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Effects of Nano Encapsulated Phytogenes on the Histology and Protein Signature of African Catfish Clarias Gariepinus (Burchell 1822)

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ABSTRACT

The effects of Nano encapsulated phytogenes on the histology of the liver, intestine, histometry and protein signature of African catfish *Clarias gariepinus* post fingerlings exposed to pathogenic stressor was assessed in this study. Three hundred fish with the average weight of 26.00 ±0.05g were fed with Nano encapsulated diets for 56 days. The phytogenes used were 0.5g of *Telfairia occidentalis* (Ugwu leaf), *Zingiber officinale* (Ginger leaf), *Moringa oleifer* (Moringa leaf) and *Ocimum gratissimum* (Scent leaf) /100g. After 56 days of feeding trial the fish were challenged with a bacteria strain *Pseudomonas fluorescence* for 14 days. The liver, intestine and muscle of the challenged fish were assessed for histopathological and SDS PAGE analyses. Histological analyses of the liver and intestine revealed less histopathological alterations in the treatments fed with Nano encapsulated diets to those fed with control positive diets. SDS PAGE analyses of the protein also revealed disintegration in the number of bands and molecular weight concentration to those fed in control positive diets. There were no significant difference (P>0.05) in the liver of the treatments fed with Nano encapsulated diets to those fed with control positive diets. Significant difference (P<.0.05) were observed in the intestine, histometry of fish fed with Nano encapsulated diets to those fed with control positive diets. There were also significant difference (P<0.05) in the protein signature of the treatments fed with Nano encapsulated diets to those fed with control positive diets. This study showed that phyto-additives diets is a potential treatments against bacterial infection in the farming of aquatic organism *Clarias gariepinus*.

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Introduction

Fish is a very important source of animal protein in human diets. As human population increases, the demand for high quality food especially from aquatic resources is needed. The production of fish through aquaculture is highly needed in order to meet up with the increased human population [1]. According to FAO (2007), aquaculture grows quickly than other agriculture sectors as it grows at a rate of 8.8% and above every year since 1970 which is greater than capture fisheries standing at 1.2%. The African catfish *Clarias gariepinus* is the most widely cultured catfish in Nigeria and Africa and third most cultured catfish species in the world [2]. Nigeria is the largest producer of *Clarias gariepinus* in the world [3]. *Clarias gariepinus* contributes immensely to the annual fresh water fish production in Nigeria [4].

Phytogenes constitute an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80% of the rural population depends on them as primary health care [5]. Plants have been used as sources of remedies for the treatment of many diseases since ancient times and people of all continents especially Africa have this old tradition. Despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Newman et al., 2000). Nano encapsulation (NE) is defined as a technology process to pack substances in miniature and provides final product functionality that includes controlled release of the core. NE technologies have the potential to meet food industry challenges concerning the effective delivery of health functional ingredients and controlled release of flavor compounds [6].

Histopathology is a branch of histology that deals with the study of disease of tissue as seen under the microscope. It is a science that studies disease pathology through morphological changes that can be seen microscopically. The separation of macromolecules in an electric field is called electrophoresis. A very common method for separating proteins by electrophoresis which uses a discontinuous polyacrylamide gel as a support medium and sodium dodecyl sulfate (SDS) to denature the proteins is called sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Polyacrylamide Gel Electrophoresis is a technique used for the separation of proteins based on their molecular weight [7]. The SDS coats the proteins, mostly proportional to their molecular weight, and confers the same negative electrical charge across

all proteins in the sample.

Materials and Methods

Experimental Site

This study was carried out at the Federal University of Technology Akure, Department of Fisheries and Aquaculture Technology, Teaching and Research farm, Obakekere.

Experimental Design

The experiment was conducted for 56 days in 15 rectangular glass tanks with four treatments and one control making 5 treatments in total with each treatment containing 3 replicates (triplicates). Each treatment was represented by different supplementations levels of Nano encapsulated Phyto-Additives. The graded levels of the Phyto-Additives was 0.00g (control), 0.5g of ugwu leaf, 0.5g Ginger, 0.5g of Moringa leaf and of 0.5g of Scent leaf per 100g for each diet denoted as Control (CTR), PT1, PT2, PT3, and PT4 in diets 1,2,3,4 and 5 for *C. gariepinus*. 15 experimental fish was randomly distributed into 15 tanks and was fed for 2 weeks with commercial diet at 5% (coppens) of their body weight for them to be acclimatized. The tanks was continuously aerated using an aerator to oxygenate the water body.

Feed Ingredients and Formulation of Experimental Diets.

The feed ingredients include; Fish meal, Soya bean meal, Groundnut cake, yellow maize, alginate, yeast, Dicalcium phosphate, Vitamin- mineral premix, Vitamin C, Groundnut oil, Phyto-additives. In this table below,
Diet A was represented by Ugwu leaf ((*Telfairia occidentalis*),
Diet B was represented by Ginger (*Zingiber officinale*),
Diet C was represented by Moringa leaf (*Moringa oleifer*),
Diet D was represented by Scent leaf (*Ocimum gratissimum*).

Table 1: The Feed Formulation of the Experimental Ingredients (g/100)

Ingredients	Control	Diet A	Diet B	Diet C	Diet D
Fishmeal(65%CP)	34	30	30	30	30
Soya bean meal (45%CP)	17	23	23	23	23
Groundnut cake (43.5%)	20	20	20	20	20
Yellow maize (10%CP)	11	11	11	11	11
Alginate	5	5	5	5	5
Yeast	3	3	3	3	3
Dicalcium phosphate	1	1	1	1	1
Vitamin C	1.5	1.5	1.5	1.5	1.5
Vitamin-mineral premix	1.5	1.5	1.5	1.5	1.5
Groundnut oil	6	6	6	6	6
Phytogenes	0	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Preparation and Dissection of Fish for Histology

Fish tissue was randomly removed for histological study and MS-222 (Tricaine methane sulphonate) at a concentration of 200 mg/l was used for terminal anesthesia of fish. After terminal anesthesia of the fish, tissue samples (liver and posterior intestine) was removed by dissection. The intestine was cut at the vent and then pulled away from the abdominal walls and finally the alimentary tract was cut free and laid out on a clean surface. Approximately 1 cm section was excised from each of the proximal, mid and distal segments of the intestine and was removed for histological processing whereas the whole liver was removed. Finally, the tissues was placed into universal bottles with Davidson's freshwater fixative.

Staining and Photo-Microscopy of Sections

After processing tissue samples, sections was stained using Cole's haematoxylin and eosin (H&E). Photomicrographs was taken using the Olympus BX 50 microscope and Olympus digital camera (Olympus, UK). Tissue sections was compared after examination under the microscope for significant differences in the morphology of the tissues.

Preparation of Polyacrylamide Gel Electrophoresis (PAGE)

Twelve percent (12%) of polyacrylamide gel was prepared by mixing 6.15ml of (22.2% acrylamide, 0.6%N: N-methylene bisacrylamide) 2.06ml of diluted water, 2.81ml of 1.5M Tris-HCL. The mixture was then transferred quickly into the gel chamber in an electrophoresis device overlaid with distilled water, and then allowed to stand for 20 minutes for polymerization to occur. After it was polymerized, the distilled water on top of the polymerized gel was carefully poured out and residual water was removed with the aid of filter paper. Thereafter, the 4% stacking gel was prepared by mixing 0.93ml of 22.2% acrylamide: 0.6%N; N- methylene bisacrylamide), 3.5ml of distilled water, 0.6ml of 0.5M Tris-HCL buffer pH 6.8, 50ul of 10% SDS, 18ul of 10% APS and 15ul of TEMED.

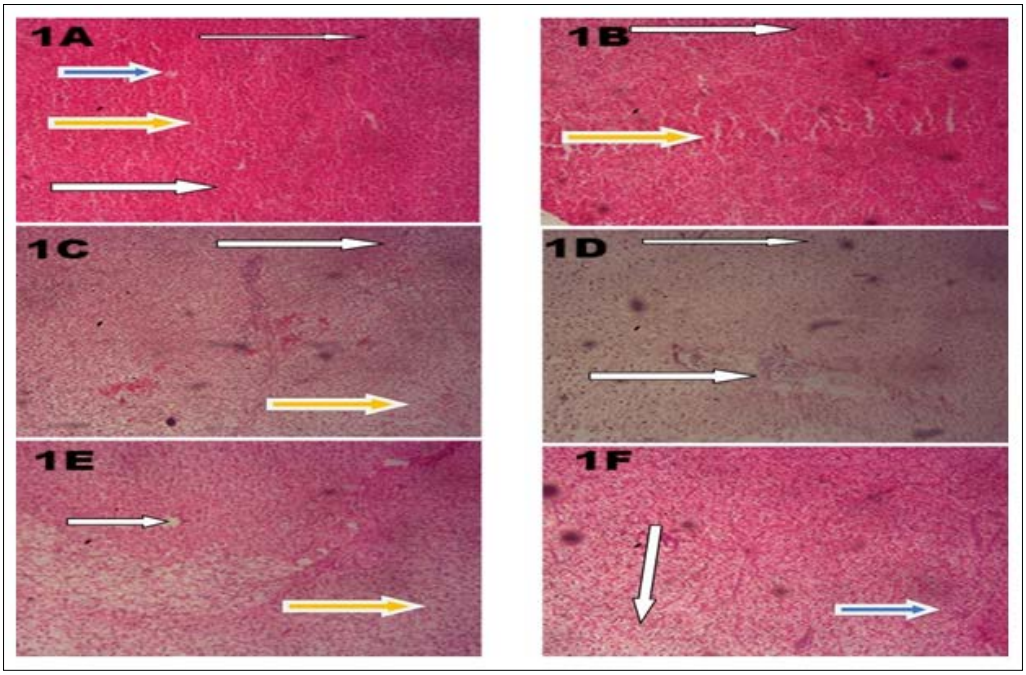
Statistical Analysis

The images of the gel and tissues was scanned and imported into gel analyzer software for the lane, band detection, and molecular weight calculation. All data was evaluated using analysis of variance (ANOVA) followed by Duncan's multiple range test. A probability level of less than 0.05 was used to indicate significance differences among the treatments. All analyses was performed using the SPSS 22.0 software.

Results

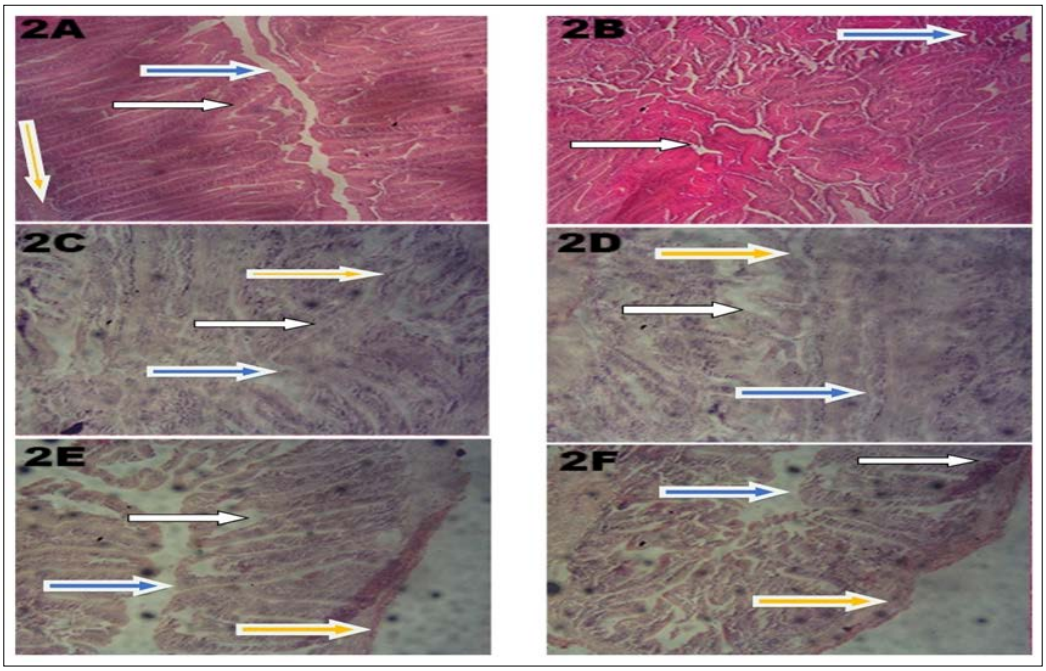
Histology of the liver

Figure 1: (1A) Photomicrograph of the liver in the control negative showing normal cell arrangement in Cytoplasm (white arrow), central vein (blue arrow), sinusoids (yellow arrow), and hepatocytes (slender arrow). (1B) Photomicrographs of the liver sections in control positive group showing cytoplasm vacoulation (white arrow), histopathology alterations in the sinusoid (yellow arrow). (1C) photomicrograph of the Liver fed with Nano encapsulated phytogenes (Ugwu leaf) showing cytoplasmic vacoulation in the hepatocyte (white arrow), sinusoids appear infiltrated (yellow arrow). (1D) photomicrographs of the liver fed with Nano encapsulated phytogenes (Ginger leaf) showing congestion of portal vein (white arrow) and degeneration of hepatocytes (slender arrow). (1E) photomicrographs of the Liver fed with Nano encapsulated phytogenes (Moringa leaf), showing central vein appear normal (white arrow), hepatocytes show cytoplasmic vacoulation (yellow arrow). Finally (1F) photomicrographs of the Liver fed with Nano encapsulated phytogenes (Scent leaf), showing normal portal vein (blue arrow), and cytoplasmic vacoulation (white arrow).



Histopathology of the Intestine

Figure 2: (2A) Photomicrograph of the intestine in the control negative group showing normal mucosal layer (white arrow), the lumen (blue arrow), and serosa layer also appear normal (yellow arrow). (2B) Photomicrograph of the intestine in the positive control group showing histopathology alterations in the mucosal layer (white arrow), and muscularis propria (blue arrow). (2C) Photomicrograph of the intestine fed with Nano encapsulated phytogenes (Ugwu leaf), showing normal mucosal layers (white arrow), lumen appear normal (blue arrow), and sub mucosal layer appear normal (yellow arrow). (2D) Photomicrograph of the intestine fed with Nano encapsulated phytogenes (Ginger leaf), showing normal mucosal layer (white arrow), the lumen appear normal (blue arrow), the sub mucosal layer also appear normal (yellow arrow). 2E) Photomicrograph of the intestine fed with Nano encapsulated phytogenes (Moringa leaf), showing normal mucosal layer, (white arrow), lumen appear normal (blue arrow), the serosa layer also appear normal (yellow arrow). (2F) Photomicrograph of the intestine fed with Nano encapsulated phytogenes (Scent leaf), showing normal muscular propria layer (white arrow), the lumen appear normal (blue arrow), serosa layer also appearing normal (yellow arrow).



SDS PAGE analysis for the Protein Signature of Clarias gariepinus Muscle Tissue

Figure 3: Electropherogram of protein profile represented as C-(control negative) have the highest number of bands with two of the bands highly concentrated, the number of bands indicates the availability of the protein present and the thickness of the bands explained high concentration of the protein. C+ (control positive) has lesser bands than control negative bands, this can be as a result of bacterial infection which results to alterations and degradation in the molecular weight of protein present. The Electropherogram of protein profile analysis of African catfish represented as G (ginger leaf), M (moringa leaf), S (scent leaf) and U (ugwu leaf) has equal number of bands but the concentrations and abundance of the protein bands differs, this could be as a result of Nano encapsulated diets present in there feeds and exposured to pathogenic stressor which results to degradations of molecular weight of protein present compare to the un treated group (control negative).

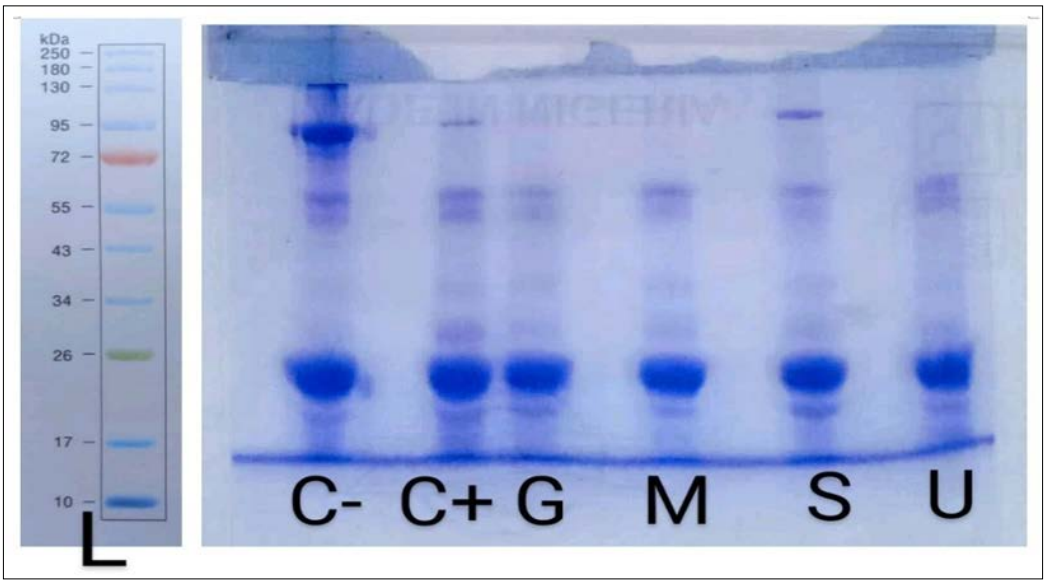


Figure 3: SDS PAGE Analyses Of *Clarias Gariepinus* Post Fingerlings Fed with Nano Encapsulated Phytogenes

C-: control negative, C+: control positive, G: Ginger leaf (*Zingiber officinale*), M: Moringa leaf (*Moringa oleifer*), S: Scent leaf (*Ocimum gratissimum*), U: Ugwu leaf (*Telfairia occidentalis*).

Discussion

The liver is composed of hepatocytes that were arranged in layers and were demarcated by endothelium. These investigations revealed that the liver of untreated fish, control negative treatment (Figure 1A) showed that the liver had normal architectural arrangement of cells as observed in the cytoplasm, hepatocytes (liver cell) and sinusoids with mild glycogen change. This mild glycogen change may be attributed to the high fat contents of catfishes. The histological sections of the negative control treatment (Figure 1B) showed histopathological alterations in the hepatocytes which results to cytoplasm vacoulation (swelling of the hepatocytes) and infiltration of the sinusoids.

The histological sections of the fish fed with Ugwu, Ginger, Moringa, Scent leaf (Figure 1C-1F) showed histopathological anomalies in the hepatocytes. The presence of cytoplasmic vacoulation of the hepatocytes could be as a result of excessive work required by the fish liver to get rid of bacterial infection from its body during detoxification (to remove a poisonous substance). This is in agreement with the report of Rapatsa and Moyo (2022) who observed vacoulation in the fish liver fed with Moringa (*M. oleifera*). It is also in line with work of Agbebi et al, who observed tubular degeneration of the liver of *C. gariepinus* fed with 10g ginger powder. The histopathological change observed in *C. gariepinus* fed with moringa leaf might be as a consequence of the unavailability of protein and amino acids that have been found with tannins or have formed indigestible complexes with polyphenols. As a results of poor digestibility, a substantial portion of the dietary nutrients was not available to the fish and subsequently excreted which explained the degeneration of the hepatocytes observed in the liver. Also the histological sections of the fish fed with ginger leaf (Figure 1D) indicated fish protein was compromised histologically, this is in alignment with the findings of (Ashade et al,) who revealed that the liver of fish fed with ginger peel had severe fatty change which lead to degeneration of the hepatocytes.

Histopathology of the intestine

The histopathology sections of the fish intestine in the control negative treatment (Figure 2A) showed normal mucosal layer with normal gland without inflammation (swollen of the body), the lumen and serosa also appear normal. The intestine of fish in the control negative treatment (Figure 2B) showed eroded mucosal layer and eroded muscularis propria respectively, this is in line with the reports of others authors who have reported a widening of the central stroma within the mucosal folding, thus leading higher amount of the connective tissue and an infiltration of inflammatory cells in the muscular propria [8].The intestine of the fish fed with (Ugwu leaf) and (Ginger leaf) shows normal mucosal layer, lumen and sub mucosal layer. This is corroborated with the work of Dauda, (2012) who observed no digestive tract damage done to the broiler chickens fed with ginger as by product meal.

The alterations in protein profile in muscle of test fish may indicate that the fish are prone to stress due to infection of bacteria strain *Pseudomonas fluorescence* causing marked alteration in the protein bands. Begum also found biochemical alterations in muscle tissue of Clarius batracus during insecticide treatment [9]. Various authors (Boone and Vijayan, 2002; Tabche et al., 2002; Ali et al., 2003) reported synthesis of stress proteins due to heavy metal treatment. When the fish is subjected to stress, a group of new or existing cellular protein synthesis may occur to combat the pathogenic effects. Such stress oriented proteins are called as stress proteins (heat shock protein which can be induced by oxidants, toxins, heavy metals, bacteria, viruses etc.).

This stress response can be assessed by the tissue/organ damage. The alterations in tissue protein level could be an important clinical indicator test for tissue specific pathogenicity. The present study demonstrated that there were definite quantitative alterations in protein bands and their intensity/ concentrations in the muscle. The protein bands in muscle showed remarkable changes in the experimental groups of fish treated with *Pseudomonas fluorescence*. These observations are similar to that of Udgata et al. who found that the antigens of *A. liquefaciens* produce a very good primary and secondary response by producing antibodies in fish [10-13].

Conclusion

The African catfish *C. gariepinus* was used in this study as a test organism. *C .gariepinus* is the most widely cultivated species in Africa due their commercial value and nutritional benefits. Plants have been used as sources of remedies for the treatment of many diseases since ancient times and people of all continents especially Africa practiced this old tradition. The current study showed that Nano encapsulated phytogenes has a beneficial effects to the well-being of the fish.

Clarias gariepinus fed with Nano encapsulated diets were able to show less histopathological alterations to those fed with control diets. Observed histopathology anomalies in the liver and intestine includes degeneration of the hepatocytes, cytoplasmic vacoulation, alteration of the sinusoids, congestion of portal vein, eroded mucosal layer and eroded muscularis propria. The histopathology changes in the liver and intestine could be as a result of medicinal effects of the phytogenes which acts as inducer in stimulating the natural or innate immune system of the exposed fish to be resistant to pathogenic stressors. However, phytogenes may contain Anti nutritional factors such as tannins, lectins which could resulted to alterations in the molecular weight of the protein to those in the control diet.

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