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Changes in the histology of *Oreochromis niloticus* Liver Fed crude extract of *Azadirachta indica* Saponins

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Abstract

Effect of sub-lethal concentrations of crude extract of Azadirachta indica saponins on liver of Oreochromis niloticus was investigated. One hundred and eighty fish were divided into 6 groups (represented by O, A, B, C, D and E) were fed for 56 days with varying sub-lethal concentrations of A. indica saponins (0.5, 1.0, 2.0, 4.0 and 8.0 g/kg with 0.0 g/kg as the control) incorporated into basal diet. The withdrawal effect of A. indica saponins was also investigated after 28 days of feeding all the fish with the control diet. Liver were sectioned into 6 µm using standard procedure for tissue sectioning. Fish fed varying concentrations varying concentrations of A. indica saponins, gradual increase in rodlet cells around the vein, dilation of the veins and thickening of the hepatocytes were observed in the liver, as the concentrations increase from 0.5 to 8.0 g/kg with. Withdrawal effects shows gradual decrease in the rodlet cells, gradual splitting of each vein into two and slight distortion in the wall of the vein were also observed. The changes observed in the vein as a result of withdrawal of A. indica saponins could be processes towards reverting to the normal shape. This study infers that, withdrawal of A. indica saponins may gradually reverse the effects on fish especially at lower concentrations.

Keywords: Effects, extracts, rodlet cells, saponins.

Introduction

Investigation into plants with medicinal and phyto-additive potentials in aquaculture is informed by their safety, little or no adverse side effect, and environmentally friendly especially when compared with the synthetic drugs. The medicinal potential of these plant materials lies in their chemical properties that usually create a specific physiological act on the living system. Saponins occur naturally, they are surface – active glycosides that foams. It is one of the many plant metabolites of great importance[1]. In fish culture, saponins extracts have been used to enhance growth and to suppress reproduction Obaroh I.O. et.al.[5]. It has also been reported to have been used to increase size, feed effectiveness and well being in ruminant animals[6].

Histological study of the liver has been suggested to be a sensitive parameter which is important for determining cellular alterations that may occur in organs of the body[7]. Liver as an organ is very sensitive to any source of pollution, and most common changes in the liver that may be observed includes; fatty degeneration of the liver, changes in parenchyma necrosis, growth of rodlet cells around the vein, irregular walls, rupturing, apparent volume reduction, greenish pigment in the interior and vacuolized of hepatocytes Wall M.E., et.al.[8,9]. *Azadirachta indica* saponins has been reported to enhance growth as well as inhibits reproduction in *Oreochromis niloticus* but the aftermath of feeding *A. indica* saponins to *O. niloticus* on the liver is not yet fully known, likewise the withdrawal effects. Thus the need for this study, to examine the effects of *A. indica* saponins on *O. niloticus* liver and to further observe the withdrawal effects of the same on the liver.

Material and Methods

Fresh leaf of *Azadirachta indica* were obtained and shade-dried for on week, after which it was ground and sieved. One hundred grammes (100g) of the ground leaf were soaked in 500 ml of absolute ethanol for 48 hours. The mixture was filtered using No 1., Whatman filter paper, the filtrate was further concentrated in a water bath until it congeal to a semi solid substance.

Crude saponins was isolated according to the methods of[10] and as modified by Wolf J.C. et.al.[11]. One hundred milliliter (100 ml) of diethyl ether was poured into the crude extract in a cornical flask, and stirred very well after which it was transferred into a separating funnel, shaken severally before allowing it to settle. On settling two layers were formed, the bottom layer containing the saponins was separated from the top layer, it was further washed several time with diethyl ether until the solvent became clear (free of the green pigment) the separated top layer was further concentrated in a water bath. 4 g of sodium chloride and 100 ml of Iso-propanol was added to the concentrated saponins content before transferring it into the separating funnel, it was shaken severally and then allowed to

settle, on settling two layers were further formed the top layer containing the crude saponins was separated from the bottom layer the crude saponins contents was further rinsed with 5 g of NaCl and 100 ml of distilled water, after which the crude saponins was concentrated in a water bath to obtained jelly-like substance.

One hundred and eighty juveniles of *Oreochromis niloticus* were grouped into 6 representing the treatments (O, A, B, C, D and E; O serves as the control). Each group had 3 replicates giving an overall of 18 replicates (10 fishes per replicate). Each of the groups were stocked in outdoor concrete tanks (2×2×1.25 m) containing 450 litres of water. Fish were fed 3 % of their total body weight, that is; 1.5 % between the hours of 0800 - 0900 h and 1.5 % between the hours of 1600 - 1700 h. Adjusted was made to the diet ratio based on the weekly weight gain obtained. The water in each tank was replaced weekly. The experimental diets were fed to the fishes for a period of 56 days, after which the control diet which also served as the basal diet was fed to all the fishes in the groups for 28 days.

Livers for histopathological examination were obtained at the termination of 56 days for the test diet and 28 days periods of feeding the fish with the control diet; two fishes were randomly selected from each replicate and sacrificed for these purpose. Tissues were immediately fixed in 10% formalin after removing them from the fish. In the laboratory tissues were progressively dehydrated in increasing grades of alcohol as follows; 10%, 30%, 50%, 70%, 90% and absolute alcohol for 5 minutes each to avoid excess hardening of the tissues, they were cleared with xylene and afterward implanted in paraffin wax for 5 hours. A microtome was used to cut a section of the tissue to 6 µm in thickness, the section was stained in haematoxylin and eosin for 5 minutes and mounted on slides with neutral Canada balsam, (Humason, 1979) and then examined under a microscope at x40 and x100 magnification. Photomicrographs were taken and then compared with that of Morrison C.M. et.al.[12,] Fiuza T.S. et.al.[13].

Results and Discussion

Effects of *A. indica* Saponins on liver of *O. niloticus*: Figure-1 shows, the histology of the livers of O. niloticus fed with different concentrations of crude extract of *Azadirachta indica* saponins. In fish fed with the control diet (Group O), the liver showed normal polyhedral hepatocytes and vein with blood spots, the liver of fish fed 0.5 g/kg diet (Group A), showed normal hepatocytes with slight production of rodlet cells around the wall of the vein, in the fish fed 1.0 g/kg diet (Group B), the liver showed slightly high production of rodlet cells around the wall of the vein (figure-1).

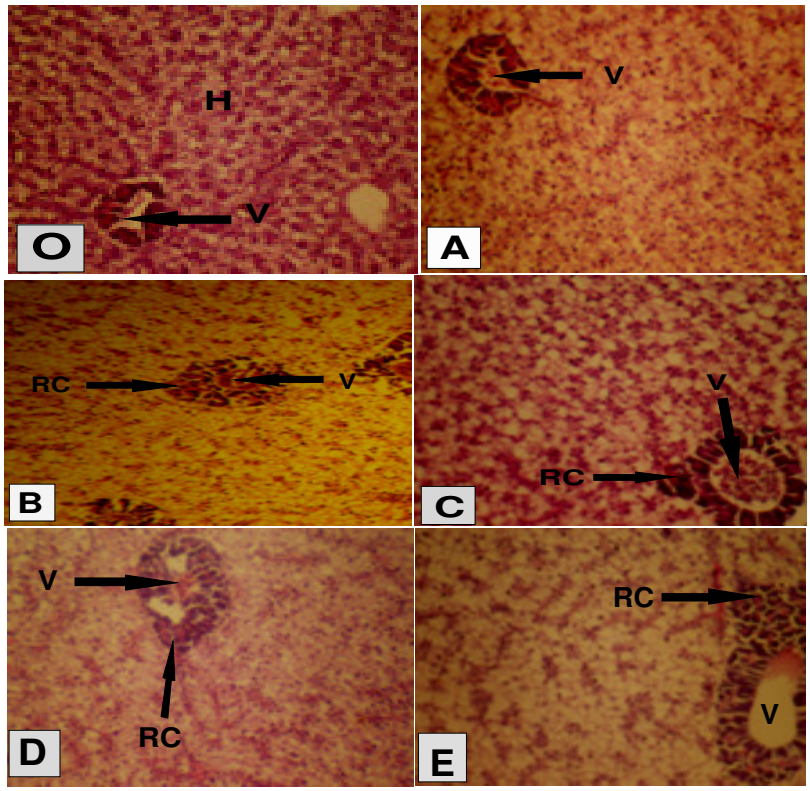

Figure-1
Histology of the livers of O. niloticus fed with different concentrations of crude extract of *Azadirachta indica* saponins

In the fish fed 2.0 g/kg diet (Group C), the liver showed normal oval shape of the vein wall with slightly high production of rodlet cells around the wall of the vein, in fish fed with 4.0 g/kg diet (Group D), the liver showed slightly high production of rodlet cells around the wall of the vein, while in fish fed 8.0 g/kg diet (Group E), the liver showed normal vein wall with slightly higher production of rodlet cells around the wall of the vein and thickening of the hepatocytes.

Figure-1: Sections of testes of *O. niloticus* fed with crude *Azadirachta indica* saponins, (Haematoxylin and Eosin stained). **O:** Fish fed with the control diet (0.0g/kg), showing normal polyhedral hepatocytes (H) and vein (V) with blood spots. Haematoxylin and Eosin stained (x150). **A:** Fish fed 0.5g/kg diet, showing slight production of the rodlet cells (x150). **B:** Fish fed 1.0g/kg diet showing slightly high production of the rodlet cells (RC) (x150). **C:** Fish fed 2.0g/kg diet showing normal oval shape of the vein with slightly high production of the rodlet cells (V) and blood congested vein (x150). **D:** Fish fed 4.0g/kg diet showing oval structure of the vein with slightly high production of the rodlet cells (x150). **E:** Fish fed 8.0g/kg diet also showing the oval structure of the vein (V) with slightly higher production of the rodlet cells (RC) and blood congested vein with thickening of the hepatocytes (x150).

Withdrawal Effects of *Azadirachta indica* Saponins on Liver: The histology of the livers of *O. niloticus* after the withdrawal of crude *Azadirachta indica* saponins is shown in figure-2. In fish fed with the control diet (Group O) the liver showed normal polyhedral hepatocytes and vein with blood spots, while fish previously fed 0.5 g/kg diet (Group A) the liver showed normal hepatocytes, vein partitioned into two with slight production of rodlet cells around the wall of the veins. Slight distortion and partitioning of the vein into two with slight production of rodlet cells around the wall of the veins were seen in the liver of fish fed 1.0 g/kg diet, while fish previously fed 2.0 g/kg diet (Group C) the liver showed distortion and partitioning of the vein with slight production of rodlet cells around the wall of the veins (Plate 20).

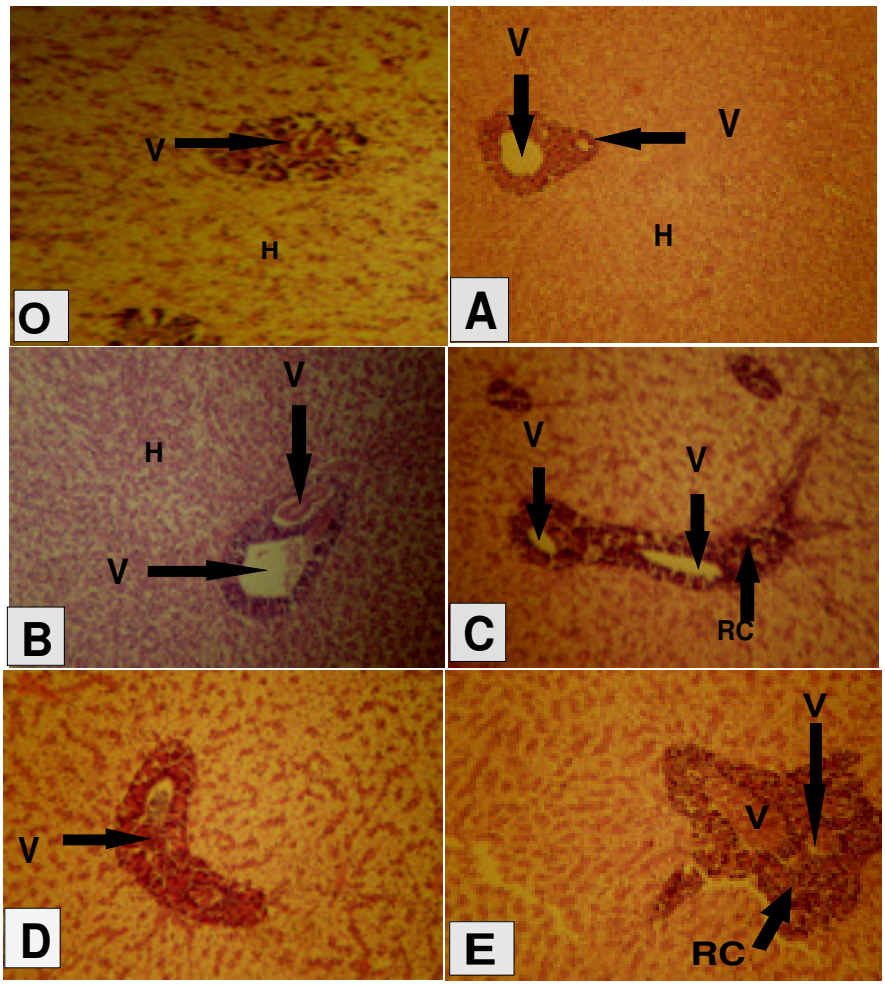

Figure-2
Histology of the livers of *O. niloticus* after the withdrawal of crude *Azadirachta indica* saponins

High distortion in structure of the vein and mild production of rodlet cells around the wall of the vein were seen in liver of fish that were fed with 4.0 g/kg diet, while fish previously fed with 8.0 g/kg diet (Group E) the liver showed distortion and partitioning of the vein into two with high production of rodlet cells around the wall of the veins (figure-2)

Plate 2: Sections of livers of *O. niloticus* fed with the control diet after the withdrawal of crude *Azadirachta indica* saponins, (Haematoxylin and Eosin stained). **O:** Fish fed the control diet (0.0 g/kg), showing normal hepatocytes (H) and vein with blood spots, (150). **A:** Fish previously fed 0.5 g/kg diet, showing two veins (V) attached to each other with slight production of the rodlet cells (RC), (x150). **B:** Fish previously fed 1.0 g/kg diet, showing slightly distorted two veins (V) attached to each other, (x150). **C:** Fish previously fed 2.0 g/kg diet, showing highly distorted two veins (V) attached to each other with mild production of rodlet cells (RC), (x150). **D:** Fish previously fed 4.0 g/kg diet, showing distortion in the oval structure of the vein (V) with slight depression at the middle and mild concentration of rodlet cells (RC), (x150). **E:** Fish previously fed 8.0 g/kg diet, showing distortion in the oval structure of the vein (V) with partial division and high concentration of rodlet cells (RC), (x150).

Discussion and Conclusion

The structural changes in the histology of the gonads and liver of *O. niloticus* fed with different concentration level of crude extracts of *A. Indica, saponins,* showed that, the control had no appreciable alteration in the histology of the livers, but at higher level of inclusion (2.0, 4.0 and 8.0 g/kg) distortion in the shape of the vein and high production of rodlet cells were observed. Dilations and blood congestion were observed in all the liver treated with the plant extracts. Similar observations were made by[9,] Sheikhlar A. et.al.[14, 5] who observed dilation of the wall of the vein and production of high number of rod let cells around the veins as the concentrations of the plant extracts were increased. This findings is in contrast to the report given by Leino R.L. et.al.[15] who observed no alteration in the liver of fish fed [7] gkg^{-1} of *Euphorbia hirta* however, they reported some abnormalities in the liver of fish that received 9 gkg^{-1} of the same plant extract. It has been suggested that rodlet cells may be arouse by certain substances which are generated due to damage to tissues or similar factors[16], high production of rodlet cells especially at high concentrations could be physiological reaction of the fish to toxic effect of the plant extract. The partial and complete splitting of the veins could be an attempt to reverse to the original size and shape. There was no mortality through the period of these studies.

This study infers that, alteration of the liver structure as a result of crude extract of *A. indica* saponins could be reversible after sometimes.

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