Abbassa Int. J. Aqua., V0l.6 No.(2) 2013. *319-347*

NEGATIVE IMPACTS OF UN-IONIZED AMMONIA (NH_3) ON HEALTH STATUS OF CULTURED *OREOCHROMIS NILOTICUS*

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Received 8/ 9/ 2013 *Accepted 15/ 10/ 2013*

Abstract

In this study, the evaluation of the negative impacts of sub-lethal concentration of un-ionized ammonia (NH_3) on the health status of *Oreochromis niloticus* (*O. niloticus*) had been conducted. Ninety *O. niloticus* fish were exposed to three levels of NH_3 (0.05, 0.1 and 0.5 $mg.l^{-1}$) for 14 days then NH_3 were adjusted to the lowest level 0.05 $mg.l^{-1}$ for another 14 days. *O. niloticus* was challenged against *A. hydrophila* at the end of the experimental period (28 days) to record mortality rates.

Results recorded that resistance of *O. niloticus* was declined and high mortality rates against challenge *A. hydrophila* especially in group exposed to high concentration of NH_3 (0.5 $mg.l^{-1}$). Decrease of RBCs, HB, PCV, MCV, MCH, MCHC, WBCs with increase in glucose and cortisol were observed along with increasing NH_3 level. The phagocytic index and phagocytic assay showed lower values with 0.5 $mg.l^{-1}$ group, while liver enzyme, urea and creatinine showed significant increase. The previous parameters came back to normal values after stopping exposure to high concentration of NH_3. The histopathological examination revealed degeneration and necrosis in gills, liver and muscles. It could be concluded that clinical findings and histopathological lesion were varied according to change in the NH_3 concentrations. Also, it was found that the lesions were aggravated with the increasing of NH_3 concentrations. Immune suppression caused by stress of NH_3 lasted for a period of time after lowering the NH_3 concentration during which *O. niloticus* were susceptible to bacterial diseases.

Key word: un-ionized ammonia, *Oreochromis niloticus*, survival rate, cortisol, liver enzyme and *A. hydrophila*.

INTRODUCTION

Fish primarily produce ammonia as a result of hepatic deamination of dietary amino acids, enzyme activity of gastrointestinal flora, nerve and muscle tissue metabolic activity (Fromm and Gillette 1968; Redner and Stickney, 1979). Usually the toxic levels of NH_3 reports between 0.6 and 2 mg l^{-1}after short time of exposure, while the maximum tolerable concentration seems to be 0.1 mg l^{-1}. Under conditions of stress, the body of the fish emits immediate responses recognized as primary and secondary responses; the primary response is release of stress hormones (cortisol) (Randall and Perry, 1992). Secondary responses occur as a consequence of the released stress hormones (Barton and Iwama, 1991), causing an increase of plasma glucose (Begg and Pankhurst, 2004). The precise mechanism of ammonia poisoning in fish is unknown, but high aqueous ammonia increases blood and tissue ammonia levels, causing elevated blood pH, osmoregulatory disturbance, increased tissue oxygen consumption, and decreased blood oxygen transport (Schwedler *et al.,* 1985). If fish are unable to excrete the metabolic waste product lead to rise in blood-ammonia levels causing damage to internal organs, (Thurston *et al.,* 1978 and Daud *et al.,* 1988). At higher levels (>0.1 mg.l^{-1} NH_3) even relatively short exposures lead to skin, eye, and gills damage, Also it causes reduction in growth rate. The histopathological changes include gill hyperplasia, hemorrhage, and telangiectasia, as well as degenerative changes in the kidneys and liver (Thurston *et al.,* 1978 and Daud *et al.,* 1988). Exposure to sub-lethal NH_3 concentrations can also increase susceptibility to bacterial, fungal, and parasitic diseases (Amin *et al.,* 1988). Ammonia exposure cause increased susceptibility to stress responses and immune mechanism impairment (Tomasso, 1994). EL-Shebly and Gad (2011) observed that

ammonia concentrations of above 0.2 mg/L in fish ponds have a tendency to harm the fishes.

So this study aimed to investigate the impacts of NH_3 exposure on survival, haemogram, immunological status of *O. niloticus*.

MATERIAL AND METHODS

1-Experimental design: A total number of ninety apparently healthy *O. niloticus* were collected from private fish farm at El-Hamol - Kafr El-Sheikh Governorate- weighing 50±3.5 gram**.** The fish were acclimated in fiberglass tanks at Kafr El-Sheikh provicinal lab, animal health research institute for 15 days to laboratory conditions. Fish were randomly distributed in glass aquarium (50 x 40 x 40 cm) containing about 60 liters of dechlorinated water and the water temperature was adjusted at 25±2.5 ^{o}C, as well as continuous oxygen supply by air pump. The fish were fed pelleted ration with daily percentage 3% of body weight six day per week. The fish were divided into 3 treatment control 1st, 2nd and 3rd (each treatment had 3 replicates exposed to different levels of NH_3 control (didn't exceed 0.05), 0.1 and 0.5 mg.l^{-1} respectively.

Experimental period divided into 2 phases (exposure and post exposure):-

1st phase (Exp): lasted for 14 days in which fish exposed to different levels of NH_3.

2nd phase (P. Exp): lasted for 14 days after exposure period.

The required level of ammonia was obtained by the addition of ammonium chloride NH_4CL 4.7 and 23.5 g to obtain 0.1 and 0.5 mg.l^{-1} NH_3 respectively at the beginning of the test (Xu *et al.,* 2005). Ammonia level was monitored using Hach kits to maintain constant level and the percentage of un-ionized ammonia added in water was calculated using the equation:-

NH_3 = (total ammonia ×percentage of ammonia in the pH, temperature, ammonia relationship tables) / 100 (Emerson *et al.,* 1975).

2- Clinical and Post mortem examination of *O. niloticus*: The collected fish were clinically examined according to Amlacher (1970). They were examined for any abnormalities including exophthalmia, skin, erosion, ulcers, hemorrhages and detachment of scales. The collected fish were opened according to method described by Amlachar (1970) internal organs were exposed by making three cuts. First from infront of Anus through abdominal cavity toward the head. Second perpendicular to the first behind the bronchial cavity. Third cut ran from anus to head parallel to the lateral line then the abdominal wall was removed and internal organs were exposed.

The survival rate percent was calculated as:

Survival rate % = (Number of live fish in specific period / Total population during that period) x 100

3-Haemogram analysis of *O. niloticus*: Red blood cell (RBCs) and White blood cell (WBCs) counts were counted by haemocytometer according to Stoskopf (1993). Blood hemoglobin (Hb) was assessed by cyanometahemoglobin method (Drubkin, 1964). In addition, M.C.V. Mean Corpuscular Volume, M.C.H. Mean Corpuscular hemoglobin and M.C.H.C. Mean Corpuscular hemoglobin concentration were calculated according to the formula mentioned by (Dacie and lewis, 1975).

M.C.V. = (PCV / RBCs) x 10 as (fl).
M.C.H. = (HB content gm/100ml/ RBCs) x 10 as (pg).
M.C.H.C. = (HB content gm/100ml / PCV) x100 as (g/dl).

4- Biochemical analysis of *O. niloticus*: Glucose was determined calorimetrically as mentioned by Trinder (1969) Cortisol was estimated using radio immunoassay technique according to the method of Pickering

and Potinger (1983) and Wedemyer (1970).The activity of the liver enzymes, Aspartate Amino Transaminase (AST) and Alanine Amino Transaminase (ALT) were determined according to (Reitman and Frankel, 1957). The activity of the serum creatinine were determined according to Henry (1974) and urea were determined according to Patton and Crouch (1977).

5- Macrophage phagocyte indices: Leukocytes isolation was performed according to the method described by Faulmann *et al.* (1983) and phagocytic activities were determined according to Kawahara *et al.* (1991). Blood was collected from the caudal vessels by syringe moisten with heparin (100 IU/ml).

Candida albicans (C. albicans) was prepared as 24 hours old culture, the number of *C. albicans* cells was counted for obtaining the required concentration $1x10^6$ yeast cells/ml. Separated peripheral blood leucocytes were adjusted to a concentration of 2.5×10^6 viable cells/ml, then to each 1 ml volume of blood leucocytes adjusted *C. albicans* suspension was added, then incubated in an incubator (CO_2 5-10 %) at 37 ^{0}C for one hour. Cover slips were stained with Giemsa stain. The phagocytic Assay was calculated according to the following equations:

Phagocytic activity = No. of Ingesting phagocytes / total No. of phagocytes.

Phagocytic index = No. of ingested *C. albicans* cells / No. of Ingesting phagocytes.

6-Challenge test: After stopping the exposure to different levels of NH_3 a total number of 30 fish (10 fish from each treatment) were injected I/P with the locally isolated and identified pathogenic *A. hydrophila* which kindly obtained from fish diseases department, animal health research institute Doki (0.3 ml of 10^8cells/ml) according to Schaperclaus *et al.*

(1992), the injected fishes were kept under observation for 14 day to record the mortality rate.

Mortality rate MR% = **(**No. of death in specific period / Total population during that period) x 100

7-Histopathological examination: Tissue specimens were taken from sacrified fish (gills, liver, spleen and kidneys) after 14 days of exposure to 0.05 , 0.1, 0.5 mg/ L ammonia , fixed in 10% buffered formalin , then processed to obtain 4 µ paraffin sections. Sections were stained with hematoxylin and eosin for microscopic examination according to (Bancroft *et al.*, 1999).

8-Statistical analysis: Duncan's Multiple Range Duncan (1955) was used to determine differences among means at significance level of 0.05. All statistics were run on the computer using the SPSS program (SPSS, 2004).

RESULTS

1- Clinical examination of *O. niloticus*:

Clinical findings of *O. niloticus* exposed to 0.5 mg.l^{-1} of NH_3 presented in (figures1&2) showed loss of appetite (presence of uneaten pellets), tail rot, blackening of skin and congested gills which indicated that *O. niloticus* were subjected to stress conditions. *O. niloticus* exposed to 0.05, 0.1 mg.l^{-1} of NH_3 showed normal clinical condition comparing with group exposed to 0.5 mg. l^{-1} of NH_3.

Data presented in Table (1) showed that survival rate of *O. niloticus* was lowered after 14 days of exposure to 0.5 mg. l^{-1} of NH_3 than those had exposed to lower concentrations of NH_3 0.05 and 0.1 mg.l^{-1}. Even after decreasing the concentrations level of all groups to 0.05 mg.l^{-1} of NH_3 still *O. niloticus* exposed to 0.5 mg.l^{-1} of NH_3 had the lowest survival rate.

Table (1): Survival rate of experimental *O. niloticus* exposed to different levels of NH_3 for 14 days (1st phase).

Item	Exp			P. Exp		
	No*	Survival	%	No	Survival	%
NH3 0.05 mg.l^{-1}	30	30	100	24	22	91.67
NH3 0.1 mg.l^{-1}	30	22	73.33	19	17	89.5
NH3 0.5 mg.l^{-1}	30	18	60	15	12	80

*No= total number of ***O. niloticus*** in group.

Table (2): Haemogram of *O. niloticus* exposed to different NH_3 concentrations (mean ± SE).

Item	0.05 mgl^{-1}		0.1 mgl^{-1}		0. 5 mgl^{-1}	
	Exp	P. Exp	Exp	P. Exp	Exp	P. Exp
RBC (X10^6/mm^3)	2.68^{a} ±0.04	2.55^{ab} ±0.03	2.17^{c} ±0.07	2.56^{ab} ±0.06	1.87^{d} ±0.06	2.44^{b} ±0.9
WBC (X10^3/mm^3)	70.9^{a} ±0.9	64.9^{b} ±1.2	60.6^{c} ±0.9	61.27^{c} ±0.6	52.9^{d} ±1.2	58.4^{c} ±1.3
HB (g/dl)	9.53^{a} ±0.16	9.35^{a} ±0.18	7.4^{b} ±0. 3	9.2^{a} ±0.18	5.9^{c} ±0.3	9^{a} ±0.11
PCV %	30.7^{a} ±0.3	28.8^{b} ±0.18	25.8^{c} ±0.26	26.4^{c} ±0.35	19.27^{d} ±0.6	26.23^{c} ±1.6
MCV(fl)	114.5^{ab} ±0.6	113.2^{ab} ±1.1	118.9^{a} ±4.6	103.4^{c} ±2.26	103.2^{c} ±0.96	107.7^{bc} ±0.09
MCH (pg)	35.6^{ab} ±0.5	36.7^{ab} ±1.1	36.1^{ab} ±2.65	36^{ab} ±2.5	32^{b} ±1.4	36.9^{a} ±0.8
MCHC (g/dl)	31^{bc} ±0.5	32.3^{ab} ±0.8	28.7^{c} ±1.46	34.8^{a} ±1.15	30.9^{bc} ±0.7	34.3^{a} ±0.8

Group with different letter within the same raw are significantly different at P< ٠.٠٥. Hb = Hemoglobin, PCV= Packed Cell Volum, MCV= Mean Corpuscular Volum, MCH= Mean Corpuscular hemoglobin and MCHC = Mean Corpuscular hemoglobin concentration.

2-Blood examination of *O. niloticus* subjected to different NH_3 concentrations:

Data represented in Table (2) showed that *O. niloticus* exposed to 0.5 mg.l^{-1} had suffered from haemolytic anaemia ,the haemogram analyses revealed significant decrease of RBCs, HB, PCV, MCV, MCH,

MCHC, WBCs compared with other groups. Fish exposed to 0.5 mg.l^{-1} of NH_3 had failed to restore normal values after 14 days of post exposure.

3-Biochemical analysis and phagocytic index & assay:

Results presented in Table (3) showed significant effect of NH_3 on stress responses. Cortisol and glucose levels had increased by exposure to different NH_3 concentrations, the significant highest level recorded by *O. niloticus* exposed to 0.5 mg.l^{-1}. There is no significant difference between 0.05 and 0.1 mg.l^{-1}groups after stopping the exposure.

Table (3): Serum glucose, cortisol, phagocytic index & assay, liver enzyme creatinine and urea of *O. niloticus* exposed to different NH_3 concentrations (mean ± SE).

Item	0.05 mg.l^{-1}		0.1 mg.l^{-1}		0. 5 mg.l^{-1}	
	Exp	P. Exp	Exp	P. Exp	Exp	P. Exp
Glucose (mg/dl)	72.3^{c} ±1.45	74.3^{bc} ±1.2	76.7^{b} ±1.4	78^{b} ±1.53	89^{a} ±0.6	77.7^{b} ±1.2
Cortisol (µg/dl)	0.8^{b} ±0.01	0.82^{c} ±0.006	0.99^{bc} ±0.04	0.88^{bc} ±0.06	1.71^{a} ±0.1	0.9^{b} ±0.12
PI	2.33^{a} ±0.67	2.33^{a} ±0.33	1.67^{a} ±0.33	2.67^{a} ±0.33	1.33^{a} ±0.33	1.67^{a} ±0.33
PA	39.3^{b} ±0.46	38.2^{b} ±0.37	33.7^{c} ±0.39	43^{a} ±0.25	24.5^{d} ±0.38	33.3^{c} ±0.12
ALT (U/l)	6.3^{c} ±0.33	8.67^{b} ±0.67	8.7^{b} ±0.88	8.7^{b} ±0.33	11.7^{a} ±0.33	8.7^{b} ±0.67
AST (U/l)	24.7^{d} ±1.76	32.3^{c} ±0.33	38^{b} ±0.6	35.3^{bc} ±0.33	42.3^{a} ±1.3	35^{bc} ±1.15
Creatinine (mg/dl)	0.75^{c} ±0.02	0.76^{c} ±0.03	0.96^{b} ±0.07	0.81^{c} ±0.02	1.43^{a} ±0.12	0.86^{b} ±0.03
Urea (mg/dl)	4.0^{c} ±0.33	4.3^{c} ±0.33	6.3^{b} ±0.33	4.3^{c} ±0.33	7.5^{a} ±0.29	5.7^{b} ±0.33

Group with different letter within the same raw are significantly different at P< ٠.٠٥. PI=Phagocytic index, PA= phagocytic assay, Alt= Alaninamino Transaminrase and Ast= Aspartate Transaminase enzyme. Significant at ($p \leq 0.05$).

Raising NH_3 concentration had an adverse effect on phagocytic index and assay the lowest recorded value in *O. niloticus* exposed to 0.5 mg.l^{-1} NH_3.

liver enzymes showed that *O. niloticus* subjected to 0.5 mg.l^{-1} had the highest ALT and AST level(11.7 U/l and 42.3 U/l) respectively, these level decreased significantly after stopping exposure to high level of NH_3 (8.7 U/l and 35.4 U/l) respectively. Creatinine and urea values indicated that high level of NH3 had an adverse effect on kidneys function and gills respectively. *O. niloticus* subjected to 0.5 mg.l^{-1} had the highest value recoded of creatinine and urea 1.43 mg/dl and 7.5 mg/dl respectively.

4- Challenge test of *O. niloticus* subjected to different NH_3 concentrations:

Findings presented in Table (4) concerning to mortality rate of *O. niloticus* after 14 days of stop the exposure to 0.05, 0.1 and 0.5 mg.l^{-1} NH_3 and injected I/P with *A. hydrophilla*. The last two groups had the highest mortality rate compared with *O. niloticus* exposed to lower concentration 0.05 mg.l^{-1} indicating adverse effect of NH_3 on the ability of *O. niloticus* to resist bacterial diseases even after lowering the level of NH_3 to 0.05 mg.l^{-1}.

Table (4): Challenge against *A. hydrophilla* of *O. niloticus* after 14 days of post exposure to different NH_3 concentrations (2^{nd} phase).

Item	Total No.	Dead No.	Sur %	MR %
1^{st}	10	7	30	70
2^{nd}	10	8	20	80
3^{rd}	10	10	0	100

1^{st}, 2^{nd} and 3^{rd} = groups of ***O niloticus*** previously exposed to 0.05, 0.1 and 0.5 mg.l^{-1} Total no. = Total number of fish, Dead no. =Dead number, Sur %= Survival rate.

5- Histopathological examination of *O.niloticus* subjected to different NH_3 concentrations:

In toxicity of *O.niloticus* with ammonia the most pathological changes appeared in gills, liver, kidney, spleen and muscles.

Gills of *O. niloticus* exposed to 0.5 mg.l^{-1} of NH_3 showed congestion in blood vessels, infiltration with inflammatory cells, degeneration and necrosis in epithelium lining lamella with telengectasis, some lamella was eroted (Fig. 4), other lamella showed hyperplasia (bladder like shaped) (Fig.5), some gills showed increase in chloride cells (Fig. 6), other lamella revealed fusion in tips associated with increase in number of chloride cells (Fig.7). Liver showed the main hepatic lessions of *O. niloticus* exposed to 0.5 mg.l^{-1} of NH_3 congestion in hepatic blood vessels and sinusoid, haemorrhag, focal infiltration with inflammatory cells (Fig.8), increase melonomacrophage center, severe vacuolar degenerative changes in hepatocytes were observed, other hepatocyte showed necrosis (Fig.9). Kidneys of group (3) given ammonia with high dose (0.5 mg.l^{-1}) revealed congestion of renal blood vessels, haemorrhage in between renal tubules (Fig.10) , some glomeruli showed edema other revealed shrinkage glomerular tuffet, some renal tubules showed degeneration and necrosis of its epithelial lining , other tubules revealed complete sloughing of its epithelium lining. (Fig.11). The histopathological finding of muscules showed splitting of muscle fiber and zenker necrosis (Fig.12 &13). Spleen of fish given high dose of unionized ammonia revealed congestion in splenic blood vessels and increase in number of melanomacrophage center (Fig.14).

Fig. (1)

Fig. (2)

Fig. (3)

Fig. (1): *O. niloticus* exposed to0.5 mg.l^{-1} NH_3 showed tail rot.

Fig. (2): *O. niloticus* exposed to0.5 mg.l^{-1} NH_3 had haemorahagic spots on liver,spleenomegaly and distended gallbladder.

Fig. (3): *O. niloticus* exposed to 0.5 mg.l^{-1} NH_3 showed swelled gallbladder and friable liver.

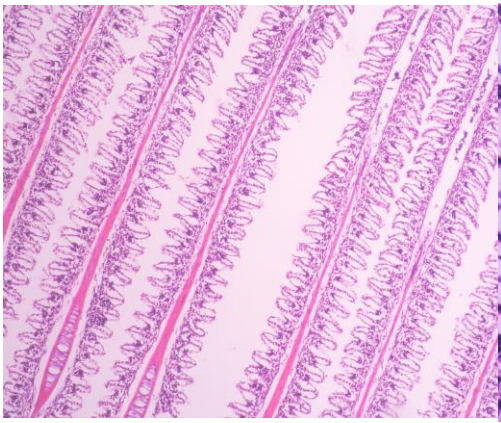
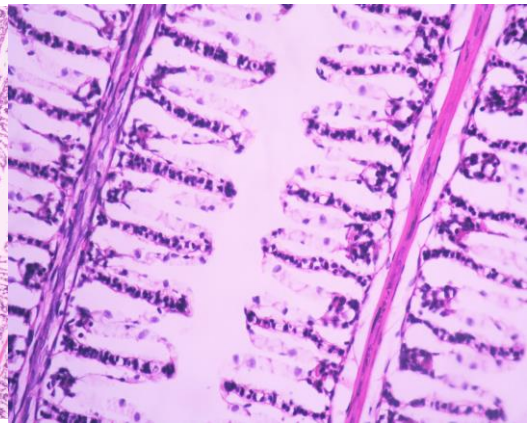


Fig. (4)



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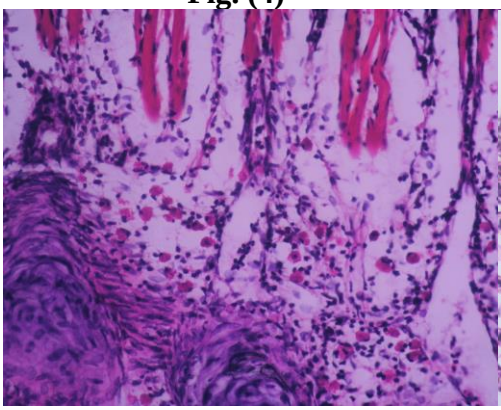

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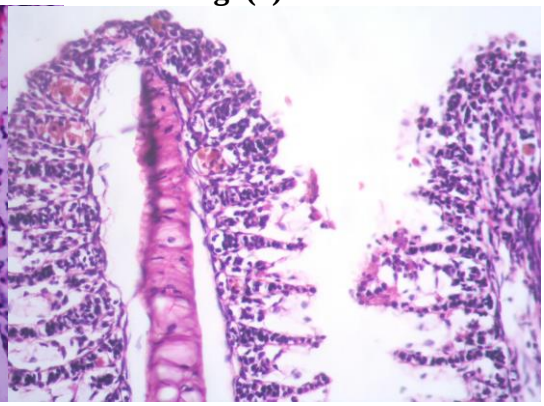


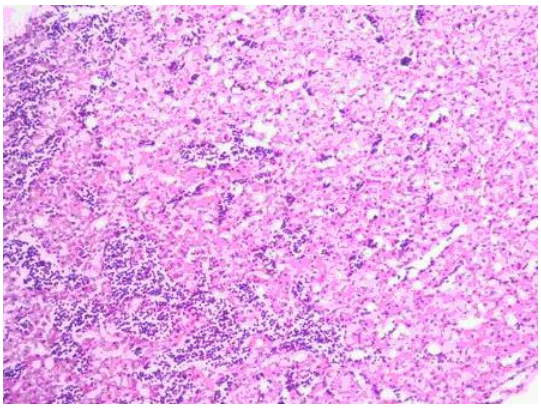
Fig. (7)

Fig. (4): Gills of *O. niloticus* exposed to 0.5 $mg.l^{-1}$ NH_3 showing degeneration and necrosis in epithelial lining secondary lamellae. H&E (X 100)

Fig. (5): Gills of *O. niloticus* exposed to 0.5 $mg.l^{-1}$ NH_3 showing hyperplastia (baldder like strcture) with epithelium cells lifting. H&E (X 400)

Fig. (6): Gills of *O. niloticus* exposed to 0.5 $mg.l^{-1}$ NH_3 showing increase in chloride cells. H&E (X 400)

Fig. (7): Gills of *O. niloticus* exposed to 0.5 $mg.l^{-1}$ NH_3 showing fusion in tips of secondary lamellae and increase in chloride cells in tips of of secondry lamellae. H&E (X 200).



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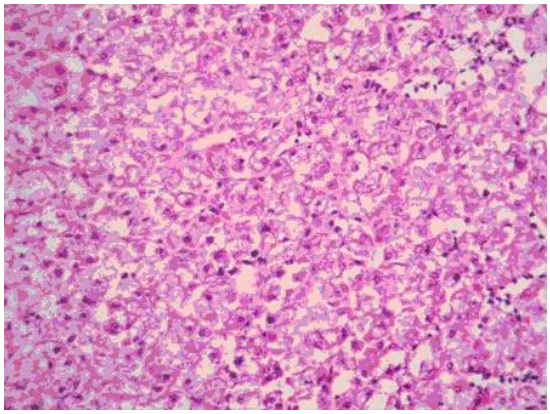

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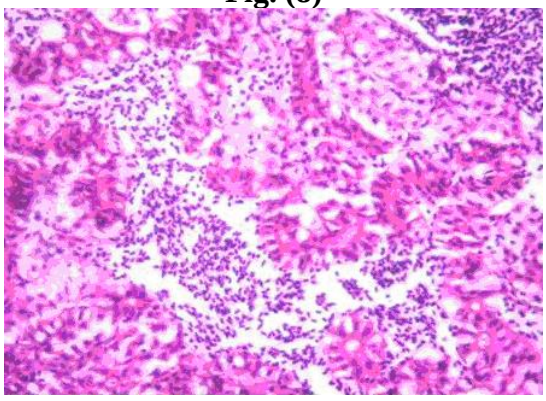

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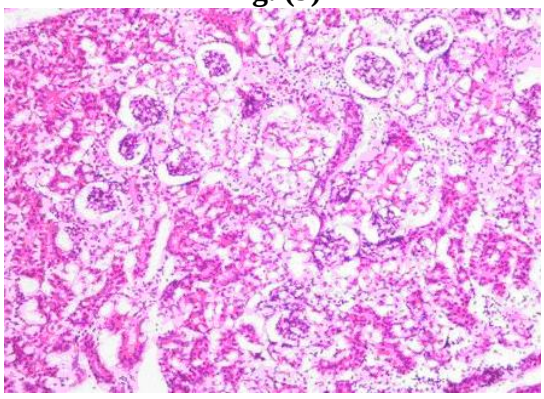


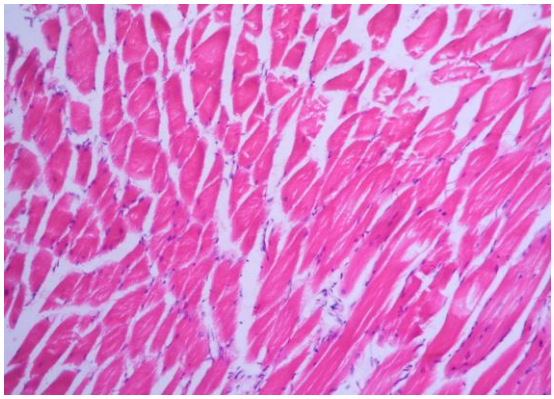
Fig. (11)

Fig. (8): Liver of *O. niloticus* exposed to 0.5 $mg.l^{-1}$ NH_3 showing infiltration with inflammatory cells in between hepatocyte . H&E (X 100)

Fig. (9): Liver of *O. niloticus* exposed to 0.5 $mg.l^{-1}$ NH_3 showing severe vacuolar degeneration of hepatocyte. H&E (X 200)

Fig. (10): Kidneys of *O. niloticus* exposure to 0.5 $mg.l^{-1}$ NH_3 showing hemorrhage in between renal tubules, degeneration and necrosis in renal tubule. H&E (X 200)

Fig. (11): Kidneys of *O. niloticus* exposed to 0.5 $mg.l^{-1}$ NH_3 showing edema in glomeruli, degeneration and necrosis in renal tubule and necrosis of melanomacrophage center. H&E (X 100)



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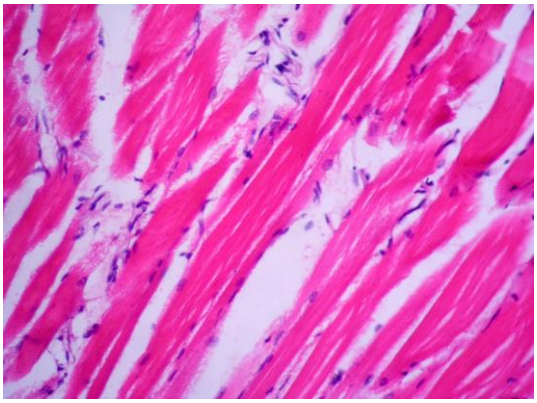

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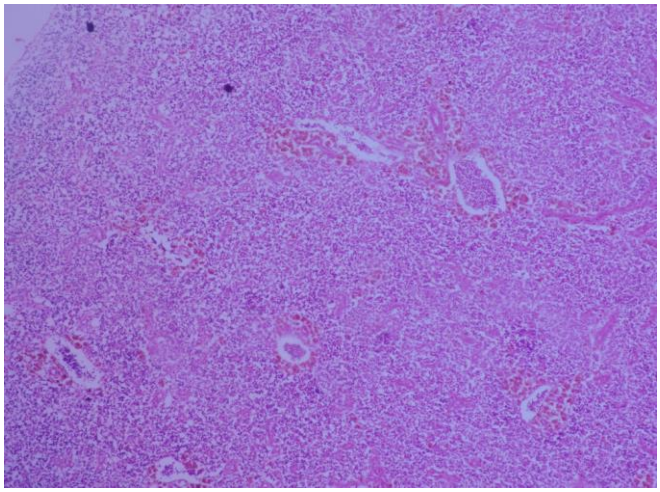

Fig. (14)

Fig. (12): Muscules of *O. niloticus* exposed to 0.5 mg.l^{-1} NH_3 showing splitting of muscle fiber. H&E (X 200)

Fig. (13): Muscules of *O. niloticus* exposed to 0.5 mg.l^{-1} NH_3 showing zenker necrosis. H&E (X 100)

Fig. (14): spleen of *O. niloticus* exposed to 0.5 mg.l^{-1} NH_3 showing congestion in blood vessels and increase number of melanomacrophage center. H&E (X 100)

DISCUSSION

Unionized form of ammonia is the most toxic form to aquatic organism, it can diffuse through cell membranes and it is highly soluble in liquids. It can cause impairment of cerebral energy metabolism, damage to gill, liver, kidney and spleen in fish, crustaceans and mollusks (smart, 1978). It is nitrogenous waste product of fish, also it is the main nitrogenous waste material excreted by gills (De Croux *et al.*, 2004). While Uzukwu (2013) mentioned that ammonia is a product of fish metabolism and is secreted across fish gills and also generated as a result of decomposition of fish faeces and uneaten feeds and in the absence of sufficient oxygen become toxic to fish.

Clinical findings of *O. niloticus* exposed to 0.5 $mg.l^{-1}$ of NH_3 showed loss of appetite (presence of uneaten pellets), tail rot and blackening of skin while post mortem examination showed congested gills, haemorrhages in liver, distended gall bladder and spleenomegally.

These results had nearly the same those obtained by Evans *et al.* (2006) who mentioned that with increased concentrations and duration of NH_3 exposure, fish became more lethargic and anorexic. Also, EL-Shebly and Gad (2011) observed changes in color of fish skin and eye subjected to NH_3 toxicity 0.4 and 0.6 $mg.l^{-1}$ and also Ortega *et al.* (2005) agreed with our findings as they mentioned that ammonia toxicity had impacts on fish behavioral changes, hyper excitability and appetite suppression. Also Miyazaki *et al.* (1984) agreed with our results as they stated that clinical and postmortem finding, showed that different sized fish were suffered from tail and fin rot, dark body coloration which may be related to the poor water quality and pseudomonas florescence infection (fin rot disease).

Mortalities could be explained by findings of Stein-Behrens and Sapolsky (1992) who stated that tissue damage has been observed in salmons, where high levels of cortisol cause death in Pacific salmon

Oncorhynchus spp by tissue degeneration and damage of homeostatic mechanisms. Results obtained by Evans *et al.* (2006) agreed with our findings as they mentioned that Nile tilapia exposed to sub-lethal NH_3 0.32 $mg.l^{-1}$ experienced sustained mortalities, resulting in cumulative mortalities of 86% for exposed fish and cumulative mortalities of 62% for control fish over 21days mortality data were not significantly different between the groups. Similar results obtained by EL-Shebly and Gad (2011) who observed accumulative mortalities of *O. niloticus* 23.7% and 43.3% occurred within 0.4 $mg.l^{-1}$ NH_3) and 0.6 $mg.l^{-1}$ NH_3.

Hematological changes in fish may be used for assessing the effects of contaminants, because blood parameters respond to low doses of pollutants as fishes exposed to metals, pesticides and effluents exhibit hematological changes, not only after laboratory exposure, but also when the exposure occurs in the field (Seriani *et al.,* 2010).

Data presented showed significant decrease in blood parameters after 14 days post exposure to high NH_3 concentration. Similar results obtained by (Atle *et al.,* 2004 and El-sherif *et al.*, 2008) as they stated that the average PCV(%) in the experimental groups was decreased with the increase of NH_3 concentrations and it was evident that Nile tilapia were anemic. Also, Ahmed *et al*. (1992) found that Nile tilapia exposed to ammonia had decrease number of RBCs count and haemolytic anaemia, leading to a significant reduction in blood oxygen content, which enhances ammonia toxicity. On the other hand, Hrubinko *et al.* (1996) found that Hb level had increased when exposed to ammonia 0.1 $mg.l^{-1}$ these different findings may be due to short period of exposure. Decrease WBCs count indicated that immunity was suppressed similar results obtained by (Pickering, 1984) stated that a reduced number of leukocytes -kind of response- were observed in described as a result of environmental stress. Also Seriani *et al.* (2011) mentioned that on the

long term, the persistence of a stress condition lead to the suppression of the leukopoietic centers, replacing the initial leukocytosis by leucopenia

Concerning glucose and cortisol levels, they showed significant increase then return to normal after stopping the exposure to ammonia. It has been suggested that after stress, the cortisol levels of fishes return to basal levels to avoid tissue damage (Bonga, 1997). These results agreed with findings of Davis and McEntire (2006) who mentioned that in experiments of acute stress, the cortisol response is rapid but regularly becomes weak or disappears some hours after the exposure to stress. While, it has been established that exposure of fish to sub-lethal levels NH_3 increases the subsequent resistance of the fish to lethal concentrations of NH_3, although the effect has only been observed to last for 2-3 days and is disappeared after 3 days (Lloyd and Orr, 1969).

Cortisol could attribute to mobilize and elevate glucose production in fish through glucogenesis and glycogenolysis pathways (Iwama *et al.,* 1999) to cope with the energy demand produced by the stressor. Exposure to sub-lethal NH_3 levels also increases tolerance to ammonia toxicity (Thurston *et al.,* 1981). Evans *et al.* (2006) who mentioned that increased concentrations and duration of NH_3 exposure, fish blood glucose levels increased significantly.

Porchas *et al.* (2009) reported that cortisol and glucose cannot be eliminated from the stress indicators list, but due to their high variability they must be complemented with other measurements such as blood-cell counts (preferably in chronic experiments), in order to have a more complete profile about the stress status of any fish. NH_3 levels greater than 1- 2 $mg.l^{-1}$ are usually lethal within 1-4 days (Meade, 1985) below this level, fish might not die, but they will be stressed (Noga, 2010).

Raising NH_3 concentration had an adverse effect on phagocytic index and assay the lowest recorded value in *O. niloticus* exposed to NH_3 0.5 $mg.l^{-1}$. Even the group exposed to the lowest concentration NH_3 0.05

mg.l^{-1} had decreased in phagocytic activities with increasing the period of exposure. These results could explain that fish had become anorexic, lowering the WBCs count and fish were under stress condition. Chronic ammonia poisoning lowers disease resistance (Walters and Plumb, 1980).

In this study the effect of NH_3 on liver enzymes showed that *O. niloticus* subjected to 0.5 mg.l^{-1} of NH_3 had the highest ALT and AST level and had decreased significantly after stopping exposure to high level of NH_3. Inhibition and induction of biomarkers –liver enzymes- is a good approach to measure potential impacts of pollutants on environmental organisms (El-Shehawi *et al.,* 2007). These results coordinate with findings of Abbas (2006) stated that liver enzyme of *Cyprinus carpio* fingerlings; ALT and AST were decreased significantly after 6 hours of exposure to 0.93 mg.l^{-1} NH_3-N at pH7.5 then increased till the end of the experiment 7 days. These increases in liver enzymes are an indication of tissue necrosis (Niels *et al.,* 1998).

Creatinine values indicated that high level of NH_3 had an adverse effect on kidneys function as shown with *O. niloticus* subjected to 0.5 mg.l^{-1} had the highest value recoded of creatinine 1.43 mg/dl. Disfunction of kidneys and leakage of these enzymes from injured tissue into blood stream (Salah El-Deen, 1999). Also Abass (2006) stated that the physiological and biochemical changes expressed of fish exposed to ammonia toxicity could be due to generalized organ system failure perior to death.

O. niloticus subjected to 0.5 mg.l^{-1} had the highest serum urea level 7.5 mg/dl indicating damages in gills The gills is the main organ of excretion of urea rather the kidney (Stoskoph, 1993) so the elevation of urea could indicate dysfunction of the gills.

Concerning to mortality rate of *O. niloticus* which previously had exposed to 0.05, 0.1 and 0.5 mg.l^{-1} of NH_3 and injected I/P with *A. hydrophilla*. The last two groups had the highest mortality rate compared

with *O. niloticus* exposed to lower concentration 0.05 $mg.l^{-1}$ indicating adverse effect of NH_3 on the ability of *O. niloticus* to resist bacterial diseases.

The same results obtained by Walters and Plumb (1980) who stated that chronic ammonia poisoning lower disease resistance. Barton (2002) reported that systemic changes in which animals may become incapable of adapting to stressors, lead to adverse effects on the animal's overall health , including their performance, disease resistance, and behavior. Also, Seriani *et al.* (2011) showed that blood immunological responses tend to be fast and may weaken the defense system, causing the animals to be more susceptible to opportunistic diseases. Also they added that the use of immunological biomarkers, together with toxicity tests, can be useful to evaluate the health of fish populations exposed to contaminated waters.

In the present study the histopathological examination of gills showed degeneration and necrosis in epithelial lining lamellae, fusion between some secondry lamellae, hyperplasia in lamellae with lifting its epithelial lining and telengecyasis, all groups of our studies showed nearly the same lesion recorded in three groups of *O.niloticus* given dose of ammonia (0.05, 0.1 and 0.5 $mg.l^{-1}$ NH_3) but differ in severity according to the dose of NH_3 given to fishes.

Our result incoordinated with those obtained by El-Shably *et al.* (2011) who recorded that slight pathological alteration in gills of *O. niloticus* exposure to (0.1 $mg.l^{-1}$ NH_3) while other two group of fish exposure to (0.2 and 0.4 $mg.l^{-1}$ NH_3) showed severe hyperplasia of gills (bladder like) with lifting its epithelium lining, fusion between lamellae, congestion in blood vessels. Also El - Sherif *et al.* (2008) reported that the pathological changes in gills differ in its severity according to the dose of NH_3 given to fish and hyperplasia in secondry lamellae of gills, degeneration and necrosis in its lining epithelial. Koca *et al.* (2005)

reported that cellular proliferation developed with secondary lamellae fusion, ballooning degenerations or club deformation of secondary lamellae of Lepomis gibbosus exposed to chemical ammonia. Krik and Lewis (1993) reported that the gills of rainbow trout exposed to (0.1 $mg.l^{-1}$) ammonia for 2 hours revealed deformity in lamellae. Smith and Piper (1975) reported that the gills lesions may cause reduced oxygen diffusion across membrane and predispose fishes to bacterial infection. Concerning to histopathological examination of liver of *O. niloticus* exposed to (0.05, 0.1 and 0.5 $mg.l^{-1}$ NH_3) showed congestion of blood vessels and sinousoid, focal infiltration with inflammatory cells, increase in melanomacrohage center , vacuolar degeneration and necrosis of hepatocyte the severity of pathological alteration differ according to the dose given , the same result recorded by (El-Sherif *et al.,* 2008; Saber *et al.,* 2004 and Thurstone *et al.,* 1984), the damage of hepatocyte confirmed by increase level of liver enzyme in serum .

In the present study the histopathological examination of kidney revealed congestion in blood vessels, hemorrhage in between renal tubule, edema in some glomeruli and shrinkage in other and degeneration and necrosis in renal tubules. nearly the same result reported by Thurstone *et al*. (1984) , but our result disagree with El-Sherif *et al.* (2008) who recorded hyaline degeneration in renal tubules and thrombus formation in renal blood vessels. The pathological changes in kidney tissue associated with increase in urea and creatinine level in serum of *O.niloticus* exposed to different concentration of unionized ammonia. Microscopical examination of muscule of fish exposure to (0.05, 0.1 and 0.5 $mg.l^{-1}$ NH_3) showed splitting of muscle fiber and zenker necrosis in muscule. Koca *et al.* (2005) recorded that the chemicals ammonia cause focal necrosis, cellular dissolution, and a decline or loss of striation in muscle fibers were seen. Taylor (2000) has shown that elevated ammonia levels in body of fish result muscle depolarization which will turn reduce the swimming performance. Our result reported increase in number of

melanomacrophage center in spleen, Ferguson (1989) recorded that increase in numbers of melanomacrophage center in spleen of fish exposed to polluted water.

CONCLUSION

It could be concluded that *O. niloticus* exposed to sub-lethal concentration of NH_3 had suffered from haemolytic anaemia and raised stress responses. Post exposure of *O. niloticus* to NH_3 stress fish did not restore normal physiological and immune status immediately. So aquaculturist must be aware that fish still under condition suitable for opportunistic pathogens.

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دراسة على التسمم بالامونيا الغير متأينه فى البلطى النيلى

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الملخص العربى

تم اجراء هذه التجربة لدراسة تأثير الامونيا الغير متأينه السامه (NH_3) بتركيزات تحت سامه علي صحة اسماك البلطى النيلى. قسمت ٩٠ سمكة بلطى نيلى الى ثلاث مجموعات كل مجموعة بها ٣٠ سمكة في ثلاث مكررات تم تعريضها الي امونيا غير متأينه (٠.٠٥ ، ٠.١ ،٠.٥ مجم /لتر) لمدة ١٤ يوم ، ثم تم خفض التركيز الي ٠.٠٥ مجم /لتر لمدة ١٤ يوم أخرى، تم اجراء عدوي صناعية ببكتريا الايروموناس هيدروفيلا في نهاية مدة التجربة علي ١٠عدد سمكة من كل مجموعة ثم تركت لمدة ١٤ يوم لتسجيل معدل النفوق. اوضحت الدراسة فقدان للشهية و قلة فى العد الكلى لكرات الدم الحمراء و البيضاء وقلة فى مستوى الهيموجلوبين ومناعة الاسماك كما لوحظ زيادة فى مستوى الكورتيزول و الجلوكوز وانزيمات الكبد والكلى. كما زاد معدل النفوق بعد الاصابة بالهيدروفيلا خاصة فى المجموعة المعرضة لتركيز ٠.٥ مجم /لتر. أوضحت نتائج الفحص الهستوباثولوجى الى ان التغيرات الباثولوجية كانت شديدة فى المجموعة المعرضة لتركيز ٠.٥ مجم /لتر فقد لوحظ من خلال الفحص زيادة فى اعداد خلايا الكلوريد بالخياشيم ، و احتقان فى الاوعية الدموية للكبد و الكلى .وجود تحلل بخلايا الكبد والكلى و الخياشيم والعضلات ، كما لوحظ زيادة فى خلايا الميلانومكروفاج فى الطحال. أثبتت الدراسة انه كلما زاد تركيز الامونيا انخفض مستوي المناعة و اصبحت الاسماك اكثرعرضة للاصابة بالامراض. كما وجد أن أستعادة الاسماك لحالتها المناعية لايتم مباشرة بعد خفض مستوي الامونيا بالماء وتكون الاسماك معرضة للاصابات البكتيرية.