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EFFECTS OF ONION *(Allium cepa)* AND CHLORAMPHENICOL ON HAEMATOLOGICAL PARAMETERS, HISTOPATHOLOGY AND SURVIVAL OF CATFISH Clarias gariepinus (burchell, 1822) SUB-ADULT INFECTED WITH *Pseudomonas aeruginosa*

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Summary

The study was carried out to evaluate the antimicrobial characteristics, immunostimulant ability and survival of *P. aeruginosa* infected *Clarias gariepinus* sub-adult exposed to chloramphenicol and *Allium cepa* (onion). Ninety *Clarias gariepinus* sub-adult fishes were used for the study. They were divided into three groups of thirty fishes each. The antibacterial efficacy of chloramphenicol and onion extract was screened against type culture of *P. aeruginosa* ATCC 27853 at 100%, 75% and 50% concentration using agar well diffusion method. Minimum inhibitory concentration (MIC) was determined using determined. *P. aeruginosa* infected fish were exposed to chloramphenicol and onion *in vivo* in prolonged bath treatment twice daily for 7 days. A total of six (6) experimental fish from the treatments were tested for their cellular immune response to *P. aeruginosa* and to the different treatment. Histological changes were evaluated before, after challenge and after treatment. Percentage survival was calculated by recording number of mortality. There was no significant ($p > 0.05$) difference in their susceptibility to the test bacteria. However, MIC for chloramphenicol and onion were greater than 50mg/ml and MBC of 50mg/ml was only obtained for onion. Haematological values of infected fish revealed significant ($p<0.05$) decrease in Parked cell volume (PCV-22.33±0.3%), haemoglobin (Hb-6.97±0.2g/dl), red blood cell (RBC-1.27±0.1x10^6/mm^3), lymphocyte (52.33±0.9%), and significant ($p<0.05$) increase in white blood cell (WBC, 17.13±0.5 x10^3/mm^3), neutrophil (47.33±1.2%) than that of control fish with PCV (34.67±5.2%), HB (9.77±0.2 g/dl), RBC (2.23±0.3 x10^6/mm^3), lymphocyte (69.00±2.3%) and WBC (10.80±0.3 x10^3/mm^3), neutrophil (30.33±2.9%) but no significant ($p>0.05$) changes were observed in all blood parameters among infected fish treated with chloramphenicol and *A. cepa*. Photomicrograph of damaged organs (gills, liver, and stomach) showed moderate regeneration of organs exposed to chlorampenicol and onion. Percentage survival was high in infected and treated experimental fish. Antibacterial potentials of onion can therefore be exploited as alternative in combating infections of *P. aeruginosa* in fish.

Key words: *Allium cepa, Clarias gariepinus, chloramphenicol, Haematology, Histology Pseudomonas aeruginosa, Survival*

Introduction

Culturing fishes in un-hygienic conditions may expose them to disease outbreak as a result of poor environmental condition and microbial agents. Of these microbial agents, bacteria pose a greater threat to aquaculture (20; 14). Bacterial infection can wipe out the entire fish population in the culture environment. In order to combat this challenge, chemotherapy has been largely used aside husbandry management practices. Different families of antibiotics have been used to achieve chemotherapeutic aims which could either be bacteriostatic or bactericidal. Chloramphenicol is generally used as fish health promoter and in major cases added in fish feed. However, long period exposures to chloramphenicol have been discovered to cause blood dyscrasias such as aplastic anaemia (18). This antibiotic has inherent toxicity resulting in immunotoxicity and recently, it has been incriminated as carcinogenic (18). Apart from the above listed, the use of chloramphenicol and other antibiotics, pose the risk of antibiotics resistance. Therefore, using immunostimulants seems to be an attractive alternative to curb fish disease outbreak (15).

The onion bulb contains numerous organic sulphur compounds, including trans-S-(1-propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, S-propylcysteine sulfoxide, and cycloallinn, flavonoids, phenolic acids, sterols including cholesterol, stigma sterol, saponins, sugars and trace of volatile oil (4). Onion contains more than 100 sulphur compounds (3). They contain chemical compounds believed to have anti-inflammatory, anticholesterol, anticancer, and antioxidant properties, such as quercetin (flavonoid). Thiosulfinates found in onion have been shown to inhibit in-vitro platelet aggregation (7). Onion is also used as antiseptic, antihelminthes, antibacterial, carminative etc. Flavonoids found in onion are chemical compounds active against microorganisms. They have been found in-vitro to be effective antimicrobial substance against a wide array of microorganisms (8).

These problems of antibiotics and the attendant positive effects of natural products such as onion, has led to this study which is aimed at comparing the effects of *A. cepa* (onion) as alternative antibiotic to chloramphenicol against *P. aeruginosa* in cat-fish (*C. gariepinus*) sub-adult.

Materials and Methods

Material, location and period of research

Experimental fish used for this study were obtained from a reputable fish farm in Abeokuta, Ogun State, Nigeria. Onion was obtained from Kuto mark*et al*so within Abeokuta metropolis. The laboratory study was conducted at the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta. The *in-vivo* experiment took place in the fish hatchery of the Department of Aquaculture and Fisheries Management, College of Environmental Resources Management of the same University between April and July, 2012.

Extraction of bio-active compounds

100g of onion bulbs were peeled, washed, cut into pieces and crushed using sterile mortar and pestle. It was then percolated in 500ml of distilled water for three (3) days with intermittent shaking. Filtration

was done using Whattman no.1 filter paper to get fine extract and then extract was collected into a conical flask. Concentration was according to the method of (10) and preserved in a refrigerator at 4^0C until further test.

Experimental fishes.

Ninety *Clarias gariepinus* sub-adult fishes were used for the study. They were divided into three groups of thirty fishes each. Each group had 3 replicates of 10 fishes. They were kept in standard aquaria of the Aquaculture and Fisheries Management, College of Environmental Resources Management of FUNAAB, Abeokuta.

Collection of bacteria

Typed culture of *P. aeruginosa* of the strain ATCC 27853 was collected on nutrient agar slant in Bijou bottle from the National Veterinary Research Institute, Vom-Jos, Plateau state, Nigeria.

In-vitro antimicrobial susceptibility testing

In-vitro antimicrobial susceptibility testing (AST) was carried out to determine the antimicrobial susceptibility of the bacteria pathogen.

Agar well diffusion method

Pure colony of *Pseudomonas aeruginosa* from original stock culture was inoculated into a nutrient broth. The inocula were seeded into a freshly prepared nutrient agar plates by spread plate method and allowed to dry. Wells of 6mm diameter were made using sterile borer into the agar media containing the bacteria inoculum and filled with different concentrations (100%, 75%, and 50%) of both chloramphenicol and onion extract. Plates were then incubated at 37^0C for 24 hours. Zone of inhibition of each of the plant extract and antibiotic was then measured using meter rule.

Minimum Inhibitory Concentration

Doubling serial dilution of each antibiotic and plant extract was prepared in 0.5% peptone water in microtitre plate. Equal volume of bacterial broth was then added into each tube. Microtitre plate was incubated at 37^oC for 24hrs. After 24 hours, glass tubes were then observed for turbidity. The last tube showing no turbidity is the minimum inhibitory concentration – the minimum concentration of antibiotics or plants extract that inhibits the growth of the bacterial.

Minimum Bactericidal Concentration

The glass tube indicating the MIC was further diluted into 5 glass tubes each glass tube was then cultured on nutrient broth agar plate and incubated at 37^oC for 24hrs and observed for growth. The plate showing no growth was recorded as the minimum bactericidal concentration.

Challenge Experiment

Experimental infection of fish was according to the method by (6). Mortalities were recorded and survivors were examined for pathological lesions.

Survival

Percentage survival was calculated by recording mortality on a daily basis after challenge.

Determination of haematological parameters

Half a millitre of blood was collected from experimental fish through the lateral line using 2ml needle and syringe. Blood was collected into an anti-coagulant treated bottle (ethylene diamine tetra acetic acid) to determine the following haematological parameters; packed cell volume (PCV), haemoglobin (Hb), red blood cell count (RBC), total white blood cell count (WBC), differential white blood cell count.

Determination of Packed Cell Volume

Capillary heamatocrit tubes, Hawksley graphic reader, plasticine and centrifuge

were used. A plain capillary tube was filled with blood up to about three quarter length of the tube. The empty end of the tube was filled using plasticine. The sealed tube was centrifuge for 5min in a Hawksley microhaematocrit centrifuge. Each tube was then read using microhaematocrit reader. The values obtained were expressed as a percentage of the total volume of blood.

Haemoglobin estimation

The haemoglobin concentration was determined spectrophotometrically according to the method of Franco, 1984 as described in Cypress diagnostic kit.

Haemoglobin was oxidised by potassium ferricyanide into methaemoglobin, which is converted to cyanomethaemoglobin by potassium cyanide. The intensity of absorbance of the cyanomethaemoglobin is proportional to the haemoglobin concentration. Two test tubes labeled blank (B) sample (S) were placed in a test tube rack. Working reagent which was prepared by adding 2 drops of reagent 1 (dyhydrogen potassium phosphate 2.00mmol/L, potassium ferricyanide 0.60mmol/L and potassium cyanide 77mmol/L) was mixed with 4.9ml of distilled water. 5ml of working reagent was dropped in to the 2 test tubes. 20 microliter of whole blood was added to the tube labeled 'S'. It was thoroughly mixed and allowed to stand for 3 min at room temperature. The absorbance of the sample was measured against the blank. The haemoglobin concentration was calculated as haemoglobin concentration (g/decimeter) = 36.77 x absorbance. 36.77 is a given multiplying factor.

Red Blood Cell Count and Total White Blood Cell Count

The red blood cell count and total white blood cell count were carried out by using the Neubauer haemocytometer.

Red Blood Cell Count

Blood was diluted in the ratio of 1:200 with Dacies fluid (99ml of 3% aqueous solution of sodium citrate and 1ml of 40% formaldehyde) which keeps and preserves the shapes of the red blood cell. Blood was drawn to 0.5mark on the red blood cell pipette. The pipette tip was wiped clean and the fluid drawn to the 101mark. The dilution was mixed and left for 3min after which the counting chamber was charged and the red blood cell counted using x40 objective of a microscope. The total number of cell counted was multiplied by 10,000 and expressed in cubic millimetre.

Total White Blood Cell Count

Using a white blood cell pipette of haematocytometer, blood was drawn to 0.5 marks and diluted at a ratio of 1:20 with white blood cell diluting fluid (2-3% aqueous solution of acetic acid to which gentian violet was added) to the 1L mark on the pipette. The blood and the fluid were gently mixed together. The counting chamber was charged with the dilution and the total white blood cell was counted using x10 objective of the microscope. The total number of cell counted was multiplied by 50 and expressed in cubic millimetre.

Differential White Blood Cell Count

A thin blood film was made by spreading a drop of blood evenly across a clean grease free slide, using a smooth edge spreader. The blood film was fixed with methyl alcohol for 3-5 min and allowed to dry. The smear was stained with giemsa stain and 100 white blood cells were enumerated and the percentage of each differential white cell was extrapolated using oil immersion objective of a microscope.

Histological Techniques

Fish organs (gills, liver and stomach) were collected from experimental fish (one fish per replicate) and kept in formalin for preservation. The organs were then fixed in 10% formalin for three days after which the tissue were dehydrated in graded levels of alcohol 70%-100% in descending order for 3 days to remove the water content and to allow paraffin wax to penetrate the tissue during embedding.

After dehydration, the tissues were cleared in xylene and infiltrated using paraffin wax for embedding. The tissues were sectioned into thin sections (5µm), by means of a rotatory microtome blade (Leica). The sections were floated on a paraffin water bath maintained at a temperature of 2-3^{0}C below melting point of the paraffin wax after which the sections were dried on a slide dryer maintained at a temperature of 3^{0}C higher than the melting point of the paraffin wax used.

After proper drying, the sections were stained with Heamatoxylin and Eosin (HandE) (5) and mounted using Histomount. Necrotic areas were photographed and read accordingly.

Challenged experimental fish were randomly placed into different aquaria at 10 *C. gariepinus* sub-adult per aquarium. Each treatment was replicated thrice.

Therapeutic effects of antibiotics and medicinal plants on infected fish

After 3 weeks of challenge and signs of infection eminent, experimental fish were exposed to both chloramphenicol and onion. Water served as positive control while infected fish without treatment as negative control. Administration was by prolonged bath treatment twice daily for 7 days. Temperature was maintained at room temperature and dissolved oxygen by regular water change. Fish were then observed for pathological changes, bacterial resistance, and subsequent healing process due to herbal treatment and antibiotics.

Statistical analysis

Data generated from this study are presented as the mean (±SE). The difference between the means in the treated groups were compared by one way analysis of variance (ANOVA) using the Statistical Packages for Social Sciences (SPSS - 17) program.

Results

In-vitro efficacy test for chloramphenicol and *A. cepa* on *P. aeruginosa* from catfish (*C. gariepinus)* sub-adult showed that both chloramphenicol and *A. cepa* inhibited the growth of the pathogen. The highest inhibition was demonstrated by chloramphenicol as shown in Table 1. The minimum inhibitory concentration (MIC) results showed that Onion extract achieved the same inhibition level (19.50±0.5) chloramphenicol achieved at 50% at 100% concentration. MBC showed no bactericidal activity for chloramphenicol (Table 2).

Results of the disease challenge test in Table 3 reveal that mortality rate was 83% in infected untreated fish while the percentage mortality in group treated with *Allium cepa* and chloramphenicol following experimental infection with *P. aeruginosa* were 30% and 27% respectively.

Table 1: Sensitivity of *P. aeruginosa* to Chloramphenicol and *A. cepa*

Treatments	**Concentrations (%)** **Mean zone of inhibition (mm)**		
	100	75	50
Allium cepa	19.04±4.0 [a]	14.5±3.0 [b]	9.5±4.0 [c]
	Concentrations (%) **Mean zone of inhibition (mm)**		
	75	50	25
Chloramphenicol	24.0±6.0 [a]	19.5±5.0 [b]	14.5±2.5 [c]

Values in the same column with different superscripts differ significantly ($p<0.05$).

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) exhibited by *P. aeruginosa* to Chloramphenicol and *Allium cepa*

Treatments	**MIC (mg/ml)**	**MBC (mg/ml)**
Chloramphenicol	0.07	0.0
A. cepa	0.19	0.05

Table 3: Mortality rate of experimental fish

Treatment	**Infected**	**Chloramphenicol**	***Allium cepa***
Number of fish challenged	30	30	30
Mortality (N)	25	9	8
Mortality (%)	83%	30%	27%

Results of haematological parameters are presented in Table 4. The results revealed that the average values of packed cell volume (PCV), erythrocyte count (RBC) and the haemoglobin (Hb) of the uninfected control fishes are significantly higher ($p<0.05$) than those of the other groups. Though not statistically significant, the average values of these three haematological parameters of the infected untreated group of fishes were lower than the groups treated with either Chloramphenicol or Onion extract.

The average value of the absolute white blood cells (WBC) of the uninfected control fishes is significantly lower ($p<0.05$) than those of the other groups except for the group treated with Onion extract. The WBC of the infected group is extremely significantly different ($p<0.001$) from all the other groups.

Table 4: Mean values of the Haematological parameters of *P. aeruginosa* infected *Clarias gariepinus* sub-adult exposed to onion and chloramphenicol

Blood Parameters	**Treatments**			
	UNINFECTED	**CHLOR**	**ONION**	**INFECTED**
=PCV (%)	34.67±5.2^{a}	23.33±2.4^{b}	24.33±2.0^{b}	22.33±0.3^{b}
=Hb (g/dl)	9.77±0.2^{a}	7.73±0.8^{b}	7.70±0.5^{b}	6.97±0.2^{b}
=RBC(x10^{12}/L)	2.23±0.3^{a}	1.63±0.1^{b}	1.40±0.1^{b}	1.27±0.1^{b}
=WBC(x10^{9}/L)	10.80±0.3^{a}	13.03±2.7^{ac}	10.77±0.7^{a}	17.13±0.5^{b}
NEU(%)	30.33±2.9^{a}	39.67±3.0^{bc}	38.00±2.7^{b}	47.33±1.2^{c}
LYM (%)	69.00±2.3^{a}	59.67±3.5^{bc}	61.67±2.7^{b}	52.33±0.9^{c}
MON (%)	0.00±0.0^{a}	0.67±0.7^{a}	0.33±0.3^{a}	0.33±0.3^{a}
EOS (%)	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}
BAS (%)	0.67±0.7^{a}	0.33±0.3^{a}	0.00±0.0^{a}	0.00±0.0^{a}

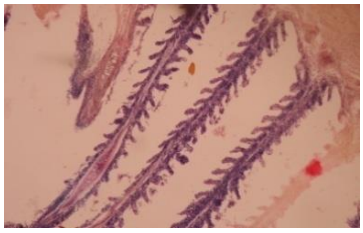
Mean values on the same row having different superscription are significantly different ($p<0.05$)

Results of histopathological test are as shown in Plate 1, 2 and 3. Infected experimental fish exposed to chlorampenicol and onion showed moderate loss of the secondary gill lamellae, regeneration of the hepatocytes with fatty degeneration in liver of infected fish exposed to onion. Stomach of infected treated fish showed rugae of sufficient height.

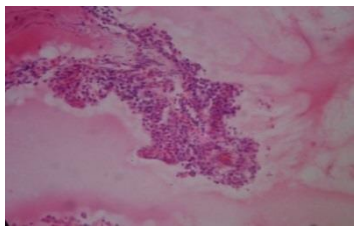
Plate 1: Comparism of the photomicrograph of organs of *P. aeruginosa* infected *Clarias gariepinus* sub-adults exposed to onion and chloramphenicol

1. Gills

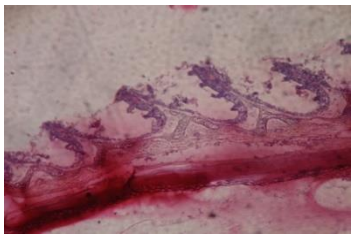
a.

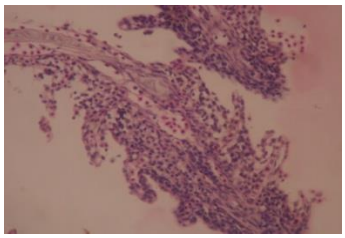


b.



c.



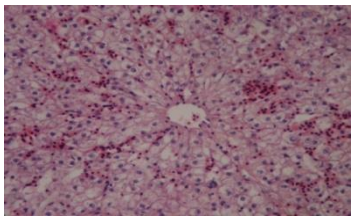
d. 

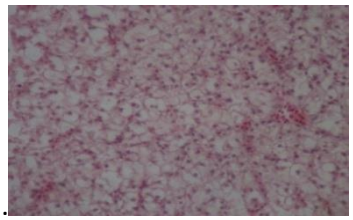
(a) Photomicrograph of the gills showed marked loss and sloughing off of the gill lamellar epithelium of infected fish. (b) Photomicrograph of the gills showed no visible lesion is observed in the gills of uninfected *C. gariepinus*. Magnification X100.
(c) Gills of infected experimental fish exposed to chloramphenicol revealed moderate to marked loss of the secondary gill lamellae, the few present appear far apart. (d) Photomicrograph of Infected fish exposed to onion showed moderate loss of the secondary gill lamellae.

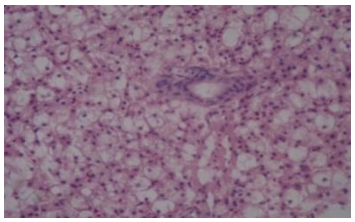
(2a) Photomicrograph of the liver showed that the hepatocytes appear finely reticulated and foamy; there are however a few foci of large cytoplasmic vacuolations of the hepatocytes; the sinusoids are moderately congested.
(b) Photomicrograph of the liver of uninfected fish showed that the liver has a few foci of hepatocytes containing variably-sized cytoplasmic vacuoles.
(c) Photomicrograph of liver of infected fish exposed to chloramphenicol revealed regeneration of the hepatocytes and hepatocytes containing variably-sized cytoplasmic vacuoles.

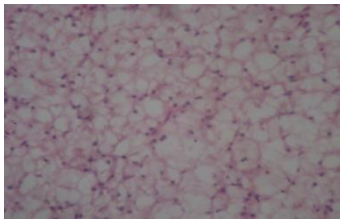
(d) Photomicrograph of the liver of infected fish exposed to onion revealed diffuse, large clear cytoplasmic vacuolations of the hepatocytes.

2. Liver

a. 

b . 

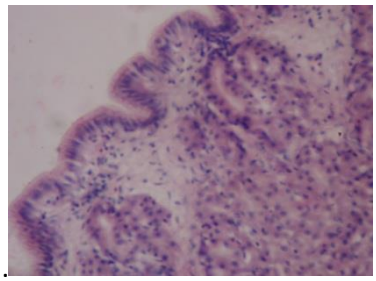
c. 

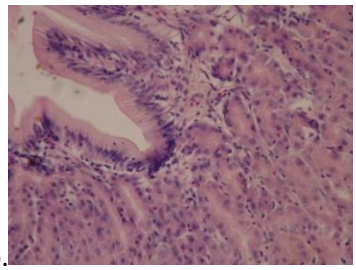
d. 

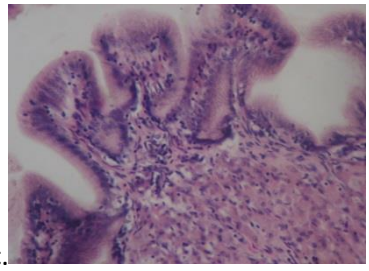
(a) Photomicrograph of the stomach of infected fish showed that the rugae are shortened; the submucosal glands are reduced in numbers; however the surface epithelial cells appear to be proliferating rapidly and immature (hyperplastic).
(b)Photomicrograph of the stomach of uninfected fish showed no visible lesions, the rugae are of sufficient

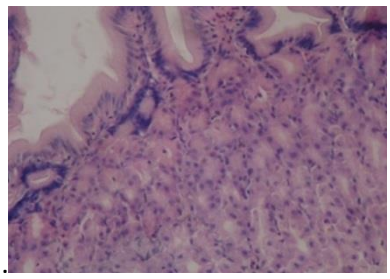
height and have a mature tall columnar surface epithelium.
(c) Photomicrograph of the stomach of experimental fish exposed to chloramphenicol revealed rugae of sufficient height and has a mature tall columnar surface epithelium.
(d) No visible lesion was observed in the stomach of infected fish exposed to onion.

3. Stomach

a. 

b. 

c. 

d. 

Discussion

Plant products have been used extensively as natural antimicrobials and antioxidants. Aqueous extract of *A. cepa* was found to be active against *P. aeruginosa.* It exhibited a high antibacterial activity against the test organism (19.04±4.0mm) and an MIC and MBC of 190mg/ml and 50mg/ml respectively. *A. cepa* when compared with chloramphenicol with MIC of 24.0±6.0mm showed no significant difference in sensitivity to the pathogen. This does not agree with the findings of (1) who observed that aqueous extract of onion did not exhibit any inhibition against *P. aeruginosa.* Findings from this work are in accordance with the research by (4) who reported that aqueous extract of onions and ginger singly showed antibacterial activity against *Staphylococcus aureus* and *P. aeruginosa*. The factors responsible for this high susceptibility of *P. aeruginosa* to aqueous extract of onion is not well known but maybe attributed to the presence of secondary plant metabolites (9).

Survival was highest in infected fish treated with both *A. cepa* and chloramhenicol when compared to that of the infected untreated fish. Similar result was reported in the findings of (18) who reported high rate of mortality in *Aeromonas hydrophila* infected compared to infected fish fed with *Allium sativum* and chloramphenicol at different levels.

There was a significant reduction in the Total red blood cell (RBC), packed cell volume (PCV), and haemoglobin (Hb) of *P. aeruginosa* infected fish compared to uninfected fish. Decreased RBC counts, heamatocrit and haemoglobin concentration indicate that RBC are being destroyed by the leucocytolitic activity usually as a result of infection or disease (12). However, there was marked improvement in the haematological parameters of theexperimental fish exposed to aqueous onion extracts and chloramphenicol. Result revealed that there was no statistically significant difference ($p>0.05$) in the PCV, Hb and RBC of infected experimental fish exposed to aqueous onion extracts and chloramphenicol. The findings is similar to the findings of (17) who reported that both garlic and chloramphenicol increased significantly all blood parameters, growth rate and also reduce bacteria count of water and fish exposed to *A. hydrophila*.

Similar result was obtained in the findings of (19) who reported that *P. aeruginisa* infected fish maintained on ginger and turmeric diet increased in total erythrocyte count, Hb and PCV.

Results from this study corroborate the findings of (18) who reported that experimental fish fed different inclusion levels of garlic and chloramphenicol increased significantly in all blood parameters compared to the control group.

Also, (9) reported significant increase in the PCV of *C. gariepinus* exposed to *Pseudomonas fluorescences* and treated with chloramphenicol when compared to infected untreated fish however, result from his work is inconsistent with the result obtained for Hb and RBC.

The mean value for total WBC (leucocyte) counts of infected fish was observed significantly higher than following exposure to aqueous onion extract and chloramphenicol. Similar result was obtained by (9) who reported a decrease in the WBC of *C. gariepinus* infected with bacteria and treated with antibiotics. This is not in agreement with the findings of (19) who observed that experimental fish *Cirrhinus mrigala* exposed to *P. aeruginosa* and maintained on ginger (*Zingiber officinale*) and turmeric diet had a higher WBC count than both the infected and uninfected fish. However, examination of the differential counts revealed that administration of treatments led to increase in lymphocyte count but decrease in neutrophil count, thus, the total WBC count could be said to have been unaltered. Neutrophils are the major granulocytes to be activated when the body is invaded by

bacteria and they provide the first line of defense against invading microorganisms (11) thus, it is therefore possible that the extract contains agents that fight against the invading pathogen thereby reducing the production of neutrophil by the bone marrow.

Tissue of experimental *C. gariepinus* exposed to *P. aeruginosa* showed varying degradation stages ranging from marked loss and sloughing off of the gill lamellae and epithelium in the gills to few foci of large cytoplasmic vacuolations of the hepatocytes and also the sinusoids were moderately congested in the liver of the infected fish and the stomach showed that rugae are shortened; the submucosal glands are reduced in numbers; however the surface epithelial cells appear to be proliferating rapidly and immature (hyperplastic). However, there was marked improvement in the tissues of experimental fish exposed to aqueous onion extract and chloramphenicol. Gills of experimental fish exposed to aqueous onion extract and chloramphenicol revealed moderate regeneration of the primary and secondary gill lamellae. Therefore, the disruption of the normal functioning of the gills caused by the invading pathogen could be said to have been ameliorated by the action of both the plant extracts and antibiotics. This is inconsistent with the findings of (16) who reported that the gills of rainbow trout (*Oncorhynchus mykiss*) exposed to b-lactams revealed a chronic bronchitis (inflammation of the mucous membrane in the airways bronchial tubes of the lungs, resulting from infection or irritation and causing breathing problems and severe coughing) with an abundant eosinophilic infiltrate

Result obtained from this study also showed that the liver of *P. aeruginosa* infected fish exposed to onion revealed severe fatty degeneration of the hepatocytes. On the other hand, few foci of hepatocytes were observed in infected fish exposed to chloramphenicol containing variably sized cytoplasmic vacuoles. This finding is consistent with the findings of earlier study by (21) who reported that the liver of Nile tilapia (*O. niloticus*) challenged with *Clostridium* and exposed to enrofloxacine showed vacuolation of the hepatic parenchyma together with slight congestion. They also reported that Nile tilapia challenged with *Pseudomonas florescence* revealed vacuolar degeneration of the hepatic parenchyma while infected fish exposed to both enrofloxacine and florfenicol revealed mild vacuolar degeneration of the hepatic parenchyma. That is with bacteria invasion; the liver undergoes different stages of enlargement and also increase in number but with the introduction of antibiotics and plant extracts, the effect is ameliorated.

No visible lesion was observed in the stomach of infected fish exposed to both aqueous onion extract and chloramphenicol. This could infer that both treatments have therapeutic capability on the text pathogen. The ability of onion to heal could be attributed to the presence of secondary plant metabolites which have both antibacterial and immunostimulant activity.

Conclusively, *Allium cepa* (onion) has been proven to possess high antibacterial activity and thus can be used in the treatment of bacteria diseases and also as immune booster to prevent bacteria (*P. aeruginosa*) invasion in cat fish farms thereby, alleviating the many side effects associated with the use of synthetic antibiotics.

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