Hepatoprotective and stress - reducing effects of dietary *Moringa oleifera* extract against *Aeromonas hydrophila* infections and transportation-induced stress in Nile tilapia, *Oreochromis niloticus* (Linnaeus 1757) fingerlings

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***Abstract*—** *The main aim of the present study was to evaluate the hepatoprotective and stress-reducing effects of Moringa oleifera extract against A. hydrophila infection and transportation- induced stress in Nile tilapia, Oreochromis niloticus fingerlings. Fish were fed diets representing different supplementation levels of Moringa oleifera leaf extract. The graded levels of M. oleifera leaf extract were 0.00g (control), 0.05g, 0.10g, 0.15g, 0.20g, 0.25g per 100g for each diet. After six weeks of the feeding trial, fish previously fed each experimental diet were exposed to pathogenic strain of Aeromonas hydrophila at a concentration of 9.3 × 10^5 CFU /mL. After bath exposure, fish from each dietary treatment was placed into the aquaria culture system. They were fed their respective diets at 5% body weight twice daily, and mortality was monitored for the remaining 4 weeks of the feeding trial. After the feeding trial, fish previously fed each experimental diet were kept in plastic tanks for a 2-hour journey. Blood and liver samples were collected for hepatocellular assessments (Aspartate transaminase (AST), Alanine transaminase (ALT), Lactate dehydrogenase (LDH) and Malate dehydrogenase (MDH) tests) and stress biomarkers (survival, cortisol and glucose). Results showed that the increases of the AST, ALT, LDH, MDH, cortisol and glucose induced by stressors were significantly reduced (P< 0.05) by supplementing the fish with M. oleifera leaf extract in the diets. Based on the result of this study, a dose of 0.10g/100g dietary Moringa leaf supplementation was sufficient as a hepatoprotective and stress reducing agent in Nile tilapia, O. niloticus.*

***Keywords*— Moringa oleifera , hepatoprotective, stress-reducing, Oreochromis niloticus, supplementation.**

I. INTRODUCTION

In aquatic environment, fish are unavoidably exposed to wide ranges of stimuli associated with environmental stress and pathological challenges (Xie et al, 2008). Stress responses provide the animal with an ability to cope in the short-term during exposure to the encounter and increase its chance of survival under adverse conditions (Rapatsa and Moyo, 2014). Physiological stress biomarkers like glucose, cortisol and lysozyme biomarkers are the primary and secondary indicators of stress and cytological damage when fish in aquaculture are exposed to certain stressful conditions (Carlos et al, 2009). Different taxa, species and stages of fish have different tolerances to stress (Adebayo, 2006). This implies that for a particular stressor, severity may vary depending on the species to which it was applied (Barton, 2002).The liver is the main organ responsible for metabolism of both endogenous and exogenous compounds and therefore it is one of the first target organs for the toxic action of stressors (Cao et al, 2016). Environmental variables, particularly nutrition, are ultimately important in affecting fish in time of stress (Barton and Iwama, 2005). Most compounds absorbed by the intestine pass through the liver, which enables it to regulate the level of many metabolites in the blood (Good, 2004). Liver injury is often instigated by the bioactivation of complex reactions involving chemically reactive metabolites, which have the ability to interact with cellular macromolecules such as proteins, lipids and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage and oxidative stress (Larrey, 2000).

Pathogenic organism can cause diseases in fish, for instance bacteria are unavoidable in fish because the fish body is made of pure protein with fatty materials which are good substrates for bacterial growth (Okaeme and Ibiwoye, 2001). Again, water in which fish develop is a favourable medium for bacterial growth (Subasinghe, 2005). In Nigeria, some of the common bacterial diseases of fish pond include *Aeromonas hydrophila* and bacterial septicemia (Okaeme, 2006). *Aeromonas hydrophila* in water-bodies and fish culture systems in Nigeria have been recognized as emerging disease problems in African catfish and tilapia production, and their effects include ulcerative dermatitis, fish egg blooming, spoiling and systemic mycoses (Awoniyi et al, 2007). Farmed fish frequently encounter and tolerate poor environmental conditions, which are well below the considered optimal (Adewolu and Adeoti, 2010). Fish undergoes physiological stress response consequent to handling and transportation procedures, such stress reduce the capacity of fish, hindering their ability to

perform essential functions (Bly and Clem, 1991; Subashinge, 2005; Welker et al, 2007).It is generally accepted that oxidative stress plays a key role in the pathogenesis of drug induced hepatotoxicity; therefore plant extracts with antioxidant properties have received a lot of attention as possible preventive and therapeutic agents against stressors and subdued immunity response which can adversely affect the liver and health of cultured fish (Ojiako, 2014).

Stressors in aquaculture are unavoidable and cause many harmful effects. Strategies to attenuate them should be considered. The use of plants extracts in aquaculture has increased rapidly for the prevention of diseases and also to avoid the indiscriminate use of antibiotics, which can lead to the development of resistant strains of pathogenic microbes (Chatterjee et al, 2006; Kaleeswaran et al, 2011). Phytogenic products and extracts are cheaper, non-toxic and biodegradable alternative to antibiotics. The anti-stress potential of plant extracts may be due to the presence of phenolic and polyphenolic compounds, and these bioactive compounds may render their effects through anti-oxidation of oxidative stress (Soosean et al, 2010). Moringa(drumstick, horse-radish) belongs to the moringaceae family, there are thirteen species of Moringa trees in the family moringaceae and *Moringa oleifera* is the most widely cultivated species (Ojiako, 2014). The *M. oleifera* tree is a single genus family of shrubs and trees cultivated across the whole of the tropical belt and used for a variety of purposes (Jahn, 1996; Becker, 2003). It can be recognized by the compound pinnate leaves, and the long narrow angular fruits containing large wind seed. Verdcourt (1993) stated that almost every part of the plant is of value for food and itis probably the most popular plant in ECHO's seedbank of underutilized tropical crops. Different parts of Moringa have shown great antioxidant activity (Anwar et al, 2007) as well as immunomodulatory function in animals (Ojiako, 2014). *Moringa oleifera* contains antioxidants which can inactivate damaging free radicals produced through normal cellular activity and from various stresses (Makanjuola et al, 2013; Rapatsa and Moyo, 2014). Traditionally, the leaves, fruits, flowers, and immature pods of this tree are edible(Ojiako, 2014). The leaves, in particular, have been found to contain phenolics and flavonoids which have various biological activities, including antioxidant, anticarcinogenic, immunomodulatory, antidiabetic and hepatoprotective functions and the regulation of thyroid status in human and animals (Hussain et al, 2014).

Tilapia is considered as an excellent species for aquaculture in tropical and subtropical regions, as a result of the high tolerance to handling, stress situations and critical conditions, tolerance to sub optimal water quality, tolerance to high stocking densities, and its fast growth (El-Sayed, 1998). More than 22 tilapia species are cultured worldwide (FAO, 2014). However, the most commercially cultured species are Nile tilapia *Oreochromis niloticus*, blue tilapia *Oreochromis aureus*, and *Tilapia rendalli*(Lim and Webster, 2006). Nile tilapia is also acceptable in term of tastes and preferences because of the white flesh, neutral taste and firm texture this is why it is often called the 'aquatic chicken' (Fitzsimmons, 2008). Tilapia is the most commonly farmed fish after carp, salmonids and catfishes (FAO, 2014). Furthermore, the polyculture of *O. niloticus* in freshwater is associated with rice fields, vegetables or livestock (Madalla, 2008).The aim of the present study was to evaluate the hepatoprective and stress-reducing effects of dietary *Moringa oleifera* extract against *A. hydrophila* infections and transportation-induced stress in Nile tilapia, *Oreochromis niloticus*.

II. MATERIALS AND METHODS

2.1 Extraction of Moringa oleifera

The leaves of *Moringa oleifera* were collected from a farm settlement at Ijare, Ondo State, Nigeria. It was identified and authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. Theleaves were destalked, washed and dried in the shade. *Moringa oleifera* leaves were ground with pestle and mortar, leaves were then extracted according to the modified method of Makanjuola et al (2013) as follows. Five hundred grams of the powdered leaf were soaked in 1.5 liter of warm water (60^{o}C). Each solution was allowed to stand for 24 hours, after which it was sieved with a muslin cloth and filtered using No 1 Whatman filter paper. The filtrate were collected in a beaker and concentrated with the aid of rotary evaporator (Resona, Germany).

2.2 Determination of main active compounds in *Moringa oleifera* leaf extract

In the present study, analytical High Performance Liquid Chromatography (HPLC) for flavonoids (Quercetin (Q) and Kaempferol (K)) was carried out on an Agilent XDB C18 column (250 mm L × 4.6 mm, 5 μm particle) and an Agilent 1100 instrument (Agilent, Palo Alto, CA, USA). Detection was made at the wavelengths of 320 nm and 250 nm respectively. HPLC conditions were as follows: The analysis was performed at a flow rate 1.5 ml per minute. The UV detector was set at *k* = 320 and 250 nm. Elution with 0.01 M acetate buffer adjusted to apparent pH 3.3 using glacial acetic acid (solvent A) and acetonitrile (solvent B) in a step gradient manner was carried out as follows: 60:40 (for 10 min), then 40:60 (for 5 min) and finally wash with 10:90 (for 10 min). All determination was performed at ambient temperature. The injection volume was 20

µl. Standard solutions of Q and K were prepared in methanol within a concentration range of 0.4–20 µg per ml for each of Q and K. Twenty µl injections were made for each flavonoid concentration. The peak height values were plotted against corresponding absorbence. The contents of the analytic were determined from the corresponding calibration curves. The contents of quercetin (Q) and kaempferol (K) were found to be 0.21 mg /g and 0.14 mg /g respectively.

2.3 Preparation of Experimental Diets

The feed ingredients were purchased at AdedomFeedmill, Ondo road, Akure, Ondo State, Nigeria. Six isonitrogenous and isocaloric diets were formulated to meet the requirements of 30% crude protein (Table 1) for *O. niloticus* fingerlings (National Research Council, 2010) using feed formulation software (WinFeed soft 2.0, USA). All dietary ingredients were weighed with a weighing top balance (Metler Toledo, PB8001 London). The ingredients were then ground to a small particle size (approximately 20 µg). Ingredients including *Moringa oleifera* extract, vitamin and mineral premix were thoroughly mixed in a Hobbart A-200T mixing machine (Hobbart Ltd London England) to obtain a homogenous mass. Alginate, *Laminaria digitata* (IGV GmbH, Germany®) was added as binder. The resultant mash was pressed without steam through a mincer using 2mm diameter die attached to the Hobbart pelleting machine. Diets were immediately air - dried, after drying the diets were broken up, sieved and stored in air-tight transparent plastic containers, labeled and stored until feeding. Standard and official methods (AOAC, 1995) were used to perform the proximate analyses of feed of fish in the study.

TABLE 1: COMPOSITION OF THE EXPERIMENTAL DIET (G/100G) CONTAINING DIETARY *MORINGA OLEIFERA* FOR NILE TILAPIA, *OREOCHROMIS NILOTICUS* FINGERLINGS

TILAPIA	MLST0	MLST5	MLST10	MLST15	MLST20	MLST25
Fish meal	11.50	11.50	11.50	11.50	11.50	11.50
Groundnut cake	19.50	19.50	19.50	19.50	19.50	19.50
Soybean meal	20.50	20.50	20.50	20.50	20.50	20.50
Yellow maize	24.50	24.50	24.50	24.50	24.50	24.50
Vegetable oil	7.00	7.00	7.00	7.00	7.00	7.00
Rice Bran	13.00	12.95	12.90	12.85	12.80	12.75
Alginate (Binder)	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin Mineral mix	2.00	2.00	2.00	2.00	2.00	2.00
Moringa extract	0.00	0.05	0.10	0.15	0.20	0.25

Composition of vitamin-mineral mix (Aquamix) (quantity/kg), Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6 mcg; Calcium; Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450. L- lysine, 10 g; Selenium, 50 ppm.

2.4 Experimental fish and feeding trial

O. niloticus fingerlings were obtained from the Hatchery unit of the Department of Fisheries and Aquaculture Hatchery, Federal University of Technology Akure, prior to the feeding trial. Fish were graded by size and groups of 15 fish of 6.00 g per replicate for *O. niloticus* were stocked into glass tanks of 60cm ×45cm×45cm dimension. A commercial diet, Nutreco ® (35% crude protein) was fed to all fish during a 2- week conditioning period. Each experimental diet was fed to six replicate groups of fish for 70days. All groups were fed their respective diets at the same fixed rate (initially 5% of body weight per day). This rate was adjusted each week. Fish were fed by 0900-1000 and 1700-1800h GMT, for 7 days each week. Growth was monitored weekly by batch weighing of fish from each tank. Dissolved oxygen was monitored using HANNA 98103SE (HANNA instruments, Rhode Island). Temperature and pH were monitored using YSI-IODO 700 Digital probe (IFI Olsztyn, Poland).

2.5 Aeromonas hydrophila challenge and transportation –induced stress

After six weeks of the feeding trial, fish previously fed each experimental diet were exposed to pathogenic strain of *Aeromonas hydrophila* (MPSTR 2143, mildly pathogenic strain, Animal care Laboratory, Ogere). This isolate was grown in brain-heart infusion broth (EM Science, Darmstadt, Germany) in a shaking bath at 27^{o}C overnight the Department of Microbiology, FUTA. The concentration of bacterial suspension was determined by the serial plate count method and diluted to 9.3×10^5 CFU (colony forming unit)/ml in fresh well water as described by Li (2005). Fish from each dietary treatment

was immersed in the bacterial suspension for 5 hours. After bath exposure, fish from each dietary treatment was placed into the aquaria culture system. Fish were fed their respective diets at 5% body weight twice daily, and mortality was monitored for the remaining 4 weeks of the feeding trial.At the end of the feeding trial, 15 fish previously fed each experimental diet from each treatment were kept in plastic tanks for a 2-hour journey. Blood samples were collected immediately after transportation for 2 hours from fish for further analyses.

2.6 Assessment of hepatocellular damage

Hepatocellular damage tests were performed at the Central Science Laboratory, ObafemiAwolowo University, Ile- Ife, Osun State. Hepatocellular stress activities were determined by Aspartate transaminase (AST), Alanine transaminase (ALT), Lactate dehydrogenase (LDH) and Malate dehydrogenase (MDH) tests according to the procedure of Hardy and Sullivan (2003). The livers of 3 fish from each treatment were removed by dissection and weighed. The tissue was homogenized with chilled 0.25 M sucrose solution in a glass tube using a mechanical tissue homogenizer. The tube was continuously kept in ice to avoid heating. The homogenate was then centrifuged (5000x g for 10 minutes at 40^0C) in a cooling centrifuge machine and stored at -20^{o}C till use. Aspartate transaminase (AST) and Alanine transaminase (ALT) were measured by the estimation of oxaloacetate and pyruvate released in a spectrophotometer at 540nm and the results were read on the calibrated graph respectively. Lactate dehydrogenase (LDH) and Malate dehydrogenase (MDH) activities were measured by the change in optical density (OD) at 340 nm for 5min. The supernatant was used directly as an LDH and MDH source in the kinetic study. LDH and MDH activities were determined following the oxidation of NADH at 340 nm in a circulating thermobath at 25^{o}C. The reaction mixture was contained in a total volume of 1 ml, 50 mM Imidazol, 1 mM KCN buffer pH 7.4 at 25^{o}C, 0.13 mM of NADH and different concentrations of pyruvate for LDH saturation plots. Substrate saturation plots for oxalacetate were determined for MDH by the oxidation of NADH at 340 nm.

2.7 Fish blood collection and analyses

At the end of the feeding trial, two fish specimen from each tank were removed for blood analyses. Blood were collected by puncture of the caudal blood vessels .This was done with the aid of 2ml disposable syringe. Serum glucose concentration was measured according to Hardy and Sullivan (2003) using Bio-La-Test oxochrome GLUCOSA (Glu 250E) based on the oxidation of glucose catalyzed by glucose oxidase to hydrogen peroxide and gluconate. Cortisol concentrations was determined in the plasma samples using enzyme linked immuno-sorbent assay ELISA (ELX-800) with a Coat Count Kit Diagnostic Products Corporation (ISELAB DRAKE LA) analysis as described by Hardy and Sullivan (2003). The standards used in the ELISA immunoassay were prepared from plasma stripped of endogenous steroids with activated charcoal. Determination of lysozyme activity was done by Turbidimetric method**.** Three fish from each treatment were carefully removed and anaesthetised with MS- 222 at 200mg / liter. Turbidimetric assay was carried out according to Lim et al (2010). Two milliliter of a *Micrococcus lysodeikticus* suspension, 0.20 mg per ml, 0.05 M sodium phosphate buffer pH = 6.2, was briefly mixed with 10 µl of lysozyme source. The decrease in absorbance (delta A) per min was recorded at 520 nm and at room temperature, 1 unit being defined as giving delta A per min = 0.001. A series of hen egg with lysozyme (HEWL, Sigma) was used as standards.

2.8 Statistical analysis

This experiment was designed with a completely randomised design (CRD) to test for significant differences in the mean of treatments. The data were expressed as mean ± standard deviation (SD).The differences between mean of treatments were considered significant at $P \leq 0.05$ by one way analysis of variance (ANOVA) using Statistica® software. Follow–up procedures were performed where significant differences occurred in the means using Tukey test.

III. RESULTS

3.1 Effects of *M. oleifera* leaf extract on hepatocellular damage indicators

Significantly higher alanine transferase, aspartate transferase, lactate dehydrogenase and malate dehydrogenase ($P< 0.05$) was recorded in fish fed the control diet compared with other dietary treatments. In the liver tissue the increases of the Aspartate transaminase (AST), Alanine transaminase (ALT), Lactate dehydrogenase (LDH) and Malate dehydrogenase (MDH) induced by *A. hydrophila* infections and transportation-induced stress were significantly inhibited ($P < 0.05$) by supplementing the fish with 0.10g, 0.15g, 0.20g, 0.25g per 100g *M. oleifera* leaf extract in the diets (Table 2).

TABLE 2
EFFECTS OF *M. OLEIFERA* LEAF EXTRACT ON HEPATOCELLULAR DAMAGE INDICATORS IN EXPERIMENTAL FISH

	MLST0	MLST5	MLST10	MLST15	MLST20	MLST25
AST (µM)	51.70 ± 2.81^{c}	45.33 ± 1.53^{bc}	30.22 ± 2.14^{a}	28.33 ± 1.53^{a}	31.22 ± 1.59^{a}	33.31 ± 1.52^{ab}
ALT (µM)	49.33 ± 2.08^{c}	22.63 ±3.78^{b}	16.77 ±1.53^{a}	15.69 ± 0.58^{a}	18.67 ± 1.15^{ab}	19.22 ± 2.03^{ab}
LDH (µM)	1.30 ± 0.04^{c}	1.08 ± 0.05^{b}	0.84 ± 0.02^{a}	0.94 ± 0.02^{a}	1.23 ± 0.05^{c}	1.30 ± 0.03^{c}
MDH (nM)	6.13 ± 0.25^{a}	3.09 ± 0.14^{c}	1.64 ± 0.13^{a}	1.71 ± 0.04^{a}	2.00 ± 0.35^{ab}	2.26 ± 0.12^{b}

^{a,b,c}values in each row with different superscripts are significantly different (P < 0.05) using ANOVA Post Hoc (Tukey test) (mean values ± SD, mean of fish from 3 replicate tanks). ALT, Alanine transferase; AST, Aspartate transferase; LDH, Lactate dehydrogenase and MDH, Malate dehydrogenase.

3.2 Effects of *Moringa oleifera* leaf extract on stress indicators and survival of experimental fish

Physiological stress biomarkers like glucose and cortisol significantly reduced with increasing Moringa supplementation (Table 3). Significantly higher survival was found in fish fed MLST10 and MLST15 diets as 95.70 and 95.70 %, respectively at the end of the feeding trial. Reduction in % survival was recorded starting from week 7 which also correspond to the beginning of the pathogenic stress challenge (Figure 1).

TABLE 3
PHYSIOLOGICAL STRESS BIOMARKERS OF *OREOCHROMIS NILOTICUS* FED THE EXPERIMENTAL DIETS

	MLST0	MLST5	MLST10	MLST15	MLST20	MLST25
Glucose (mg/dl)	112.67 ± 2.52^{e}	100.33 ± 1.53^{d}	73.67 ± 3.51^{c}	53.33 ± 1.53^{a}	54.09 ± 1.48^{a}	64.67 ± 2.08^{b}
Cortisol (ng/ml)	204.01 ± 3.58^{d}	156.30 ± 1.58^{c}	142.19 ± 1.98^{b}	141.12 ± 2.00^{ab}	133.17 ± 1.40^{a}	134.74±1.53^{a}

^{a,b,c,d,e}values in each row with different superscripts are significantly different (P < 0.05) using ANOVA Post Hoc (Tukey test) (mean values ± SD, mean of fish from 3 replicate tanks).

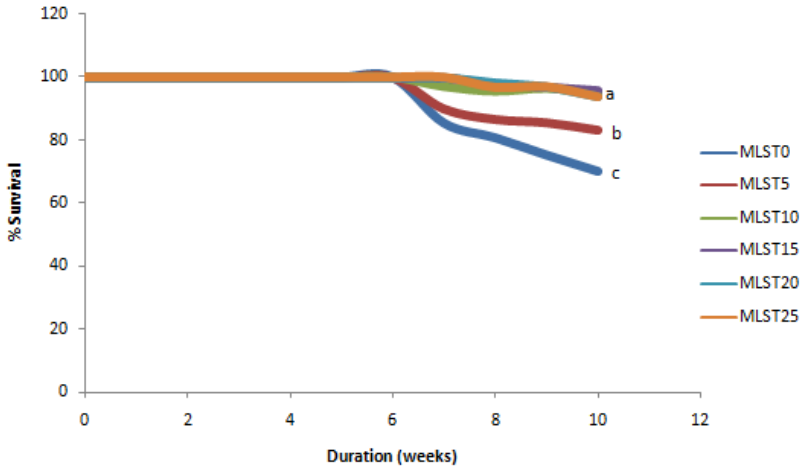

FIGURE 1: % SURVIVAL OF O. NILOTICUS FED THE EXPERIMENTAL DIETS FOR 10 WEEKS .^{a,b,c} VALUES IN EACH ROW WITH DIFFERENT SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P < 0.05) BY USING ANOVA POST HOC (TUKEY TEST).

IV. DISCUSSION

In this study, significantly elevated activities of in cellular enzymes AST, ALT, LDH and MDH activities observed in fish exposed to *Aeromonas hydrophila* and transportation-induced stressindicated that environmental stressors caused liver injury. Several studies on various stressors like handling (Carey and McCormick, 1998), transportation (Barton et al, 1988), stocking density (Chatterjee et al., 2006) and pathogenic stress (Tekle and Sahu, 2015) reported reduction in cellular enzymes AST and ALT activities when fish diets were supplemented with plant extracts. In the present study, amino-transferase activities were found highest in the control group compared to the other dietary groups. The higher activity of AST and ALT indicates the mobilization of aspartate and alanine via gluconeogenesis for glucose production to cope with stress (Barton and Iwama, 2005), which also reflected in higher glucose level observed in the control group in this study. It has also been reported that

increase in the activity of cellular enzymes (AST and ALT) is an indicator of cellular damage in stressed fish (Soosean et al, 2010). Chatterjee et al, (2006) reported that transaminase activity increases during stress. Elevated level of transaminase activity during stress would lead to increase feeding of ketoacids into TCA cycle, thereby affecting oxidative metabolism (Tekle and Sahu, 2015). Moringa leaf supplementation significantly reduced the activities of AST and ALT suggesting that Moringa leaf protected the membrane integrity of the liver cells against stressors. Many studies have shown that an important mechanism of the hepatoprotective effects may be related to an antioxidant capacity to scavenge reactive oxygen species (Cao et al, 2016). Hence, as there was less cellular activity in the Moringa supplemented groups, it can be inferred that addition of Moringa plant extracts reduced stress and improve growth and health of fish in the present study.

Fish fed the control diet in this study showed higher LDH activity than fish fed the Moringa supplemented diets. The higher LDH activity in the control group is attributed to the production of preferred substrate (Lactate) for gluconeogenesis (Chatterjee et al, 2006). Generally, LDH and MDH activities increases in stress condition (Barton and Iwama, 2005). Significantly lower LDH and MDH activities in the treatment groups suggested that there was stress mitigating effect of Moringa in *O. niloticus*. This is in agreement with the findings of Tekel and Sahu, (2015), which reported that the MDH activity in *O. niloticus* fingerlings subjected to pathogenic stress was higher in the control than fish treated with *M. oleifera* flower. Therefore, the lower LDH and MDH activity in *O. niloticus* fed dietary Moringa leaf supplemented diets showed that Moringa has the ability to ameliorate the effects of stressors used in the present study. The energy demands of fish increases during stress thereby increasing the MDH activity. The higher activity of MDH indicates greater activity of TCA cycle due to increased energy demands (Wedemeyer et al, 2000). The finding in the current study is supported by the high AST and ALT enzyme activities and the hepatic cellular damage leading to the increased synthesis of these enzymes in the liver of *O. niloticus* in the control treatment. The water quality parameters monitored during the present study were adequate for *O. niloticus* growth and it is in agreement with the report of Lim and Webster (2006) for the production of *O. niloticus.* Therefore the differences reported in the physiology of fish in the present study could not be traced to the water quality parameters.

Glucose and cortisol are important endogenous stress indicators in cells (Kaleeswaran et al, 2011), and their activities are key indicators of the immunity of the fish (Oliva-Teles, 2012).Supplementation of Moringa leaf decreased the level of glucose in fish fed the experimental diets in this study. However, the highest glucose, cortisol and cellular enzymes levels were found in the control group. This result is in agreement with many studies that reported the role of plant extracts in stimulating the immune system by modulating the activity of metabolic and antioxidative stress enzymes. For example, Kaleeswaran et al, (2011) reported positive effects of *Cynodon dactylon* (L.) on the innate immunity and disease resistance of Indian major carp, *Catla catla*. Tekle and Sahu (2015) reported the ameliorative effects of Moringa flower on *O.niloticus* subjected to *Aeromonas hydrophila* induced stress. *M. oleifera* plant has been widely reported to contain constituents such as nitrile, glycosides and quercetin (Ojiako, 2014) which are believed to be responsible for enhancing immunity against oxidative stress and microbial diseases. In a previous in vitro study on *O. niloticus* by Tekle et al (2015), ethanolic flower extract of *M. oleifera* showed potent antioxidative and antimicrobial activities. The role of the bioactive compounds in the extract is to boost the non-specific defence system including the lysozyme activity, phagocytic activity and the respiratory burst activity of the immune cells (Tekle et al, 2015). Therefore the presence of potent antioxidants in Moringa supplemented diets was helpful in reducing the negative effects of stressors in *O. niloticus*. In the present study, exposure of fish to stressors increased the stress biomarkers as evidenced by high glucose, cortisol and lysozyme concentration in the fish blood therefore rendering the fish immune-compromised. However, the activities of these stress indicators were significantly reducedwith supplementation of Moringa leaf leading increased survival in fish. This result is in agreement with many studies that reported the role of plant extracts in stimulating the immune system and reducing the percentage mortality of fish infected with pathogenic strain of bacteria and by modulating the activity of metabolic and antioxidative stress enzymes. In the present study, fish in MLST10 and MLST15 groups exhibited the highest post-challenge survival (95.70 %) and (95.13%) respectively. Similarly, dietary administration of guava at 30 mg/ kg for 45 days significantly enhanced the resistance of *L. rohita* to *A. hydrophila* infection (Giri et al, 2015). It was also reported that extracts of Moringa leaves penetrate the lipid bilayer of the cell membrane, rendering it more permeable, and promote the scavenging of ROS; also by up regulation of antioxidant defenses (Hammed et al, 2015). Hammed et al, (2015) also reported that the presence of potent antioxidants in Moringa leaf can be correlated with increase in antibody production which helps in the survival and recovery of fish. In the present study, supplementation of Moringa leaf at the dose of 1.00g/kg in the diet was sufficient to induce hepatoprotective and ameliorative effects against stress -induced liver damage in fish in a dose dependent manner. The hepatoprotective action of Moringa leaf was probably related with its eliminating free radical, maintaining the integrity of the hepatocyte membrane

and increasing the antioxidant enzyme activities, inhibiting ROS damage. Supplementation of Moringa leaf may potentially be used as a hepatoprotective and stress reducing agent for improved performance fish for especially during stress periods.

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