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Research Article
Effects of *Moringa oleifera* Saponins Extract on Histology of Liver and Intestine of *Clarias gariepinus*

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Abstract

Background and Objective: The use of phytoadditives in fish culture is on the rise due to their greater advantages over the synthetics drugs. *Moringa oleifera* saponins has a positive effect on fish growth, however little is known about its effects on liver and intestine. Thus, this study was aimed at investigating the effects of crude extract of *Moringa oleifera* saponin on liver and intestine histology of *Clarias gariepinus*. **Materials and Methods:** Crude saponins were isolated from *Moringa oleifera* leaf using standard procedures. Two hundred and forty fish divided into 4 groups (A, B, C and D) and fed for 60 days with varying concentrations of the crude extract (0.0, 0.5, 1.0 and 1.5 g kg^{-1}) incorporated into basal diet. At the end of the study, liver and intestines from fish in each group were sectioned using standard procedure. **Results:** Photomicrograph impression of the fish liver in the control group revealed normal central vein and fats cells. While in the other groups deposit of fat cells distorting the normal architecture of the liver and fibrosis were observed as the concentration of saponin increases. The intestine of fish in the control group revealed normal mucosal lining. While in the other groups normal mucosal lining and gradual increase in the number of inflammatory cells in submucosa were observed as the concentration of saponins increases. **Conclusion:** This study infers that crude extract of *Moringa oleifera* saponins affects the structure of the liver and causes inflammation in the intestine of *Clarias gariepinus* especially at high concentrations.

Key words: *Moringa oleifera*, saponins, liver, intestines, histology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aquaculture plays a vital role in income generation, food security and poverty mitigation[1]. Fish is the cheapest source of animal protein, thus it is in high demand. In order to meet up with the demand there is need to increase productivity by enhancing growth rate.

Over the years attention has been drawn to the use of phytoadditives in the formulation of fish diet in order to enhance growth or to inhibit reproduction for maximum growht[2-7]. This is because plant materials had been observed to be safe, cheap and environmentally friendly when compared with synthetic substances used as growth promoters. The intestine and liver are organs important in digestion and absorption of food nutrients, thus monitoring of these organs is necessary[8]. In view of the increasing use of plant extracts as potential alternative for synthetic hormones and chemotherapeutics, it is necessary to scientifically investigate the effects of plant extracts on the organs of fishes. Appreciable growth response was observed when *Moringa oleifera* leaves were fed to fish[9]. El Tazi[10] also observed promising effects of *M. oleifera* on rabbit. Obaroh *et al.*[7] reported increase in growth rate when *M. oleifera* leave extract was fed to *C. gariepinus* at varying concentrations. Although positive effects of *Moringa oliefera* leave extract on growth of *Clarias gariepinus* was reported. However, there is little or no detailed information on the effects of crude extract of *Moringa oleifera* saponin on *Clarias gariepinus.* Thus, this study was aimed at assessing the effects of crude extract of *Moringa oleifera* saponin on the histology of liver and intestine of *Clarias gariepinus*, baseline data would be provided for further study.

MATERIALS AND METHODS

Experimental site: The experiment was conducted at the Fisheries and Hydrobiology Research Unit, Department of Animal and Environmental Biology, Kebbi State University of Science and Technology, Aliero. Aliero is located in Sudan Savannah vegetation zone of Nigeria it is on latitude 113°S. 12.44°N latitude 36°W. 42°E.

Acquisition, identification and processing of *Moringa oleifera* leaves: *Moringa oleifera* leaves were obtained within the campus by using clean sharp knife. The plant was authenticated at the Herbarium of Plant Science and Biotechnology Department. Leaves were washed with distilled water, shed-dried and ground to powder using pestle and mortar after which they were sieved. One hundred grams of ground leaves of *Moringa oleifera* was weighed and transferred into a conical flask containing 250 mL ethanol. The mixture was covered with cotton wool and capped with aluminum foil for 24 h and kept under room temperature. After 24 h the mixture was filtered using No. 1 Whatman filter paper, the filtrate was further concentrated in water bath.

Extraction of crude saponins: Saponins content of the leaf crude extract was isolated according to the methods of Wall *et al.*[11] and as modified by Hostettmann *et al.*[12]. The crude extract of *M. oleifera* was mixed with 100 mL of diethyl ether in a conical flask and stirred steadily, the homogenous mixture was transferred into a separating funnel and then shaken for 3 min. The separating funnel with it content was mounted on a tripod stand and allowed to settle for 30 min. Afterwards the mixture separated into 2 layers, the lower layer which consist of the crude saponins was removed and treated with diethyl ether to clear off the pigments. Furthermore, 4 g of NaCl and 100 mL of Iso-propanol was introduced into the crude saponins, the mixture was vigorously shaken and allowed to stand for 1 h and subsequently 2 layers were established. The first layer which is the crude saponins was removed. The crude saponin was purified with 5 g of NaCl and 100 mL of distilled water before reducing it to a jelly like substance in water bath.

Preparation of experimental diet: A basal diet of 40% crude protein was prepared. *Moringa oleifera* saponin extract at 0 g kg^{-1} (Diet 1), 0.5 g kg^{-1} (Diet 2), 1.0 g kg^{-1} (Diet 3) and 1.5 g kg^{-1} (Diet 4) were added separately to the basal diet. Feed compositions were measured and mixed properly with the help of an electric feed mixer (Kenwood). The mixed dough were subjected to pelleting using an electric feed pelletizer with 2 mm diameter diet. The pelleted feeds were sundried and stored until the commencement of the feeding trial.

Acquisition of experimental fish: A total of 240 *C. gariepinus* fingerlings of approximately equal body weight were purchased from a private hatchery in Jega Local Government area. The fingerlings were transported to the Fisheries and Hydrobiology Research Unit of Department of Animal and Environmental Biology, Kebbi State University of Science and Technology, Aliero, Nigeria. The experimental fishes were adapted to their environment for one week and they were fed with the basal diet during this period (40% crude protein).

Experimental design: Two hundred forty fingerlings were distributed into 4 rectangular concrete tanks with 60 fingerlings in each tank. The set up was in a completely

randomized designed layout having 4 treatments representing the experimental diets with 3 replicates per treatment. Fishes in each treatment were given feed representing 5% of their body weight for a duration of 12 weeks (starting from 5th May-28th July, 2019) of the feeding trial period. The daily ration was split into 2 and given twice daily at 9:00 am and 5:00 pm. The ration were adjusted weekly based on the new weight gain in each concrete tank. Uneaten feeds together with faecal residues were siphoned out, tanks were cleaned every week and refilled with fresh water.

Histological examination: Eight fish samples (2 per group) were randomly selected and sacrificed using an overdose of 2-phenoxy ethanol. Fish were dissected and after dissection liver and intestine were fixed in 10% neutral buffered formalin. Transverse sections of the central portion of the organ samples were dehydrated using ethanol, embedded in paraffin, sectioned at 4-5 and stained with haematoxylin and eosin (Merck) for histological examination[13]. After the sectioning and staining, slides were examined under a light microscope (CH-2 Olympus-Japan). Photomicrograph impression of each slide was taken and interpreted.

RESULTS

Effects of *Moringa oleifera* saponin on *Clarias gariepinus* liver: Results on the effects of crude extract of *Moringa oleifera* saponin on histology of liver of *Clarias gariepinus* are presented in Fig. 1-4. The group of fish fed with the control diet (0.0 g kg^{-1}, Group A) showed normal central vein and fats cells (Fig. 1). Fish fed with 0.5 g kg^{-1} (Group B) of *M. oleifera* saponins showed deposit of fat cells which alters the normal architecture of the liver (Fig. 2). Fish fed with 1.0 g kg^{-1} (Group C) of *M. oleifera* saponins showed parenchymal and fibrosis with deposition of fat (Fig. 3). While fish fed with 1.5 g kg^{-1} (Group D) of *M. oleifera* saponins showed deposit of fat cells distorting the normal architecture of the liver (Fig. 4).

Effects of *Moringa oleifera* saponin on *Clarias gariepinus* intestine: Fish fed with control diet (0.0 g kg^{-1} Group A) showed normal mucosal lining (Fig. 5). Fish fed with 0.5 g kg^{-1} (Group B) of *M. oleifera* saponins showed inflammatory cells and normal mucosal lining (Fig. 6). Fish fed with 1.0 g kg^{-1} (Group C) of *M. oleifera* saponins showed normal mucosal lining with scanty

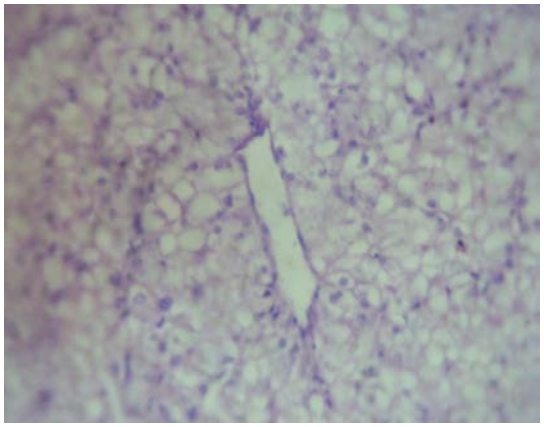

Fig. 2: Photomicrograph of liver section showing deposit of fat cells altering the normal architecture of the liver (0.5 g kg^{-1})
(×200)

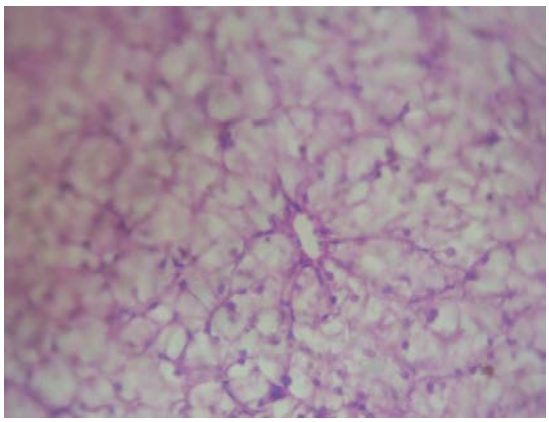

Fig. 1: Photomicrograph of liver section showing central vein and fats cells (0 g kg^{-1})
(×200)

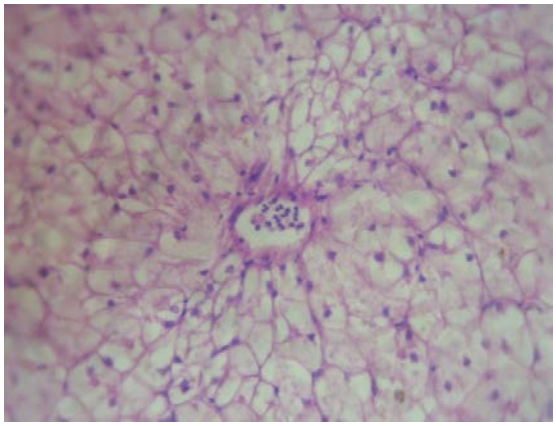

Fig. 3: Photomicrograph of liver section showing parenchymal and fibrosis with fat deposit (1.0 g kg^{-1})
(×200)

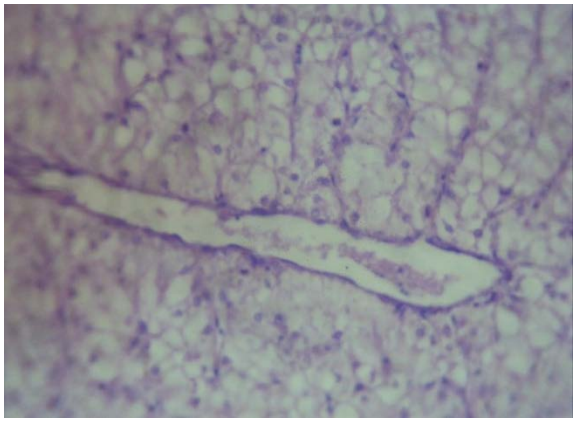

Fig. 4: Photomicrograph of liver section showing deposit of fat cells with distortion of the normal architecture of the liver (1.5 g kg^{-1})
(×200)

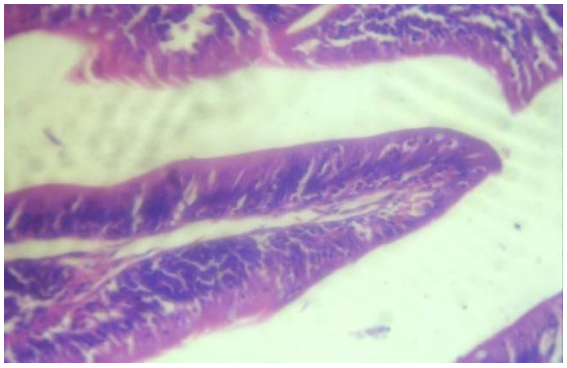

Fig. 5: Photomicrograph of intestinal section showing normal mucosal lining (0 g kg^{-1})
(×200)

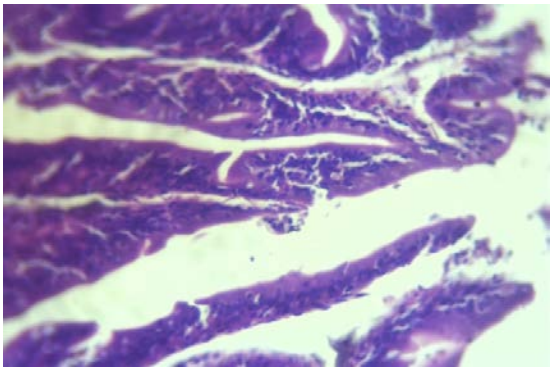

Fig. 6: Photomicrograph of intestine section showing inflammatory cells and normal mucosal lining (0.5g kg^{-1})
(×200)

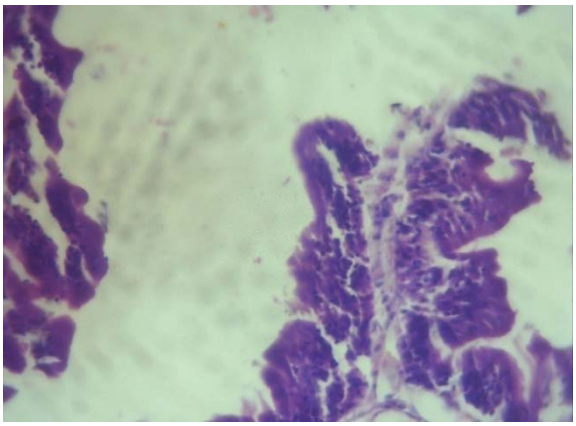

Fig. 7: Photomicrograph of intestinal section showing normal mucosal lining with scanty inflammatory cells in submucosa indicating inflammation of the intestine (1.0 g kg^{-1})
(×200)

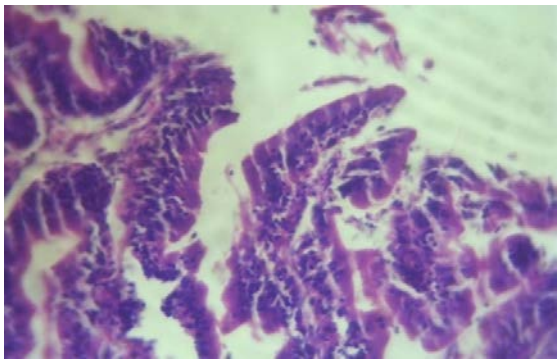

Fig. 8: Photomicrograph of intestinal section showing disorganized structure and high number of inflammatory cells in submucosa (1.5 g kg^{-1})
(×200)

inflammatory cells in submucosa indicating inflammation of the intestine (Fig. 7). While fish fed with 1.5 g kg^{-1} (Group D) *M. oleifera* saponins showed normal mucosal lining with disorganized structure and higher number of inflammatory cells in submucosa (Fig. 8).

DISCUSSION

This study revealed that varying concentration of crude extract of *Moringa oleifera* saponin alters the normal architecture of *Clarias gariepinus* liver with parenchymal and fibrosis at higher level of inclusion. Similar changes were observed by Ayotunde *et al.*[14], who studied the histological

changes in *Oreochromis niloticus* exposed to aqueous extract of *Moringa oleifera* seeds powder and observed dis-arrangement of hepatic cell and necrosis. Sheikhlar *et al.*[15] also observed distortion in the normal architecture of the liver as the concentrations of the plant extracts were increased. However, this findings is in contrast to the report given by Leino[16] who observed no alteration in the liver of fish fed 7 g kg^{-1} of *Euphorbia hirta*, but they reported some abnormalities in the liver of fish that received 9 g kg^{-1}. Obaroh and Nzeh[17] reported distortion in the normal structure of the liver vein and production of rodlet cells.

The results on histology of *Clarias gariepinus* intestine showed that crude extract of *Moringa oleifera* saponin affects the mucosal lining of the intestine with several inflammations cells at higher level of inclusion. In a similar study, Bhattacharjee and Das[18] reported vacuolation of epithelial cell, proliferation of goblet cells, necrotic areas and inflammatory cell infiltration in intestine of *Channa punctate* exposed to 0.03 g L^{-1} lindane. Chowdhary *et al.*[19] also observed alteration in the intestinal architecture in fish fed with plant protein. In contrast, Estruch *et al.*[20] reported no significant alteration in gut histology of gilthead seabream (*Sparus aurata*, L.) fed with high plant protein based diets. It has been reported that inflammation of the mucosa wall is a result of the immune system attacking a harmless virus, bacteria or food in the gut[21]. However, further study is needed to be done on the long term effects of this extract on liver, intestine and biochemical indices of the fish.

CONCLUSION

It can be concluded that lower concentration crude extract of *Moringa oleifera* has mild effects on the liver and intestine of *Clarias gariepinus.* However, at higher concentrations severe effects were observed both on the liver and the intestine of the fish. Based on the findings from this research, it is hereby advised that, crude extract of *M. oleifera* saponins should be used at a minimal concentration.

SIGNIFICANCE STATEMENT

At the end of this study it was generally observed that at high concentration crude extract of *M. oleifera* saponins has a negative impact on the liver and intestine of *C. gariepinus* thus the statement that plants material are 'safe' may not be at the long run as their safety is dose dependent. It is hoped that these findings will assist researchers in unveiling the hidden danger in the use of plant extracts particularly *Moringa oleifera* extract especially at a much higher concentrations. Thus the new theory that the safety of plant extracts is dose/concentration dependent may be arrived at.

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