DELETERIOUS EFFECTS OF ACUTE AMMONIA EXPOSURE IN CULTURED
OREOCHROMIS NILOTICUS

M. I. Hanna¹, S. A. EL-Maedawy²; A. M. Kenawy³; S.M. Gergis⁴

¹Department of Fish Diseases and Management, Faculty of Vet. Medicine, Cairo University.
²Department of Biochemistry and Toxicology, Animal Health Institute, Dokki, Giza.
³- Department of Hydrobiology, National Research Center, Dokki, Giza. Akenawy70@yahoo.com
⁴- Department of Cell Biology, National Research Center, Dokki, Giza.

ABSTRACT
Ammonia is the principal nitrogenous waste product of fishes and it is also the main nitrogenous waste material excreted by gills beside urea and amines. This study was planned to determine the effect of acute ammonia exposure on cultured Oreochromis niloticus. Fish of nearly the same weight were divided into four groups. The 1st group was served as a control group, the 2nd exposed to 2.5mg/L of total ammonia nitrogen (TAN) (0.16 NH₃ mg/L), the 3rd exposed to 5.0 mg/L of total ammonia nitrogen (TAN) (0.32 NH₃ mg/L) and the 4th exposed to 10.0 mg/L of total ammonia nitrogen (TAN) (0.65 NH₃ mg/L) at water pH 8 and water temperature 28 °C for six days. Results revealed that acute ammonia exposure was associated with fish mortalities, clinical abnormalities in the form of convulsions, hyperexcitability, signs of asphyxia and sluggish fish reflexes. Histopathological examination showed lamellar hyperplesia with fusion of the secondary gill lamellae, hydropic degeneration in the liver, hyperactivation of the melanomacrophage center of the spleen, glomerulonephritis and cerebral oedema with neuronal degeneration. Also significant increase in lipid peroxidation product (malondialdehyde), nitric oxide, cortisol and micronucleus count were recorded. It was concluded that acute ammonia exposure had several deleterious effects on cultured Oreochromis niloticus.

Key words : toxic ammonia, O.niloticus, micronucleus assay, deleterious effects

1-INTRODUCTION
Ammonia is a common aquatic pollutant that can enter natural aquatic systems via discharges from wastewater treatment plants, degradation of nitrogen-containing organic matter, fertilizer runoff, and industrial sources. Ammonia is also the main nitrogen waste material in teleosts, and is generated as a product of protein catabolism (Randall and Wright 1987). Under intensive culture conditions, if water flow is restricted or inadequate, the presence of uneaten food and organic waste can lead to increased levels of ammonia in the water, which may reach levels that adversely affect the physiology of the fish.

Two forms of ammonia occur in water, un-ionized (NH₃) and ionized (NH₄⁺) ammonia, and the relative proportion of each form is dependent on pH, temperature and salinity (Bower and Bidwell 1978). The un-ionized form of ammonia (NH₃) is highly toxic to fish, while the ammonium ion (NH₄⁺) is much less so. Un-ionized ammonia mainly enters fish via the gills, as it can readily pass through the gill epithelium (Hampson, 1976), which, however, is rather impermeable to ionized ammonia (Sheehan and Lewis 1986).

Exposure of fish to high levels of ammonia therefore results in a rapid increase in plasma levels of the compound (Person-Le Ruyet et al. 2003), and it might result in net accumulation of ammonia at toxic levels in the fish (Rasmussen and Korsgaard 1998).

The toxic levels of un-ionized ammonia for short-term exposure usually are reported to lie between 0.6 and 2 mg/l, while some consider the maximum tolerable concentration to be 0.1 mg /l (Pillay, 1992).

Many studies confirmed the validation of micronucleus assay in fish as a monitor for the occurrence of aquatic genotoxic agents through the observation of close association between the frequency of micronucleus and the degree of pollution (Compana et al.,2001).
So, this work was planned to study the harmful effects of short-term ammonia poisoning in cultured *Oreochromis niloticus* through recording of clinical abnormalities, histopathological changes, biochemical alterations and genotoxicity associated with ammonia poisoning.

2-MATERIALS & METHODS

2-1-Fish and rearing conditions:

Hundred *Oreochromis niloticus* fish weighing 90.0 ± 10.0 g was obtained from a private fish farm. Fish were transported in plastic buckets supplemented with battery aerators to the lab. Fish were acclimated in fully prepared glass aquaria supplemented with air pumps containing dechlorinated tap water for 10 days before the experiment commenced. During acclimation, fish were fed on a commercial ration. Fish were examined clinically to assure the absence of any abnormalities or external signs according to the methods described by Amlacher (1970).

2-2-Experimental design:

Eighty *Oreochromis niloticus* of almost the same weight and size were categorized into four groups. The 1st group was served as a control group, the 2nd exposed to 2.5 mg/L of total ammonia nitrogen (TAN) (0.16 NH₃ mg/L), the 3rd exposed to 5.0 mg/L of total ammonia nitrogen (TAN) (0.32 NH₃ mg/L) and the 4th exposed to 10.0 mg/L of total ammonia nitrogen (TAN) (0.65 NH₃ mg/L) at water pH 8 and water temperature 28°C according to EPA (1984) for six days. The experimental technique followed that of APHA (1975), Reish and Oshida (1987), and ISO (1996). The experimental medium was changed every 24 h with fresh solution. Water was aerated by compressed air to maintain the oxygen concentration at 6 ± 0.5 mg/L, water temperature was maintained constant at 28°C using thermostatic water heater and water pH is maintained at pH 8. Ammonium chloride (NH₄Cl) was used as a source of ammonia. All chemicals used were analytical reagent grade. Serum samples were collected from different groups at the start and end of the experiment (0 and 6 days) and stored in deep freezer at -80°C.

2-3-Clinical investigation and Post Mortem examination: The exposed fish were kept under proper observation during the period of experiment for any external clinical abnormalities, PM lesions or deaths according to the method described by Amlacher (1970).

2-4-Biochemical examination: Blood samples were allowed to clot at room temperature and centrifuged at 3000 r.p.m for 15 min. to separate serum. Also blood samples were collected using sodium citrate as anticoagulant and centrifuged to separate plasma. Serum and plasma samples were used to determine:

2-4-1- Lipid peroxidation product: malondialdehyde was measured according to the method described by Albero et al. (1986).

2-4-2- Cortisol: The quantification of cortisol in the plasma was carried out by means of a commercial ELISA kit (RADIM SpA, Rome, Italy). (Yalow and Berson, 1971).

2-4-3- Nitric oxide (NO) assay: the nitrite level in the collected serum samples was calculated according to the method described by Green et al. (1982).

2-5-Micronucleus test: One thousand erythrocytes were examined for every fish to determine the percentage of cells containing micronuclei (Al-Sabti and Metcalfe, 1995).

2-6-Histopathological studies: Tissue samples were processed by conventional method, sectioned at 5 µm and stained with Haematoxylin and Eosin (Roberts, 2001).

2-7-Statistical analysis: The results obtained in this study were statistically analyzed according to Petrie and Watson (1999). All data were subjected to one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test for comparison among treatment means using SAS, Version 8.2 (2001).

3- RESULT AND DISCUSSION

Fish exposed to different concentrations of ammonia moved rapidly, lost equilibrium in water and began to sideways swimming contrary to the control group. An increase in their movements, convulsions, spiral swimming, efforts to swallow air from the surface of water, increase in ventilation and death were observed in varied degrees in a concentration dependent manner (photos 1&2). Post mortem examination revealed an increase in the amount of mucus secretion in the gills and on the body surface, congestion in the gills (photo,3) and darkening in the eye and on the skin. Mortality was recorded only in 10 ppm exposed group and attained 50% by the end of the experiment. Acute ammonia toxicity can cause an assortment of clinical signs in fish, the most severe of which include convulsions, coma, and death (Randall and Tsui 2002), as well as behavioral changes such as...
hyperexcitability and appetite suppression (Ortega et al., 2005). The recorded clinical signs were recorded by Knoph, 1992.

The results represented in the table (1), described the acute exposure of fish to toxic ammonia at different concentrations (0, 0.16 mg/l, 0.32 mg/l and 0.64 mg/l). Toxic ammonia exposure induced an increase in malondialdehyde and nitric oxide levels, similarly to that can be observed in other fish (Bosoi and Rose 2012). Acute ammonia intoxication diminishes the activities of antioxidant enzymes and increases superoxide formation. These effects could play a role in the mechanism of ammonia toxicity. It has been shown that ammonia toxicity is mediated by activation of NMDA receptors. Ammonia intoxication also induces a depletion of glutathione and an increase in lipid peroxidation (Jayakumar, 1999). One important molecular consequence of oxidative stress obtained in table (1) is the activation of intracellular signaling cascades. Numerous studies indicate that reactive oxygen species (ROS) activate protein kinases (Czaja et al., 2003). In particular, p38MAPK, JNK, and ERK1/2 are phosphorylated by exposure to exogenous H2O2. One possible explanation for the biphasic phosphorylation of ERK1/2 is that the first peak of ERK1/2 phosphorylation might be attributable to ROS generation (Clausen et al., 2004). The second peak might be attributable to the inactivation of mitogen-activated protein kinase phosphatase, which might occur in the second phase of ERK1/2 phosphorylation (Berlett and Stadtman, 1997). The early phosphorylation of MAPKs by ammonia appear to be critical for the ammonia-induced astrocyte dysfunction. An increase of ammonia in experimental animals or treatment of cultured astrocytes with ammonia generates reactive oxygen and nitrogen species in the target tissues, leading to oxidative/nitrosative stress (ONS) (Skowrońska et al., 2012). Interrelated mechanisms underlying this response include increased nitric oxide (NO) synthesis which is partly coupled to the activation of NMDA receptors and increased generation of reactive oxygen species by NADPH oxidase. ONS and astrocytic swelling are further augmented by excessive synthesis of glutamine (Gln) which impairs mitochondrial function. Ammonia-induced ONS results in the oxidation of mRNA and nitration/nitrosylation of proteins which impact intracellular metabolism and potentiate the neurotoxic effects (Skowrońska et al., 2012).

The results of this study showed that, acute exposure to toxic ammonia induced an increase in the cortisol levels, similarly to what can be observed for other fish species (Person-Le Ruyet et al., 1998). Given the increase in the levels of cortisol, it is evident that the acute (12 and 24 h) exposure to ammonia activated the hypothalamus–pituitary–interrenal (HPI) axis, initiating a typical stress response, in the Senegalese sole. Taking into account that the cortisol is the main responsible for the effects of stress, it is normal to think that different physiological functions, such as growth, reproduction or the effectiveness of the behavioural responses (Galhardo and Oliveira, 2009), could be negatively affected in the ammonia-stressed sole. Less data are available for fish, to declare the increase in the cortisol levels although there is some evidence suggesting a possible relationship between the increase in the plasma concentration of cortisol and the increase in the 5-HT activity (serotonin) or in the ratio of 5-HIAA/5-HT (Mancebo et al., 2007), or in the of 3, 4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxy-3-indoleacetic acid (5HIAA), DOPAC/DA and 5-HIAA/5-HT ratios (Gesto et al., 2008). Although the evidence is indirect, we propose that the increase in the plasma levels of cortisol, induced by exposure to ammonia, may modulate the cerebral activity of the 5-HT and DA systems. Evidently, further studies are required to clarify the possible relationship between cortisol and monoamnergic transmission in fish (Weber, 2011).

The results of micronucleus assay in control and ammonia exposed groups were summarized in table (2) and photo (4). Results showed that there was a significant increase of micronucleus production in different toxic ammonia exposed groups when compared to the control group in a concentration and time dependent manner. Among current cytogenetic techniques, micronuclei and some other nuclear abnormalities are considered to be sensitive indicators of genotoxicity and cytotoxicity. Micronuclei estimation in fish has been shown to be a better parameters than chromosomal aberrations in the environmental studies under laboratory and field conditions (Metacalfe, 1998). The gill cells were chosen to estimate the micronucleus formation because many studies recorded that gill cells are more sensitive than haemopoietic cells to micronucleus inducing agents (Hayashi et al., 1998). The occurrence of genetic damage in O.niloticus exposed to ammonia was illustrated by high frequency of micronucleus in ammonia exposed fish for 2, 4 and 6 days compared to the control group in a concentration and time dependent manner. These results are similar
to those obtained by Abumourad et al. (2012). Also an increase in micronucleus frequency with time was found by Ramirez and Garcia (2005) in zebra fish coincide with our results.

The histopathological observations for both control and ammonia exposed Nile tilapia fish with representative images of the tissues displayed in plates A-C. Control individuals did not show any pathological changes in the tissues examined by the light microscope (plate A, a,b,c,d and e). Most differences between control and fish exposed to 2.5 mg/L of total ammonia nitrogen (TAN) (0.16 NH₃, mg/L) (1st gp) were detected. Gills revealed telangiectasis and hyperemia in the branchial and lamellar blood vessels (A,1), lamellar hyperplasia which leading to fusion of secondary lamellae began from the base to the apex of the lamellae with degenerative changes in the epithelial lining of the secondary lamellae (A,2). Liver lesions consisted of hydropic degenerations and cloudy swelling in the hepatocytes with focal aggregation of melanomacrophage cells in between the hepatocytes (A,3). Focal areas of necrosis, mononuclear inflammatory cells and hyperplasia in the wall of the bile duct were also detected (A,4), while the kidneys displayed glomerulonephritis (A,5), vacuolar degenerative changes in the tubular epithelium and slight congestion (plate A,6). Spleen showed hyperactivation of the melanomacrophage centers with slight congestion in splenic blood vessels (A,7). The brain exhibited slight congestion (A,8). Lateral muscle and the skin of the fish exposed to ammonia showed no histological differences compared to the control groups.

All the pathological alterations showed a relationship with prevalence increasing with increasing ammonia concentration and exposure time.

More advanced pathological changes than in the 1st gp were observed in the fish exposed to 5.0 mg/L of total ammonia nitrogen (TAN) (0.32 NH₃, mg/L) (2nd gp). Gills showed sever congestion (B,1) associated with severe hyperplasia (B,2). The liver showed severe congestion and hemorrhages (B,3) with area of haemolysis in between the hepatic parenchyma (B,4). The kidney had hyperplasia in the wall of renal blood vessels associated with necrosis in the heamopiotic interstitial tissues, peritubular and periglomerular oedema and degenerative changes in the endothelial linning the glomerular tuft (B,5&6). The spleen showed severe congestion and hemorrhages, hyperplasia in the wall of splenic blood vessels, hyperactivation of melanomacrophage centers and focal area of depletion of lymphocytic tissues (B,7). The brain showed severe oedema in the cerebellum and neuronal degeneration (B,8).

Microscopical observation in the fish exposed to 10.0 mg/L of total ammonia nitrogen (TAN) (0.65 NH₃, mg/L) (3rd gp) showed, moreover the previous changes the gills exhibited more congestion (C,1) with complete fusion of the secondary lamellae leading to complete obliteration of the inter-lamelar space associated with severe degenerative and necrotic changes in the respiratory epithelium (C,2). The Liver had swollen, degenerated and necrotic hepatocytes with losing adenoid structure (C,3), infiltration of mononuclear inflammatory cells in between the hepatic parenchyma and vacuolar degeneration and necrosis in acinar cells of pancreas (C,4). The kidney showed aggregation of melanomacrophage cells in between the interstitial heamopiotic tissues specially at the necrotic areas with sever degenerative and necrotic changes in the tubular epithelium and heamopiotic tissues (C,5) with more pronounced peri-tubular and peri-glomerular oedema (C,6), The spleen showed the same pictures mentioned before with more extent. Brain showed neuronal degeneration (C,7), oedema and focal glaiosis (C, 8).

This study presents results concerning the sublethal effects of ammonia on Nile tilapia with respect to tissue histopathology. Person Le Ruyet et al. (1998) have shown that NH₃ entered fish within 15 min exposure. The first effects of contaminants usually occur at cellular and sub-cellular levels, starting from the first hour of contamination (Metcalfe, 1998). Flis (1968) reported that chronic ammonia exposure might damage gills, liver and kidney, which may predispose the fish to numerous infections. In the present study, the important histopathological effects of sublethal ammonia on the gills were chloride hyperplasia, telangiectasis on lamella and hyperemia on epithelium. Gills are a well known target organs in fish, being the first to react to unfavorable environmental conditions. Several authors have reported similar alterations on the gills of different fish species exposed to ammonia Redner and Stickney (1979) observed aneurysms, lamellar capillary congestion and hemorrhaging of Tilapia after acute (2.4 mg / NH₃–N) and chronic (0.43– 0.53 mg / l NH₃–N) ammonia exposure (Kucuk, 1999). Larmoyeux and Piper (1973) determined aneurysms and fused lamella of rainbow trout (Salmo gairdneri) gill epithelium cells. Furthermore, Kirk and Lewis (1993) reported that the gills of the rainbow trout exposed to 0.1 mg / l ammonia for 2 h exhibited deformation of the lamellae.
Salin and Williot (1991) observed that Siberian sturgeon (Acipenser baeri) (270 g) exposed to more than 60 mg/l of ammonia reveal a modification of the epithelium of the secondary lamellae and the base of the filament is slightly turgescent. Similar results were also confirmed by Mitchell and Ceceh (1983) with channel catfish (Ictalurus punctatus), Malik et al. (1986) with common carp (Cyprinus carpio) and Cardoso et al. (1996) with Lophiosilurus alexandri. In the present study, cloudy swelling and hydropic degenerations on the liver were observed where liver being the main organ of various key metabolic pathways, toxic effects of chemicals usually appear primarily in the liver. Ammonia can be carried by the hepatic portal vein to the liver as a nutrient and enter liver metabolic pathways (Kucuk, 1999). Ammonia exposure causes liver glycogen vacuolation due to disruption of energy production (Thurston et al., 1978). The most frequently encountered types of degenerative changes are those of hydropic degeneration, cloudy swelling, vacuolization and focal necrosis on fish exposed to different kinds of contaminants (Hinton and Lauren, 1990). Wajsbrot et al. (1993) observed clear signs of liver pathology in gilthead sea bream (Sparus auratus) after 20 days of exposure to 13 mg/l 1 TA-N (0.7 mg l_1 NH3–N). In our study, kidney tissues displayed glomerulonephritis and hyperemia after being exposed to different concentrations of sublethal ammonia concentrations where the kidney is a one of the major organs of the toxic effects. Thurston et al. (1978) observed hydropic degeneration in the kidney of cutthroat trout after exposure to 0.34 mg l_l NH3–N. There was hyperactivation of melano-macrophage centers also diffuse infiltration with melanophores within the spleenic tissue. The intensity of coring cells increased in melano-macrophage centers as a result of stress condition where the ACTH (adreno – corticotrophic hormone) released. This hormone produces a rapid dispersion of melanin granules in many fish species, thus pigment changes could be attributed to high level of ACTH associated with stress condition (Hanna, 2004). Concerning the histopathological changes in the brain which may be resulted from the toxic effect of ammonia or the hypoxic effect which resulted from gill destruction. These findings accentuates the findings of Ricardo et al., (2011).

4- CONCLUSION
The clinical signs, post mortem lesions, histopathological changes and biochemical findings recorded in the present study contribute to understanding the ammonia toxicity mechanism in fish. The present study proved that ammonia had genotoxic effect.

5-REFERENCES


Table 1. The toxic effects of acute ammonia exposure on Cortisol, malondialdehyde and Nitric oxide levels:
Means with different subscript letters are differed significantly at P<0.05

<table>
<thead>
<tr>
<th>ppm/group</th>
<th>Cortisol (µg/dl)</th>
<th>Malondialdehyde (µMol/l)</th>
<th>Nitric oxide (mMol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>43.77 ± 3.22a</td>
<td>1.36 ± 0.05a</td>
<td>4.97 ± 0.48a</td>
</tr>
<tr>
<td>2 days</td>
<td></td>
<td></td>
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<tr>
<td>2.5 ppm TAN</td>
<td>141.14 ± 5.21b</td>
<td>2.41 ± 0.37b</td>
<td>74.07 ± 6.64b</td>
</tr>
<tr>
<td>5 ppm TAN</td>
<td>162.03 ± 6.94c</td>
<td>2.71 ± 0.24c</td>
<td>103.12 ± 9.64c</td>
</tr>
<tr>
<td>10 ppm TAN</td>
<td>199.48 ± 6.07d</td>
<td>5.26 ± 0.36d</td>
<td>146.37 ± 6.64d</td>
</tr>
<tr>
<td>4 days</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2.5 ppm TAN</td>
<td>103.54 ± 3.71b</td>
<td>3.47 ± 0.42</td>
<td>62.75 ± 4.04b</td>
</tr>
<tr>
<td>5 ppm TAN</td>
<td>185.77 ± 6.56c</td>
<td>3.83 ± 0.54c</td>
<td>129.88 ± 9.96c</td>
</tr>
<tr>
<td>10 ppm TAN</td>
<td>225.46 ± 6.89d</td>
<td>5.14 ± 0.09d</td>
<td>158.73 ± 9.70d</td>
</tr>
<tr>
<td>6 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 ppm TAN</td>
<td>106.15 ± 7.69b</td>
<td>2.31 ± 0.29b</td>
<td>67.44 ± 6.08c</td>
</tr>
<tr>
<td>5 ppm TAN</td>
<td>160.56 ± 4.26d</td>
<td>4.59 ± 0.40d</td>
<td>134.62 ± 9.70</td>
</tr>
<tr>
<td>10 ppm TAN</td>
<td>206.32 ± 1.45d</td>
<td>4.95 ± 0.52d</td>
<td>133.61 ± 9.70d</td>
</tr>
</tbody>
</table>
Table 2. Mean values and percentages of micronuclei incidence in control and toxic ammonia exposed O.niloticus for 2, 4 and 6 days.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total No. of examine d fish</th>
<th>Total No. of PCE₅*</th>
<th>Total No. of Mn.</th>
<th>Micronucleus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>5000</td>
<td>10</td>
<td>(2.0 ± 0.14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>2.5 ppm TAN for 2 days</td>
<td>5</td>
<td>5000</td>
<td>30</td>
<td>(6.0 ± 0.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.60</td>
</tr>
<tr>
<td>5 ppm TAN for 2 days</td>
<td>5</td>
<td>5000</td>
<td>35</td>
<td>(7 ± 0.19)</td>
</tr>
<tr>
<td>10 ppm TAN for 2 days</td>
<td>5</td>
<td>5000</td>
<td>35</td>
<td>(7 ± 0.20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.70</td>
</tr>
<tr>
<td>2.5 ppm TAN for 4 days</td>
<td>5</td>
<td>5000</td>
<td>30</td>
<td>(6.0 ± 0.24)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.60</td>
</tr>
<tr>
<td>5 ppm TAN for 4 days</td>
<td>5</td>
<td>5000</td>
<td>40</td>
<td>(8.0 ± 0.20)</td>
</tr>
<tr>
<td>10 ppm TAN for 4 days</td>
<td>5</td>
<td>5000</td>
<td>40</td>
<td>(8.0 ± 0.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.80</td>
</tr>
<tr>
<td>2.5 ppm TAN for 6 days</td>
<td>5</td>
<td>5000</td>
<td>35</td>
<td>(7 ± 0.19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.70</td>
</tr>
<tr>
<td>5 ppm TAN for 6 days</td>
<td>5</td>
<td>5000</td>
<td>40</td>
<td>(8.0 ± 0.26)</td>
</tr>
<tr>
<td>10 ppm TAN for 6 days</td>
<td>5</td>
<td>5000</td>
<td>45</td>
<td>(9.0 ± 0.30)</td>
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<td></td>
<td></td>
<td></td>
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<td>0.90</td>
</tr>
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PCE₅ = Polychromatic erythrocytes. Mn = Micronuclei.

Means with different subscript letters are differed significantly at P<0.05.

Photo (1): Oreochromis niloticus showing surfacing and gasping of air in association with exposure to 5 ppm of TAN for 4 days.
Photo (2): Oreochromis niloticus showing surfacing, gasping of air and accumulation of fish around the source of air in association with exposure to 10 ppm of TAN for 6 days.
Photo (3): Oreochromis niloticus showing gill congestion in association with exposure to 10 ppm of TAN for 6 days.
Photo (4): Blood film stained with Giemsa stain showing micronucleus in association with exposure to 10 ppm of TAN for 6 days.
Plate A, a,b,c,d& e as control, Oreochromis niloticus of the first gp Fig. 1: Gills after 4 days of exposure showed telangiectasis and hyperemia in the branchial and lamellar blood vessels H&E, X400. Fig.2: Gills after 6 days of exposure showed lamellar hyperplasia and fusion of secondary lamellae began from the base to the apex of the lamellae with degenerative changes in the epithelial lining the secondary lamellae H&E, X400. Fig. 3: Liver after 4 days of exposure showed hydropic degenerations, cloudy swelling in the hepatocytes with focal aggregation of melanomacrophage cells in between the hepatocytes H&E,X400. Fig.4: Liver after 6 days of exposure showed focal areas of necrosis, mononuclear inflammatory cells and hyperplasia in the wall of the bill duct were also detected H&E, X400. Fig. 5: kidneys after 4 days of exposure showed glomeru-lonephritis H&E, X400. Fig. 6: kidneys after 6 days of exposure showed vacuolar degenerative changes in the tubular epithelium and slight congestion H&E, X400. Fig. 7: Spleen after 6 days of exposure showed hyperactivation of the melanomacrophage centers with slight congestion H&E, X400. Fig. 8: Brain after 6 days of exposure showed edema in the cerebellum and slight congestion.
**Plate B: Oreochromis niloticus** in the Second gp. **Fig.1:** Gills after 4 days exposure showed severe congestion H&E, X400. **Fig. 2:** Gills after 6 days exposure associated with severe hyperplasia H&E, X400. **Fig. 3:** Liver after 4 days exposure showed severe congestion H&E, X400. **Fig. 4:** Liver after 6 days exposure showed area of haemolysis in between the hepatic parenchyma H&E, X400. **Fig. 5&6:** Kidney after 6 days exposure showed hyperplasia in the wall of renal blood vessels, necrosis in the haemopoietic interstitial tissues, peritubular and periglomerular aedema and degenerative changes in the endothelial lining the glomerular tuft H&E, X400. **Fig. 7:** Spleen after 6 days exposure showed severe congestion, hemorrhages, hyperplasia in the wall of splenic blood vessels, hyperactivation of melanomacrophage centers and focal area of depletion of lymphocytic tissues H&E, X400. **Fig. 8&9:** Brain after 6 days exposure showed severe edema in the cerebellum and neuronal degeneration H&E, X400.
Plate C: *Oreochromis niloticus* in the 3rd gp. Fig. 1: Gills after 4 days of exposure showed more congestion, H&E, X400. Fig. 2: Gills after 8 days exposure showed complete fusion of the secondary lamellae, complete obliteration of the inter-lamellar space, severe degenerative and necrotic changes in the respiratory epithelium H&E, X400. Fig. 3: Liver after 4 days of exposure showed swollen, degenerated and necrotic hepatocytes with losing adenoid structure H&E, X400. Fig. 4: Liver after 6 days of exposure showed infiltration of mononuclear inflammatory cells in between the hepatic parenchyma and vacuolar degeneration and necrosis in acinar cells of pancreas H&E, X400. Fig. 5: Kidney after 4 days exposure showed aggregation of melanomacrophage cells in between the interstitial haemopiotic tissues specially at the necrotic areas with severe degenerative and necrotic changes in the tubular epithelium and haemopiotic tissues H&E, X400. Fig. 6: Kidney after 6 days of exposure showed with more pronounced peri-tubular and peri-glomerular edema H&E, X400. Fig. 7: Brain after 4 days exposure of showed neuronal degeneration H&E, X400). Fig. 8: Brain after 6 days exposure of showed oedema and focal gliosis H&E, X400).