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Histopathology And Activities Of Proteases, Aminases And Glutamate Dehydrogenase In *Clarias Gariepinus* Fingerlings Exposed To Linear Alkyl Benzene Sulphonate (LAS)

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Abstract

Fingerlings of the African catfish, *C. gariepinus* (mean weight 13.13±2.77g) were exposed to linear alkyl benzene sulphonate (0.0166mg/L) under laboratory conditions. The tissues (liver, gill, muscle and kidney) enzyme parameters were analysed after 0hr (initial) 2hr, 12hr and 24h exposure. The increased neutral and alkaline proteases activities in the different tissues of *C. gariepinus* indicates the damage caused due to impairment of energy supply and alkaline proteases activity indicates higher protein degradation. Therefore, the proteins are denatured leading to more activation of protease. During the toxic exposure of LAS, the aspartate aminotransfarase (AAT) and alaninetransfarase (ALAT) were enhanced which led to incorporation of amino acids into TCA cycle for energy production. Whereas, glutamate dehydrogenase activity similarly enhanced the elevated level of amino acids, transaminases which possibly suggest the utilization of protein under stress condition of fish. Possible Impaired respiration was evident due to the toxic insult of LAS to the gill which showed severe damage such as filaments with disjointed lamella. In the liver, there was mild vacuolation and congestion of the liver tissue. The kidney showed sequensiation of the kidney architecture with accumulation of hyaline droplets in the tubular epithelial cell. These changes occur predominantly in the 24hr exposure. LAS is toxic to fingerlings fish *C. gariepinus* are more susceptible to LAS, therefore their use on/near fish farm or in area close to aquatic environment should be discouraged.

Keyword: Linear alkyl benzene sulphonate, proteases, aminases, glutamate dehydrogenase, histopathology

Introduction

Water is essential natural resources of living organisms. Man depends on it for various purposes like drinking, cooking, irrigation and even disposal of waste products. The aquatic environment plays an important role in the life of all living organism due to its physicochemical and biological properties and formation of food webs. The environment is getting polluted due to entry of different polluting effluents into the water bodies through domestic, agrochemicals, fertilizers, pesticides and industrial discharge etc. These polluting agents are toxic and deteriorate the water quality by changing its physicochemical nature that cause an ecological imbalance leading to stress of different kinds on aquatic organism (Kamble and Tapale, 2011). Detergents are cleaning products derived from synthetic organic chemicals. The cheapness of detergent production from petrochemical sources with its ability to foam when used in acid or hard water gives it an advantage over soaps (Okpokwasili and Nwabuzor, 1988). Surfactant is the components mainly responsible for the cleaning action of detergents. In commercial detergents, the surfactants component is between 10 and 20%. The other components includes bleach, filler, foam, stabilizer, builders, perfume, soil suspending agents, enzymes, dyes, optical brightners and other materials designed to enhance the clearing action of the surfactant (Ogundiran *et al*, 2010). Generally detergents are xenobiotics components which are usually washed into water bodies and are made up of several compounds of which the active components are the surface-active agents or surfactants (Ruiswell *et al*, 1992). Surfactants are of various types which are used in the formulation of detergents; the LAS (Linear alkyl benzene sulphonate) are the most widely used (Mcavoy *et al*, 1997). Contamination of aquatic phase by detergents has been reported in aquatic organism such as fishes (Adewoye, and Fawole, 2002; Ogundiran *et al*, 2007, 2009, 2010). The pollutants build up in food chain and are responsible for the adverse effects and death in aquatic organism (Farkas *et al*, 2002).

Fish can serve as bioindicators of environmental pollution (ecotoxicological studies) and therefore can be used for the assessment of the quality of aquatic environment (Lopes *et al*., 2001). Since they are directly exposed to chemicals resulting from agricultural production via surface runoff of water or indirectly through the food chain of ecosystem. The choice of the test object depends on its geographic distribution, its availability in good number round the year and its sensitivity to the toxicant (ELFAC, 1983).

However, African catfish (*Clarias gariepinus*) is of great commercial importance and it is the most common

freshwater widely consumed in Nigeria (Olaifa *et al*, 2004). It can therefore be a good model to study responses to various environmental contaminants due to two reasons; first this species of fish exhibits anatomical and physiological changes at the level of both respiratory and circulatory systems, owing to the presence of ramifying organ in the preribranchial activity for air breathing. Secondly, this specie apart from the fact that it is found in African rivers also lives in temporary puddles forming in desert areas after rainy inundation, in which a large amount of pollutant rapidly accumulate (Forombi *et al.,* 2008).

There is mounting evidence that serum enzymes [alanine aminotransfarase (ALAT, formerly SGPT); aspartate amino-transfarase (ASAT, formerly SGOT); lactate dehydrogenase (LDH); creatine kinase (CK)] possess special diagnostic magnitudes to several pollutant categories (Krajnoviae-Ozretiae and Ozretiae, 1987; Nemcsol *et al*., 1987; Adhan et al., 1997 and Adhan, 2010). Anil Kumar *et al* (2010) has reported the impact of thiametoxan on the enzymatic activities of *Channa punctatus*. Recent studies reported the impact of lether dyes on total proteins of *Cirrihinus mrigala* (Afaq and Rana, 2009).

Histopathology was selected because of its usefulness as a diagnostic tool in the search for target tissues and mechanisms of action of environmental contaminants (Hinto *et al*, 1992). The study focused on gills because they are the main target for many aquatic pollutants in general and surfactant in particular (Kikuchi *et al*., 1975) as well as the most seriously affected organ due to direct contact with the aquatic environment (Mishra *et al*., 1985). Also the complex and interconnected functional-structural features of the gill epithelium make it an excellent model system for examining the effects of dissolved substances on tissues (Evans, 1987). In fish, surfactants have shown to produce damage in the gills, skin and pharynx (Bardach *et al*., 1965). However, liver of fish can be considered a target organ to pollutants, alterations in its structure can be significant in the evaluation of fish health (Myers *et al*., 1988) and exhibits the effects of a variety of environmental pollutants (Hintons *et al*., 1992). Moreover, the liver has played a major role in complex enzymatic processes of tetraiodothyronine (Thyroxine), tri-iodothyronine (T_4-T_3) conversion. The metabolic rate of hepatocytes is certainly modulated by thyroid hormones. Thyroid dysfunction may perturb liver function and liver diseases affect thyroid hormone metabolism (Malik and Hodgson, 2002). The liver was examined because it plays primary roles in the metabolism and excretion of xenobiotics compounds with morphological alteration occurring in some toxic conditions (Rocha and Montero, 1991). Monitoring of histological alterations in fish liver is a highly sensitive and accurate way to assess the effects of xenobiotic compounds both in the field and in the laboratory. Therefore, it is imperative to focus more attention on the function of the liver when affected by the detergent. From the foregoing, the present study focused on the proteases, aminases and glutamate dehydrogenase in *C. gariepinus* exposed to Linear alkyl benzene sulphonate (LAS).

Materials and Methods

The fish sample *Clarias gariepinus,* weighing 13.13±2.27g were obtained from Aqua fish farm Anambra State, Nigeria and were treated with 0.05% $KMnO_4$ solution for 2 min to avoid any dermal infections. They were further acclimated for 14 days and container was covered on top with nylon mesh tied firmly around the top of the container with rubber band to prevent the fish from jumping out. They were fed daily with commercial feed *(Multifeed).* Linear Alkyl benzene sulphonate (LAS CII - 12) popularly called klin was purchased from Onitsha market, Anambra State. Dechlorinated tap water with 99.9 % purity was used as dilution for the control experiment. The water met the biological criteria of the dilution water. The physicochemical properties of the water was checked and given in Table 1. The LC_{50} of The compound LAS 0.0166mg/L was determined for 24hr by the method of Finney (1986). Batches of 5 fishes were exposed to 2, 12 and 24hr for sublethal concentration at 0.005mg/L along with control fish in separate tanks consisting of six litres of water.

The fish were anaesthesized using MS-222 and the gill, kidney, muscle and liver were excised and used for the assay. For assaying neutral and alkaline proteases, 10% tissue homogenates were prepared in ice cold distilled water and centrifuged at 3000rpm for 15minutes. A clear cell free supernatant was used for the assay of proteases by the method of David and Smith (1955). Neutral protease activity was assayed at pH 7.0 using phosphate buffer and alkaline protease activity at pH 9.0 with carbonate bicarbonate buffers and 10mg of denatured heamoglobin protein was used as substrate. However, the glutamate dehydrogenase (GDH) was assayed by homogenating 10% tissue in ice cold 0.25m sucrose solution and was centrifuged at 3000 rpm for fifteen (15) minutes. The clear cell supernatant was collected and used for GDH assay using the method of Lee and Lardy (1965). Furthermore, buffer and enzyme, 0.1 micromolecules of NAD^+ and 2 micromoles of INT in addition to the substrate were added to the reaction mixture. Reitman and Frankel (1957) were adopted in the assay of AAT and ALAT activity were 10% homogenates were prepared in ice cold 0.25m sucrose solution and centrifuged at 3000 rpm for 15minutes. The supernatant was used for the enzyme source.

For histological examination, the gill, liver and kidney were fixed in 10% neutral buffered formalin and examined using light microscope and photomicrography. Sections of 3-5µm thickness were stained with heamatoxylin and eosin histological examination.

Results

The enzyme activities of neutral and alkaline protease, AAT, GDH and ALAT (Table 2) were found to be increased in liver, gill, muscle and kidney tissues of *Clarias gariepinus* exposed to LAS for 0, 2, 12 and 24hr. It was evident that neutral proteases showed a slight increase but not significant ($p>0.05$). In tissues of liver, muscle, kidney and gill but were not significant ($p>0.05$). At the same time, the alkaline proteases increased in the liver, muscle, kidney and gill of the experimental fish but showed no significant difference ($p>0.05$). However, the liver, gill, kidney and muscle of *C. gariepinus* AAT enzyme evidently increased across the exposure period while the ALAT enzyme activity was more in the liver, kidney, gill and muscle. Hence, glutamate dehydrogenase showed a marked increase in enzyme activity throughout the exposure period (0-24hr) in *Clarias gariepinus* exposed to LAS but showed no significant different ($p>0.05$)

No recognizable changes were observed in the gills of the control fish. The control gill consisted of a primary filament and secondary lamellae as shown in Fig 4. Disjointed lamella epithelium was evident in LAS exposed to *C. gariepinus* (Fig 1).

The histology of control fish liver revealed normal typical paranchymatous appearance Fig 5. The liver was made up of hepatocytes that were polygonal cells with a central spherical nucleus and densely stained nucleolus. However, mild vacuolation of the hepatocytes was evident Fig 2. Similarly, no recognizable changes were observed in the kidney of the control fish as shown in Fig 6. The kidney tissue from *Clarias gariepinus* exposed to LAS showed no significant pathological damages which depict the kidney tubules that were intact, Fig 3.

Discussion

The increased proteases both neutral and alkaline in all the tissues of *Clarias gariepinus* exposed to LAS was in consonance with Anil kumar et al., 2010 who reported increased proteases in the tissue of *Channa punctatus* (Bloch) exposed to thiametoxan. This alteration in the proteolytic activities might be due to LAS intoxication which enables the increased degradation of protein to yield excess energy that might sequester the toxic impact of LAS. Moreover, the ALAT and AAT enhanced enzymatic activity might be attributed to the stress caused by the toxicant which may affect the intake of food by fish thereby requiring additional energy to overcome the toxic perturbation of LAS (Schullman *et al*, 2002). It can be observed that transaminase activities may be enhanced with proteolytic activities. Fish can be seen to utilize the free amino acid from amino acid pool for energy production due to elevated level of transaminases. This agrees with the findings of (Abdul Naveed *et al*., 2009, Anilkumar, 2010) that made similar observation on the toxic influence of pesticides on fishes. The change in transaminase activities may be due to possible change in protein metabolism/or aminoacid metabolism, tissue turnover and increased respiration in *Clarias gariepinus* exposed to LAS. This also agrees with Nivedita *et al*, (2002), who reported similar findings of *Cirrhina mrigala* exposed to diethyl phthalate (DEP) indicating that transaminase activity is enhanced in the liver due to DEP toxic insult. Similarly, Philip and Rajasree (1996) reported elevated of the transaminases, aspartate aminotranfarase and alanine aminotransfarase and glutamate dehydrogenase indicating active transamination and oxidative deamination in *Cyprinus carpio* exposed to cypermethrin. They also noted increase in total protein contents in all the tissues (liver, gill, brain and muscle) with a decrease in amino acids and protease activity. In the present study, Glutamate dehydrogenase enzyme activity was increased in the tissues (liver, gill, kidney and muscle) of *C. gariepinus* due to increased rapid utilization of amino acids and onset of detoxification mechanism with possible operation of oxidative deamination under toxic impact of LAS. This coincides with the findings of (David and Micheal, 2005; Prashanth, 2006; and Ganesh *et al*, 2006). This can be observed as increased level of oxidation of amino acids trying to sequester the potential deleterious toxic effect of linear alkyl benzene sulphonate. It can be deduced that LAS intoxication in *C. gariepinus* not only enhanced proteases and at the same time ameliorated amino acid transaminases and glutamate dehydrogenase. This can suggest the possible rapid utilization of proteins when the fish was under toxic stress of LAS.

Gill tissue has its own importance while assessing the toxicity of surfactants. The large surface area of the tissue coupled with its important role in respiration and osmoregulation made it ideal for examining the toxic effects. In the present study damages of the gills indicated that the LAS caused impairment in gaseous exchange efficiency of the gills. The major changes noted were disjointed lamellar within the exposure period. This coincides with the findings of Part *et al.* (1985) who reported the viability of gills which deteriorated rapidly during 60 min of exposure to 100 micromoles/litre of linear alkyl benzene sulfonate and to nonyl phenol. Histopathological changes of gill such as hyperplasia and hypertrophy, epithelial lifting, aneurysm and increase in mucus secretion have been reported after the exposure of fish to a variety of noxious agents in the water, such as herbicides, phenols and heavy metal (Nowak,1992) . Liver is especially useful organ in assessing the possible impact of pollutant in fish due to the fact that chemical tends to concentrate there. This is also a major site for biotransformation of toxic chemicals which usually makes them less toxic and more easily excreted. In the study

of Risbourg and Bastide (1995), the exposure of fish to atrazine herbicide increased in the size of lipid droplets, vacuolisation in the liver. The most frequent encountered types of degenerative changes are those of hydropic degeneration, cloudy swelling, vacuolization and focal necrosis. This also agrees with Babu *et al*. (2007) in the exposure of fish to fenevalerate on the liver tissues of *Cirrhinus mrigala*, when necrosis of tubular epithelium and pycnotic nuclei in the hematopoietic tissue occurred. Mild vacuolation of the hepatocytes and congestion in the liver tissues was observed, probably resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver. The inability of fish to regenerate new liver cells may also have led to necrosis. Oyinidira *et al* 2010 reported major histological abnormalities observed in liver such as cellular necrosis, cellular infilteration, congestion of the central vein was similarly evident in *C. gariepinus* exposed to LAS. The kidney of *Clarias gariepinus* fingerlings exposed to LAS showed intact tubules and sequensiation of kidney architecture with accumulation of hyaline droplets in the tubular epithelial cells. Oulmi *et al*. (1995) studied the effect of linuron herbicide on the rainbow trout (*Oncorhynchus mykiss*).Their results showed small cytoplasmic vacuoles, nuclear deformation in the epithelium of the first and second segments of the proximal tubule. The kidney cells (hepatocytes) were observed to have been massively destroyed. The renal corpuscles of the kidney were scattered resulting in the disorganization and consequently obstruction to their physiological function. Some of the kidney cells were found clogging together while they were disintegrated in some tissues of the organ. This also agrees with the findings of Omoniyi *et al.* (2002) and Rahman *et al*.(2002). Lesions in the kidney tissues of fish exposed to deltamethrin in the epithelial cells of renal tubule, pyknotic nuclei in the hematopoietic tissues, dilution of glomerular capillaries, degeneration of glomerulus were observed (Elif, 2006). The comprehensiveness of the study carried out on LAS provides documentation of the environmental safety of LAS and its effect on the physical and physiological alterations in fishes in the aquatic environment and to ascertain when the aquatic organism e.g fish in the aquatic habitat exceeds their safe limit.

Conclusion

Detergent effluents can induce damages to the tissue and organ, which might make all the living entities in polluted environment vulnerable to diseases, and eventually leads to death. Therefore, there is need for the adoption of proper effluent treatment technology which would ensure proper treatment of industrial effluents prior to their discharge into the environment. Although, in a developing country like the Nation Nigeria, several numbers of legislations exist on the quality assurances of water resources but such legislations are rarely followed and yet Industrial growth and its associated environmental problems such as water and sediment contamination are fast increasing. So there is the need for us to imbibe from such developed nations where environmental monitoring agencies are more effective and environmental laws and legislations are strictly followed.

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There is no conflict of interest among the authors

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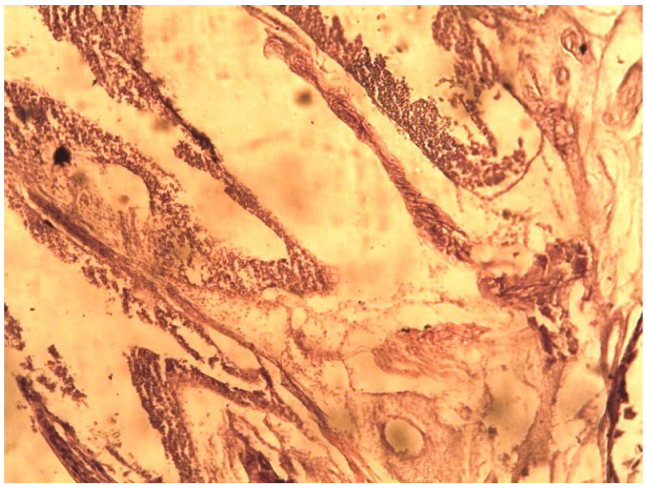

Figure 1 Gill section of *C. gariepinus* exposed to LAS for 24hr mag (H&E) x40. Filaments are not enlarged; they are narrow with disjointed lamella epithelium

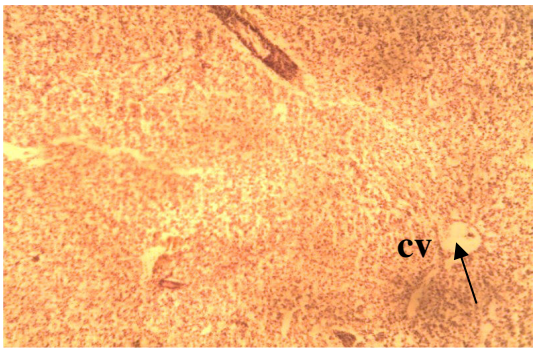

Fig 2. Histologic section of liver of *Clarias gariepinus* treated with LAS for 24hr showing mild vacuolation of hepatocytes.Note the central vein (V). (H and E) mg x100

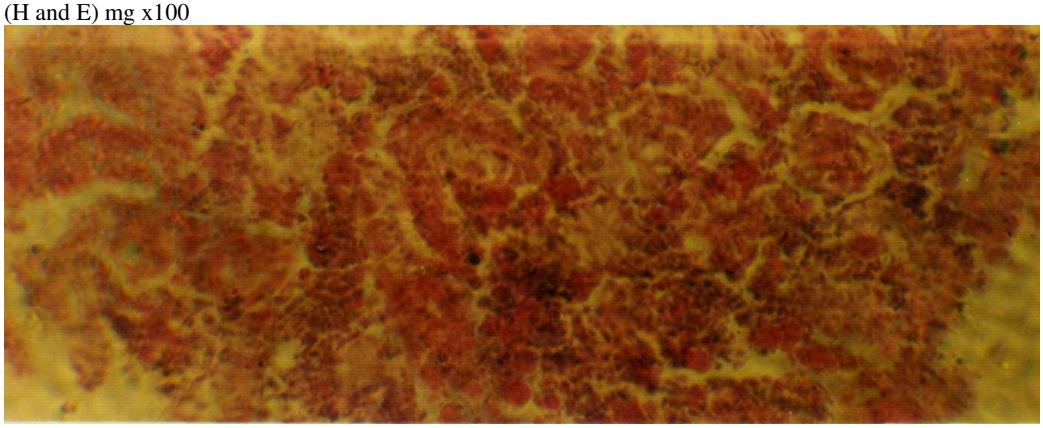

Fig 3. Kidney section of *C. gariepinus* exposed to LASFor 24hr. Tubules are intact and sequensiation of kidney architecture. mag (H&E) x40

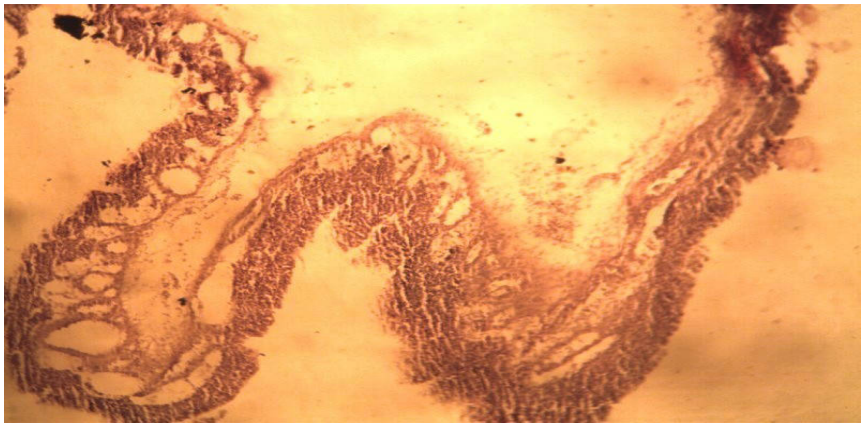

Fig 4. Control gill showing Normal filament and lamella Epithelium Mag(H&E) x400

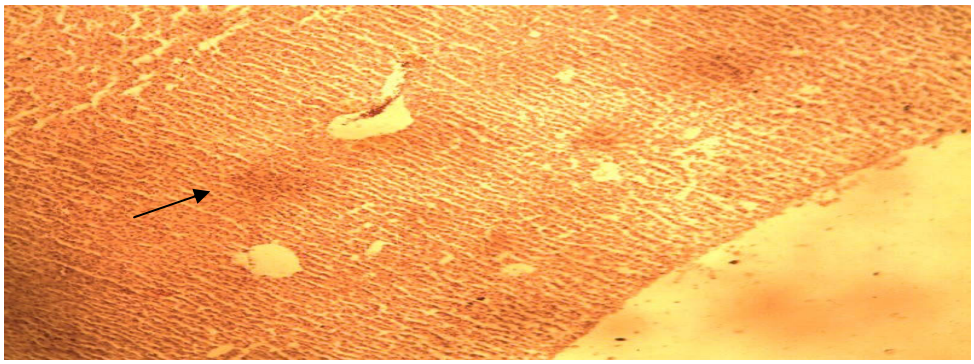

Fig 5. Control liver showing normal histology and central vein (black arrow). Mag (H&E) x400 .

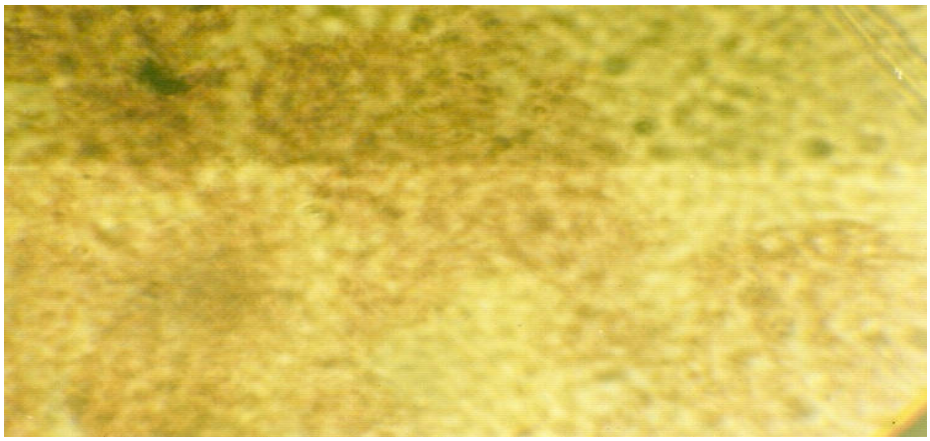

Fig 6. Control kidney showing intact tubules Mag (H&E)x400

Table 1. Physicochemical parameters of the experimental water

Parameters	Values
Temperature	20.1^{o}C±2.5
pH	6.9±2.1
Free carbon dioxide(mg/L)	10.0±2.1
Calcium (mg/L)	4.9±1.2
Bicarbonte (mg/L)	1.42±0.1
Total alkalinity (mg/L) as ($CaCO_{3)}$	6.9±1.1
Sulphate (mg/L)	7.1±2.1
Nitrates (mg/L)	3.4±0.2
Iodine (mg/L)	0.01±0.00
Chlorides (mg/L)	37.0±3.1
Dissolved oxygen (mg/L)	9.2±2.1
Biological oxygen demand (BOD)	1.6±0.1

Mean±SD, 3 individual observation

Table 2. Effect of LAS on the Activities of proteases, aminases and GDH in *Clarias gariepinus* (Burchell) (n=5).

Parameters	Tissue	Start (Initial)	Control	2hr	12hr	24hr
Neutral protease mM of tyrosine equivalent/mg protein/hr	Liver	0.28±0.00^{ns}	0.29±0.10^{ns}	1.29±0.50^{ns}	1.32±0.45^{ns}	1.49±0.49^{s}
	Muscle	0.19±0.10^{ns}	1.29±0.10^{ns}	1.31±0.12^{ns}	1.39±0.20^{ns}	1.59±0.31^{s}
	Kidney	0.76±0.12^{ns}	0.74±0.01^{ns}	1.02±0.14^{ns}	1.23±0.30^{ns}	1.28±0.2^{ns}
	Gill	1.19±0.31^{ns}	1.24±0.11^{ns}	1.33±0.25^{ns}	1.37±0.46^{ns}	1.47±0.81^{s}
Alkaline proteases mM of tyrosine equivalent/mg protein/hr	Liver	1.34±0.21^{ns}	1.31±0.11^{ns}	1.31±0.14^{ns}	1.36±0.20^{ns}	1.45±0.21^{s}
	Muscle	1.21±0.33^{ns}	1.21±0.20^{ns}	1.24±0.10^{ns}	1.28±0.24^{ns}	1.41±0.34^{s}
	Kidney	0.15±0.01^{ns}	1.07±0.10^{ns}	1.12±0.24^{ns}	1.17±0.36^{ns}	1.21±0.30^{ns}
	Gill	1.20±0.32^{ns}	1.22±0.41^{ns}	1.19±0.01^{ns}	1.17±0.02^{ns}	1.31±0.20^{s}
AAT mM pyruvate formed/mg protein/hr	Liver	2.11±0.12^{ns}	2.14±0.51^{ns}	2.11±0.14^{ns}	2.23±0.19^{ns}	3.23±0.29^{ns}
	Muscle	1.59±0.13^{ns}	1.61±0.19^{ns}	1.72±0.29^{ns}	2.01±0.31^{ns}	2.13±0.46^{ns}
	Kidney	0.17±0.21^{ns}	1.09±0.14^{ns}	1.13±0.21^{ns}	1.16±0.36^{ns}	1.32±0.38^{ns}
	Gill	2.12±0.32^{ns}	2.12±0.64^{ns}	2.20±0.31^{ns}	2.31±0.36^{ns}	2.38±0.45^{ns}
ALAT mM pyruvate formed/mg protein/hr	Liver	5.12±1.00^{ns}	5.01±1.02^{ns}	6.14±1.31^{ns}	6.39±1.81^{ns}	6.84±1.00^{s}
	Muscle	4.65±0.39^{ns}	4.65±0.81^{ns}	4.79±0.41^{ns}	4.89±1.06^{ns}	5.09±0.82^{ns}
	Kidney	2.51±0.71^{ns}	2.51±0.94^{ns}	2.61±0.82^{ns}	2.70±1.11^{ns}	2.87±0.99^{ns}
	Gill	4.67±0.19^{ns}	4.67±0.79^{ns}	4.72±0.67^{ns}	4.89±1.09^{ns}	5.07±1.11^{ns}
GDH mM formazen/mg protein/hr	Liver	0.32±0.01^{ns}	0.33±0.02^{ns}	0.43±0.01^{ns}	0.49±0.03^{ns}	0.57±0.01^{ns}
	Muscle	0.22±0.01^{ns}	0.22±0.01^{ns}	0.31±0.01^{ns}	0.35±0.01^{ns}	0.47±0.03^{ns}
	Kidney	0.25±0.01^{ns}	0.23±0.01^{ns}	0.29±0.01^{ns}	0.33±0.011^{ns}	0.37±0.012^{ns}
	Gill	0.32±0.01^{ns}	0.31±0.07^{ns}	0.33±0.03^{ns}	0.42±0.07^{ns}	0.45±0.05^{ns}

Mean±SD 5 individual observation, ns=not significant, s=significant