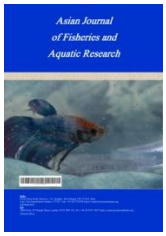

Asian Journal of Fisheries and Aquatic Research

15(6): 148-156, 2021; Article no.AJFAR.79502
ISSN: 2582-3760

Effect of Dietary *Persea americana* on the Organosomatic Indices, Diseases Resistance and Liver Histopathlology of *Clarias gariepinus* Expose *to Klebsiella pneumoniae*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJFAR/2021/v15i630360
Editor(s):
(1) Prof. Ahmed Karmaoui, University Moulay Ismail, Morocco.
Reviewers:
(1) Ni Made Ayu Suardani Singapurwa, Warmadewa University, Indonesia.
(2) Shayenne Elizianne Ramos, Escola Superior de Cruzeiro, Brazil.
Complete Peer review History, details of the editor(s), Reviewers and additional Reviewers are available here: https://www.sdiarticle5.com/review-history/79502

Original Research Article

Received 22 October 2021
Accepted 24 December 2021
Published 26 December 2021

ABSTRACT

The experiment was carried out to evaluate the prophylactic efficiency of dietary powdered *Persea americana* leaves on the organosomatic indices, disease resistance, and liver histopathology of *Clarias gariepinus* exposed to *Klebsiella pneumoniae.* Five (5) different isonitrogenous diets with varying percentages of *P. americana* powered leaves inclusion were formulated as follows: Do(0%); D1(3%); D3 (6%); D3 (9%) and D4 (12%). One hundred and fifty (150) *C. gariepinus* were distributed in five groups in triplicates of ten (10), and fed diets Do-D4 accordingly. After eight (8) weeks of feeding, they were injected intraperitoneally with *klebsiella pneumoniae* at days 1, 7, 14 and 21. After twenty eight (28) days post-infection period, three fish from each of the triplicate were sacrificed for evaluation of the Hepatosomatic index (HSI); Cistosomatic index (CSI), and Spleenosomatic index (SSI), and the liver was taken to the laboratory for histopathological analysis. The survival rate was calculated in each of the groups and the disease resistance was determined. At the end of the experiment, serious ulcerations were observed on the fish fed Do and infected with *K. pneumonia*, while the fish fed D1 – D4 were ulcer- free. The result reveals that the SSI and CSI were similar in all the groups (Do-D4), but the HSI was higher (P>0.05) in the group fed Do (control) compared to the group fed D1-D4. The survival rate and the diseases were

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Lower (<0.5) in the group Do compared to the group fed D1-D4, though it was not dose dependant. The result of the histopathology shows that the liver of the fish fed Do has bloodstain in the portal vein and sinusoid, while the liver of the fish fed D1-D4 have no bloodstain. The experiment reveals that *Persea americana* powdered leaves as applied is an anti-liver inflammatory herb, disease resistant and anti-bacterial.

Keywords: Aquaculture; histology; organosomatic Indices; disease and klensilla pneumonia.

1. INTRODUCTION

Aquaculture is the rearing of aquatic organisms in conducive and controlled environments or enclosures. It is one of the fast-growing food sectors in the world [1]. The demand for fish and fish products has been on the high side due to population increase, and this cannot be met by the supply from natural water bodies. Environmental degradation and population increase have affected aquaculture in developing countries [2], but agriculture and science have been developing ways to enhance productivity in aquaculture to meet up the high population demand and reduce the effects of environmental degradation [3]. Nutrients contained in fish and fish products could go a long way to solve the world's malnutrition problems [4], and more than half a billion people all over the world are relying on aquaculture and fisheries for a living [5].

One of the problems associated with aquaculture practice is the presence of microorganisms. And this is a result of so many factors, including maladministration of feed [6]. The presence of micro-organisms makes fish susceptible to diseases, which cause death and retard growth [7], and most of these diseases are caused by bacteria, viruses, fungi, parasites, etc. [8]. There is a serious need to improve disease resistance in aquaculture to enhance feed acceptability and growth [9,10].

Synthetic drugs and chemicals have been used in aquaculture for the prevention and eradication of diseases, but their uses are associated with disadvantages such as polluting the environment, depositing on fish flesh, drug resistance, etc [10]. The use of herb and herbal products is fast replacing the use of chemicals, because it does not pollute the environment, deposit on fish flesh, and are not immunospecific [10].

The presence of disease or pathogen causing disease can be assessed in fish in so many ways: Haematological analysis [11,12]; Organosomatic indices [13,14], Biochemical parameters [15,16] Histopathological analysis [17] among others.

Several herbs and herbal extracts have shown their potency as antibacterial in aquaculture, they include *Caricapapay* aqueous root extracts [18], *Persea amerciana* aqueous leaves extracts [19], *Azardirachta/curcuma lionga* aqueous extracts [20], etc.

The *Persea americana* leaves extracts have been reported to contain medicinal phytochemicals [21, 22]. This research work tends to assess the efficacy of *P. americanan* powered leave as in *Clarias gariepinus* infected with *Klebsiella pneumonia.*

2. MATERIALS AND METHODS

2.1 Experimental Fish

The experimental fish was purchased in Idi-Onyana farms along Abua Ahoada road in Abua/Odual Local Government Area of Rivers State.

2.2 Experimental Diets

Persea americana leaves were harvested within Port Harcourt, Rivers State, Nigeria. They were air-dried to constant weight, grounded to powder, sieved, and stored. The sieved powdered leaves were added to 38.35%Cp formulated diets Do, at 3%(D1), 6% (D2), 9% (D3), and 12% (D4) respectively following the method of [23].

2.3 Experimental Procedure

150 catfish (117.80 ± 0.11g and 25.88 ± 0.14cm) were stocked in the experimental tanks in triplicate at 10 fish per tank. Feeding commenced 24 (twenty-four) hours after stocking, and the fish were fed with the experimented diets (Do – D4) accordingly at 5% body weight per day, two (2) times daily. After 8 weeks of feeding, the fish were infected

intraperitoneally with *klebsiella pneumoniae* at days 1, 7, 14 and 21 with 1.5ml of $1.9x10^5$ *cfu*/ml of overnight grown *K. pneumonia.* After 28 days post-infection, the liver, spleen, heart of three fish in all the triplicates were harvested for organosomatic indices, the liver was also analyzed for histopathology, and the disease resistance ability and survival rates were determined in each group

2.4 Determination of Organosomatic Indices

This was determined using the formula

$$\frac{Weight\ of\ Organ}{Weight\ of\ fish} x100 \quad [15]$$

2.5 Histopathological Analysis

The fish liver was taken to the laboratory in sample bottles containing 10% formalin solution. The samples (liver) were manually processed and trimmed using a rotary microtone (LEICA RM 2125 RTS), manufactured by LEKA Brosysteo, Buffalo Grove, U.S.A. Tissues were dewaxed, stained in hematoxylin and eosin for a display of tissue architecture. Stained slides were examined under light microscope at x 10 magnification.

2.6 Anti-pathogenic Ability (Disease Resistance)

This was determined as relative percentage survival, using the formular:

$$RSP=1-\ \frac{\%\,Mortality\ in\ treated}{\%\,Mortality\ in\ control} x100 \quad [24]$$

Where RSP = Relative Survival Percentage

2.7 Data Analysis

The collected data were analyzed using SPSS statistics software 17.0 windows. A one-way analysis of variance (ANOVA) was employed to reveal a significant difference between control and treated groups. Tukey's multiple comparison test was applied to separate treatments with significant differences [25].

3. RESULTS

The result of the Organosomatic indices is shown in Table 1, there was no significant difference in the spleenosomatic and cardiosomatic indices in the experimental fish fed Do – D4. The hepatosomatic index was significantly higher in fish fed Do, but significantly the same in fish fed D1 – D4 diets. The result for the disease resistance fluctuated across the diets, but it was significantly lower in the fish fed Do (control) followed by the fish fed D1 and significantly the same in the fish fed D2 – D4 (Table 2).

Plates 1 (a-f) shows the liver histopathology of the experimental fish infected with *K. pneumonia.* **1a** is the liver of fish fed Do without infection, it has normal portal vein (P) and there are patches of liver vacuolations; **1b** is the liver of fish fed Do and infected with *K. pneumonia*, it has blood- stained portal vein with micro liver vacuoles and sinusoid; **1c** is the liver of fish fed D1 and infected with *K. pneumonia*, it has central vein (CV) with no liver vacuoles and sinusoid; **1d** is the liver of fish fed with D2 and infected with the *K. pneumonia*, it has micro liver vacuoles and sinusoid;.**1e** is the liver of fish fed D3 and infected with *K. pneumonia,* it has a portal vein, micro vacuoles, and sinusoid; while **1f** is the liver of fish fed D4, it has patches of liver vacuolations. Fig. 1 shows a picture of fish fed Do and infected with *K. pneumonia* and Fig. 2 shows a picture of fish fed supplemented diets and injected with *K. pneumonia.*

4. DISCUSSION

4.1 Behaviour Observation

There were no ulcerations on the body of the fish fed D1 – D4 after the infection with *K. pneumonia,* but there was serious ulceration in the fish fed Do and exposed to *K. pneumonia.* Similar result was obtained when *C. gariepinus* was challenged with *P. aeruginosa* and exposed to *Carica Papaya* aqueous root extracts [11]. [26] also reported similar result when *punica* peel and oxytetrayclia were administered on *A. hydrophila* infected *C. gariepinus*. This could be as a result of the phytochemicals present in the *P. americana* powdered leaves that are antibacterial [27-29] also reported that phenotic compounds (as contained in *P. americana* in this work) seriously inhibit microbial activities. The result obtained in this work could also be as a result of improved thrombocytes formation in fish fed D1 – D4, which is believed to prevent injuries in infected fish [30;13].

4.2 Organosomatic Indices

Organosomatic indices such as HSI, SSI, and CSI are used to evaluate the health status of fish and other organisms [31,14,32]. Some of the factors that affect the organ somatic indices in fish include water quality, feed type, presence of pathogen/disease, etc. [14,32,13]. The result of the organ somatic indices after the period of infection in this work reveals that there was no significant difference in the CSI and SSI in the experimental fishes but the HSI value was higher (P>0.05) in the fish fed Do compare to the fish fed D1 – D4. This result is in agreement with the reports of [33] who reported an increase in HSI when *Rattus rattus* was infected with *cysticeness fasciolaris* and [13] when *C. gariepinus* fed dietary mango back were experimentally infected the *P. aeruginosa.* [15] also reported an increase in the size of the liver, spleen, and intraperitoneal fat in the control, when *C. gariepinus* was challenged with *P. aeruginesa* and exposed to *P. americana* aqueous extracts.

Table 1. Organosomatic indices of some organs of *Clarias gariepinus* fed with Avocado pear leaf Supplemented diets and infected with *Klebsiella pneumonia* (Mean ±SD)

Parameters	Diets				
	D0	D1	D2	D3	D4
HSI	2.00±0.25 [a]	1.71±0.09 [b]	1.06±0.21 [b]	1.19±0.02 [b]	1.20±0.08 [b]
CIS	0.08±0.02 [a]	0.15±0.00 [a]	0.78±0.01 [a]	0.10±0.02 [a]	0.06±0.05 [a]
SSI	0.06±0.04 [a]	0.08±0.04 [a]	0.06±0.02 [a]	0.07±0.01 [a]	0.03±0.02 [a]

Means within the same roll with different superscripts are significantly different (p<0.05)
HSI: Hepetasomatic Index; CSI: Cardiosomatic Index and SSI: Spleenosomatic Index

Table 2. Percentage survival and relative survival percentage (Disease Resistance) of *Clarias gariepinus* fed with avocado pear leaf supplemented diets and infected with *Klebsiella pneumonia* (Mean ±SD)

Parameters	Diets				
	D0	D1	D2	D3	D4
% Surv	66.66±5.77 [c]	90.00±10.00 [b]	96.66±5.77 [a]	96.66±5.77 [a]	93.33±11.54 [a]
RSP	0.00±0.00 [d]	69.33±33.85 [c]	91.66±14.43 [a]	91.66±14.43 [a]	77.66±38.68 [b]

Means within the same roll with different superscripts are significantly different (p<0.05) % Surv: Percentage Survival; RSP: Relative Survival Percentage

Fig. 1. Fish Fed Do and Infected with *K. pneumonia*

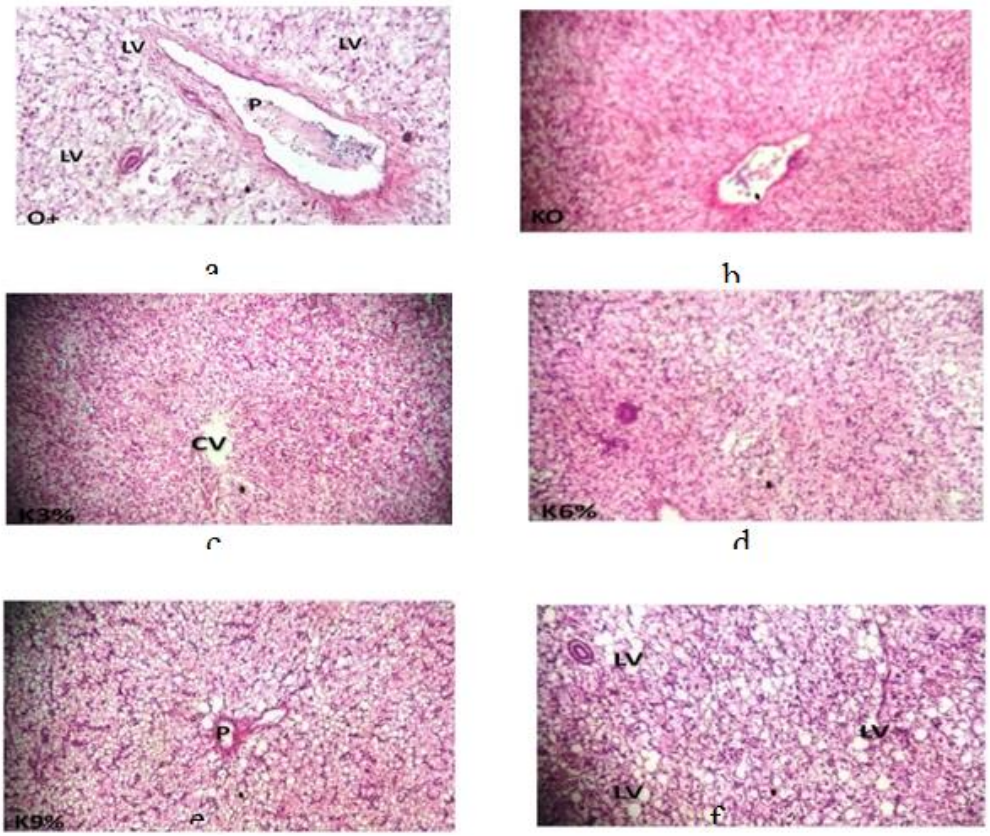

Plate 1. Pathology of liver of *C.gariepinus* fed the experimented diets and exposed to *Klebsiella Pneumonia*

a: liver of fish fed (Do) 0% leaf inclusion diet without infection
b: liver of fish fed (Do) 0% leaf inclusion diet exposed to *K.pneumonae*
c: liver of fish fed (D1) 3% leaf inclusion diet exposed to *K.pneumonae*
d: liver of fish fed (D2) 6% leaf inclusion diet exposed to *K.pneumonae*
e: liver of fish fed (D3) 9% leaf inclusion diet exposed to *K.pneumonae*
f: liver of fish fed (D4) 12% leaf inclusion diet exposed to *K.pneumonae*

Fig. 2. Fish Fed *P. americana* supplemented diet and infected with *K. pneumonia*

The increased HSI in the infected fish fed Do (control) and infected with *K*. *pneumoniae* could be as a result of glycogen depletion [34] arising from the restlessness and rapid opercula movement observed in the fish fed Do when they were injected with the *K. pneumonia* and depletion of glycogen leads to liver inflammation [35]. Lack of glycogen also leads to the metabolic formation of fat in the liver [36] and this could lead to liver weight increase. The lower HSI in the fish fed D1-D4 compared to fish fed Do could be as a result of the antibacterial activities in the experimental herb [37;18]. It could also be a result of the anti- inflammatory effects of flavonoids and Saponins present in the *P. americana* powdered leaves [38; 15].

4.3 Liver Histopathology

There was a high presence of liver vacuoles in the liver of fish fed 0% leaf inclusion diet without exposure to *K. pneumonia* (positive control), but the liver of the fish fed 0% leaf inclusion diet and exposed to *K. pneumonia* (negative control) had no vacuoles, but minor necrosis. This result is similar to the result of [38] when *Lactobacillus acidophilus* was used as a biocontrol agent against *Clarias gariepinus* juveniles infected with pathogenic bacteria (*Aeromodas hydrophila*). The liver of the fish fed D1 – D4 and exposed to *K. pneumonia* had little presence of liver vacuolation, with more in the fish fed D4. The high presence of liver vacuolation in the positive control could be as a result of the presence of high glycogen due to energy intake and use, as a ($P<0.05$) in fish fed Do compare to the fish fed D1-D4, though it was not dose dependent. The result is similar to the reports of [13] who reported the effect of mango bark extract on *C. gariepinus* infected with *P. aeruginosa*; [46] who reported the effect of neem leaf powder on common carp infected with *A. hydrophila*. [47] also reported high disease resistance when *P. aeruginosa* infected *C. gariepinus* was exposed to *Carica papaya* aqueous extracts and attributed the results to the presence of phytochemicals such as phenols. The high disease resistance in the fish fed D1-D4 could be as a result of the phytochemicals such as flavonoids and other alkaloids present in the *P. americana* leaves that are bactericidal and bacteriostatic [18; 21].

4.4 Disease Resistance

The disease resistance ability which was calculated as relative percentage survival (RSP), and the percentage survival were low ($P<0.05$) in fish fed Do compare to the fish fed D1-D4, and it increased as the percentage inclusion of *P. americana* leaves increased in the diets. The result is similar to the reports of [13] who reported the effect of mango bark extract on *C. gariepinus* infected with *P. aeruginosa*; [46] who reported the effect of neem leaf powder on common carp infected with *A. hydrophila*. [47] also reported high disease resistance when *P. aeruginosa* infected *C. gariepinus* was exposed to *Carica papaya* aqueous extracts and attributed the results to the presence of phytochemicals such as phenols. The high disease resistance in the fish fed D1-D4 could be as a result of the phytochemicals such as flavonoids and other alkaloids present in the *P. americana* leaves that are bactericidal or bacteriostatic [18; 21].

5. CONCLUSION

The result of this research work shows that powered *P. americana* is a good prophylactic agent against ulceration and other skin diseases in *Clarias gariepinus* that may arise from the attack of *K. pneumonia*. Despite being a good antibacterial as seen as the result of the disease resistance in this work, *P. americana* powdered leaves have also proven to be a good anti-inflammatory and anti-necrosis agent in *C. gariepinus* liver in the presence of pathogens such as *K. pneumonia. P. americana* powdered leaves should also be tested for their efficacy against other fish pathogens, to enhance their usage in aquaculture. To this end, we advised that *P. americana* trees should be planted in our farms for the availability of the leaves when needed.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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