Nigerian J. Anim. Sci. 2021, 23 (3): 90-98

Toxicological Effects of Ricinus communis Seed Oil on Hepatic and Ovarian Architecture of Female Oreochromis niloticus (Linnaeus, 1758) Broodstock

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***Target Audience:** Fish Breeders, Fisheries Scientists and Researchers.*

Abstract

This study focuses to evaluate the toxicological effects of phytochemical constituents of seed oil extract of Ricinus communis on Nile tilapia. Phytochemical constituents of oil extracted from the seed of mature Ricinus communis plants were screened. Sexually mature female Nile tilapia were oral gavage the seed oil at sub-lethal doses of 1,000mg/kg, 1250mg/kg and 1,500mg/kg body weight and observed for 72 hours post-treatment. Each treatment group was replicated three times. Histological microtome sectioning and staining was carried out to aid microscopic examination of hepatic and ovarian tissues. Increase in the number of rodlet cells and distortion of the vein walls in the fish hepatic tissues were observed with increase in the sublethal concentrations of the seed oil extract, while mild to severe necrosis and granulation of the interstitial tissues were observed in the ovarian tissues as the concentration of the seed oil extract increased from 1000 to 1,500mg/kg. Changes in the liver's histology revealed mild sub-lethal toxicity effect of R. communis seed oil at moderate level of inclusions. Severe alterations in the ovarian architecture probably denote the antifertility potency of R. communis seed oil linked to the presence of steroids.

***Key words:** Histology; Oreochromis niloticus; Ovary; Ricinus communis; Toxicology*

Description of Problem

Oreochromis niloticus (Nile tilapia) is of economic importance in tropical and subtropical countries and considered mostly as an essential food fish nowadays. It is an African freshwater fish that spawns and produces offspring with ease which makes it a good fish to farm. However, this trait of being highly prolific also creates problems such as stunted growth associated with overcrowding (1) which reduce productivity. The characteristic prolific breeding nature of Nile tilapia usually leads to over population in the culture system and stunting in growth of the fish species (2) as a result of depletion of natural food organisms in the pond (1). Unfortunately, available reproduction control methods such as; intermittent harvest, high density effect, manual sexing, use of predators, cage culture, sterility and hybridization (3), to solve this quagmire do not yield optimum results (4), plus they come with adverse side effects. There exists lack of utilization of abundantly available plant materials conferring exceptional reproduction control prowess with very minimal or no side effect (2).

Ricinus communis (Castor bean) is well adapted to the climatic conditions of many parts of the world, but it is however native to Africa (5). A readily available plant with low nutritional competition, containing rich

quantity of steroids, alkaloids (6) and ricinoleic acid (used in contraceptive jelly) could take the place of synthetic and inadequate fish contraceptives with negligible side effect. Also, ricin-A chain (RAC) from the seed extract acts as abortifacient in rabbits (7). The use of castor plant to treat reproductive health related disorders by several ethnic groups in India has been reported (8).

Ricin, found in the protein bodies of the endosperm of castor seeds, is one of the most effective phytotoxins found in nature (9), and has been reported to be toxic. Ricin levels in the seed are said to increase with seed maturation and dehydration (10). Castor seed ingestion has been reported to cause ricin poisoning (11). Reported data show that the median lethal oral dose (LD_{50}) of ricin poisoning in mice is 30ppm of body weight, whereas the lethal oral dose in humans has been estimated to be 1-20ppm of body weight (12). It is important to note that following extraction of castor oil, the toxic glucoprotein ricin is left in the press-cake/castor bean meal. Owing to castor oil seed's significant crude protein content of above 20%, its dietary potentials was investigated (13); the authors recorded significant weight gain in African catfish fingerlings fed varying inclusion levels of Castor oil seeds boiled at 100°C for 50 minutes.

Castor bean seed yields vicious, pale yellow non-volatile and non-drying oil which is one of the few naturally occurring highly pure glycerides due to ricinoleic acid taking a bulk 90% of its fatty acid composition (14). The oil is inexpensive and environmentally friendly. Castor seed oil has been recorded to cause infertility in human (15).

Based, on the foregoing suggestions by various researchers and a dearth of information on the toxicity effects of Castor seed oil on fish, this study will establish basal scientific information on the safety of R. communis seed oil on O. niloticus; taking into cognizance the major organ of detoxification and the organ impacted for contraception; basically, the ovaries.

Materials and Methods

Collection, identification and processing of Castor bean

Mature castor beans in their containing capsules were plucked from Castor plant stands within Zuba, Abuja and were identified at the Herbarium Unit of the Department of Botany, Ahmadu Bello University, Zaria, with the voucher number 01910. The seeds were ground into a pulp, after removing the covering capsules. The pulp (250g) was put through Soxhlet extraction and afforded a slightly cloudy almost colourless oily extract (Castor oil) which was weighed and the percentage calculated and recorded. The oily extract was stored in a dark cupboard till it was needed for administration. Tween 80 solubilized oily extract in water was used during administration, with water serving as the vehicle.

Phytochemical Screening

The extracted Castor seed oil was taken to National Research Institute for Chemical Technology (NARICT), Zaria for phytochemical analysis which included test for alkaloids, tannin, flavonoids, steroids and saponins.

Alkaloids: Ten drops of Meyers reagent was added to 0.5 ml of each extract in a test tube. Cream, yellow or white indicate the presence of alkaloids (16).

Tannins: 0.5 ml extract of solution, 10 ml of distilled water and 1 to 2 drops ferric chloride were added to filtrate. A blue or green black coloration indicates the presence of tannins (17).

Flavonoids: 0.1 ml of each extract was dissolved in NaOH solution. The presence of yellow solution, which disappeared on addition of HCl, indicates the presence of flavonoids (16).

Steroids**:** Concentrated H_2SO_4 (1 ml) was added to 1 ml of test extract. Red colour indicates the presence of steroid ring.

Saponin: 0.5 ml of each extract was shaken with 10 ml of distilled water in a test tube for 5 minutes, the production of frothing which persisted on heating indicate the presence of saponins (16).

Source of Nile tilapia

Sexually mature male and female Nile tilapia between the ages of 4-5 months, weighing 6.5-8.5 g were bought from Bawa farms Ltd., Nuhu wali Street, Rigasa, Kaduna State. They were acclimatized in experimental tanks for 72 hours and fed Ad libitum after purchase. A total of 4 male and 21 female Nile tilapia were used for this study.

Experimental set-up

Two main experiments were staged; pilot study (preliminary study to determine the non-lethal concentration) and contraceptive (oily extract of R. communis) exposure.

Determination of non-lethal concentration

In a quest to determine the toxicity of R. communis seed extract on female Nile tilapia, to establish the LD_{50} and to select the most suitable concentration and doses to be used for further study, three groups of three female Nile tilapia each were orally gavage, following standard procedure (18). This was done at 5000, 2000 and 1000mg/kg body weight respectively, of 500 mg/mL concentration of oily extract-water solution. The fish were observed for 72 hours for any sign of toxicity and death due to the oily extract, as every other factor was kept at optimum. At the end of the study, the lethality status and appropriate doses to be used for further studies was determined. Thirty percent (30%) of the highest non-lethal dose was determined and used as the upper limit of administered doses (19).

Administration of Ricinus communis oil

Fishes were stocked in outdoor plastic tanks supplied with 10 litres of water. In this phase, a total of 16 fish (1male and 3 females per group) were used. The fish were grouped into T_1, T_2, T_3 and C where the females were orally gavage with 1500, 1250 and 1000mg/kg body weight (as derived from the pilot study) respectively, of 500mg/mL concentration of oily extract-water solution of R. comminus seed oil, using Tween 80 as a solubilizer and water as the vehicle. This served as the experimental treatment groups while fish in group C were not administered anything and served as control group. After administration, the female fishes were mixed with the males, with one male fish in every group, to enhance reproductive cues through an exposure window of 72 hours.

Histological examination of hepatic and ovarian tissues

Fresh parts of ovaries and liver removed were preserved and fixed in 10% formalin, and was taken to the Histology Laboratory of Department of Biology for sectioning. The portions of the ovaries and liver removed were put into different concentrations of alcohol, 50% for 2 hours, 70% for 2 hours, 95% for 2 hours and absolute alcohol for 4 hours to effect dehydration in the tissue and were later put into 12.5 chloroform + 37.5 alcohol for 2 hours, 25 chloroform + 25 alcohol for 2 hours, 37.5 chloroform + 12.5 alcohol and pure chloroform to get rid of the alcohol. Histological sectioning was carried out. Haematoxylin and Eosin was used to

stain the ovarian and hepatic tissues to aid gross and microscopic examination (20).

Results and Discussion

A percentage yield of 39.2 of oil was obtained from 250g of intact seeds of R. communis. The quantity of oil afforded by the seeds of R. communis fell within the range of 35% to 55% of the weight of the seeds reported by some authors (21, 22). The result of the preliminary phytochemical screening is shown in Table 1. The seeds have high concentration of steroids and fair concentration of alkaloids. These two phyto-components identified in the seed oil suggest that R. communis can be a potential source of natural antibiotic (23). Other phytochemicals viz; flavonoids, tannins and saponins that were tested for were either below detectable levels or not present. In addition to the phytochemicals detected in this study, two co-researchers detected tannins, saponins, terpenoids and cardiac glycosides in R. communis seed oil (24). This disparity is probably due to the Gas Chromatography-Mass Spectrophotometry (GC-MS) method employed by the researchers for screening.

Table 1: Phytochemicals of Ricinus communis seed oil

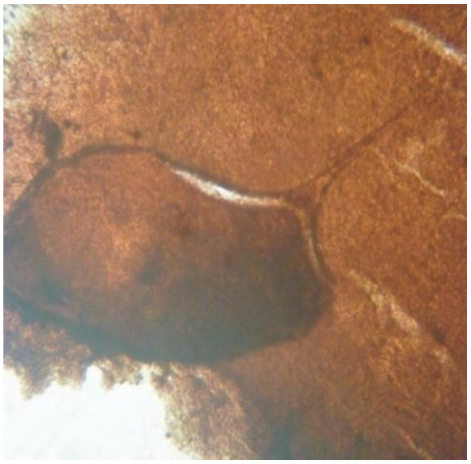
Phytochemical constituents	Present or absent
Alkaloids	++
Flavanoids	-
Tannin	-
Steroid	+++
Saponin	-

Key: +++ = highly present
++ = fairly present
- = absent

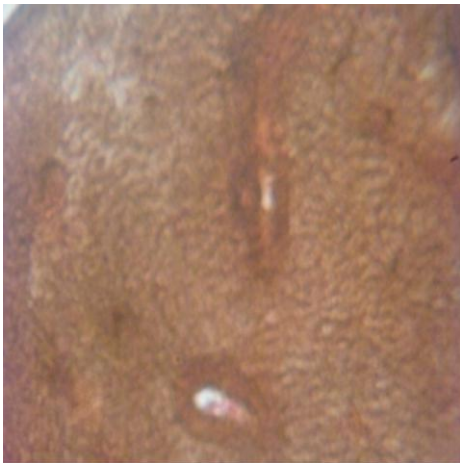
Ricinus communis seed oil posed toxic effect on the liver (Plate I A-D) as all the samples at various inclusion levels showed varying degrees of pathological lesions, disintegration of cells, hydropic degeneration and eventually necrosis. Increase in the rodlet cells observed in this study also suggests possible toxic effect of the plant seed oil extract at higher levels of inclusion. The histopathological study of O. niloticus liver showed increase in the alteration of the hepatic tissues as the seed oil extract inclusion level increased. Gradual distortion in the structure of the veins and increase in the number of rodlet cells were observed in the liver of the fish that received different inclusion levels of the oil extract, suggested that the rodlet cells (RCs) may be stimulated by certain substances produced as a result of tissue injury or related factors, and is reminiscent of leukocyte responses and various chemothatic stimuli (25). Certain steroids have been reported to be connected to hepatotoxicity (26), this is contrary to hepatoprotective activity of ethanol extracts of the leaves of Castor plant reported (27).

Ricinus communis seed oil posed greater toxic effect on the ovaries (Plate II A-D), these architectural distortions increased with dose. Mild to severe necrosis, hyperplasia and atrophy were observed in the ovaries of the treated fish. Granulation of the interstitial tissues was observed in the ovaries as the concentration of the seed oil increased from 1000 – 1500mg/kg. These findings are in tandem with those of (28) on Tilapia zillii

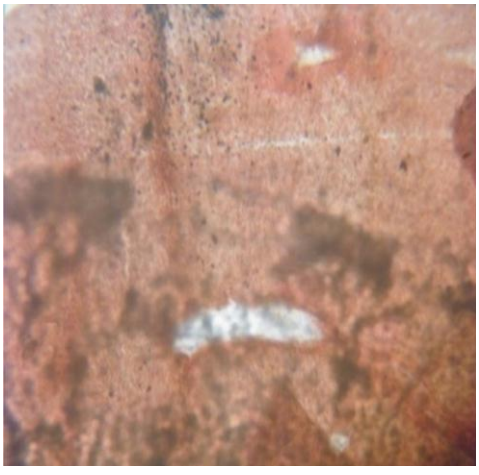
(Gervais) fed Neem (Azadirachta indica) leaf meal diets.



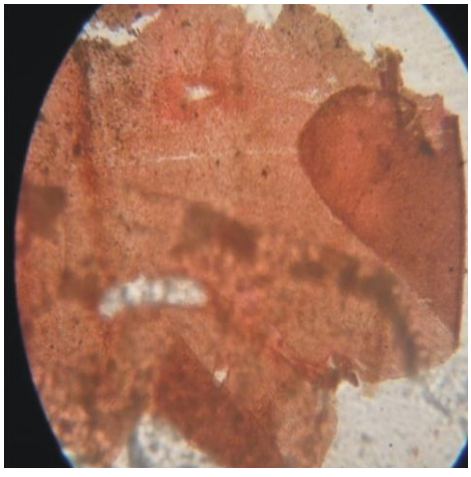
(A) Control liver



(B) T1 liver



(C) T2 liver



(D) T3 liver

Plate I: Cross section of hepatic tissues of O. niloticus administered varying concentrations of Ricinus communis seed oil extract (Haematoxylin and Eosin stained).
A- Control group
B- T_1 (1000mg/kg)
C- T_2 (1250mg/kg)
D- T_3 (1500mg/kg)

Control gonad (A)

T1 gonad (B)

T2 gonad (C)

T3 gonad (D)

O. niloticus administered varying concentrations of Ricinus communis seed oil extract (Haematoxylin and Eosin stained).
A- Control group
B- T_1 (1000mg/kg)
C- T_2 (1250mg/kg)
D- T_3 (1500mg/kg)

Pathological alterations observed in the hepatic and ovarian tissues viz; lesions, disintegration of cells, hydropic degeneration, increase in the rodlet cells, infarction on exposure to R. communis seed oil observed in this study suggest possible toxic effects of the plant extract at higher levels of inclusion. Changes in the architecture of the interstitial tissues of the ovaries were more pronounced than those observed in the hepatic tissues, this suggests that toxicity was not pronounced; the liver being the major organ expected to be impacted. Therefore, the severe necrosis, hyperplasia, atrophy and granulation of the ovarian tissues are probably linked to the high levels of steroids in the Castor seed oil.

Conclusion and Applications

1. The 39.2% oil yield from 250 g of intact seeds of R. communis is representative of the known range of its containing oil.
2. In light of this study, the toxicity status of the seed oil of R. communis on female Nile tilapia has been determined which provides relevant data on the negligible toxicity of the oil on Nile tilapia.
3. Ricinus communis seed oil contains alkaloids and steroids, which were observed to interfere mildly with the architectural structures of the liver of O. niloticus even though these changes were not severe enough to lead to mortality.
4. Changes in the architecture of the interstitial tissues of the ovaries were more pronounced than those observed in the liver, thus suggesting the action of steroids as the concentration of the seed oil increased from 1000 – 1500 mg/kg.

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