxidation of liver and kidney of the exposed fish. This finding supports the finding of [7,8], who reported significant increase in LPO of fish exposed to DEP at the same time, increased protein level observed in this study contradicts the report of Henna and Ramtey [8] who reported decreased protein content at 40 ug/nl of DEP concentration. Oxidative stress occur if the activity of the antioxidant defense enzyme such as SOD, CAT, GPx, enzymes changes by environmental pollution induces the production of reactive oxygen species. [9]. However, Alzebeta et al. [10] indicated that the activity of SOD in fish can increase or decrease after exposure to various xenobiotics. The present result is in good accord with the findings of Umamaheswari and Senthilnathan [9] who have evinced significant decrease ($P<0.001$) in the SOD activity of muscle and CAT activity of muscle and liver after 60 days DEP exposure. The decreased CAT and SOD activity of the liver and kidney in this study is well supported by Alzebeta et al. [10]. The

decreased CAT activity in the liver and kidney of *C. gariepinus* exposed to DEP agrees with the findings of Kang et al. [7] who reported elevation of CAT activity by 100 mg/kg DEP

Table 1. Changes in the total protein, lipid peroxidation and antioxidant enzymes in the liver of *C. gariepinus* exposed to DEP$^{0-21}$

Parameters	Concentrations (µg/L)	0	7	14	21
		Duration of exposure (days)			
`Total protein	Control	6.39±0.5 ^{a}	6.29±0.9^{b}	6.71±0.18^{ab}	6.71±0.43^{b}
	0.01	6.83±0.5^{c}	6.17±0.32^{a}	7.08±0.53^{b}	8.67±0.10^{a}
	0.03	6.57±0.1^{b}	6.31±0.03^{b}	6.26±0.9^{a}	8.94±0.004^{d}
	0.05	6.82±0.4^{c}	6.91±0.4^{c}	7.77±0.18^{c}	7.59±0.004^{b}
Lipid peroxidation	Control	0.510±.002^{a}	0.89±0.003^{a}	0.36±0.01^{a}	0.46±0.002^{a}
	0.01	0.49±0.10^{a}	0.55±0.063^{b}	0.24±0.02^{c}	0.23±0.002^{c}
	0.03	0.49±0.03	0.48±0.003^{b}	0.32±0.00^{b}	0.25±0.021^{c}
	0.05	0.39±0.001	0.45±0.034^{b}	0.32±0.002^{b}	0.58±0.34^{b}
SOD	Control	78.47±10.12^{c}	78.48±13.2^{c}	86.83±10.28^{a}	87.25±19.25^{a}
	0.01	59.94±11.67^{c}	50.46±10.2^{a}	53.75±13.76^{d}	52.21±11.91^{b}
	0.03	56.94±10.2^{c}	51.45±9.67^{a}	63.82±11.96^{b}	51.08±16.12^{b}
	0.05	62.12±14.21^{b}	44.78±11.89^{a}	56.83±10.65^{c}	86.32±19.81^{a}
CAT	Control	1.86±0.01^{a}	1.76±0.02^{a}	1.93±0.02^{a}	1.74±0.01^{c}
	0.01	1.58±0.08^{b}	1.49±0.07^{b}	1.27±0102^{d}	1.25±0.01^{a}
	0.03	1.76±0.085	1.73±0.012^{a}	1.86±0.012^{b}	54.33±11.98^{b}
	0.05	1.75±0.02^{a}	1.71±0.02^{a}	179.23±27.10^{c}	89.39±16.85^{a}
GPx	Control	446.18±23.19^{d}	481.26±19.18^{d}	390.65±56.03^{c}	381.16±45.38^{d}
	0.01	315.33±34.36^{a}	324.07±33.42^{a}	322.56±29.17^{a}	356.75±25.24^{b}
	0.03	411.26±41.35^{c}	401.77±34.40^{c}	342.04±37.26^{b}	306.65±35.14^{a}
	0.05	349.62±34.54^{b}	352.70±22.54^{b}	347.67±31.29^{b}	370.66±31.07^{c}

*Each value is a mean±SE of five individual observations. *Means within the same column followed by different letters are significantly different (P=.05). *Means within the same column followed by the same letters are not significantly different (P≠.05)*

Table 2. Changes in the total protein, lipid peroxidation and antioxidant enzymes in the kidney of *C. gariepinus* exposed to DEP$^{0-21}$

Parameters	Concentrations (µg/L)	0	7	14	21
		Exposure duration (Days)			
Total protein	Control	6.88±0.106^{d}	6.42±0.4^{b}	6.59±0.36^{b}	6.30±0.10^{a}
	0.01	6.62±0.26^{b}	6.62±0.12^{c}	6.81±0.10^{b}	8.55±0.35^{bc}
	0.03	6.52±0.14^{a}	5.92±0.03^{a}	5.82±0.11^{a}	8.76±0.15^{cb}
	0.05	7.14±0.03^{c}	7.44±0.03^{d}	7.69±0.72^{c}	8.17±0.58^{b}
Lipid peroxidation	Control	0.48±0.002^{c}	0.46±0.003^{b}	0.38±0.002^{a}	0.41±0.004^{a}
	0.01	0.44±0.003^{d}	0.54±0.002^{a}	0.25±0.005^{c}	0.22±0.003^{c}
	0.03	0.55±0.002^{a}	0.42±0.002^{c}	0.32±0.009^{b}	6.22±0.002^{c}
	0.05	0.53±0.002^{b}	0.54±0.001^{a}	0.32±0.001^{b}	0-31±0.002^{b}
SOD	Control	84.21±12.59^{a}	82.45±19.80^{a}	88.65±19.09^{a}	85.41±19.20^{a}
	0.01	55.54±21.32^{c}	63.25±13.91^{c}	54.72±11.8^{c}	56.26±10.02^{c}
	0.03	58.59±11.74^{b}	68.95±21.74^{b}	65.40±12.51^{b}	52.26±10.02^{b}
	0.05	53.69±14.72^{a}	63.25±9.34^{c}	52.47±9.23^{d}	68.83±15.92^{b}
CAT	Control	1.76±0.24^{a}	1.76±0.06^{a}	1.94±0.09^{a}	1.82±0.01^{a}
	0.01	1.36±0.04^{c}	1.37±0.02^{c}	1.34±0.04^{c}	1.26±0.28^{c}
	0.03	1.75±0.01^{b}	1.72±0.08^{b}	1. 87±0.07^{a}	1.43±0.03^{b}
	0.05	1.75±0.03^{b}	1.74±0.02^{a}	1.63±0.54^{a}	1.42±0.81^{b}
GPx	Control	462.69±39.23^{b}	451.24±49.674^{b}	390.57±46.39^{b}	441.53±65.40^{d}
	0.01	308.57±34.67^{b}	308.57±56.16^{a}	340.67±30.03^{a}	338.42±24.01^{b}
	0.03	257.83±45.22^{a}	308.68±29.03^{b}	346.9±34.01^{b}	327.30±32.13^{a}
	0.05	423.62±20.8 ^{c}	374.56±23.34^{c}	343.9±24.67^{ab}	361.38±21.21^{c}

*Each value is a mean±SD of five individual observations. *Means within the same column followed by different letters are significantly different (P=.05). *Means within the same column followed by the same letters are not significantly different (P≠.05)*

in *Paralicthys olivaceus*. The decline in hepatic antioxidant enzyme activity observed in this study partially agrees with that of Kathya et al. [11] who observed significant decline in round up transorb exposed to *Prochilodus lineatus* (1 mg/L and 5 mg/L) after 6 and 24 hours of exposure no significant alteration in the hepatic SOD and CAT activity. They have further assumed that H_2O_2 is responsible for reduction in SOD activity and also may be due to superoxide which is probably not being neutralized efficiency by SOD. In parallel to the present finding, GPx activity depleted in the liver and kidney of *C. gariepinus* exposed to DEP. This observation agrees with the present findings of Guluzar and Mustafa [12] who have noticed decreased GPx hepatic activity in *Oreochromis niloticus* chronically exposed to both Ca, Zn, and Fe, on the contrary, Basha and Rain [13] suggested that higher GPx hepatic activity in *O. mossambicus* exposed to cadmium. Reduction in GPx prevents the formation or radical intermediates by oxygen reduction mechanism Chung et al. (2001). In parallel to the present study, Kang et al. [7] observed elevated/or enhanced GPx actively in the liver and muscle of olive flounder *Paralichthys olivaceus* exposed to 110-910 mg/kg DEP.

Indiscriminate release of pollutants into the environment may disturb the ecological balance of the earth. DEP is one such chemical reported to influence the aquatic biota. There is still need to checkmate the illicit disposal of DEP made in the waterways which could cause stress in fish. There is need for more studies in the oxidative stress, antioxidant status, and biochemical alterations by DEP to fish species.

5. CONCLUSION

Diethylphthalate has been seen to be toxic to the environment and most especially the aquatic organism found in aquatic environment. The effect of different doses of diethyl phthalate on the lipid peroxidation and some antioxidant enzymes of *C. gariepinus* fingerlings have been studied. The use of diethyl phthalate as an ingredient in a variety of cosmetic formulations etc. are likely to be the primary sources to aquatic environment pollution, when indiscriminately disposed. There is need to create awareness on the possible physiological alterations in the aquatic biota e.g. fish when been exposed to constant DEP through runoff.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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