World Applied Sciences Journal 35 (1): 88-95, 2017
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2017.88.95

Effects of Dietary *Moringa oleifera* Extract Against *Aeromonas hydrophila* Infection and Transportation-Induced Stress in African Catfish *Clarias gariepinus* (Burchell, 1822) Fingerlings

Gbadamosi Oluyemi Kazeem[*], *Fasakin E. Adedayo and Adebayo Olabode Thomas*

Department of Fisheries and Aquaculture Technology,
Federal University of Technology, Akure, Ondo State, Nigeria

Abstract: The main aim of the present study was to assess the hepatoprotective and stress-reducing effect of *Moringa oleifera* extract against *Aeromonas hydrophila* infection and transportation- induced stress in African catfish, *Clarias gariepinus* fingerlings. Fish were fed diets representing different supplementation levels of *M. 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 *A. hydrophila* at a concentration of 9.3×10^5 CFU /mL. After bath exposure, fish from each dietary treatment were placed into the aquaria culture system. They were fed their respective diets at 5% body weight twice daily 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 histological examination. Results showed that the increases of the AST, ALT, LDH, MDH and hepato-histological insults 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 hepatoprotective and stress reducing agent in *C. gariepinus* fingerlings.

Key words: Hepatoprotective · Stress – Reducing · *Moringa oleifera* · *Aeromonas hydrophila* · African Catfish · Extracts

INTRODUCTION

Stress and stress-related diseases are currently a much discussed topic in animal including fish husbandry and research [1-3]. 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 [4-6]. Environmental variables, particularly nutrition, are ultimately important in affecting fish in time of stress [6]. 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 [7-9].

Farmed fish frequently encounter and tolerate poor environmental conditions, which are well below the considered optimal [10, 11]. 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 [10, 12]. 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 [13, 14]. Phytogenic products and extracts are cheaper, non-toxic and biodegradable alternative to antibiotics.

Corresponding Author: Oluyemi Gbadamosi, Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Ondo State, Nigeria.

The *Moringa 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 [15, 16]. [17] 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. 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 [18]. Different parts of Moringa have shown great antioxidant activity [19] as well as immunomodulatory function in animals [18]. It can be recognized by the compound pinnate leaves and the long narrow angular fruits containing large wind seed. *Moringa oleifera* contains antioxidants which can inactivate damaging free radicals produced through normal cellular activity and from various stresses [4,20,]. Traditionally, the leaves, fruits, flowers and immature pods of this tree are edible [18]. 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 [21].

The African catfish, *Clarias gariepinus*is the most important fish species cultured in Nigeria; it grows rapidly, it is disease and stress resistant, sturdy and highly productive in polyculture with many other fish species [22]. This species has shown considerable potential as a fish suitable for use in intensive aquaculture [23]. *C. gariepinus* production is considered to be the fastest growing segment of the Nigeria aquaculture industry over the past decade [22]. More investors are entering catfish *C. gariepinus* farming in Nigeria as there exists a large unmet demand and market prices of catfish *C. gariepinus* which are more than those of other species [23]. The African catfish (*C.gariepinus*) is the leading aquaculture species in Nigeria [24, 25]. 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 African catfish, *Clarias gariepinus*.

MATERIALS AND METHODS

Extraction of *Moringa oleifera* Leaf: The leaves of *M. 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. The leaves were destalked, washed and dried in the shade. *M. oleifera* leaves were ground with pestle and mortar, leaves were then extracted according to the modified method of [20] as follows. Five hundred grams of the powdered leaf were soaked in 1.5 liter of warm water (60°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).

Preparation of Experimental Diets: The feed ingredients were purchased at Adebom Feedmill, Ondo road, Akure, Ondo State, Nigeria. Six isonitrogenous and isocaloric diets were formulated to meet the requirements of 40% crude protein (Table 1) for *C. gariepinus* fingerlings [26] 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, 2010) were used to perform the proximate analyses of feed of fish in the study.

Proximate Analyses of Moringa leaf and experimental feed: Standard and official methods [27] were used to perform the proximate analyses of Moringa leaf and feed of fish in the study. Formulated feed were blended to a homogeneous mince using a meat grinder (Binatone, UK) with a 4 mm diameter orifice plate. A sub-sample of Moringa leaf extract and feed were taken and stored for estimation of dry matter which was determined after drying in the oven (Gallenkamp, UK) at 105°C for 24 h. The remaining homogenates were dried in the oven and used for all subsequent analyses. Ash content was calculated by weight loss after incineration in a muffle furnace (Carbolite, UK) for 12 h at 550°C. A Parr bomb calorimeter was used to calculate gross energy content, this method measures energy content by combustion under an atmosphere of compressed oxygen with benzoic

Table 1: Composition of the experimental diet (g/100g) containing dietary *Moringa Oleifera* for African catfish, *Clarias gariepinus* fingerlings

	MLSC0	MLSC5	MLSC10	MLSC15	MLSC20	MLSC25
Fish meal (68 % CP)	23.50	23.50	23.50	23.50	23.50	23.50
GNC (48 % CP)	29.00	29.00	29.00	29.00	29.00	29.00
Soybean meal (42 % CP)	20.50	20.50	20.50	20.50	20.50	20.50
Yellow maize	10.50	10.50	10.50	10.50	10.50	10.50
Vegetable oil	7.00	7.00	7.00	7.00	7.00	7.00
Rice Bran	5.50	5.45	5.40	5.35	5.30	5.25
Alginate	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 leaf extract	0.00	0.05	0.10	0.15	0.20	0.25
Proximate composition of experimental diets fed to*Clarias gariepinus* (% dry matter basis)						
Crude protein	39.98	40.06	40.09	40.20	40.19	40.22
Lipid	10.05	10.23	10.15	10.41	10.42	10.45
Crude fibre	5.63	5.72	6.01	6.31	6.94	6.69
Ash	8.93	8.33	8.54	9.06	9.13	9.74
Dry matter	92.37	92.19	91.06	90.14	90.18	90.07
Nitrogen-free extract (NFE)	27.78	27.85	26.27	24.21	23.50	22.90
Gross Energy (kJ/g)	15.81	15.79	15.88	15.93	15.96	16.04

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.

acid as a standard. The Kjeldahl technique was used to measure crude protein. In this technique, the nitrogen (N) content was determined and multiplied by a conversion factor of 6.25.

Experimental Fish and Feeding Trial: *C. gariepinus* 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 10.00±0.05 g per replicate for *C. gariepinus* 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. 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).

Physico-Chemical Water Parameters: 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).

***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°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 [28]. 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 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. The liver samples were collected immediately after transportation for 2 hours from fish for further analyses.

Assessment of Hepatocellular Damage: 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 [29]. 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°C) in a cooling centrifuge machine and stored at -20°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°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°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.

Histopathological Examination: The liver were collected and fixed in Davidson's freshwater fixative by 24h then rinsed and put into 70% ethanol until dehydrated in graduated ethanol 50–100%,cleared in xylene and embedded in paraffin. Sections of 5 lm thickness were prepared, stained with haematoxylin and eosin (H&E) dye. Photomicrographs were taken with the aid of Olympus digital camera (Olympus, UK) at 50 µm. Tissue sections were compared after examination under the microscope, for significant differences in the morphology of the tissues.

Statistical Analysis: The data were expressed as mean±standard deviation (SD).The differences between mean of treatments were considered significant at $P < 0.05$ 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.

RESULTS

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).

Histology of the Liver of *Clarias Gariepinus* Fed the Experimental Diets: Histology of the liver of *C. gariepinus* fed the experimental diets is shown in Figure 1A-E. In fish fed the MLSC0 diet, disorganised sinusoids (arrow), less nuclei and highly vacuolated cytoplasm (circles) was observed (Figure 1A). Liver of fish fed the MLSC5 diet showed erosion of the hepatocytes. However, fish fed MLSC10 to MLSC25 diets showed more nucleated hepatocytes (arrow) (Figure 1C-F).

Table 2: Effects of *M. oleifera* leaf extract on hepatocellular damage indicators in experimental fish

	MLSC0	MLSC5	MLSC10	MLSC15	MLSC20	MLSC25
AST (µM)	60.28±2.05^{d}	47.51±1.70^{c}	30.35±1.09^{ab}	28.65±1.17^{a}	30.02±1.20^{a}	34.04±0.82^{b}
ALT (µM)	51.53±1.58^{e}	27.16±1.70^{d}	17.78±1.24^{ab}	16.61±1.25^{a}	19.26±1.69^{c}	19.18±1.03^{bc}
LDH (µM)	1.33±0.06^{e}	1.12±0.05^{c}	0.85±0.02^{a}	0.94±0.05^{b}	1.28±0.01^{d}	1.35±0.13^{e}
MDH (nM)	6.13±0.05^{d}	3.12±0.16^{c}	1.84±0.03^{a}	1.73±0.04^{a}	2.12±0.15^{b}	2.26±0.12^{b}

^{a,b,c,d,e,f} 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.

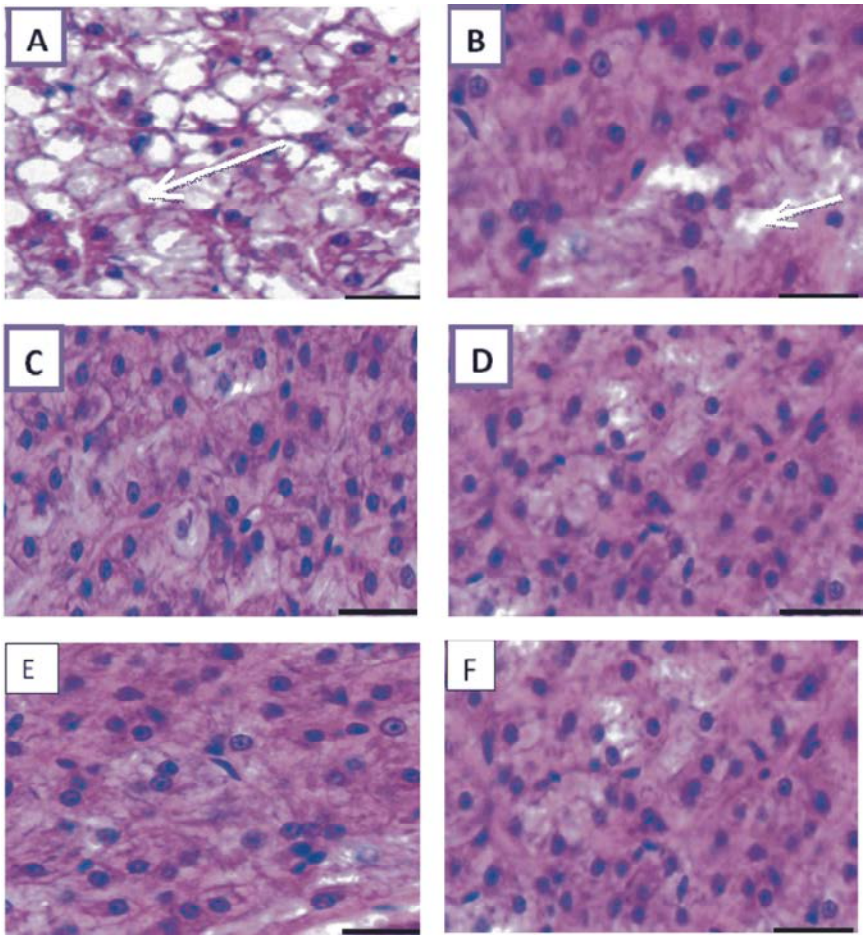

Fig. 6: (A). Liver of fish fed the MLSC0 diet showing disorganised sinusoids (arrow), less nuclei and highly vacuolated cytoplasm (circles). **(B)** Liver of fish fed the MLSC5 diet showing erosion of the hepatocytes and vacuolated cytoplasm (arrow) **(C)** Liver of fish fed MLSC10 diet showing more nucleated hepatocytes (arrow). **(D)** Liver with hepatocytes in fish fed MLSC15 diet showing nucleated hepatocytes. **(E)** Liver of fish fed MLSC20 diet showing nucleated hepatocytes. **(F)** Liver of fish fed MLSC25 diet showing nucleated hepatocytes (scale bar = 50 µm).

DISCUSSION

Significantly elevated activities of cellular enzymes AST, ALT, LDH and MDH observed in fish in the control group exposed to *Aeromonas hydrophila* and transportation-induced stress indicated that stressors caused liver injury in the present study. Stressors like transportation, stocking density and pathogenic stress have been reported to cause hepatocellular damage in Nile tilapia by increasing the activities of cellular enzymes AST and ALT [30]. Soosean *et al.*[31] also reported that increase in the activity of cellular enzymes (AST and ALT) is an indicator of cellular damage in stressed fish. 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 [32]. Elevated level of transaminase activity during stress would lead to increase feeding of ketoacids into TCA cycle, thereby affecting oxidative metabolism [30]. 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. Cao *et al.* [32] stated that an important mechanism of the hepatoprotective effects may be related to an antioxidant capacity to scavenge reactive oxygen species. 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. Generally, LDH and MDH activities increases in stress condition [6]. Significantly lower LDH and MDH activities in the Moringa treated groups suggested that there was stress mitigating effect of Moringa on the liver of fish in the current study. This is in agreement with the findings of Tekle and Sahu [30], 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 *C. gariepinus* fed dietary Moringa leaf supplemented diets showed that Moringa has the ability to ameliorate the effects of stressors used in the present study. Furthermore, various histopathological changes were noticed in the liver of *C. gariepinus* fed the control and MLSC5 diets which were not observed in fish fed the other Moringa supplemented diets (Figure 1). These changes were as follows: the cord-like parenchymal structures of the liver were lost, resulting in disorganised sinusoids and highly vacuolated cytoplasm with loss of nuclei. Highly vacuolated cytoplasm, deformed sinusoids and vacoulation in the hepatocytes were observed as signs of physiological dysfunction in unhealthy *Cyprinus carpio* [33]. The hepatocytes of *C. gariepinus* showed apparently normal structures in fish fed MLSC10 and MLSC15 diets. Fish fed MLSC10, MLSC15 and MLSC20C diets did not show any apparent histopathological changes in the liver, as the histology of the liver of fish in these dietary treatments showed normal hepatocytes, numerous nuclei and cytoplasmic organelles suggesting that the inclusion of Moringa in fish fed MLSC10, MLSC15 and MLSC20 diets had positive effects on the liver of tilapia in this study.

This results of the present study 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 Ilavenilb and Ravikumara [14] reported positive effects of *Cynodon dactylon* (L.) on the innate immunity and disease resistance of Indian major carp, *Catla catla*. [30] 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 [14] which are believed to be responsible for enhancing hepatoprotection, immunity against oxidative stress and microbial diseases. Therefore the presence of potent antioxidants in Moringa supplemented diets was helpful in reducing the negative effects of stressors in *C. gariepinus*. Hammed *et al.*[22] 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 during stressful periods.

CONCLUSION

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 fish performance and health especially during stress periods.

REFERENCES

1. Guo, Q., F. Liu, F.H. Shi, Y. Yu, Y. Wang and W.F. Shen Lv, 2016. Effects of astaxanthin and emodin on the growth, stress resistance and disease resistance of yellow catfish (Pelteobagrus fulvidraco). Fish and Shellfish Immunology, 51: 125-135.
2. Conte, F.S., 2004. Stress and the welfare of cultured fish. Applied Animal Behaviour Science, 86(3): 205-223.
3. Xie, J.K., S. Jun and F. Jian, 2008. Effects of anthraquinone extract from rhubarb, Rheum officinale Bail on the crowding stress response and growth of common carp, Cyprinus carpio var. Jian. Aquaculture 281: 5-11.
4. Rapatsa, M.M. and N.A.G. Moyo, 2014. Effect of Moringa oleifera on the histology, haematology and growth of Oreochromis mossambicus in aquadams® fertilised with chicken manure in South Africa. African Journal of Aquatic Science, 39: 295-303.
5. Liu, Z., Y. Xie, L.Song, Z. Weng and S. Liu, 2015. Hsp90, Hsp60 and sHsp families of heat shock protein genes in channel catfish and their expression after bacterial infections. Fish and Shellfish Immunology, 44: 642-651.
6. Barton, B.A. and G.K. Iwama, 2005. Simple field methods for monitoring stress and general condition of fish. Aquaculture Research 26: 273-282.

7. Good, J.E., 2004. Vegetable Oils: Effects on Fish Health. A thesis presented for the degree of Doctor of Philosophy to the University of Stirling Institute of Aquaculture University of Stirling,Stirling, Scotland, UK.
8. Larrey, D., 2000. Drug-induced liver diseases. Journal of Herpetology, 32: 7-88.
9. Okaeme, A.N. and T.I.I. Ibiwoye, 2001. Hints on disease problems, prevention and control in the culture of tilapias and Clariid species in fresh water system in Nigeria. Technical Report Series, 18(ISSN 00331-6734): 1-9.
10. Subashinge, R.T., 2005. Physiological responses and depression of humoral components of the immune system in gilthead sea bream (*Sparus aurata*) following daily acute stress. Canadian Journal of Fisheries and Aquatic Science, 52: 2339-2346.
11. Adewolu, M.A. and A.J. Adoti, 2010. Effect of mixed feeding schedules with varying dietary crude protein levels on the growth and feed utilization of Clarias gariepinus (Burchell, 1822) fingerlings. Journal of Fisheries and Aquatic Sciences, 5: 304-310.
12. Welker, T.L., C. Lim, M. Yildirim-Aksoy, M. Shelby and P.H. Klesius, 2007. Immune Response and Resistance to Stress and Edwardsiella ictaluri Challenge in Channel Catfish, Ictalurus punctatus, Fed Diets Containing Commercial Whole-Cell Yeast or Yeast Subcomponents. Journal of the world Aquaculture Society, 38(1): 24-35.
13. Chatterjee, N.A.K., Pal, Das, T. Manush, S.M. Sarma, K. Venkateshwarlu and S.C. Mukherjee, 2006. Secondary stress response in Indian major carps Labeo rohita (Ham), Catla catla (Ham) and Cirrhinus mrigala (Ham) fry to increasing packing densities. Aquaculture Research, 37: 472-476.
14. Kaleeswaran, B., S. Ilavenilb and V. Ravikumara, 2011. Dietary supplementation with *Cynodon dactylon* (L.) enhances innate immunity and disease resistance of Indian major carp, Catla catla (Ham.). Fish and Shellfish Immunology, 31: 953-962.
15. Jahn, S.A.A., 1996. Using Moringa seeds as coagulants in developing countries. Journal on Management Operations, 13: 43-50.
16. Becker, K., 2003. Moringa oleifera ; an underutilized tree with amazing versatility. Cambridge University Press, Cambridge, 28: 45-56.
17. Verdcourt, B., 1993. A synopsis of the Moringaceae. Kew Bull., 40(1): 1-15.
18. Ojiako, E.N., 2014. Phytochemical analysis and antimicrobial screening of Moringa Oleifera leaves extract. The International Journal of Engineering and Science. 3(3): 32-43.
19. Anwar, F., S. Latif, M. Ashraf and A.H. Gilani, 2007. Moringa oleifera: a food plant with multiple medicinal uses. Phytotherapy Research, 21(1): 17-25.
20. Makanjuola, O.O., E.O. Dada and F.O. Ekundayo, 2013. Antibacterial actitvities of Moringa oleifera on coliforms isolated from some surface waters in Akure, Nigeria. FUTA Journal of Research in Sciences, 1: 63-71.
21. Hussain, S., F. Malik and S. Mahmood, 2014. Review: an exposition of medicinal preponderance of *Moringa oleifera* (Lank.), PakistanJournal of Pharmaceutical Sciences, 27(2): 397-403.
22. Hammed, A.M., A.O. Amosu A.F. Awe and F.F. Gbadamosi, 2015. Effects of Moringa oleifera leaf extracts on bacteria (Aeromonas hydrophila) infected adults African mud cat fish. International Journal of Current Research, 7, (10), 22117-22122. Available online at http://www.journalcra.com.
23. Adebayo, O.T., 2006. Reproductive performance of African clariid catfish, Clarias gariepinus broodstocks on varying maternal stress. Journal of Fisheries International, 1: 17-20.
24. FAO., 2014. Food and Agricultural Organisation of the United Nations: The state of world Fisheries and Aquaculture 2014, Rome, Italy.
25. Fagbenro, O.A., A.M. Balogun and C.N. Anyanwu, 1992. Optimal dietary protein level for Heterobranchus bidorsalis fingerlings fed compounded diets. Isreali Journal of Aquaculture Bamidach, 44: 87-92.
26. NRC, 2011. National Research Council. Nutrient requirement of fish. National Academy Press. Washington D.C, PP: 114.
27. AOAC, 1995. Association of Official Analytical Chemists Official Methods of Analysis. (16th edition.) Arlington, VA, USA. pp: 1056.
28. Li, L., M. Kobayashi, H. Kaneko, H. Nakajima-Takagi, Y.Y. Nakayama and M. Yamamoto, 2008. Molecular evolution of Keap1:Two Keap1 molecules with distinctive intervening region structures are conserved among fish. Journal of Biological Chemistry, 283(6): 3248-3255.
29. Hardy, R.W. and C.V. Sullivan, 2003. Pathogenesis of infectious diseases of fish. Annual Review Microbiological 40: 479-502.

30. Tekle E. W. and N. P.Sahu, 2015. Growth and immunodulatory response of Nile tilapia, Oreochromis niloticus fingerlings to ethanolic extract of Moringa oleifera flower. IJSRP5(7): 285-296.
31. Soosean, C., K. Marimuthu, S. Sudhakaran and S.R. Xavier, 2010. Effect of mangosteen (*Garcinia mangostana* L.) extracts as a feed additive on growth and haematological parameters of African catfish (Clarias gariepinus) fingerlings. European Rev. Med. Pharmacological Science, 14: 605-611.
32. Cao, L., J. Du, Ding, W. Rui, J, Liu, Y, Xu, P, Teraoka and H. G,Yin, 2016. Hepaprotective and antioxidant effects of dietary Angelica sinensis extract against carbon tetrachloride-induced hepatic injury in Jian Carp (*Cyprinius carpio* var. Jian). Aquaculture Research 47: 1852-1863.
33. Venkatesan, S., K. Pugazhendy, M. Meenambal, M. Sangeetha, D. Vasantharaja, C.K. Jayachandren and S. Prabakaran, 2012. Protective role of Spirulina on the variation of haematological parameter induced by herbicide atrazine in the fresh water fish *Cyprinus carpio* (Linn). International Journal of Pharmaceutical & Biological Archives, 3(1): 249-254.