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Effect of some Medicinal Herbal Plants on Hematology of African Catfish *Clarias gariepinus*

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Abstract: This study deals with the evaluation of the effect of two natural medicinal plants, *Zingiber officinale* (ginger) and *Moringa oleifera* (moringa) on hematological parameters of African catfish, *Clarias gariepinus*, as an effective solution for improving fish aquaculture and fish health. A total of 200 specimens of *Clarias gariepinus* were divided into four groups, control (G1), 0.1 g/L of ginger water extract (G2), 0.1 g/L of thyme water extract moringa (G3) and 0.05 g/L of ginger water extract mixed with 0.05 g/L of moringa water extract (G4) groups. Each fish group was placed in 250 liters of water tank from River Nile for 4 weeks. The present results displayed positive effects of ginger and moringa supplements on growth performance and health status when added to the diet of common catfish in all treatments. They improved the growth rate of common catfish and improved blood parameters (eg, hemoglobin count, RBC count, WBC count). The current findings demonstrated that ginger and moringa supplements had a positive effect on health status as a natural source for fish aquaculture.

keywords: Ginger, Moringa, *Clarias gariepinus*, serum collection, biochemical analysis.

1.Introduction

The African catfish, *Clarias gariepinus* Burchell 1822 is a widely distributed fish food in Africa with a high growth rate and excellent meat quality. It has enormous potential for use in human nutrition as a source of protein and energy, and its lipids are an excellent source of polyunsaturated fatty acids, particularly what are known as omega-3 fatty acids. Parasites have the potential to drastically impact the aquaculture of this fish species and therefore, the health of this fish is of major concern [1].

The development of alternative practices for disease management poses a significant challenge. Recently, research has begun to demonstrate the positive effect of the application of phytochemicals and herbal products in fish culture [2].

Medicinal plants can affect the hematological parameters and parasitological activity of fish [3]. In general, plant extracts containing abundant proteins, carbohydrates, vitamins, lipids, and minerals have an important role in enhancing growth performance and modifying the physiological function of fishes [3].

Hematologic evaluation is one of the diagnostic tools for the identification of diseases. A clinical hematology evaluation of normal variation from intrinsic or extrinsic factors or diseases affecting blood cells and counts may be required. Most of the fish have nucleated erythrocytes which play an important role in oxygen transport which depends on the amount of hemoglobin concentration within the cell and the gas-exchange mechanism. Similar to other vertebrates, fish leucocytes are involved in defense mechanisms and provide protection against infections [4].

Zingiber officinale (ginger, Zingiberacae) is a medicinal plant that has been widely used for infectious diseases and helminthiasis [2]. Ginger at 1000 mg/35L inclusion level increased erythrocyte count, hematocrit, hemoglobin, and lymphocytes count in *Clarias gariepinus* juveniles. The increase in hematological indices reflects immune system stimulation and the function of organs related to

blood cell formation such as the thymus, spleen, and bone marrow [5].

Moringa oleifera (moringa, Moringaceae) is one of the important plants that can be used for food and traditional medicine. Previous research has shown that the leaves, seeds, roots, barks, and flowers of this plant contain a variety of bioactive substances with nutritional and therapeutic benefits. Furthermore, this plant's use in the treatment of parasitic diseases such *filariasis*, *leishmaniasis*, *trypanosomiasis*, *schistosomiasis*, and malaria suggests that it naturally has antiparasitic properties [6, 7].

The aim of this study is to evaluate the effect of natural medicinal plants, *Zingiber officinale* and *Moringa oleifera* on hematology of *C. gariepinus* as an effective solution for improving fish aquaculture.

2. Materials and methods

2.1. Sampling of the African fish, *Clarias gariepinus*

A total of 200 specimens of the African catfish, *Clarias gariepinus* Cuvier and Valenciennes, 1840 were collected from the River Nile in El-Mansoura City (El-Dakahlia Governorate, 120 Km north to Cairo, Lower Egypt, coordinates: 31° 7' 30" N, 31° 25' 49" E).

Fishes were placed in the laboratory in a plastic tank aquarium supplied with 250 l of water tank from the River Nile, which was equipped with an air pump and an electric filter in order to be as similar to its natural environment as possible.

2.2. Sampling of experimental plants and preparation of extracts

Fresh rhizomes of ginger (*Zingiber officinale*) and fresh plant leaves of moringa (*Moringa oleifera*) were collected. Plant parts were cleaned, cut into small pieces, minced and weighed then well crushed and the stock solution of each was prepared by diluting 100 g of each in 1 L distilled water.

2.3. Grouping of experimental fish

The fish were separated into four groups, and each group was given the following treatment for one month in a separate tank as the following:

A- The control group (G1), in which fish were cultured in tanks without the inclusion of any supplementary factors during the study period.

B- *Zingiber officinale* group (G2), in this group amount of 0.25 L of prepared *Z. officinale* juice forming 0.1 g/L in the tank water of was added to the tank twice weekly for four weeks.

C- *Moringa oleifera* group (G3), in this group amount of 0.25 L of prepared *M.oleifera* juice forming 0.1 g/L in the tank water of *M. oleifera* was added to the tank twice weekly for four weeks.

D- *Zingiber officinale* & *Moringa oleifera* group (G4), in this group amount of 0.125 L of prepared *Z. officinale* juice forming a concentration of 0.05 g/L in the tank water of the aqueous extract mixed with a same concentration of *M. oleifera* (0.05 g/L) were added to the tank twice weekly for four weeks.

2.4. Haematological analysis and blood collection

Fish were randomly selected from control and experimental aquaria. Blood was withdrawn from the caudal blood vessels in two portions; one with anticoagulant for evaluating blood parameters and the other without anticoagulant for separating serum by letting the blood clot at room temperature in a slanting position. Fish were restrained using a hand towel and their dorsal section, which exposed their ventral portion. Using a sterile 3G needle and a 5 ml syringe, blood was extracted from the fish. 3–4 cm away from the vaginal entrance, a puncture was made to avoid mucus contamination. A needle was inserted perpendicular to the vertebral column of the fish and gradually dragged downward until blood began to flow. It was slowly aspirated until 3 ml of blood had been obtained. Blood samples were put in sanitized, plain plastic containers very away after collection. The hematology lab received blood samples right away for biochemical evaluation.

2.5. Determination of the haematological parameters

Hematological assays were performed using whole blood. Using an upgraded Neubauer hemocytometer, the total number of

erythrocytes (RBCs) and leukocytes (WBCs) was counted. Utilizing the cyanmethemoglobin method outlined by Van Kampen and Zijlstra [8] In small capillary hematocrit tubes, hematocrit (Hct) was determined using a microhaematocrit centrifuge at 3000 rpm for 15 minutes. According to Gupta [9], three blood indices were calculated: Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), and Mean corpuscular haemoglobin concentration (MCHC) according to Stoskopf [10].

2.6. Serum collection and biochemical analysis

In sterile plastic test tubes without an anticoagulant, the blood was drawn. The tubes were stored at room temperature in a slanting wood rack so that blood might coagulate. The clot of blood was centrifuged at 3500 revolutions per minute for 15 minutes (rpm). The serum, a transparent liquid, was pipetted into a clean, sterilized vial for further examination.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated from serum using commercial kits (Bio-diagnostic, Egypt). The concentration of Albumin was determined according to Doumas et al. [11] using commercial kits (Biodiagnostic Company, Egypt). Kidney function tests; urea and creatinine were determined by Jaffe spectrophotometric method. Analysis was done with the use of semi-automatic biochemistry analyzer (Erba Chem 7, India). Procedure for the analysis and reagents used was done according to the manufacturer's instruction.

2.7. Statistical data analysis

In the experiment, a One-way ANOVA test using SPSS (version 25 for Windows) was used to detect differences between hematology during the experiment in different groups. If significant differences were detected, multi-range comparisons (least significant difference) were selected from PostHoc tests (Tukey HSD) on the same statistical package to detect differences between infestation levels for in each group. Blood samples were collected via caudal vein puncture as described by Kori-Siakpere et al. [12].

3. Results

3.1. Hematological parameter finding Hematology:

The results of hematology revealed highest values for Hb, was observed in G3 (١٢,٥٤± 1.86 g/dl) when compared to G2, G4 and G1. On the other hand, RBCs, HCT and PLT showed its highest value in G4 (2.43 ± 0.33 $x100^3$ cells/µl, ٣٦,٤٣ ± 4.47 % and 51.83 ± 35.87 10^3/µl, respectively). However, highest values for WBC was observed in G2 (٢٤٠,٣٣± 30.41 $x10^3$ /mm^3) when compared to other groups as shown in Table (1).

Statistical analysis (One-way ANOVA) indicated that HGB showed a very high significant difference between different groups ($p \leq 0.001$). Regarding the Multiple Range Comparisons (PostHoc tests: Tukey HSD) detected very high significant differences between G1 with G3 and G4 and G2 with G 3 ($p \leq 0.001$).

Additionally, Statistical analysis (One-way ANOVA) indicated that RBCs and HCT showed a very high significant difference between different groups ($p \leq 0.001$). Regarding the Multiple Range Comparisons (Post Hoc tests: Tukey HSD) detected very high significant differences between G1 with G3 and G4 and high significant differences between G1 with G2 ($p \leq 0.01$).

On the other hand, Statistical analysis (One-way ANOVA) indicated that WBCs showed a very high significant difference between different groups ($p \leq 0.001$). Regarding the Multiple Range Comparisons (PostHoc tests: Tukey HSD) detected very high significant differences between G1with G2, G3 and G4 ($p \leq 0.001$).

Finally, Statistical analysis (One-way ANOVA) indicated that PLT showed a significant difference between different groups ($p \leq 0.05$). Regarding the Multiple Range Comparisons (PostHoc tests: Tukey HSD) detected significant differences between G1 with G4 ($p \leq 0.05$).

3.2. Biochemical analysis for experimental groups:

The results of biochemical analyses revealed that the liver function tests (GPT and GOT) and kidney function tests (urea and creatinine) showed their highest values in control group

(G1) (٢٤٠,٢٣ ± 31.35 U/ml, 135.04 ± 22.56 U/ml, 17.54 ± 2.07 mg/dl and 0.95 ± 0.07 mg/dl, respectively) when compared to G2, G3 and G4. On the other hand, the liver function (albumin) showed its highest value in G2 (1.24 ± 0.12 g/dl) when compared to other groups as shown in Table (2).

Statistical analysis (One-way ANOVA) indicated that GPT, GOT, albumin, urea and creatinine showed a very high significant difference between different groups (p≤0.001).

Table (1): Blood picture analysis of *Clarias gariepinus* in the four studied groups.

Blood Parameter	G1	G2	G3	G4
HGB(g/dl)	6.76±1.61	8.60± 1.68	١٢,٥٤±1.86	١٠,٦٣± 1.56
RBC(x100^3 cells/µl)	1.46±0.43	2.15± 0.25	٢,٢٤±0.25	٢,٤٣± 0.33
HCT(%)	22.07±7.11	32.49± 4.40	٣٥,٢٧±6.51	٣٦,٤٣± 4.47
MCV(fl)	155.30±21.80	151.17± 8.86	١٦٤,٠١±12.25	١٥٠,٦٤± 12.44
MCH(pg)	48.07± 9.21	39.81± 3.65	٥٤,٠٩± 9.66	٤٧,٣٠± 6.41
MCHC(g\dl)	34.70± 6.44	26.37± 2.69	٣٥,٣١± 6.49	٣١,٣٤± 3.15
WBC(x10^3 /mm^3)	139.63±17.67	240.33± 30.41	٢١٤,٣٩±11.65	٢٢٧,٢٩±37.28
PLT(10^3/µl)	9.43± 3.78	37.86± 4.73	٢٢,٩١± 6.04	٥١,٨٣±35.87

Table (2): Biochemical analysis (liver and kidney functions) of the *Clarias gariepinus* in the four studied groups.

Biochemical Analyses	G1	G 2	G 3	G4
GPT(U/ml)	٢٤٠,٢٣±31.35	١٣٣,٢٩± 71.91	٩٧,٧٠± 58.32	١٠٣,٤٤± 16.80
GOT(U/ml)	١٣٥,٠٤± 22.56	٣٦,٦٣± 26.16	٤٠,٢١± 21.00	١٧,٣٠± 5.23
Albumin(g/dl)	١,٠٦± 0.06	١,٢٤± 0.12	١,٤٦±0.16	١,٤١± 0.15
Urea(mg/dl)	١٧,٥٤± 2.07	١٠,٥٦± 3.88	٤,٤٥±1.48	٨,٥٣± 2.54
Creatinine (mg/dl)	٠,٩٥± 0.07	٠,٣٤± 0.05	٠,٣٦±0.07	٠,٢٢± 0.12

Regarding the Multiple Range Comparisons (PostHoc tests: Tukey HSD) of GPT detected very high significant differences between G1with G3 and G4 (p≤0.001) and high significant differences between G1 with G2 (p≤0.01). Regarding the Multiple Range Comparisons (PostHoc tests: Tukey HSD) of GOT detected very high significant differences between G1 with G2, G3 and G4 (p≤0.001). However, regarding the Multiple Range Comparisons (PostHoc tests: Tukey HSD) of albumin detected very high significant differences between G1 with G3 and G4 (p≤0.001) and high significant differences between G2 with G3 (p≤0.01).

Additionally, regarding the Multiple Range Comparisons (PostHoc tests: Tukey HSD) detected very high significant differences between G1 with G2, G3 and G4 and G2 with G3 (p≤0.001) and high significant differences between G3 with G4 (p≤0.01).

Finally, regarding the Multiple Range Comparisons (PostHoc tests: Tukey HSD) detected very high significant differences between G1 with G2, G3 and G4 in addition to

G2 with G3 (p≤0.001) and high significant differences between G3 with G4 (p≤0.01).

4. Discussion

The ability of fish to adapt to their environment and their nutritional state as well as their health status can all be evaluated using haematological and biochemical examinations [3].

According to previous haematological investigations, erythrocytes are a significant and valid signal of a variety of stress causes. Additionally, RBCs carry haemoglobin (Hb), which carries oxygen. The amount of oxygen that reaches tissue relies on the age of RBCs and the amount of Hb. This study's findings revealed a highly significant difference between groups (p≤0.001). The fish treated with an aqueous extract of *Moringa oleifera*, had the greatest levels of RBC, Hb and HCT levels compared to the ginger group and the control group. The improvement of haematological indicators indicates that the immune system is being stimulated and that the organs involved in the production of blood cells, such as the thymus, spleen, and bone marrow, are functioning normally [13].

The first line of defense against infectious pathogens, tissue damage, and inflammatory processes is made up of white blood cells (WBC). This study indicated a very high and

significant difference between groups. The rise in WBC (leucopomia) in fish could be related to an increase in leucocyte synthesis in the kidney and possibly the spleen as a defense mechanism against parasites. This increase in white blood cells in the circulatory system contributed to the better survival rate of the experimental fish [14].

In the same respect, similar findings were made by Haghighi and Mostafa [14], who reported that *Oncorhynchus mykiss* fed with the supplement of powdered ginger rhizome showed a significant immune-stimulatory effect and increased WBC values when compared to the control.

Measurement of plasma biochemical parameters is mostly used in clinical diagnosis of fish physiology to determine the general status of health [15]. GPT and GOT are enzymes found primarily in liver cells, and their presence in blood plasma indicates a problem with the liver cells [16, 17]. The tests for these enzymes are means of evaluating liver function. This gives an evaluation of the health condition of the liver in terms of performance. These enzymes are associated with liver parenchymal cells. When body tissues or an organ such as the heart or liver are destroyed, their additional levels are released into the bloodstream. The amount of each in the blood is directly related to the extent of the tissue damage [18].

The findings of this study for GPT and GOT showed a very high significant difference between different groups compared to the control. Results showed that, there was a marked decrease in level of GPT and GOT of all treated groups along the whole of experiment period than control. These results indicate that the fish treated have healthy liver tissue than control at the same condition. Similarly feeding of catfish with *Z. officinale* incorporated diet at dose of 0.5g with other herpes resulted in lower level of GPT and GOT in ginger treated group than control infected groups [19].

An increase in serum albumin levels in fish is assumed to be linked to a higher innate immune response [20]. The present study found that fish treated with ginger and moringa raised albumin levels in all treated groups over the course of the experiment, with a very significant difference between groups. These findings are nearly identical to those found in Asian sea bass fed ginger for 15 days [21].

The urea is a byproduct of protein metabolism. The urea test aims at measuring the nitrogen in the circulatory system originating from the waste product urea. This examination is designed to evaluate the efficiency of kidneys.

Creatinine is a substance that is produced by the body during the metabolic process. The body eliminates creatinine through the kidneys filtration process. Creatinine measurements provide a precise evaluation of the efficiency of renal filtration processes are functioning. Kidney function decreases as creatinine level in the blood system increases [22]. The current study's findings revealed a very high significant difference between groups which indicates the effective impact of moringa and ginger on the treated groups.

Similar findings were published by Khali and El-houseny [23], who reported decreased level of serum creatinine when *C. gariepinus* infested with gill monogenea was exposed to ginger bath treatment. The current study contrasts with one by Ayotunde et al. [24], who found that fish exposed to acute and chronic concentrations of *M. oleifera* had elevated levels of creatinine and uric acid. The glomerular filtration rate, which is affected by diseased kidney alterations, may be affected by the toxic substances in *M. oleifera.*

4. References

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