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Effect of Ammonium Chloride on Antioxidants Activity of Nile tilapia *(Oreochromis niloticus)*

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Abstract: The purpose of this study was to find a sensitive biomarker to evaluate the effects of ammonium chloride (NH_4Cl) on Nile tilapia (*Oreochromis niloticus*). Tilapia exposed to different concentrations of $NH_4Cl$for 2 weeks. The resultsof Catalase (CAT, E.C.1.1.1.1.6), glutathione peroxidase (GPer, E.C.1.1.1.1.9), superoxide dismutase (SOD, E.C.1.1.5.1.1) activity and malonyldialdehyde (MDA) concentration in plasma, liver and muscle tissues were increased significantly ($P \leq 0.05$) compared to untreated tilapia. But the total protein, lipids, carbohydrates in liver and muscle tissues were decreased significantly ($P \leq 0.05$) compared to untreated tilapia. Form the obtained results, blood and tissue catalase, glutathione peroxidase and superoxide dismutase activities of Nile tilapia were more sensitive to the chemical environmental stress than the classical hematological indicators.

Key words: Nile tilapia (*Oreochromis niloticus*) · Ammonium chloride · Enzymes · Catalase · Glutathione peroxidase · Superoxide dismutase · Malonyldialdehyde

INTRODUCTION

Ammonia in water occurs in two forms, which together are called total ammonia nitrogen (TAN). Chemically, these two forms are represented as NH_4^+ and NH_3. NH_4^+ is called ionized ammonia and NH_3 is called un-ionized ammonia (UIA). NH_3, un-ionized ammonia, is the more toxic form to fish. Both water temperature and pH affect which form of ammonia is predominant at any given time in an aquatic system [1]. Ammonia and urea are the two main nitrogenous end products of protein catabolism excreted by teleost fish [2], with ammonia usually representing 75 to 90 % of nitrogenous excretion [3]. Ammonia is mainly excreted as the un-ionized form NH_3. In seawater, NH_3 ionizes to form NH_4^+ and proportion of the two forms depends upon pH, temperature and, to a lesser extent, salinity [4, 5]. The NH_3 molecule is non-polar and readily soluble in lipids. It is 300 to 400 times more toxic than NH_4^+ [6,7] Under intensive rearing conditions and particularly when effluent water is re-used, ambient ammonia concentrations may reach levels that limit fish survival and growth [7]. However, «safe levels» for growth, usually extrapolated from LC_{50} data, are reported to range from 0.05 to 0.2 mg l^{-1} UIA-N, depending on species, age and environment, oxygen concentration, pH [3, 8]. Lethal concentration for 50% of the population (96-h LC_{50}) have been reported to be 1.7 mg l^{-1} UIA-N (40.0 mg l^{-1} TAN) in sea bass juvenile [9]. Tilapia-ammonia tolerance has been documented to be as high as 2.4 mg l^{-1} (LC_{50}, 48h) in unacclimated fish and 3.4mg l^{-1} (LC_{50}, 48h) in fish acclimated to a sub-lethal level of ammonia [10]. The lethal concentrations (LC_{50}) of unionized ammonia (NH_3) for the larvae and fingerlings of Tilapia (*Oreochromis niloticus L.*) for 48 h LC_{50} values were determined as 1.009±0.02 mg/l for larvae and 7.40 ± 0.01 mg/l for the fingerlings. [11]. Ammonia causes stress and damages gills and other tissues. Fish exposed to low levels of ammonia over time are more susceptible to bacterial infections, have poor growth and is a killer when present in higher concentrations [12]. Environmental ammonia concentrations increase due to ammonia excretion or because of the breakdown of organic matter in the water and the Sublethal UIA are cause behavioral, physiological and histological changes infish and have

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several possible mechanisms of toxicity. These mechanisms include causing water and mineral imbalances, decreasing blood pH, altering cardiac function and affecting ATP levels [13]. Environmental contaminants may also enhance the generation of Reactive oxygen species (ROS). Thus, transition metals (Cu, Fe) catalyze the production of hydroxyl radicals through the Fenton reaction, while biphenyls, quinones and nitro aromatics produce superoxide by redox cycling [13]. ROS like the superoxide radicals (O^{--}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) are continuously being formed during normal aerobic metabolism. Toxic forms of activated oxygen react with cellular components resulting in oxidation of protein, DNA damage and as well as peroxidation of unsaturated lipids in cell membranes. Fish have extensive defense systems, consisting of antioxidant enzymes, such as Glutathione peroxidase, Superoxide dismutase and catalase, nutritional antioxidants, such as vitamin E and carotenoids [14]. ROS which induces oxidative stress and can result in damage to cell membranes, inactivation of enzymes and damage to genetic material and other vital cell components. Radical damage can be significant because it can proceed as a chain reaction [15]. The enzymes include radical scavenging enzymes such as (CAT) and (SOD) acting on H_2O_2 and O_2^-, respectively and (GPer), which scavenges H_2O_2 and lipid hydroperoxides [14].

Consequently, mortality can occur due to severe destruction by massive radicals generated from acute stresses or long-term chronic stresses [15]. Ammonia, a chief constituent of fertilizers when present in high levels is quite toxic to most organisms and it must be either continuously eliminated or converted into less toxic compounds to prevent a build up to harmful concentrations within the body [16]. Studies on the protection of anti-oxidants against oxidative damage can be conducted by pretreating the animals with antioxidants then subjecting them to oxidative stress induced by oxidants or toxic substances [17]. The formation of ROS is following acute ammonia intoxication in the brain of mammalian models [18, 19]. Ammonia exposure induced oxidative stress in brain and gills of the high ammonia tolerant fish mudskipper, *Boleophthalmus boddarti* [20]. The enzymatic or non-enzymatic scavengers protect cells against oxidative damage [21]. Nile tilapia *(Oreochromis niloticus)* exposed to sublethal concentration of total ammonia the result showed that, lipid peroxidation, superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, glutathione reductase, were significantly increased in liver and white muscle of fish exposed to both low and high ammonia levels [22]. Ammonia LC_{50} was found to be 18.8 mg/L and $1/6^{th}$ of the LC_{50} Fishes were exposed 14 days to concentration, 3.24 ppm was selected as sub lethal concentration and fishes were exposed to 14 days, activity levels of Arginase, Xanthine oxidase, Superoxide dismutase, Catalase enzyme levels were significant increase in the brain tissue of the *Cyprinus carpio* [23]. Chronic effect of ammonia on *Cyprinus carpio* increased activity levels of, Glutathione peroxidase, Glutathione-s-Transferase, Glutamate dehydrogenase, Superoxide dismutase enzyme [24].In addition, GPer, CAT, SOD participates in cell membrane protection and cell detoxification from xenobiotic through reduce the oxidative properties [25]. The acute selenium exposure decrease hepatic GSH, LPO and GPer activities significantly varied with treatment [26]. The liver and kidney glutathione transferase, glutathione reductase and glutathione peroxidase were higher in *O. niloticus* captured from all the polluted areas compared to the control [27]. It is contended that the nucleophilic glutathione reacts with electrophilic carbon atoms [28]. Glutathione may also bind with non-carbon atoms such as cadmium, zinc, lead and mercury [29] and with ammonia [30]. The MDA is one of the end products of lipid peroxidation processes, its levels were significantly higher in tissues of the *C. trutta* from the contaminated station ($P \leq 0.05$). Also CAT activity was significantly increased in the gill tissue at the contaminated station ($p<0.05$). But GSH levels were lower in tissues of the *Capoetat rutta* collected at the contaminated station ($P \leq 0.05$) [31]. A battery of antioxidant enzymes increased simultaneously in contaminated fishes, including glutathione peroxidase, superoxide dismutase, catalase, glucose-6-P dehydrogenase and glutathione reductase [32]. Heavy metals which may cause oxidative stress in the *Clarias gariepinus* when collected from Ogun River [33].Lipid peroxidation expressed by MDA, (GPer), (CAT) and (SOD) could be used as biomarkers for heavy metals pollution in fish [34]. GPer provides cellular protect ion by assisting in the breakdown of peroxides and by reduction of disulfides [35]. By alternate concentrations of dissolved oxygen, he found that liver of pacu has the highest antioxidant glutathione peroxidase activity [36]. Reduced glutathione and glutathione-S-transferase in the liver of a freshwater climbing perch *Anabas testudineus* (Bloch) exposed to common industrial pollutants, decrease in reduced glutathione and an increase in glutathione-S-transferase activity [37]. CAT activity in serum, liver and muscle tissues of Nile tilapia significantly

increased after exposed to Cu and Zn [38]. When antioxidant defenses are impaired or overcome, oxidative stress may produce DNA damage, enzymatic inactivation and peroxidation of cell constituents, especially lipid peroxidation [39]. Toxicity biomarkers, such as malondialdehyde, have been also proposed to reflect the oxidative status of exposed species [40]. MDA is used as marker of oxidation of membrane phospholipids through lipid peroxidation. An increase in MDA levels in organisms can be related to degradation of an environmental site by decreasing the water quality [41]. Significant increases in lipid peroxidation product MDA, nitric oxide, cortisol and micronucleus count were recorded, in cultured *Oreochromis niloticus* during acute ammonia exposure [42].

Oxidative damages affect nucleic acids, proteins, lipids and carbohydrates [43]. A sub-lethal ambient NH_4Cl concentration on the total autolysis of protein in different tissues of the Indian air-breathing murrel, Chauna punctatos (Bloch), has been demonstrated ammonia induced increased breakdown of proteins [44]. Increasing UIA-N concentrations resulted in decreasing of hematocrit and hemoglobin parameters [45]. Arillo *et al*. [46] opined that accumulation of amino acid in trout liver was due to the ammonia-induced enhancement of proteolysis through increased lysosomal liability and enzymatic activity. Das *et al.* [47] reported that increased energy demand might increase protein consumption, a process where protein is converted into energy and therefore the protein serum will be reduced. Meanwhile, total protein, triglyceride, cholesterol, levels in the blood serum showed downward regulation under the same experimental conditions [48]. Common carp was exposed to ammonia there was no significant difference in cholesterol level between experimental groups and control fish [49]. Stressors have been correlated with reduced body lipid content in fish [50]. Triglyceride and cholesterol are energy based substances that are basically derived from lipid absorption [51]; their levels in blood serum have been associated with stress management [52, 53]. Vijayan *et al.* [54] reported a reduction in triglyceride level when brook charr (*Salvelinus fontinalis*) was exposed to a stressful situation that triggered higher energy demand. Da Rocha *et al.* [55] who also reported the animals could have utilized substantial amount of metabolizable energy in their response to the stressful condition. The decreased trends of triglyceride and cholesterol was observed in Senegalese sole; *Solea sene galensis* [56]. According to Casillas *et al.* [57] serum total protein, can give clue to liver damages in fish and increases in AST and ALT activities could have resulted from the long stay in chronic NH_3 and NO_2. The decrease in tissue lipid and protein might be partly due to their utilization in cell repair and tissue organization with the formation of lipoproteins, which are important cellular constituents of the cell membrane and cell organelles present in cytoplasm [58]. Biochemical alterations in protein, carbohydrate and lipids changes in gills, hepatopancreas and visceral mass of freshwater mussel, *L. marginalis* exposed to sub lethal concentrations of Ammonia [59]. Both fry and fingerling of *Cyprinus carpio* were exposed to ammonia, the total carbohydrate content decreased in fish throughout the time course of the study against the normal fish [60].The purpose of this study was to obtain reference values of some biochemical sensitive biomarker in tilapia used in measuring stress of ammonia.

MATERIALS AND METHODS

This study was carried out at the indoor wet lab of Aquaculture research center, department of reproductive physiology, Agriculture research center, Egypt, in order to evaluate the effect of different levels of NH_4Cl on some antioxidant enzymes of Nile tilapia (*O. niloticus*).

Experimental Fish: Nile tilapia (*O. niloticus*) fingerlings with mean average weight of 50.55±3.10 gbody weight were obtained from Fish Research Center. Fish were homogenous in size, body weights and apparently healthy. They were fed on the same diet used in this study for 2 week, prior to adapt them for the experimental conditions. Fifteenglass aquaria (40×70×60 cm) with capacity of 60 L. De-chlorinated water in aquaria was aerated by a constant supply of compressed air pump and was exchanged daily [61].

Experimental Design: This experiment was devoted to study the effect of NH_4Cl on some biochemical sensitive biomarker of *O. niloticus* as catalase, glutathione peroxidase and superoxide dismutase and malonyldialdehyde in blood and liver tissues. The fish were stocked at five different levels of NH_4Cl i.e. 0.0, 2.0, 4.0, 8.0 and 10.0 mg/L, with three replicates for each treatment. NH_4Cl solution was added gradually into the water to increase the NH_4Cl concentration [62] and HCl was used to obtain pH 6 and 7 [61]. Tilapia was stocked for 7 days at a rate of 10 fish per aquarium. Commercial diet containing 26.58% crude protein was used through the experiment period with daily ration rate 3% of fish weight in the morning (10.00 AM).

Sample Collection: At the end of experiment, blood and liver and muscle tissue samples were collected, blood collected from the caudal vein of fish after exposure to NH_4Cl for 2 weeks as well as from the control group fish. The blood left to clot then centrifuged at 3000 rpm for 15 minutes. The separated serum samples were stored at-20°C for biochemical analysis. Fish were sacrificing freshly after collection of blood samples immediately, the liver and tissues were tacked and homogenized, then divided into two parts, the first part was, centrifuged (at 3500rpm for 30-minutes) and the supernatant stored at-20°C until used for biochemical analysis and the second part was used without centrifugation for determination of MDA.

Blood Measurements: GPer (E.C.1.1.1.1.9), activity was determined by measurement of the reduced glutathione substrate (GSH) remaining after the action of the enzyme using the combined methods of Chiu *et al*. [63] with Ellmans reagent in presence of cumene hydroperoxide as a secondary substrate. The activity of SOD (E.C.1.1.5.1.1) was determined spectrophotometrically at 480 nm by the epinephrine method by Misra and Fridovich [64] and it was expressed in units of enzyme activity/ gm of liver tissues (U/g wet wt)/ ml of blood serum (U/ml blood serum). The activity of CAT (E.C.1.1.1.1.6) was determined spectrophotometrically at 240 nm by Beers and Sizer [65] and it was expressed in bergmyer units/ Bu/g wet wt. or mg protein. MDA was determined according to the method described by Nair and Turner [66], MDA formed in the course of lipid peroxidation was determined with 2-thiobarbituric acid [TBA]. 0.5 ml homogenate without filtration was taken and 4.5 ml of TBA reagent was added. The mixture was heated using water bath for 20 min, centrifuged at 2500 rpm and read at 532 nm. Total protein content in blood serum was determined by the Biuret method described by Wootton [67]. Total carbohydrates content was estimated by the method of Carrol *et al.,* [68]. Anthrone method and Sulfo-phospho-vanillin method of Barnes and Blackstocle [69]for determination of total lipids.

Statistical Analysis: The data obtained in this study wereanalyzed by one-way ANOVA Procedure of Statistical Analysis System [70]. Means were compared by Duncan's new multiple range test [71].

RESULTS

Antioxidant enzymes activity in blood serum of fish treated with different concentration of NH_4Cl, such as Glutathione peroxidase, (28.28±0.32 U/ml), Superoxide dismutase, (214.23±1.81 U/ml) and Catalase enzyme (62.38±1.72 mmol/ml) showed significant ($P \leq 0.05$) increase in their activity compared to control groups as in (Table 1), also the end product of fatty acid destruction the Malonyldialdehyde (MDA) concentration (47.85±1.16 nmol MDA/ml) showed significant ($P \leq 0.05$) increase in blood serum of Nile tilapia treated with NH_4Cl compared to fish without treated NH_4Cl (Table 1).

Antioxidant enzymes activity in liver tissues of Nile tilapia (*O. niloticus*) treated with NH_4Cl, such as Glutathione peroxidase, (57.60 ±0.13 U/g), Superoxide dismutase, (309.23± 2.50U/g) and Catalase enzyme (90.72±1.54 mmol/g) showed significant ($P \leq 0.05$) increase in their activity compared to control groups as in (Table 2), also the MDA concentration (58.61±1.13 nmol MDA/g) showed significant ($P \leq 0.05$) increase in blood serum of Nile tilapia (*O. niloticus*) treated with NH_4Cl compared to fish without treated NH_4Cl (37.60±1.33nmol MDA/g) (Table 2).

Antioxidant enzymes activity in muscle tissue of fish treated with NH_4Cl, such as Glutathione peroxidase, (20.56±0.27 U/g), Superoxide dismutase, (296.82±1.95 U/g) and Catalase enzyme (76.38± 1.72 mmol/g) showed significant ($P \leq 0.05$) increase in their activity compared to control groups as in (Table 3), also the end product of fatty acid destruction the MDA concentration (37.85±1.13 nmol MDA/g) showed significant ($P \leq 0.05$) increase in blood serum of Nile tilapia (*O. niloticus*) treated with NH_4Cl compared to fish without treated NH_4Cl, (Table 3).

The blood serum glucose level of Nile tilapia (*O.niloticus*) after treated with different concentration of NH_4Cl showed significant ($P \leq 0.05$) increases (88.19±1.3mg/dl), compared to the control fish group (39.16±1.2mg/dl), but the results of total protein concentrations in serum of Nile tilapia groups treated with different NH_4Cl showed significant decreases (2.83±0.27g/dl), compared to the control fish group, (Table 4). The serum total lipids, (418.36±0.41mg/dl), serum total cholesterol (238.96±0.91 mg/dl) and serum triglycerides (91.23±0.58 mg/dl), were significantly ($P \leq 0.05$) increases observed in Nile tilapia (*O.niloticus*) treated with different concentration of NH_4Cl compared to the control fish group, (Table 4).

Total carbohydrate (36.44±1.22 mg/g), total protein (12.83±0.27 mg/g), total lipids (35.36±0.41 mg/g) level in muscles of Nile tilapia (*O.niloticus*) after treated with different concentration of NH_4Cl showed significant ($P \leq 0.05$) decrease, compared to the control fish group, (Table5).

Table 1: Effect of different concentrations of NH_4Cl on Antioxidants GPer, SOD, CAT, MDA in blood serum of Nile tilapia (*O. niloticus*)(Mean+SE)

	Experimental groups				
Measurements parameters	Normal	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Gper activity U/ml blood serum.	19.61 ±0.13^{e}	21.75 ±0.34^{c}	20.94 ±0.19^{d}	25.66± 0.21^{b}	28.28± 0.32^{a}
SOD activity U/ml blood serum.	152.52± 1.25^{f}	168.41 ± 1.24^{d}	206.25 ±1.95^{c}	212.22± 1.77^{b}	214.23± 1.8 ^{a}
CAT activity mmol/ml blood serum.	45.68 ± 0.91^{d}	52.36 ± 0.57^{c}	55.42 ± 1.41^{b}	60.54± 1.32^{a}	62.38±1.72^{a}
MDA nmol /ml blood serum.	35.73 ±1.19^{c}	37.34 ±1.35^{c}	40.18 ±1.23^{b}	45.75 ±1.42^{a}	47.85 ±1.16^{a}

Mean with the same letter for each parameter at the same row is not significantly different.
Highly significant difference between groups ($p<0.01$).

Table 2: Effect of different concentrations of NH_4Cl on Antioxidants GPer, SOD, CAT, MDA in liver tissues of Nile tilapia (*O. niloticus*) (Mean+SE)

	Experimental groups				
Measurements parameters	Normal	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Glutathione peroxidase activity U/g wet.Wt. In liver.	29.65 ±0.39^{d}	35.51 ± 0.23^{c}	41.53 ±0.44^{b}	56.72 ±0.18^{a}	57.60 ±0.13^{a}
Super Oxide Dismutase activity U/g wet. Wet.Wt. In liver.	225.54 ±1.92^{e}	265.54 ±1.68^{d}	278.63 ±2.60^{c}	309.23±2.50^{a}	303.19±2.14^{b}
Catalase activity mmol/g wet.Wt. In liver.	71.23± 1.31^{d}	76.53± 1.23^{c}	83.45± 0.92 ^{b}	89.62± 1.34 ^{a}	90.72±1.54 ^{a}
Malonyldialdehydenmol MDA /g Wet.Wt. In liver	37.60 ±1.33^{d}	43.55 ±1.23^{c}	47.33 ±1.14^{b}	58.61 ±1.13 ^{a}	56.71 ±1.22^{a}

Mean with the same letter for each parameter at the same row is not significantly different.
Highly significant difference between groups ($p<0.01$).

Table 3: Effect of different concentrations of NH_4Cl on Antioxidants GPer, SOD, CAT, MDA in muscles of Nile tilapia (*O. niloticus*)(Mean+SE)

	Experimental groups				
Measurements parameters	Normal	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Gper activity U/g muscle tissues.	16.81 ±0.14^{b}	20.71 ±0.34^{a}	17.54 ±0.18^{b}	20.56± 0.27^{a}	19.24± 0.36^{a}
SOD activity U/g muscle tissues.	192.34± 1.28^{d}	250.61 ± 1.44^{c}	296.82 ±1.95^{a}	270.63± 1.70^{b}	271.28± 1.51^{b}
CAT activity mmol/g muscle tissues.	55.68±0.91^{d}	62.36±0.57^{c}	69.42±1.41^{b}	74.54±1.32^{a}	76.38±1.72^{a}
MDA nmol /g muscle tissues.	26.53±1.14^{c}	29.34±1.33^{c}	33.18±1.23^{b}	36.75±1.32 ^{a}	37.85±1.13^{a}

Mean with the same letter for each parameter at the same row is not significantly different.
Highly significant difference between groups ($p<0.01$).

Table 4: Effect of different concentrations of NH_4Cl on blood glucose, total protein, triglycerides, cholesterol, total lipids in blood serum of Nile tilapia (*O. niloticus*) (Mean+SE)

	Experimental groups				
Measurements parameters	Normal	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Blood glucose mg/dl serum	39.16±1.2^{e}	44.31± 1.3^{d}	50.18±1.3^{c}	65.16 ±1.2^{b}	88.19±1.3^{a}
Total protein gm/dl serum	5.64±0.27^{a}	4.32±0.43^{b}	3.13 ±0.38^{b}	2.73±0.27^{c}	2.83±0.27^{c}
Triglycerides mg/dl serum	76.35±1.22^{d}	80.94±1.28^{c}	86.43±0.94^{b}	89.44±0.73^{a}	91.23±0.58 ^{a}
Cholesterol mg/dl serum	159.35±0.88^{e}	187.53±1.80^{d}	198.45±0.79^{c}	229.96±0.91^{b}	238.96±0.91^{a}
Total lipids mg/dl serum	379.75±0.28^{e}	387.83±1.64^{d}	389.55±0.67^{c}	399.54±0.53^{b}	418.36±0.41^{a}

Mean with the same letter for each parameter at the same row is not significantly different.
Highly significant difference between groups ($p<0.01$).

Table 5: Effect of different concentration of NH_4Cl on total carbohydrates, total protein, total lipids in liver tissues of Nile tilapia (*O. niloticus*) (Mean+SE)

	Experimental groups				
Measurements parameters	Normal	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Total carbohydrates mg/g liver	80.38±1.28^{e}	60.47 ±1.51^{d}	55.65 ±1.24^{c}	42.66±1.36^{b}	36.44±1.22^{a}
Total protein gm/g liver	26.72 ±0.49 ^{a}	19.32±1.43 ^{b}	18.13 ±0.38 ^{b}	15.73 ±0.27 ^{c}	12.83 ±1.27^{c}
Total lipids mg/g liver	110.75±0.28 ^{e}	87.83±1.64^{d}	56.55 ±0.67^{c}	49.54 ±0.53^{b}	35.36 ±0.41^{a}

Mean with the same letter for each parameter at the same row is not significantly different.
Highly significant difference between groups ($p<0.01$).

Table 6: Effect of different concentration of NH_4Cl on total carbohydrates, total protein, total lipids in muscle tissues of Nile tilapia (*O. niloticus*) (Mean+SE)

	Experimental groups				
Measurements parameters	Normal	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Total carbohydrates mg/g muscles	28.12±1.24^{e}	23.17 ±1.36^{d}	19.15 ±1.34^{c}	16.16±1.43^{b}	14.19±1.52^{a}
Total protein mg/g muscles	25.82 ±0.39^{a}	22.32±0.43^{b}	20.13 ±0.38^{b}	18.73 ±0.27^{c}	16.83 ±0.27^{c}
Total lipids mg/g muscles	10.75±0.28^{a}	7.23±1.64^{b}	5.15 ±0.67^{c}	4.54 ±0.53^{d}	4.36 ±0.41^{d}

Mean with the same letter for each parameter at the same row is not significantly different.
Highly significant difference between groups ($p<0.01$).

Total carbohydrate (14.19±1.52mg/g), total protein (16.83±0.27mg/g), total lipids (4.36±0.41mg/g) level in muscles of Nile tilapia (*O.niloticus*) after treated with different concentration of NH_4Cl showed significant ($P \leq 0.05$) decrease, compared to the control fish group, (Table 6).

DISCUSSION

Glutathione peroxidase activity in serum was (28.28±0.32 U/ml), liver and muscle tissue homogenates (57.60±0.13 U/g), (20.56±0.27 U/g) showed significant increase in fish groups treated with NH_4Cl compared to the control group,(Tables 1,2 and 3).These results were similar to Nile tilapia exposed to sublethal concentration of total ammonia the glutathione peroxidase was significantly increased in liver and white muscle of fish exposed to both low and high levels [23]. Chronic effect of ammonia on *Cyprinusc arpio* increased activity levels of, Glutathione peroxidase enzyme. In addition, GPer, participates in cell membrane protection and cell detoxification from xenobiotic through reduce the oxidative properties [25]. GPer activity in serum and tissues of Nile tilapia significantly increased after exposed to Cu and Zn [38]. GPer provides cellular protection by assisting in the breakdown of peroxides and by reduction of disulfides [35].

Superoxide dismutase activity in serum was (214.23±1.81 U/ml), liver and muscle tissue homogenates (309.23±2.50 U/g), (271.28±1.51U/g) showed significant increase in fish groups treated with NH_4Cl compared to the control group tables [1,2 and 3].These results were similar to the Nile tilapia *(Oreochromis niloticus)* exposed to sublethal concentration of total ammonia the result showed that superoxide dismutase was significantly increased in liver and white muscle of fish exposed to both low and high levels [23].The Superoxide dismutase enzyme levels were significant increase in the brain tissue of the *Cyprinus carpio* exposed to sub lethal concentration of [24]. Chronic effect of ammonia on *Cyprinus carpio* increased activity levels of Superoxide dismutase enzyme which participates in cell membrane protection and cell detoxification from xenobiotic through reduce the oxidative properties [25]. SOD activity observed in this study might be to detoxify the super oxide anion radicals' in order to arrest the radial damage to cellular organization SOD activity in serum, liver and muscle tissues of Nile tilapia significantly increased after exposed to Cu and Zn [38]. Similar response of SOD activity also reported by [72], in *Cyprinus carpio* treated with mixed polluted yellow river of China, thus fish seem to tolerate low levels of ammonia and were able to protect themselves by detoxifying it.

Catalase activity in serum was (62.38±1.72 mmol/ml), in liver and muscle tissue homogenates (90.72±1.54 mmol/g), (76.38±1.72 mmol/g) showed significant increase in fish groups treated with NH_4Cl compared to the control group tables [3, 4 and 5]. These results were similar to the Nile tilapia *(Oreochromis niloticus)* exposed to sublethal concentration of total ammonia, showed that catalase was significantly increased in liver and white muscle of fish exposed to both low andhigh levels compared to control [23]. Activity levels of Catalase enzyme levels were significant increase in the brain tissue of the *Cyprinus carpio* compared to control [24]. Chronic effect of ammonia on *Cyprinus carpio* increased activity levels of, antioxidant Catalase, participates in cell membrane protection and cell detoxification from xenobiotic through reduce the oxidative properties [25]. CAT activity in serum, liver and muscle tissues of Nile tilapia significantly increased after exposed to Cu and Zn [38].

MDA in serum was (47.85±1.16 nmolMDA/ml), in liver and muscle tissue homogenates (56.71 ±1.22 nmol MDA/g), (37.85±1.13nmol MDA/g),) showed significant increase in fish groups treated with NH_4Cl compared to the controlgroup tables [1, 2 and 3]. Toxicity biomarkers, such as malondialdehyde (MDA), have been also proposed to reflect the oxidative status of exposed species [41].These results were similar tothe result of Hegazi *et al*. [23], when Nile tilapia exposed to sublethal concentration of ammonia the MDA was significantly increased in liver and white muscle of fish in both low and

high levels.MDA in serum, liver and muscle tissues of Nile tilapia significantly increased after exposed to Cu and Zn [38]. The MDA levels were significantly higher in tissues of the *C. trutta* from the contaminated station ($P \leq 0.05$) compared to those collected from the uncontaminated station [31]. An increase in MDA levels in organisms can be related to degradation of an environmental site by decreasing the water quality [40]. A significant increase in MDA was recorded, in cultured *Oreochromis niloticus* during acute ammonia exposure [42].

Blood serum glucose level of Nile tilapia (*O.niloticus*) after treated with different concentration of NH_4Cl showed significant ($P \leq 0.05$) increases (88.19±1.3mg/dl), compared to the control fish group (39.16±1.2mg/dl) (Table 4), this condition was attributed to effects of ammonia on the antioxidant system in â cell of pancreas to prevent insulin creations and also increase breakdown of storage glycogen. These results agree with those of significant increases in cortisol of cultured *Oreochromis niloticus* during acute ammonia exposure [42]. High ammonia concentrations elevated levels of circulating corticosteroids [62] Serum glucose levels, an indicator of cortisol released in blood were increased as stocking density increased [48].

Total carbohydrate (14.19±1.52mg/g), level in muscles and liver (36.44±1.22mg/g), of Nile tilapia (*O.niloticus*) after treated with different concentration of NH_4Cl showed significant ($P \leq 0.05$) decrease, compared to the control fish group (28.12±1.24mg/g), (80.38±1.28mg/g), respectively (Tables 5 and 6). Oxidative damages affect on carbohydrates catabolism an increase breakdown of storage glycogen [43]. Both fry and fingerling of *Cyprinuscarpio* were exposed to ammonia solution for 14 days, the total carbohydrate content decreased in ammonia fry and fingerling of fish throughout the time course of the study against the normal fish [60].

Total protein concentrations in serum (2.83±0.27g/dl), liver (12.83±0.27mg/g) and muscle (16.83±0.27mg/g), tissues of Nile tilapia (*O.niloticus*) groups treated with different NH_4Cl showed significant decreases compared to the control fish group (5.64±0.27 g/dl), (26.72±0.49 mg/g), (25.82 ±0.39 mg/g) respectively (Table 5 and 6). Serum protein is a fairly labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors. High serum protein levels have been reported due to improve liver and other organs functions which synthesized plasma protein, but increase breakdown of protein catabolism for energy production and increase gluconeogenesis. Oxidative damages affect nucleic acids and proteins [43]. Decreased trend of protein at the higher concentration of ammonia confirmed the resultsobtained by SahaandDas [44], they said that sub-lethal ambient NH_4Cl concentration on the total autolysis of protein in different tissues of the Indian air-breathing murrel,Chaunapunctatos (Bloch), has been demonstrated ammonia induced increased breakdown of proteins. Increasing UIA-N concentrations resulted in decreasing of hematocrit and hemoglobin parameters [45]. Arillo *et al.* [46] opined that accumulation of amino acid in trout liver was due to the ammonia-induced enhancement of proteolysis through increased lysosomal liability and enzymatic activity. Das *et al.* [47] have reported that increased energy demand might increase protein consumption, a process where protein is converted into energy and therefore the protein serum will be reduced. When increased stocking density, total protein levels in the blood serum showed downward regulation [48].

Total lipids, (418.36±0.41mg/dl), total cholesterol (238.96±0.91 mg/dl) and triglycerides (91.23±0.58 mg/dl), in serum were significantly ($P \leq 0.01$) increases observed in Nile tilapia (*O.niloticus*) treated with different concentration of NH_4Cl compared to the control fish group, (Table 4). The increase of total lipid, cholesterol and triglycerides in blood serum of *O. niloticus*treated with different concentration of NH_4Cldue to increase breakdown of lipids catabolism for energy production and increase gluconeogenesis. This results are in agreement with the study of common carp was exposed to ammonia there was no significant difference in cholesterol level between experimental groups and control fish [49]. Stressors have been correlated with reduced body lipid content in fish [50].Triglyceride and cholesterol are energy based substances that are basically derived from lipid absorption in the intestines and liver fatty acid metabolism [51]; their levels in blood serum have been associated with stress management [52,53].Vijayan *et al.* [54] reported a reduction in triglyceride level when brook charr (*Salvelinusfontinalis*) was exposed to a stressful situation that triggered higher energy demand; and was further supported by Da Rocha *et. al.,* [55], who also reported significant change in the above parameter in matrinxã (*Bryconcephalus*) after handling and acute crowding stress.Total lipids level in liver (35.36 ±0.41mg/g) and muscles (4.36 ±0.41mg/g) of Nile tilapia (*O.niloticus*) after treated with different concentration of NH_4Cl showed significant ($P \leq 0.01$) decrease, compared to the control fish group, (Tables 5 and 6).The results confirmed to oxidative damages effect on lipids liability

[43].Stressors have been correlated with reduced body lipid content in fish [50].According to Ramesh *et al.* [59], lipid content shows that decreased in visceral and hepatopancreas of *L. marginalis*compared to control, during exposed to Ammonia. The decrease in tissue lipid and protein might be partly due to their utilization in cell repair and tissue organization with the formation of lipoproteins, which are important cellular constituents of the cell membrane and cell organelles [58].

CONCLUSION

Ammonia is one of the serious problems in freshwater fish, some Oxidative changes takes place under the effect of ammonia toxicity, GPer, SOD, CAT, activity and MDA concentration in serum and tissues are thought to be involved in the Antioxidant system and carbohydrate, protein and lipids are the main biochemical compounds is apparently disrupted by ammonia toxicity. The present study, it is concluded that ammonia is highly toxic has a profound influence on the antioxidants activity and on the metabolism of Nile tilapia.

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