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ORIGINAL ARTICLE **OPEN ACCESS**

Biochemical alterations in *Clarias gariepinus* (Burchell, 1822) exposed to four environmentally significant pharmaceuticals

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ABSTRACT
Pharmaceuticals are a fast emerging class of environmental contaminants eliciting concern. This is primarily because of their relatively unknown effects on non-target organisms, and probable deleterious effects on ecosystem health and complexity. The latter scenario is further thrown into sharp relief due to their temporally continual therapeutic and restorative use, and the development of new and more potent varieties. A local fish species, Clarias gariepinus (Burchell, 1822) was exposed to four individual pharmaceuticals detected in surface waters from Lagos, Nigeria (chloramphenicol, diclofenac, erythromycin, and sulphamethoxazole) for 9 days at concentrations exceeding ecotoxicological effect concentrations in literature. Ecotoxicological effects were assayed using the hepatic enzymic damage markers, alanine amino transferase (ALT), and aspartate amino transferase (AST). All four toxicants individually elicited quantifiable increases in measured enzymes even at the lowest concentrations mirroring measured environmental concentrations (MECs) of 0.36, 0.27, 1.0, and 1.5 µg/l (micrograms per litre). AST and ALT control concentrations were 133.7 IU/l, and 36.74 IU/l respectively. An ALT concentration maximum across toxicants of 137.7 IU/l was recorded for erythromycin on day 6 of exposure in the100 µg/l concentration group, representing a percentage increase of approximately 275%. Concentration based variations were inconsistent across groups and toxicants. On the basis of aminotransferase concentrations in plasma, a suggested hepatoxicity gradient for individual agent severity is proposed i.e. ERY > DFC > SMX > CPC. The study validates the sensitivity and suitability of these markers for the detection of the exposure of feral fish to minute quantities of aberrant pharmaceutical residues in the aquatic environment bordering cities.
Keywords: *Ecotoxicology, Pharmaceuticals, Clarias gariepinus, Lagos lagoon, Nigeria.*

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INTRODUCTION

The presence of pharmaceutically active compounds (PhACs) in the environment has been severally documented [1, 2], and a plethora of literature reporting the analytical detection of these compounds presently exists. In contrast, literature documenting the effects of pharmaceuticals on biota are fewer though increasing. Pharmaceuticals, classified as "emerging" contaminants, alongside personal care products and nutriceuticals, post administration/use, necessarily end up in the environment, and ultimately in water at very low concentrations in the nanogram to microgram range, well below defined acute toxicity concentrations levels for humans. Due to their ubiquitous usage, and also to the large and ever growing number of proprietary drug preparations in daily use, a "cocktail" of human and veterinary pharmaceuticals contaminate surface waters worldwide. Currently available commercial water treatment methods have been shown to be unable to effectively clear fluid sewage influents of pharmaceutical residues, and some are entirely unchanged by such processes [3-5]. In third world urban situations, waste water treatment is inadequate in terms of sewage treatment facility (STF) numbers and facility effectiveness.Thus, such pharmaceutically active compounds (PhACs) could potentially enter surface waters without any preliminary treatment, and the effects consequent on this fact could potentially be even more profound than realized at present. Due to the miniscule environmental quantities recorded, it could be wrongly surmised that these residues would have little effect on resident flora and fauna for which they were not originally intended for, but there are uncertainties and concerns about the effects of chronic exposures to environmentally active pharmaceutical residues [6]. This because, lethality consequent on acute toxicity is highly improbable. Rather, the effects of pharmaceuticals on aquatic species or ecosystems are presumably subtle, inapparent, and protracted, relative to the lifespan of the

affected organism. In recent times, there has been an increased interest in the effects of pharmaceuticals, human and veterinary on fish [7, 8]. And methods including histological examination of some target organs and tissues in tandem with measurements of plasma/serum enzymic alterations have been shown to be suitable for this purpose, alongside batteries of other markers for toxicity assessment. The serum transaminase enzymes, alanine amino transaminase (ALT) and aspartate amino transaminase (AST) are sensitive markers of anthropogenic exposure stress in fish and are regarded as sensitive markers of hepatocellular damage, though AST is considered less specific for hepatotoxicity, its measurements are complementary to ALT. This study is designed to investigate the sensitivity and degree of discernment of each enzyme to establish them as sensitive markers of aquatic pharmaceutical contamination.

MATERIALS AND METHODS

Appropriate pre-determined volumes of each pharmaceutical solution were dispensed into test tanks using a microtitre pipette, the agitatory swimming activity actions of the fish serving to mix the test solutions adequately. In the single individual entity tests each pharmaceutical was applied to tanks .in three concentrations, each with a replicate tank containing the same number of fish and pharmaceutical concentration per agent. Used concentrations were, measured environmental concentration (MEC), MEC X 10, and MEC X 100. For instance, the MEC for diclofenac was 0.27 µg/l which was taken as the first exposure concentration was then multiplied by 10^{th}, and 100^{th}factors as stated above to get 2.7 and 27µg/l (Table 1).

Table 1: Measured environmental concentrations (MEC) of assayed pharmaceuticals.

Compound	MEC
Chloramphenicol	0.36
Diclofenac	0.27
Erythromycin	1.0
Sulfamethoxazole	1.5

Following exsanguination on collection dates, whole blood was allowed to stand for two hours at 4 ^{0}C. Blood specimens were then centrifuged at 500 RPM for 30 minutes. Plasma fractions were collected from each blood specimen and harvested plasma stored at -20 ^{0} C until required. Prior to analysis, aliquots were prepared from thawed plasma into Eppendorf tubes (1.5 ml). Plasma aliquots were analysed for levels of the transaminases (TA's), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatise (ALP) using colorimetric methods based on reagents contained in kits manufactured by Randox^R (UK) for the quantitative *in vitro* determination of the target transaminase enzymes. Individual specimen absorbance's were then read against the absorbance of the sample blank using a Beckmann-Coulter®DU-720 UV/VIS photo spectrometer at a specified wavelength of Hg 546 nm.

RESULTS

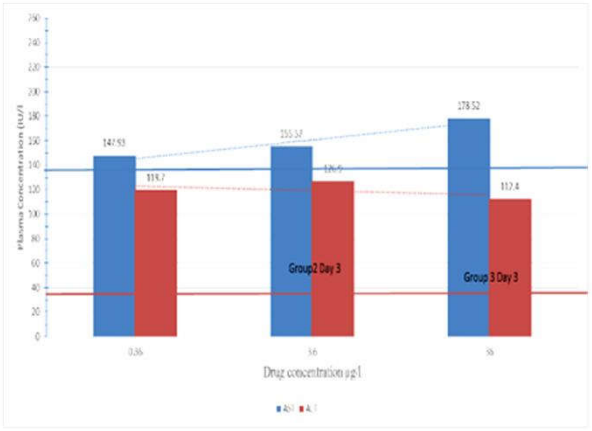
Aspartate amino Transferase (AST) and Alanine amino Transferase control concentrations were 133.7 IU/l, and 36.74 IU/l respectively. AST and ALT levels were generally increased and sustained throughout the exposure . On the basis of percentage deviations of AST from the normal, erythromycin was deemed the most hepatotoxic of the toxicant series with a maximum increase of 102% on day 9 in the maximum (100 µg/l) group. Plasma ALT levels were more highly elevated than for AST. This was expected as ALT is deemed a far more sensitive marker for liver damage. An ALT concentration maximum across toxicants of 137.7 IU/l was recorded for erythromycin on day 9 of exposure in the highest concentration group (100 µg/l), representing a percentage increase of approximately 275%. Concentration based variations were inconsistent across groups and toxicants. In the combination toxicant exposure group maxima and minima for AST and ALT were 199.9 and 147.9 IU/L, and 119.7 and 110.6 IU/l respectively. A clear duration mediated trend in transaminase concentrations was clearly discernible in this group as toxicant concentrations remained constant throughout the exposure period.

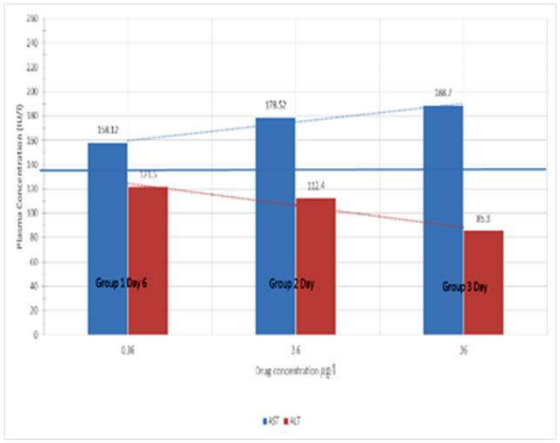
DISCUSSION

The several routes of the largely uncontrolled entry and egress of several chemical contaminant groups including the pharmaceuticals, into the environment, and biotic networks following human use, is widely documented, and continues as an important subject for interdisciplinary scientific inquiry in the life sciences. An earlier study (Olarinmoye *et al.*, 2016) documented the presence of pharmaceuticals from several therapeutic classes in some environmental matrices especially surface water (Lagos lagoon) in Lagos, Nigeria.

Presences were quantified in miniscule amounts in the range of millionths of a gram per litre (µg/L). In the Lagos study referred to, four pharmaceuticals (chloramphenicol, Diclofenac, erythromycin, and Sulfamethoxazole in order of magnitude) were found at concentrations exceeding ecotoxicological effect concentrations and erythromycin concentrations were highest recorded for Africa. Concentration maxima recorded in the study were for erythromycin (1.0 µg/L) and sulphamethoxazole (1.5 µg/L).The levels of the latter pharmaceutical entities and their residues in Lagos waters are pointers to their widespread consumption and inherent individual agent environmental persistence.

The serum transaminase enzymes, alanine amino transaminase (ALT) and aspartate amino transaminase (AST) are sensitive markers of anthropogenic exposure stress in fish and are regarded as sensitive markers of hepatocellular damage, though AST is considered less specific for hepatotoxicity. However, its measurements complementary to ALT. In recent times, there has been an increased interest in the effects of pharmaceuticals, human and veterinary on fish [7, 8], and the measurements of plasma/serum enzymic alterations have been shown to be suitable for this purpose alongside batteries of other markers for toxicity assessment. Persistent elevations of either transaminase could be indicative of progressive injury. False negatives are possible if specimen collection timing allows clearance of elevated circulatory levels. In this study, ALT levels were elevated up to two hundred fold and these levels were sustained in most cases for the entire experimental period, an indication of sustained, though non-lethal, and, presumptively reversible liver damage during the exposure period. While several studies exist which document *in vitro* measurements of transaminase enzymes in fish exposed to xenobiotic contaminants, relatively fewer exist which report responses to concentrations similar to measured environmental concentrations. In the present study, all the toxicants increased both ALT and AST plasma levels even at lowest concentrations. The present study has established that exposure of fish to pharmaceuticals exclusively at concentrations mimicking actual measured concentrations, is capable of eliciting quantifiable tissue and plasma derangement in key organs. The study has not however being able to establish patently clear concentration/duration response trends, a phenomenon not unknown as can be gleaned from similar studies [9-11]. The latter conclusion could be explained on the bases of idiosyncratic subject responses to similar levels and concentrations of a particular toxicant, and relatively smaller number of analysed subjects compared to natural situations where scales of exposure are mediated by the latitude of available migratory space.





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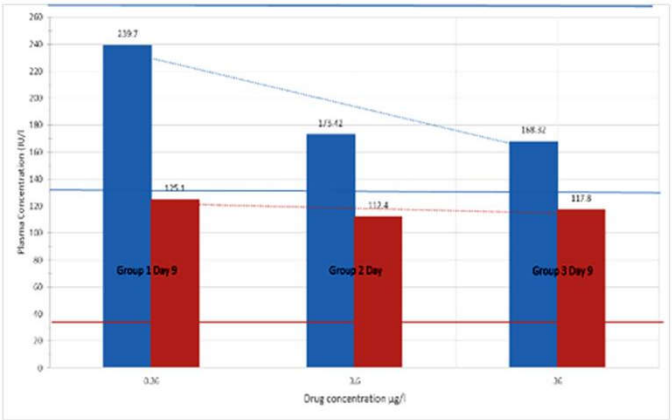

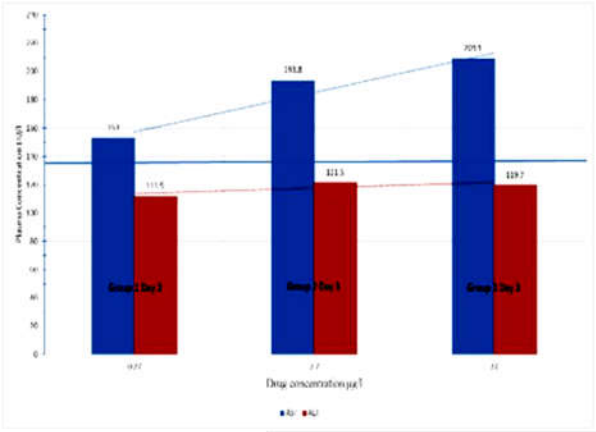

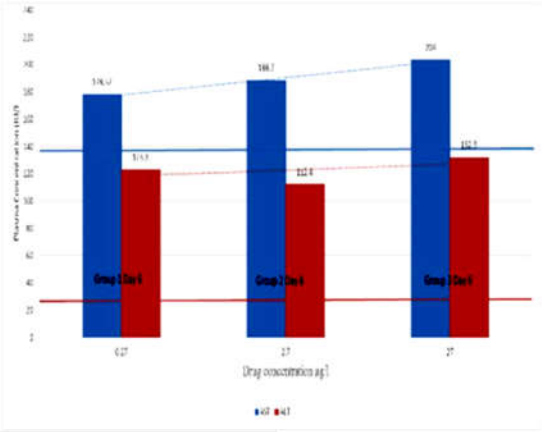

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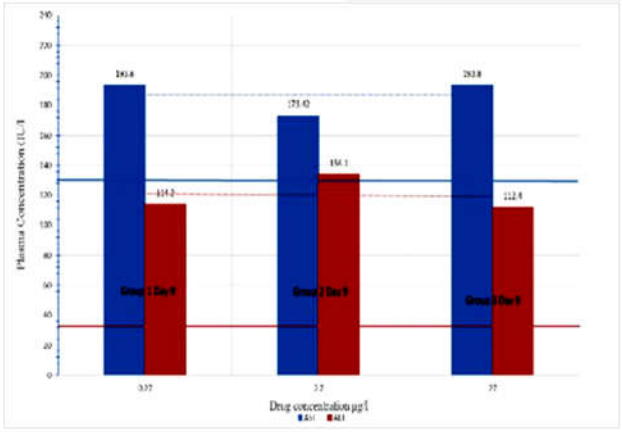

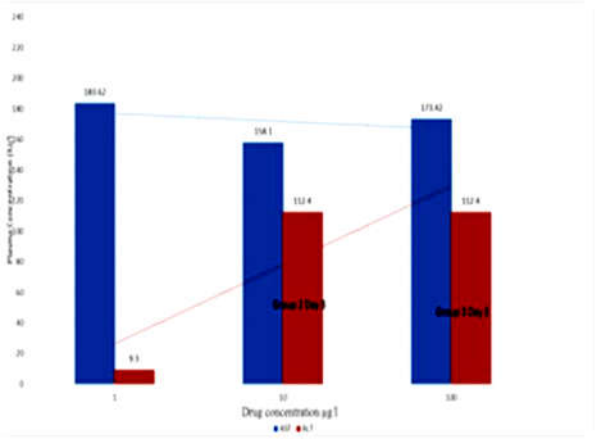

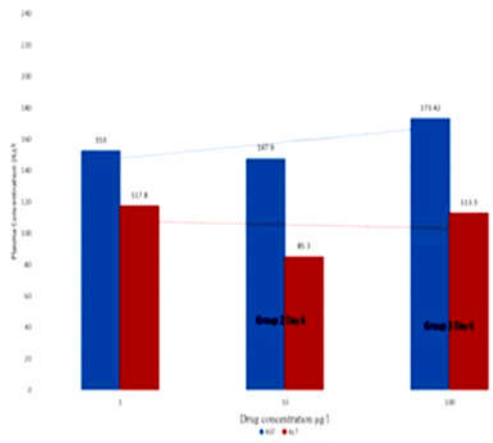

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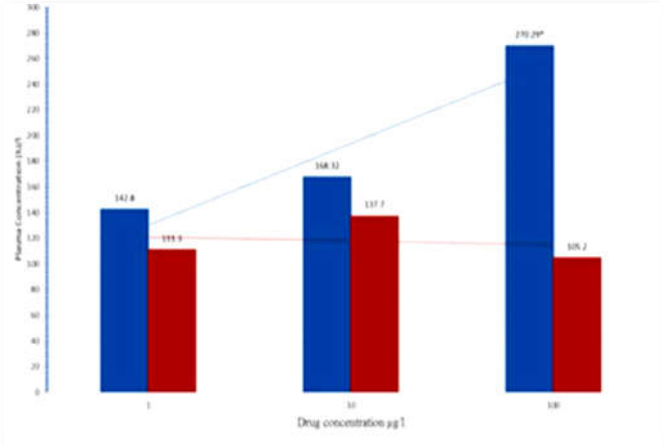

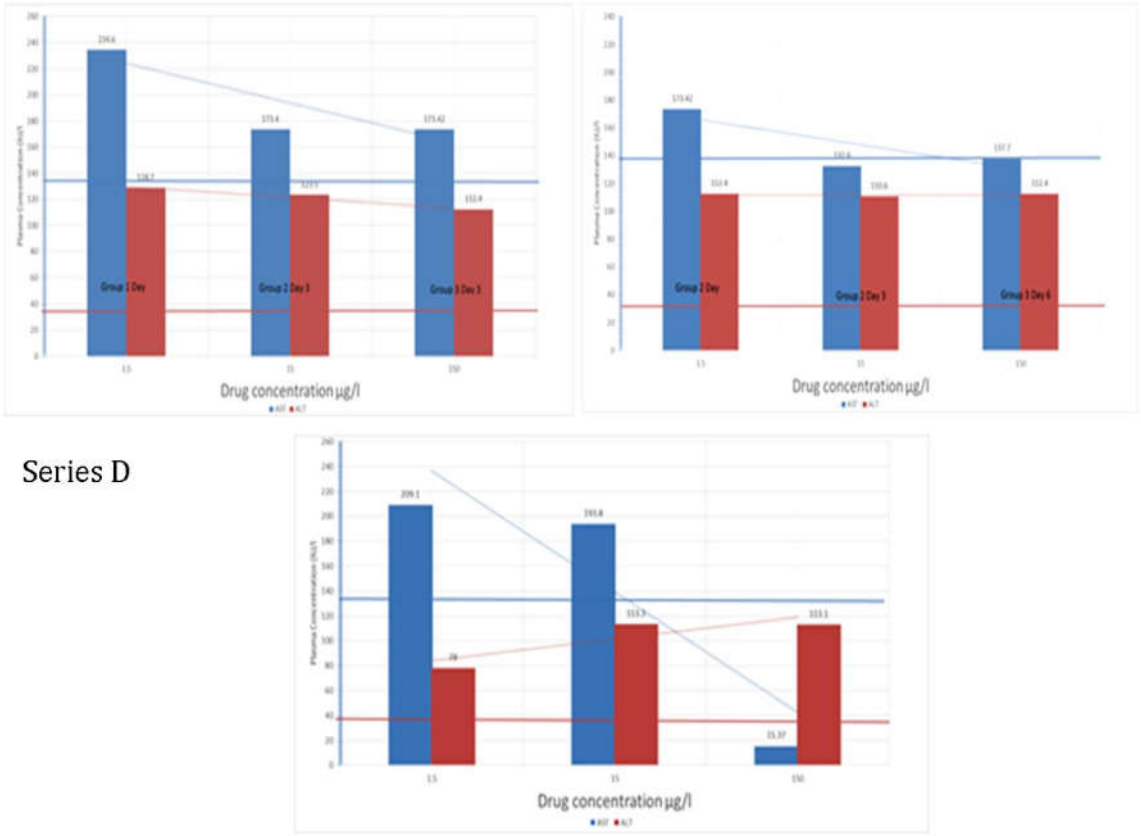

Figure 1: Depicting AST and ALT concentrations for each day (entire duration-9 days) of exposure drug Series A to D(Chloramphenicol, Diclofenac, Erythromycin, and Sulfamethoxazole) respectively per concentration group. Both AST and ALT were significantly elevated even at lowest exposure concentrations and levels exceeded control (solid blue and red lines respectively).

The environmental presences of several pharmaceutical classes, and individual therapeutic products, has become a scientifically proven reality. The pertinent contemporary questions are, whether the effects attributed to pharmaceutical exposure of non-target species is sufficient to justify of the frenetic activity, and costs of inquiry currently ongoing in the ecotoxicological scientific community? And as adjunct, is this activity limited to bioindicator discovery and characterisation for the satisfaction of the scientist's curiosity and publication needs? –science for its own sake? Secondly, are the syndromes popularly put across as the main outcome concerns for the health and wellbeing of the human population as end consumers of the fisheries- organic bioaccumulation and biomagnification of pharmaceutical residues in fish products and their transmittal to humans, as examples, sufficiently worthy of public dissemination without such simple instructive qualifying statements as "A person would have to eat hundreds of thousands of fish dinners to get even a single therapeutic dose" for perspective?. Do current levels of contamination pose relevant risks to the maintenance of current exploitable stocks, and future recrudescence of already exploited ones? These questions while cursorily "heretic", are asked, basically to streamline and ensure the high throughput of immediately useful environmental data, pertinent to the insurance of human and animal environmental welfare.

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