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Toxicity of *Raphia vinifera,* P. beauv fruit extracts on biochemical composition of Nile Tilapia (*Oreochromis niloticus,* Trewavas)

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Abstract

Biochemical compositions (total protein = TP, total lipid = TL, globulin = Gl, albumin = Al and albumin/globulin = A/G ratio) of *Oreochromis niloticus* liver, kidney and brain exposed to 0.5 ppm aqueous extracts of *Raphia vinifera* (AERV) were investigated. The various biochemical compositions fluctuated with time of exposure to AERV. TP ranged between 0.91-4.64 mg/dl, Al between 0.41 – 3.02 mg/dl, Gl between 0.70-3.40 mg/dl, A/G between 0.20-3.50 mg/dl and TL between 571.3-998.2 mg/dl. Significant ($P<0.05$) decrease in TP, Gl and TL values of the liver over either the brain or the kidney at 21-day duration in AERV, indicated high energy requirement for detoxification function of the liver. With these biochemical compositions in *O. niloticus*, it is inferred that their uses in the fish varied according to production in the tissue and relative effect of AERV, which produced fish's physiological dysfunction.

Key words: Toxicity, *Raphia vinifera*, Biochemical, *Oreochromis niloticus, B*otanical

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INTRODUCTION

Agriculture is the main economic activity in the South-west Nigeria. However, expanding agriculture causes problems with infringement on protected lands and improper soil use in areas with difficult topographic conditions. Majority of practitioners are artisanal farmers, who often engage in the use of pesticides to control weeds and insect pests in their farms[1]. Organisms are then exposed to several kinds of pesticides, which may lead to toxicological, synergetic, and additive interactions[2].

Detailed knowledge of the various pesticides in use is limited due to lack of control from appropriate authorities. Some of the few known pesticides like DDT, aldrin, gammalin 20, primextra, gramoxone and heptachlor which have been banned by the Federal Ministry of Environment and Water Resources, Nigeria are highly toxic to humans and to aquatic environment[3]. In place of these organochlorines and organophosphate pesticides are biodegradable botanicals, which are plant derivates, containing active alkaloids. Plants that contain such active ingredients that are toxic to fish are known as plant piscicides. Among plant piscicides found in the South-west Nigeria is *Raphia vinifera*.

Raphia vinifera (P. Beauv) Family: Palmae, known as bamboo palm, is particularly abundant along the creeks of Niger Delta, Cross River, Lagos and Ikorodu in Nigeria[4]. The nut contains bitter oil, which has the property of stupefying fish[5]. Nile tilapia, *Oreochromis niloticus* (Trewavas) Family: Cichlidae is a hardy and economically important food fish, which is predominantly cultured in South-west Nigeria.

Many toxicity studies had been carried out on the use of pesticides on fish but very few works were done on the use of plant piscicides on fish biochemical compositions. This study therefore focuses on toxicity of *Raphia vinifera* on biochemical composition of Nile tilapia, *Oreochromis niloticus*

MATERIALS AND METHODS

Preparation of the botanical stock

Fresh fruits of *R. vinifera* were collected from Ajibode Farm, Ibadan. In the laboratory they were washed, oven dried (60^{o}C) for 96 hours, ground into fine powder and stored in a deep freezer at 4^{o}C. Aqueous extraction of the ground powder of *R vinifera* (AERV) was obtained by mixing 100g in 10 litres of water at room temperature, 23 ± 0.5 ^{0}C [6] and allowed to stand for 24 hours. 500 ml or 0.5 ppm AERV concentration was used for the sub-lethal toxicity test.

Collection, transportation and acclimation of test fish

One hundred fingerlings of Nile tilapia, *O. niloticus* (TL 8.7 $\pm$ 0.3 cm) were procured from Oyo State Fish Production Farms, Secretariat, Ibadan, Nigeria. The fingerlings were transported in two aerated polythene bags to the laboratory where they were acclimated to laboratory conditions for 14 days in two rectangular tanks (80L capacity) containing dechlorinated tap water. During this period, fish were fed 2% of their body weight twice daily using Sanders Commercial supplementary feeds (30% protein content) and used water was changed every other day.

Test procedure

Six rectangular glass tanks (10.46L) were used. Scrubbing and washing of the tanks after each use were according to screening toxicity test procedure[7]. Feeding of fish was stopped 24 hours prior to toxicity testing. Ten fish per tank arranged in pair (i.e. treatment and control tanks) were set out in triplicates. The control tank contained extract- free water only. The test media were replaced with freshly prepared concentrations of the same quality every 48 hours to maintain the requisite level and potency of the concentrations. The experiment lasted for 21 days. Two fish per tank were removed every 7-day interval, narcotised with 40% ethyl alcohol soaked cotton tampon put on the gills under the opercula cover and dissected. Three organs (Kidney, liver and brain) were collected separately from treated and control fish and homogenised. The contents were emptied into small bottles, corked and stored in the deep freezer at 4^{o}C for 24 hours.Five biochemical parameters investigated were globulin, total lipids, albumin/globulin ratio, total protein and serum albumin. Total protein and lipids were determined using

Biuret reaction[8]. Serum albumin concentration was determined by the Bromocresol green binding method[9]. Globulin was calculated by subtracting the concentration of albumin from that of the total protein[10]. Albumin/globulin ratio was also determined using the same method[10].

One-way analysis of variance at 50% probability was used to validate the variability within and between each treatment group.

RESULTS

Results of the biochemical parameters tested are shown in Figure 1a-e. Total protein (TP) of the liver (control) was the highest (4.64 mg/dl) while that of the kidney (21-day) was the lowest (0.91mg/dl). 0Liver TP significantly ($P < 0.05$) reduced from 3.90mg/dl on the 7th day to a lower value (1.60 mg/dl) on the 21st day; and further reduced to the lowest value (1.46 mg/dl) in the reversed experiment. Brain TP was significantly lower (1.69 mg/dl) in the control experiment and increased from 2.03 mg/dl on 7th day to 3.89 mg/dl on the 14th day. At the 21st day, however, TP was reduced to 1.0 mg/dl, while the reversed TP increased to 2.53 mg/dl. The TP of the kidney was small, ranging from 0.91 mg/dl (21day) to 1.71 mg/dl (control) and a reversed value of 0.92 mg/dl (Fig 1a).

Albumin (Al) of the kidney reduced from 0.64 mg/dl (control) throughout the duration of exposure and reversed experiment. All the Al values of the kidney were significantly ($P < 0.05$) lower than either of the liver or the brain. Brain Al increased from 1.14 mg/dl in the control experiment to 3.02 mg/dl after 14 days and drastically decreased to 0.47 mg/dl on the 21st day and rose slightly (1.18 mg/dl) in the reversed experiment. Liver Al decreased from 1.24 mg/dl (7-day) to 0.54 mg/dl in the reversed experiment (Fig 1b).

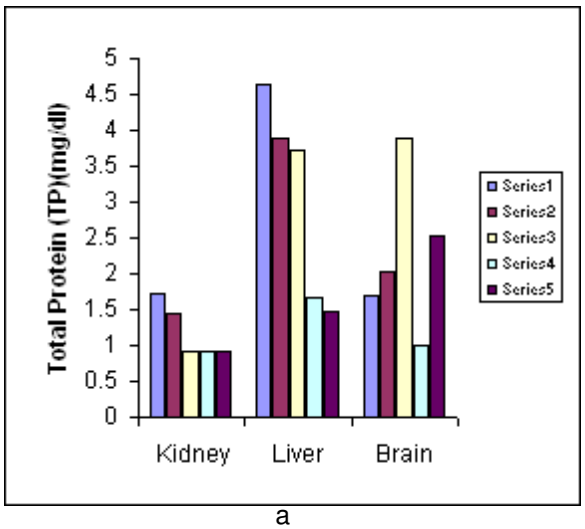
On the 7th and 21st day of experiment, globulin (Gl) of both kidney and liver decreased from 0.59 mg/dl and 3.39 mg/dl to 0.51 mg/dl and 0.79 mg/dl respectively, while in the reversed experiment the values were 0.55 mg/dl and 0.92 mg/dl respectively (Fig 1c). The control values were 1.07 mg/dl in the Kidney and 1.35 mg/dl in the liver. Globulin of the brain increased from 0.55 mg/dl in the control experiment to a higher value (1.35 mg/dl) in the reversed experiment with the lowest value (0.53 mg/dl) recorded on the 21st day.

Albumin/globulin (A/G) ratio of both kidney and brain (Fig Id) increased significantly ($P<0.05$) from 0.61 mg/dl and 2.07 mg/dl (control) to 1.2 mg/dl and 3.5 mg/dl (14-day) respectively. The highest values recorded on the 14th day in both organs decreased to 0.94 mg/dl and 0.89 mg/dl on the 21st day of the experiment reaching a lower value of 0.80 mg/dl and 0.88 mg/dl respectively in the reversed experiment. A/G ratio of liver decreased from 0.40 mg/dl (control) to 0.20 mg/dl (7-day) and 0.30 mg/dl (14-day) before increasing to 1.21 mg/dl (21-day) and 0.61 mg/dl (reversed).

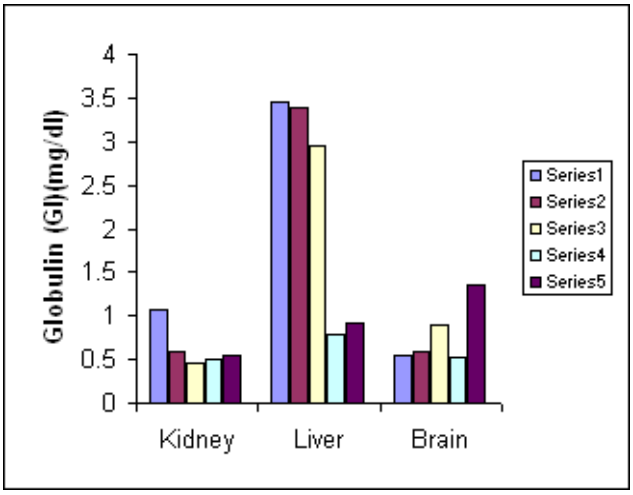
In Fig Ie, total lipid (TL) of the kidney increased from 571.3 mg/dl (control) to 713.5 mg/dl (21-day) and 611.4 mg/dl (reversed). Brain TL also increased from 634 mg/dl (control) to 746.0 mg/dl (7-day), 714.5 mg/dl (21-day) and 644.2 mg/dl (reversed) and reduced to 573.3 mg/dl (14-day) (Fig 1e). Liver TL was higher (998.2 mg/dl) in the control and highest in all the three tissues. The 21-day and reversed experiment recorded decreasing TL values.

DISCUSSION

The significantly reduced total protein values of the kidney and liver from the control experiment throughout the 21-day exposure indicated liver and kidney dysfunction. The least value obtained at 21-day exposure might be attributed to terminal depletion of body reserves of TP[11,12]. The significant increase in brain TP of *O. niloticus* till 14-day showed the activation of the nervous system in combating stress induction of the botanical. However, the significant reduction of TP in the brain of the fish might cause brain malfunction[12]. Reduced albumin (Al) values of the liver and kidney of *O. niloticus* at 7-day exposure showed the stressful condition of fish in AERV. The increase at 14 and 21-day exposure might be a result of production and synthesis of Al far more than the value used for energy supply[13]. The geometric increase of brain Al at 14-day might be explained in the same manner as TP, while its drastic reduction at 21-day might be due to loss of homeostasis in fish[11,14].



a



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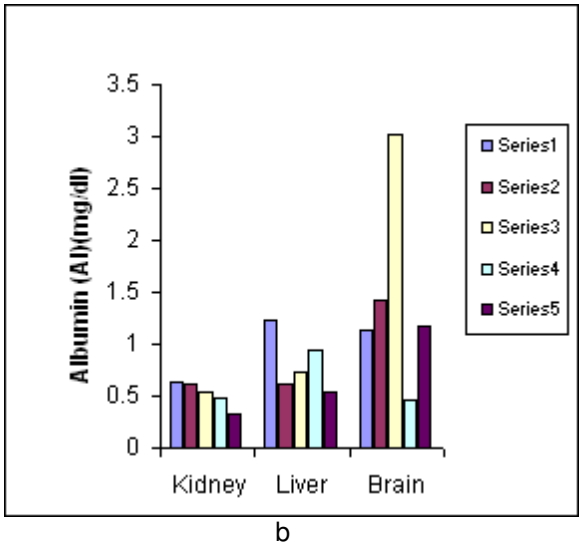

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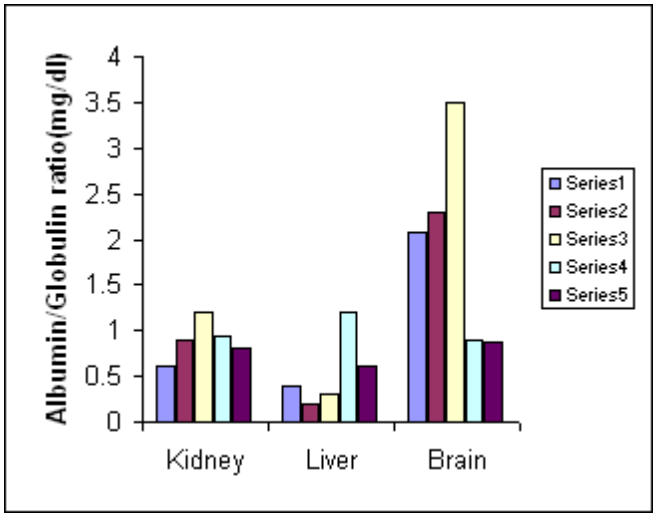

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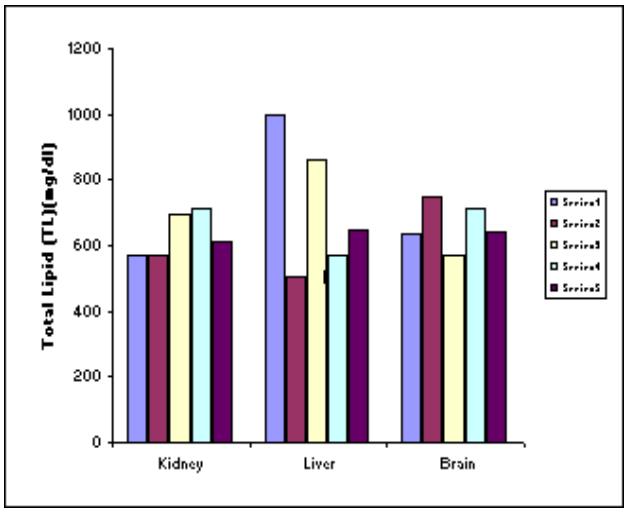

e

Key

Series 1	-	Control
Series 2	-	7-day
Series 3	-	14-day
Series 4	-	21-day
Series 5	-	Reversed

Figure 1 (a-e): Biochemical parameters of *Oreochromis niloticus* tissues exposed to 0.5ppm aqueous extracts of *Raphia vinifera* (AERV) for 21 days.

The significantly higher value of Gl in the liver of *O. niloticus* than the Gl value of its brain or kidney emphasized the importance of liver as an organ of detoxification and globulin as energy buffer for liver function[13,15]. The significant reduction of kidney Gl might account for kidney damage and paralysis of fish excretory system[15]. Slight increase in Gl value of the brain in 21-day exposure further confirmed fish nervous system remedy for effective co-ordination of its activities[12].

The albumin/globulin (A/G) ratios of the liver of *O. niloticus* that were significantly reduced throughout the 21-day exposure period further affirm the importance of these biochemicals in liver function as a detoxifying organ. Also, the significant decrease in A/G ratio of the kidney might have caused kidney malfunction resulting in poor excretion or metabolism of AERV from fish body. Similar observation was made in a North Sea population of viviparous blenny (*Zoarces viviparus*) exposed to chemical effluents[15].

The increased value of total lipid (TL) in the brain of *O. niloticus* might be regarded as a turnover process for an effective brain metabolism. Similar process was reported in the study of biochemical and physiological effects of bleached pulp mill effluents in perch (*Perca fluviatilis*)[16]. Massive production of TL in brain and kidney might be explained either as a result of alteration in plasma ion levels or a change in immunological responses and alteration of carbohydrate metabolism of the fish[17-19]. Therefore this increased TL in fish organs might be joint products of reduced lipid secretion into the plasma, increased lipid synthesis and increased lipid removal rate from circulation to the tissues.

In conclusion, the biochemical composition of the liver, brain and kidney of *O. niloticus* exposed to 0.5ppm AERV showed physiological changes, which might have affected the general health status of the fish thereby confirming the piscicidal importance of this plant.

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