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Comparative effect of Hibiscus Rosa sinensis flowers and leaves hydroethanolic extracts on survival, growth and gonads development of Oreochromis niloticus (Linnaeus, 1758)

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Abstract

The aim of this study was to evaluate the efficacy of Hibiscus Rosa sinensis flowers and leaves hydroethanolic extracts on survival, growth and gonads development of Oreochromis niloticus. To this end, 840 fingerlings of Oreochromis niloticus with an average weight of 7.5 ±1.01g were randomly distributed in 21 tanks with a capacity of 40L each, placed in an earthen pond of approximately 150 m^2 (fed by ground water), at a density of 40 fingerlings per tank. These different fishes were fed with 7 experimental feeds, including 01 control and 06 based on H. rosa sinensis leaves and flowers hydroethanolic extracts at doses of 100, 150 and 200 mg/kg of feed (for the leaves) and 100, 150 and 200 mg/kg of feed (for the flowers) respectively. After 45 days post-treatment, survival and zootechnical growth parameters were assessed. Histological examination of the gonads was used to determine the impact of the various treatments on gonads development at 45 and 60 days post-treatment respectively. The results showed that the dose of extract did not significantly affect fish's mortality. Analysis of the growth parameters of fishes from the different group treated with different doses of H. Rosa sinensis leaves and flowers hydroethanolic extracts revealed that the dose of 100mg/kg of H. rosa sinensis leaves extract had a greater significant effect compared with the other treatments applied. Analysis of the Gonado-somatic index at 45 days post-treatment revealed significantly ($p < 0.001$) lower gonad weight/mass ratios in males treated with the various doses of H. rosa sinensis leaves and flowers hydroethanolic extracts compared with sample from control group (0.415 ± 0.031%). However, at 60 days post-treatment, satellite males from control group and those from group treated with 100mg/kg of flowers and leaves extract recorded a higher Gonado-somatic Index than the other treatments, with Mean values of 0.415 ± 0.022%, 0.431 ± 0.044% (FL'100) and 0.387 ± 0.016% (LE'100) respectively. On the other hand, in females, those treated with a dose of 200mg/kg of flowers recorded a higher Gonado-somatic Index than the other treatments, with a mean value of 1.34 ± 0.37%. Observation at 45 days post-treatment of the testicular structures of males from group treated with extracts showed a dose-dependent alteration in spermatogenesis marked by destruction of spermatozoa. However, in satellite males from group treated with 100 mg/kg of H. rosa sinensis leaves and flowers hydroethanolic extracts, spermatozoa were restored. In satellite females, the integrity of ovarian tissue was only restored in females treated with 100mg/kg of H. Rosa sinensis leaves and flowers hydroethanolic extracts.

Keywords: Hibiscus Rosa sinensis; Oreochromis niloticus; Hydroethanolic extract; Survival; Growth; Gonads development

Introduction

In many developing countries, fish represents an important source of protein of good dietary quality and moderate price [1]. It continues to be one of the most traded food commodities in the world, with more than half of exports by value coming from developing countries. Faced with increasing demand for fisheries resources and stagnating or even declining fisheries resources, aquaculture has expanded and intensified in almost every region of the world. Among the main farmed species, tilapia occupy a prime position and are the group of fish whose production has seen the strongest growth over the last ten years, taking all aquatic species together [2]. With world production estimated at over 4.3 million tons a year, they represent a considerable resource for human consumption, particularly in developing countries. After carp, they constitute the second major group in world aquaculture [3]. In recent years, tilapias have become the predominant species in African commercial fish farming [4]. Of these tilapia species, Oreochromis niloticus is the best known and most widely used, having been the subject of huge research and extension program in Africa and around the world. This species has long been heralded as the gem of African fish farming because of its high market demand, ease of 2 reproduction and rearing, high growth rate and, above all, its relatively plastic diet. However, the development of this production is faced with a major problem, which is paradoxically linked to the high reproduction rate of the species. Tilapias reach sexual maturity early and are able to reproduce at relatively small sizes of 8 to 13cm [5]. This early maturation and their continuous reproduction (once a

Month or even more if the eggs are removed from the oral cavity and if the temperature and photoperiod conditions are favourable) cause rapid overpopulation of the ponds and dwarfism (reduced growth) of the individuals, thus having a negative impact on the production yield on the farms [6,7]. To circumvent these constraints linked to anarchic reproduction and improve yields by producing high-growth individuals, various practices exist and have been developed, including manual sexing, polyculture with predatory species, culture of monosex

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male populations (by hormonal inversion either by administering androgens via the diet or by balneation, masculinisation via thermal shocks, hybridisation, genetic approaches to producing YY males or YY supermales) [8-10]; sterilization (through the use of irradiation, chemosterilants and other reproductive inhibitors), intermittent/ selective harvesting, the use of slow maturing tilapia species, among others [11]. However, all these population control methods have their shortcomings and limitations. It is therefore necessary to examine a less costly and appropriate technology for solving the problem of uncontrolled tilapia farming using biological inhibiting agents. Indeed plant extracts contain various bioactive principles such as alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids, essential oils which have been reported to promote various activities such as anti-stress, growth stimulation, appetite stimulation, tonicity and immunostimulant and antimicrobial properties during fish production. Therefore, plant extracts could be used as safe alternative agents to control tilapia early maturity and prolific reproduction in production systems [12].

A member of the Malvaceae family, Hibiscus rosa sinensis Linn is a glabrous shrub widely cultivated in the tropics as an ornamental plant with a variety of flower shapes and colours [13].

The various parts of this plant have been reported to have various medicinal properties (hypoglicaemic, antitumour, antioxidant, antihypertensive, antipyretic, anti-inflammatory, analgesic, antibacterial, menstrual cycle regulator, contraceptive, etc.) [14-18]. This would justify its use by natural health practitioners [13]. Flavonoids, tannins, terpenoids, saponins and alkaloids are the main phytochemical compounds present in various extracts from the leaves, seeds, bark and stem of Hibiscus rosa-sinensis and are most likely responsible for their biological activities [19]. The flavonoids contained in H. rosa-sinensis extracts are thought to induce anti-implantation and anti-spermatogenic effects in animals [20-23] ,Several authors have demonstrated the anti-fertility, antispermatogenic, anti-oestrogenic and abortive activity of extracts of different parts of the H. rosa-sinensis plant [13,21,24,25]. Vasudeva and Sharma [26] reported post-coital activity of ethereal extract of Hibiscus rosa sinensis roots administered orally to female colony-bred albino rats (Wister strain) and adult albino mice. Jegede [27] studied the control of reproduction in Oreochromis niloticus using Hibiscus rosasinensis (Linn) leaf powder as a reproductive inhibitor. In the course of his work, he showed that a dose of 4.0g/kg administered for 60 days induced disintegration of the spermatids and necrosis of the testes, as well as severe atresia of the ovarian follicles. Kissi and Asoumada [28] evaluated the contraceptive effect of benzene extracts from the flowers of H. Rosa-sinensis on the oestrous and reproductive cycle of juvenile Nile tilapia. . At the end of the study, a disturbance in the oestral cycle was observed in the subjects after 30 days of treatment. In addition, a reduction in the weight of the ovaries, uterus and pituitary gland was observed. The inclusion of H. Rosa-sinensis flower extracts in the diet at a dose of 4g/kg also reportedly induced masculinisation of Nile tilapia fry, producing up to 73.13 ± 6.38% male individuals [29]. However, no comparative study on the efficacy of H. rosa-sinensis flowers and leaves hydroethanolic extracts on the inhibition of gonad development in Oreochromis niloticus (Linn. 1758) juveniles has been carried out. Hence the interest of this research works. various parts of this plant have been reported to have various medicinal properties (hypoglicaemic, antitumour, antioxidant, antihypertensive, antipyretic, anti-inflammatory, analgesic, antibacterial, menstrual cycle regulator, contraceptive, etc.) [14-18] this would justify its use by natural health practitioners [13]. Flavonoids, tannins, terpenoids, saponins and alkaloids are the main phytochemical compounds present in various extracts from the leaves, seeds, bark and stem of Hibiscus rosa-sinensis and are most likely responsible for their biological activities [19]. The flavonoids contained in H. rosa-sinensis extracts are thought to induce anti-implantation and anti-spermatogenic effects in animals [20-23], Several authors have demonstrated the anti-fertility, antispermatogenic, anti-oestrogenic and abortive activity of extracts of different parts of the H. rosa-sinensis plant [13,21,24,25]. Vasudeva and Sharma [26] reported post-coital activity of ethereal extract of Hibiscus rosa-sinensis roots administered orally to female colony-bred albino rats (Wister strain) and adult albino mice. Jegede [27] studied the control of reproduction in Oreochromis niloticus using Hibiscus rosasinensis (Linn) leaf powder as a reproductive inhibitor. In the course of his work, he showed that a dose of 4.0g/kg administered for 60 days induced disintegration of the spermatids and necrosis of the testes, as well as severe atresia of the ovarian follicles. Kissi and Asoumada [28] evaluated the contraceptive effect of benzene extracts from the flowers of H. rosa-sinensis on the oestrous and reproductive cycle of juvenile Nile tilapia. At the end of the study, a disturbance in the oestral cycle was observed in the subjects after 30 days of treatment. In addition, a reduction in the weight of the ovaries, uterus and pituitary gland was observed. The inclusion of H. rosa-sinensis flower extracts in the diet at a dose of 4g/kg also reportedly induced masculinisation of Nile tilapia fry, producing up to 73.13 ± 6.38% male individuals [29]. However, no comparative study on the efficacy of H. rosa-sinensis flowers and leaves hydroethanolic extracts on the inhibition of gonad development in Oreochromis niloticus (Linn. 1758) juveniles has been carried out. Hence the interest of this research works.

Materials and Methods

Experimental Site

The experiment was carried out from 20th January to 20th July 202" in the technical installation of the Green Hope farm located in the locality of Bibé belonging to the Mbankomo District, Mefou and Akono Department, Centre Region of Cameroon and whose geographical coordinates are as follows: 3°43' - 3°45' North Latitude, and 11°24' -11°27' East Longitude, with an average altitude of 764 m Above sea level [30]. The climate of Bibé is subequatorial with tropical tendencies, with four seasons of unequal duration: a long dry season from November to mid-March; a short rainy season from mid-March to mid-June; a short dry season from mid-June to mid-August; and a long rainy season from mid-August to the end of October. The average temperature is around 22.5°C.

Collection and selection of Oreochromis niloticus

840 fingerlings of Oreochromis niloticus with an average weight of 7.5 ± 1.01g from an initial stock of the Green hope farm were used to carry out this work. Acclimatization took place over 14 days. During this period, the fishes were fed ad libitum 3 times a day with a commercial feed name GOUESSANT containing 46% protein and 10% lipids. The photoperiod was 12L/12D. The physicochemical parameters were monitored on a daily basis in order to resolve any problems that might affect the development or growth of the fishes.

Collection of plant material

1.198kg of leaves and 0.598kg of fresh flowers of Hibiscus rosa sinensis were collected from the stems of the plant in their natural habitat nearby the Green Hope farm located in the locality of Bibé (Figure 1). After harvesting, the leaves and flowers were dried for 21 days in the shade out of the sun, as drying in the sun could cause

Figure 1: Harvesting and drying plant material **A)** harvesting of plant material; **B)** weighing of Hibiscus rosa sinensis flowers; **C)** Drying 1 - Leaves 2 – Flowers.

photoreactions that could alter molecules of certain active ingredients [31]. The dried leaves and flowers were then ground into powder using a mechanical mill. The powder obtained was stored in a hermetically sealed jar. It was then taken to the laboratory for extract preparation.

Preparation of Hibiscus Rosa sinensis leaves and flowers hydroethanolic extracts

566g of leaf powder and 133.2g of flower powder from Hibiscus Rosa sinensis respectively were taken and introduced into a beaker containing 3500 ml of ethanol diluted to 30%. The mixture was stirred for 48 hours using a mechanical shaker at 250 rpm. The supernatant was collected and the total volume extracted was filtered using a filter cloth. The extract obtained was then evaporated to dryness under pressure at 45°C using a rotary evaporator. The yield of evaporated dry extract on an initial weight basis was calculated using the following equation:

R (%) = (W1×100)/W2 where W1: weight of the extract after evaporation of the solvent; W2: dry weight of the initial sample. The different yields obtained from the extraction process were as follows: 10.55% for the leaf extract and 15.65% for the Hibiscus rosa sinensis flower extract.

Phytochemical characterisation of Hibiscus rosa sinensis leaves and flowers hydroethanolic extracts

Phytochemical Screening

The phytochemical analysis was carried out on the basis of characteristic colour tests to identify the main chemical groups. The hydroethanolic extracts of Hibiscus rosa sinensis flowers and leaves 4 were analyzed. The different chemical groups were characterized using the techniques described in the work of [32-34].

Identification of tannins (Reaction with 1% ferric chloride)

To 1ml of extract in a test tube was added 2ml of water followed by one or two drops of 1% ferric chloride. The appearance of a blue, blue-black or black color indicates the presence of gallic tannins; a green or dark green color indicates the presence of catechic tannins.

Detection of Flavonoids (sodium hydroxide test)

A few drops of a 10% NaOH solution are added to a tube containing 3ml of the extract solution. A yellow-orange colour indicates the presence of flavonoids.

Identification of anthocyanins

To 5ml of 5% extract, were added 5ml of 10% H_2SO_4, then a base (5 drops of 25% NH_4OH). If the coloration becomes more pronounced with acidification and then turns violet-blue in a basic medium, this indicates the presence of anthocyanins.

Detection of Leucoanthocyanins

To 1ml of extract, 5 ml of hydrochloric alcohol was added. The whole mixture was heated in a water bath for 15 minutes. A cherry red or purplish colour indicates the presence of leucoanthocyanins.

Detection of alkaloids (Buchard reaction)

To 1ml of each solution, 2 drops of Bourchard's reagent (iodine-iodide reagent) are added. The observation of a reddish-brown precipitate indicates a positive reaction.

Detection of steroids (Salkowski test)

5 drops of concentrated H_2SO_4 were added to 1ml of extract. A red coloration in each extract indicates the presence of steroids.

Detection of saponins (Foam Index)

0.1g of extract was dissolved in a test tube containing 10 ml of distilled water. The tube was shaken vigorously lengthwise for 30-45 seconds and then left to stand for 15 minutes. The height of the foam is measured. The persistence of foam more than 1 cm high indicates the presence of saponins.

Detection of saponins (Foam Index)

0.1g of extract was dissolved in a test tube containing 10ml of distilled water. The tube was shaken vigorously lengthwise for 30-45 seconds and then left to stand for 15 minutes. The height of the foam is measured. The persistence of foam more than 1cm high indicates the presence of saponins.

Identification of polyphenols (reaction with ferric chloride ($FeCl_3$)

A drop of 2% alcoholic ferric chloride solution is added to 2 ml of extract. A more or less dark blue-black or green color indicates a positive reaction.

Phytochemical quantification

Determination of total alkaloids

The assay was performed using the spectrophotometric method described by Sreevidya & Mehrotra [35]. A quantity of 5mL of extract solution was taken and the pH was maintained between 2 and 2.5 with dilute HCl. 2ml of Dragendorff's reagent was added and the precipitate formed was centrifuged. The centrifugate was checked for complete precipitation by adding Dragendorff's reagent and the centrifuged mixture was decanted completely. The precipitate was washed with alcohol. The filtrate was discarded and the residue was then treated with 2ml of di-sodium sulphate solution. The brownish-black precipitate formed was then centrifuged. Completion of precipitation was checked by adding 2 drops of disodium sulphate. The residue was dissolved in 2ml of concentrated nitric acid,

Warming if necessary. This solution was diluted to 10ml with distilled water. Then 1ml of this diluted solution was taken and 5ml of thiourea solution was added. The absorbance was measured at 435nm. The standard curve was made from a stock solution of atropine at 10mg/l with a range from 0 to 1mg/ml. Absorbances were read using a spectrophotometer at 435nm against the white tube prepared under the same conditions by replacing the sample with distilled water. The alkaloid content of the samples was estimated from the linear regression line and expressed in gram equivalents of atropine per 100g of powder.

Total flavonoids determination

The method described by Patricia et al. [36] was used for the determination of total flavonoids. In a 25ml flask, 0.75 ml of 5% (w/v) sodium nitrite (NaNO2) was added to 2.5ml of extract. 0.75ml of 10% (w/v) aluminium chloride (AlCl3) was added to the mixture and incubated for 6 minutes in the dark. After incubation, 5 ml of sodium hydroxide (1N NaOH) was added and the volume made up to 25ml. The mixture was shaken vigorously before being assayed using a UV-visible spectrophotometer. The reading was taken at 510nm. Trials were carried out in triplicate. Flavonoid content was expressed as milligram quercetin equivalent per gram extract (mg Qc-eq/g extract). Quercetin was used here as the reference standard for quantifying total flavonoid content. The total flavonoid content (concentration) was calculated using the formula: £=/; £: Content or concentration (mg. AG/g or mg.Qc/g dry extract); C: concentration of the sample given by the spectrophotometer (mg/mL); V: volume of the prepared solution (mL); D: dilution factor; m: mass of the extract (g).

Determination of total polyphenols

The method described by Patricia et al. [36] was used to determine total polyphenols. A volume of 2.5ml of diluted (1/10) Folin-Ciocalteu reagent was added to 30µL of extract. The mixture was kept for 2 minutes in the dark at room temperature, and then 2 mL of sodium carbonate solution ($75g.L^{-1}$) was added. The mixture was then placed for 15 minutes in a water bath at 50°C, and then rapidly cooled. Absorbance was measured at 760nm, using distilled water as the blank. A calibration line was performed with gallic acid at different concentrations. Each analysis was performed in triplicate and the polyphenol concentration was expressed in milligrams per milliliter of gallic acid equivalent extract (mg/mL). Gallic acid was used here as the reference standard for quantifying total polyphenol content; this quantity was expressed in milligrams of gallic acid equivalent per gram of extracts (mg.eq.GA/g extract). Total polyphenol contents (concentrations) were calculated using the formula: £=/; £: Content or concentration (mg.GA/g or mg.Qc/g dry extract); C: concentration of the sample given by the spectrophotometer (mg/mL); V: volume of the prepared solution (mL); D: dilution factor; m: mass of the extract (g) 6

Preparation of experimental feed

Three doses were prepared from the hydroethanol extract of H. rosa sinensis leaves initially prepared, namely: D1=100mg/kg; D2=150mg/kg; D3=200mg/kg. Considering the extraction yield of 10.55%, the doses applied were as follows: D1 = 10.55mg/kg; D2=15.825mg/kg; D3= 21.1mg/kg. To prepare the dose of D1=10.55mg/kg, 1055 mg of crude extract was taken and 100ml of 75% ethanol was added. As for the other doses, D2=15.825mg/kg and D3 = 21.1mg/kg, 1582mg and 2110mg were respectively taken from the crude extract to which 100ml of 75% ethanol was added. As with the leaf extract, three doses were prepared from the hydroethanol extract of H. rosa

sinensis flowers, namely: D'1=100mg/kg; D'2=150mg/kg; D'3=200 mg/kg Considering the extraction yield, which was 15.65%, the doses applied were as follows: D'1 = 15.65mg/kg; D'2=23.47mg/kg; D'3= 31.3mg/kg. To prepare the dose of D'1 = 15.65mg/kg, 1565mg of crude extract was taken and 100ml of 75% ethanol was added. As for the other doses, namely D2 = 23.47mg/kg and D3 = 31.3mg/kg, 2347mg and 3130mg were respectively taken from the crude extract to which 100ml of 75% ethanol was added. Seven (07) experimental feeds corresponding to the different treatments were prepared using a base feed: GOUESSANT, a commercial floating granulated feed with a diameter of 2mm, containing 46% protein. The experimental control feed consisted solely of the commercial feed. The preparation of the different extract-based experimental feeds involved impregnating the feeds with different doses of extracts using the feed-extract mixing technique. After initial homogenization, a volume of 250ml of 95% ethanol per kg of feed was added to ensure better distribution of the extract in the feed. The experimental feeds were then dried on transparent cloths for 48h to evaporate the ethanol. This process was carried out away from the sun and at room temperature to preserve its effectiveness [37]. Each test food was stored in hermetically sealed, labeled boxes.

Experimental procedure

840 Oreochromis niloticus fingerlings with an average weight of 7.5 ± 1.01g were placed in 21 tanks with a capacity of 40L each, in an earthen pond of approximately $150m^2$ (fed by groundwater), at a density of 40 fingerlings per tank and subjected to natural temperature and light conditions (Figure 2). The quantity of feed distributed was set according to the average biomass of fingerlings per week. The fingerlings were fed 5% of their Ichtyo-biomass for the first four weeks of the experiment, and 4% for the last three weeks, according to Mareck's rationing table. The daily ration was divided into 3 meals, from 07:30 to 17:30 with an interval of 5.5 hours [38] and adjusted every 10 days after the various control fisheries according to changes in the biomass of the subjects. Every morning and evening at 8AM and 4PM respectively, the physico-chemical parameters of the water (temperature, pH, and dissolved oxygen) were taken. These parameters, which provide information on water quality, were monitored regularly to ensure optimum rearing conditions for Oreochromis niloticus . The survival and growth of the subjects were monitored from the second week of experimentation, respectively by counting the dead individuals counted and by weighing a sample of 30 individuals taken at random from each of the treatments, at the end of the treatments and then every fortnight until the end of the experiments. The growth performance of Oreochromis niloticus juveniles at the end of this experiment (in terms of Average Weight Gain (AWG), Daily Weight Gain (DWG), Specific Growth Rate (SGR)), Total Fish Length (TL), Condition Factor (CF) and Survival Rate (SR) were determined using the following formulae borrowed from various authors [27,39-43]. These different parameters were

Calculated at the end of the experiment. These formulas are as follows:

Average Weight Gain: AWG (g) = (Average Final Weight - Average Initial Weight) (g)

Figure 2: Experimental set-up. **A)** Installation of the experimental set-up; **B)** experimental set-up protected by a wire mesh to control treatment LE100 = Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of 100mg/kg of feed; LE150 = Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of l50mg/kg of feed; LE200 = Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of 200mg/kg of feed; FE100 = Treatment with hydroethanolic extract of H. rosa sinensis flowers at a dose of 100mg/kg of feed; FE150 = Treatment with hydroethanolic extract of H. rosa sinensis flowers at a dose of 150mg/kg of feed; FE200 = Treatment with hydroethanolic extract of H. rosa sinensis flowers at a dose of 200mg/kg feed.

Specific Growth Rate (SGR) given by SGR (%.d-1) = 100. (lnWf - ln Wi). t-1 with Wi: Initial average weight (g) Wf: Final average weight (g)

Feed Conversion Ratio (FCR): = Rd. (Bf - Bi) -1 with Bi: Initial biomass (g) Bf: Final biomass (g) and Rd: Ration or quantity of feed consumed or distributed (g); 7 Survival Rate (%) = 100x (final number of individuals/initial number of individuals). Condition factor (K): = (Wt/ Lt3)×100 (where P = total weight, Lt = total length)

At 45 and 60 days post-treatment, a sample of 5 males and 5 females was taken at random from each treatment. Males and females were separated using the manual sexing method described by Orobiyi [44]. For this purpose the fishes were previously euthanized by an overdose of benzocaïne (400mg.L^{-1}). After dissection, the gonads (testes and ovaries) were removed and separated by treatment to avoid any confusion. Those different organs were weighed in order to determine the gonadosomatic index of the different subjects according to the treatments. The gonadosomatic index was determined by the following formula: Gonadosomatic Index (GSI) = (whole organ mass (g)/animal mass (g))×100. The various organs were then sectioned and fixed in formalin for 24 hours in a saline formalin solution consisting of equal volumes of 10% formalin and 0.9% NaCl. Histological sections 8 µ thick were prepared using standard procedures as described by Smith and Bruton [45]. Photomicrographs were taken using a Leitz microscope (Ortholux) and a camera. The process of inhibition of gonadal development was assessed on the basis of the alterations to the gonadal structures on the histological sections.

Statistical analysis

Results are expressed as mean ± standard deviation. The homoscedacity and normality of the datasets were checked beforehand using Hartley's test. Once the conditions of normality and homoscedacity had been met, a one-way analysis of variance (one-factor ANOVA) was used to analyse the differences between the treatments. The 2-to-2 comparisons were made using the post-test for multiple comparisons of means (Turkey test). Differences were considered significant at $p < 0.05$. Statistical tests were performed using Graphpad Prism 8.0.1.244 software.

Results

Phytochemical characterisation of Hibiscus rosa sinensis leaves and flowers hydroethanolic extracts

Qualitative phytochemical screening revealed the presence of phenolic compounds, flavonoids, alkaloids and tannins in the hydroethanolic extracts of Hibiscus rosa sinensis leaves and flowers. Anthocyanins and leucoanthocyanins were absent in both extracts. Steroids and saponins were only present in the leaves and flowers extracts respectively. Quantitative evaluation of the extracts revealed that flavonoids were the most important compounds in both flowers and leaves hydroalcoholic extracts, with mean values of 113.12±2.33mg/g extract for the leaves and 113.12±0.71mg/g extract for the flowers. The lowest levels of alkaloids were observed in both extracts, with mean values of 9.28±0.02mg/g extract for leaves and 9.37±0.00mg/g extract for Flowers.

Effects of Hibiscus rosa sinensis leaves and flowers hydroethanol extracts on survival and growth parameters of O. niloticus

A comparative analysis of the different survival rates at 60 days post-treatment of Oreochromis niloticus juveniles from control group and group treated with different doses (100mg/kg; 150mg/kg; 200mg/kg respectively) of H. rosa sinensis leaves and flowers hydroethanolic extract revealed a significant difference ($p < 0.05$) between treatments. Fishes from control group and those from group treated with 100mg/kg of H. rosa sinensis leaves hydroethanolic extract obtained the highest survival rates (100±0%), while the lowest was obtained from fishes from group treated with 100 mg/ kg of H. rosa sinensis flowers hydroethanolic extract with an average value of 95±2.12 %. Growth characteristics varied significantly according to the treatment applied. A comparative analysis of the control group and group treated with H. rosa sinensis leaves hydroaethanolic extract at different doses (100, 150, 200mg/kg) and those treated with H. rosa sinensis flowers hydroaethanolic extract (at respective doses of 100, 150, 200mg/kg) of the different progeny shows a significant difference ($p < 0.05$) between the treatments. The results show that the control group treatment had a significantly greater effect ($p < 0.05$) than the other treatments applied in terms of Average Weight Gain, Average Daily Gain, Specific Growth Rate and Condition Factor. The respective mean values were 18.6±1.69g (AWG), 0.46±0.06 g/d (ADG), 3.11±0.00%/d (SGR) and 2.07±0.28 (K). A comparative analysis between the different group treated with leaves and flowers hydroethanolic extracts shows that treatment at the dose of 100 mg/ kg had a higher effect than the other doses with regard to Average Weight Gain (12.4±1.13 g), Average Daily Gain (0.31±0.03 g/d) and Condition Factor (1.88±0.23). However, the group treated with doses of 150 and 200mg/kg of H. rosa sinensis leaves hydroethanolic extract, as well as those from group treated with doses of 100, 150 and 200mg/kg of H. rosa sinensis flowers hydroethanolic extract, had a similar effect on both Average Weight Gain (with respective averages of 10.8±0.98 g (LE150), 10.49±0.96g (LE200) and 11.7±1.07g (FL100), 9.4±0.88 g (FL150) , 9±0.82g (FL200)) and Average Daily Gain (with respective values of 0.27±0.02g/d (LE150) ; 0.26±0.02g/d (LE200) and 0.23±0.02 g/d (FL150); 0.22±0.02g/d (FL200)). In the same way, fishes fed feed based on H. rosa sinensis leaves and flowers hydroethanolic extract presented similar Specific Growth Rates, with mean values of: 2.44±0.00%/d; 2.23±0.00%/d; 2.18±0.00 %/d and 2.35±0.00%/d; 2.03±0.00 %/d; 1.97±0.00%/d respectively for treatments LE100, LE150, LE200 and FL100, FL150, FL200. A comparative analysis of the Consumption Index of the different group fed feed based on H. rosa sinensis leaves and flowers hydroethanolic extracts compared with the control group showed a significant difference ($p < 0.05$) between the different treatments. In fact, the fishes from the control group had a low Consumption Index (0.83±0.16%) compared with the other treated group. The highest value was obtained with the FL200 treatment, with an average value of 1.32%. However, comparing the different group treated with H. rosa sinensis leaves and flowers extracts, the treatment with the lowest value was the 100 mg/kg leaves extract treatment, with an average of 1.09±0.08%.

Effects of Hibiscus rosa sinensis leaves and flowers hydroethanolic extracts on Gonadosomatic Index of males and females at 45 and 60 days post-treatment

An analysis of the Gonado-somatic Index at 45 days post-treatment of males from group treated with different doses of H. rosa sinensis leaves and flowers hydroethanolic extracts compared with control group showed a significant difference ($p < 0.05$) between treatments (Figure 3). Fishes fed with diets based on Hibiscus rosa sinensis leaves and flowers hydroethanolic extracts showed significantly ($p < 0.001$) lower gonad/mass weight ratios compared with fishes of the control group, whose mean value was the highest (with a mean of 0.415±0.031%). However, a comparative analysis of the group treated with different doses of hydroethanolic extracts revealed a significantly greater effect

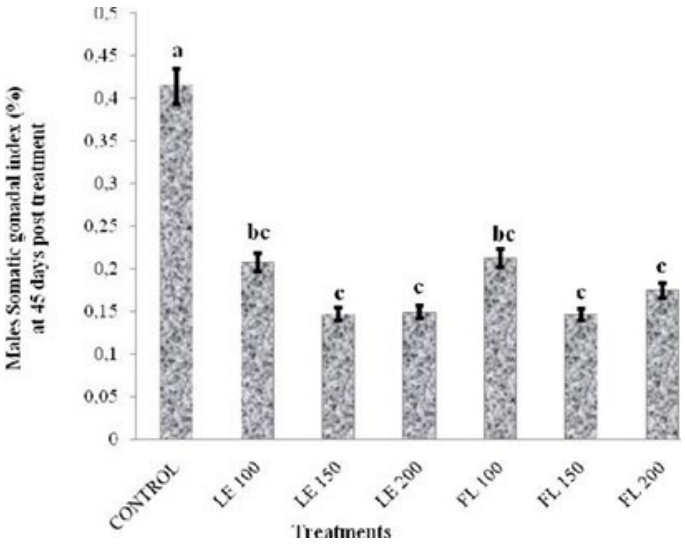

Figure 3: Somatic gonad index of males treated with different doses of hydroethanolic extract of Hibiscus rosa sinensis flowers and leaves at 45 days post-treatment compared with males from the control group. Vertical bars with the same letter are not significantly different ($p < 0.05$; ┬ = standard deviation of the mean (n=3)).

LE100 = Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of 100mg/kg feed;

LE150 = Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of 150mg/kg feed;

LE200 = Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of 200mg/kg feed;

FE100 = Treatment with hydroethanolic extract of H. rosa sinensis flowers at a dose of 100mg/kg feed;

FE150 = Treatment with hydroethanolic extract of H. rosa sinensis flowers at a dose of 150mg/kg feed;

FE200 = Treatment with hydroethanolic extract of H. rosa sinensis flowers at a dose of 200 mg/kg feed

of the group treated with doses of 100 mg/kg of H. rosa sinensis leaves and flowers hydroethanolic extracts (with respective averages of 0.21±0.16% (LE100) and 0.213±0.03% (FL100). On the other hand, group treated with doses of 150 and 200 mg/kg of H. rosa sinensis leaves hydroethanolic extract and group treated with the same doses of flowers hydroethanolic extract had a similar effect. The respective averages were 0.147 ± 0.066% (FE150); 0.149±0.039% (FE200) and 0.146±0.060% (FL150); 0.174±0.045% (FL200). Similarly, an analysis of the Gonado-somatic Index at 60 days post-treatment of satellite males from group treated with different doses of hydroethanolic extracts of H. rosa sinensis leaves and flowers compared with control group showed a significant difference ($p < 0.05$) between treatments (Figure 4). The control group and those treated with 100mg/kg of flowers and leaves extract recorded a higher Gonado-somatic Index than the other treatments, with mean values of 0.415±0.022% (Control group), 9 0.431±0.044% (LE'100) and 0.387±0.016% (FL'100) respectively. However, the LE'150, LE'200 and FL'150, FL200 treatments had similar effects. Analysis of the Gonado-somatic Index of females at 60 days post-treatment revealed a significant difference ($p < 0.05$) between the different treatments (Figure 5). Females treated with the 200mg/kg flowers extract recorded a higher Gonado-somatic Index than the other treatments, with an average of 1.34±0.37%. However, group fed with 100, 150 and 200mg/kg of H. rosa sinensis leaves hydroethanolic extract and group fed with 100 and 150mg/kg of H. rosa sinensis flowers hydroethanolic extract had a similar effect.

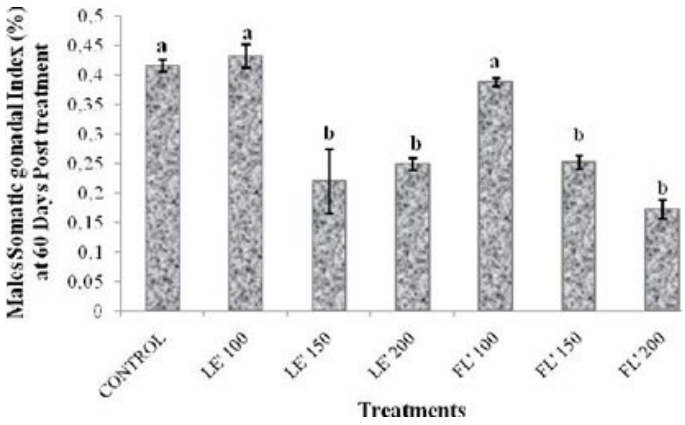

Figure 4: Somatic gonad index of satellite males treated with different doses of hydroethanol extract of Hibiscus rosa sinensis flowers and leaves at 60 days post-treatment compared with males from the control group.

Vertical bars with the same letter are not significantly different ($p<0.05$; ┬ = standard deviation of the mean (n=3))

LE'100= Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of 100mg/kg feed;

LE'150= Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of l 50mg/kg feed;

LE'200 = Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of 200mg/kg feed;

FE'100 = Treatment with hydroethanolic extract of H. rosa sinensis flowers at a dose of 100mg/kg feed;

FE150 = Treatment with hydroethanolic extract of H. rosa sinensis flowers at a dose of 150mg/kg feed;

FE'200 = Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of 200mg/kg feed

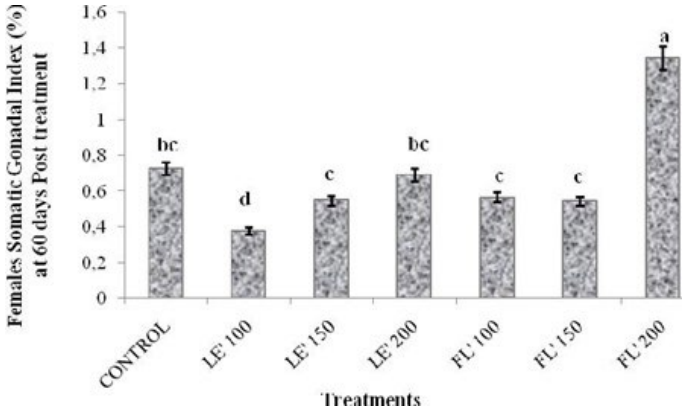

Figure 5: Gonado-somatic index of satellite females treated with different doses of hydroethanol extract of Hibiscus rosa sinensis flowers and leaves at 60 days post-treatment compared with females from the control group.

Vertical bars with the same letter are not significantly different ($p<0.05$; ┬ = standard deviation of the mean (n=3)).

LE100= Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of 100 mg/kg feed;

LE150= Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of l 50 mg/kg feed;

LE200 = Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of 200 mg/kg feed;

FE100 = Treatment with hydroethanolic extract of H. rosa sinensis flowers at a dose of 100 mg/kg feed;

FE150= Treatment with hydroethanolic extract of H. rosa sinensis flowers at a dose of 150 mg/kg feed;

FE200= Treatment with hydroethanolic extract of H. rosa sinensis leaves at a dose of 200 mg/kg feed

Effects of H. rosa sinensis leaves and flowers hydroethanolic extracts on Oreochromis niloticus male and female gonad structure

An analysis at 45 days post-treatment of the histological sections of the male and female gonads from the different group treated with H. rosa sinensis leaves and flowers hydroethanolic extracts compared with the offspring from the control group reveals a differentiation of the gonadal structures in both males and females. Analysis of the testicular structures of males from control group reveals a normal microstructure of the testis, with seminiferous tubules (showing germline cells at different stages of development and organised centripetally) and conjunctivo-vascular tissue (containing clusters of Leydig cells). On the other hand, the testicular structures of males from group treated with H. rosa sinensis leaves and flowers hydroethanolic extracts showed a dose-dependent alteration in spermatogenesis marked by destruction of the spermatozoa. However, satellite males (at 60 days posttreatment) from group treated with 100mg/kg of H. rosa sinensis leaves and flowers hydroethanolic extracts showed a restoration of spermatozoa (Figure 6). Analysis of histological sections of the female gonads at 60 days post-treatment revealed a healthy ovarian structure in females from control group, with oocytes cells at different stages of development. Satellite females from group treated with H. rosa sinensis leaves and flowers hydroethanolic extracts underwent changes to their ovarian structures (damaged oocytes). These changes were more marked in females treated with 150 and 200mg/kg. However, the integrity of ovarian tissue was only restored in females treated with 100mg/kg of H. rosa sinensis leaves and flowers hydroethanolic extract (Figure 7).

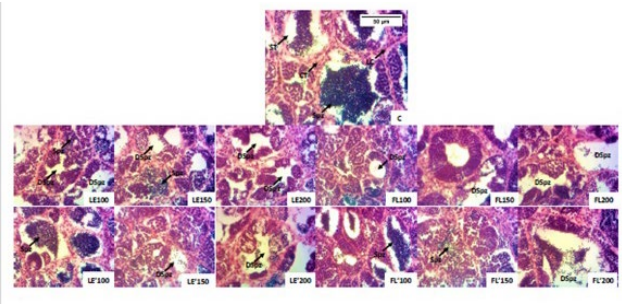

Figure 6: Microphotographs of the testes of Oreochromis niloticus treated with different doses of hydroethanolic extracts of H. rosa sinensis leaves and flowers compared with males from control group (X250); staining with haematoxylin-eosin. Fish fed with the control diet (0.0g/kg) showing the normal testicular tissues with the spermatozoa well distributed, (x250);

LE100, Fish Treated with hydroethanolic extract of H. rosa sinensis leaves at a dose of 100 mg/kg diet showing destroyed Spermatozoa

LE150, Fish Treated with hydroethanolic extract of H. rosa sinensis leaves at a dose of 150 mg/kg diet, showing mild Spermatozoa damaged

LE200, Fish Treated with hydroethanolic extract of H. rosa sinensis leaves at a dose of 100 mg/kg diet, showing destroyed Spermatozoa

FL100, Fish treated with hydroethanolic extract of H. rosa sinensis flowers at a dose of 100 mg/kg diet, showing destroyed Spermatozoa

FL150, Fish treated with hydroethanolic extract of H. rosa sinensis flowers at a dose of 150 mg/kg diet, showing destroyed Spermatozoa

FL200, Fish treated with hydroethanolic extract of H. rosa sinensis flowers at a dose of 100 mg/kg diet, showing Destroyed Spermatozoa

LE'100, Satellite male issue of the group treated with hydroethanolic extract of H. rosa sinensis leaves at a dose of 100 mg/kg diet, showing destroyed Spermatozoa

LE'150, Satellite male' issue of the group treated with hydroethanolic extract of H. rosa sinensis leaves at a dose of 150mg/kg diet, showing destroyed Spermatozoa

LE'200, Satellite male issue of the group treated with hydroethanolic extract of H. rosa sinensis leaves at a dose of 200mg/kg diet, showing destroyed Spermatozoa

ST = Seminiferous tube; CvT = Connective vascular tissue; Spz = Spermatozoa; LC = Leydig Cell; Dspz = Destruction of spermatozoa

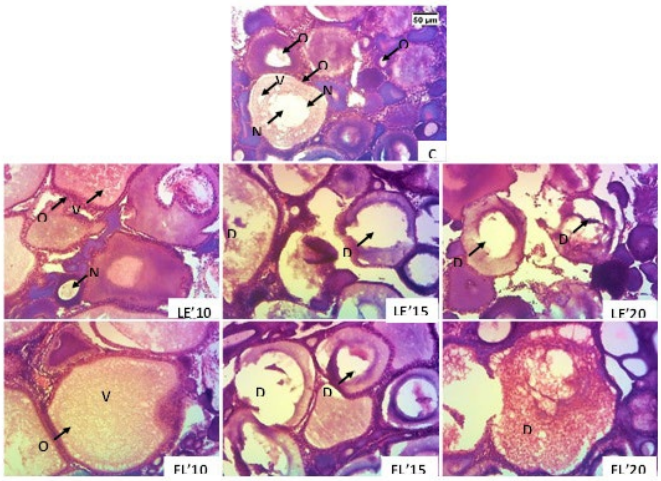

Figure 7: Microphotographs of the ovaries of Oreochromis niloticus treated with different doses of hydroethanolic extracts of H. rosa sinensis leaves and flowers compared with females from control group (X250); staining with haematoxylin eosin. Fish fed with the control diet (0.0g/kg), showing normal ovarian tissues at the ripe stage of the oocytes. LE'100: Fish Treated with hydroethanolic extract of H. rosa sinensis leaves at a dose of 100mg/kg diet, showing restoration of the oocytes, (x250).

LE'150 Treated with hydroethanolic extract of H. rosa sinensis leaves at a dose of 100 mg/kg diet, showing damaged of the oocytes

LE'200: Treated with hydroethanolic extract of H. rosa sinensis leaves at a dose of 100 mg/kg diet, showing mild atresia of the oocytes

FL'100: Treated with hydroethanolic extract of H. rosa sinensis flowers at a dose of 100 mg/kg diet, showing restoration of the oocytes

FL'150: Treated with hydroethanolic extract of H. rosa sinensis flowers at a dose of 150 mg/kg diet, showing damaged oocytes

FL'200: Treated with hydroethanolic extract of H. rosa sinensis Flowers at a dose of 200 mg/kg diet, showing damaged oocytes

OD: Oocytes development; Vi =Vitellus; DO = Damaged Oocytes; VG: Vitellus Granules; Nu = Nucleus

Discussion

The main physico-chemical parameters measured during this experiment were Temperature, pH, Dissolved Oxygen, Nitrates and Nitrites. The average values for temperature, pH and dissolved oxygen remained relatively stable over the experimental period. The same applies to Nitrates and Nitrites. In fact, the temperature values oscillated from 21.12±1.08°C to 24.09 ± 1.04°C, while pH values varied from 7.06±0.14 to 7.13±0.22. Mean Dissolved Oxygen values varied from 5.24±0.2 to 5.13±0.5 mg/l. Nitrate and nitrite values ranged from 0.25±0.07 to 0.26±0.08 mg/L for nitrite (NO^{2-}) and from 9.74±0.45 to 13.86±0.71 mg/L for ammonium ions (NH^{4+}). These main temperature and Dissolved Oxygen values presented are within the acceptable standards for rearing O. niloticus as reported by Omitoyin [46], since the optimum temperature for O. niloticus growth is between 24 and 28°C, while the pH is between 7 and 8. The optimum dissolved oxygen concentration is 5mg/l. The concentration of nitrogenous waste in particular nitrates (NH^{4+}) and total ammonia must be kept below the so-called critical threshold of O. niloticus. To this end, it must not exceed 5mg/l for nitrates (NH^{4+}), 500 mg/l for nitrites (NO^{2-}) and 15mg/l for total ammonia [47]. Phytochemical screening revealed the presence of phenolic compounds, flavonoids, alkaloids and tannins in H. rosa sinensis leaves and flowers hydroethanolic extracts. The same applies to steroidsand saponins. However, anthocyanins and leucoanthocyanins were non-existent in both extracts. Quantitative analysis revealed the preponderance of flavonoids in both extracts,

with mean values of 113.12±2.33 for the leaves and 113.12±0.71 for the flowers. These results are in line with those 10 obtained by Pekamwar et al. [48], who detected the presence of saponins, tannins, steroids and flavonoids in H. rosa sinensis leaves, flowers and roots extracts. Indeed, Jegede [27] reported that flavonoids induced anti-implantation and anti-spermatogenic effects in fishes fed with diet supplemented with H. rosa sinensis powder. In addition, alkaloids have been shown to damage germ cells and seminiferous tubules in rats [49]. The presence of these phytochemicals could therefore inhibit the reproductive process in Oreochromis niloticus. Analysis of the different survival rates at 60 days post-treatment of Oreochromis niloticus fishes from control group and group treated with different doses (100 mg/kg; 150 mg/kg; 200mg/kg respectively) of H. rosa sinensis leaves and flowers hydroethanolic extracts revealed a significant difference ($p < 0.05$) between treatments. The results show that the average survival rates varied from 95±2.12% to 100±0%. These high values of survival rates in all group treated with hydroethanolic extracts of H. rosa sinensis leaves and flowers as well as control group show that these treatments could not have a deleterious effect on the survival of the various offspring. However, survival did not change depending of treatment in group treated with H. rosa sinensis leaves hydroethanolic extracts or those treated with flowers hydroethanolic extracts. These results show that the dose of extract does not significantly affect juvenile mortality. These results are higher to those obtained by Akoha [50] whose work aimed to evaluate the effect of H. rosa sinensis leaves ethanolic extract on the survival and growth characteristics of O. niloticus larvae; with a survival rate values ranging from 81.5% to 95%. This difference could be associated with the different ages of the fishes used and the different doses of extracts applied during the experiments. In fact, the extracts applied had a differential effect on the survival of the offspring. Similarly, sensitivity to a treatment depends on the stage of development. Larvae are more sensitive than juveniles, which has an impact on their survival. Growth characteristics varied significantly according to the treatment applied. A comparative analysis of control group and group treated with H. rosa sinensis leaves hydroethanolic extract at different doses (100 mg/kg; 150 mg/kg; 200 mg/kg) and those treated with H. rosa sinensis flowers extract (at respective doses of 100mg/kg; 150mg/kg; 200mg/kg) of the different offspring showed a significant difference ($p < 0.05$) between treatments. These results show that the dose of extract applied had an effect on the growth performance of Oreochromis niloticus juveniles. However, the fishes from the control group obtained significantly higher growth performances compared with the other group treated with H. rosa sinensis leaves and flowers hydroethanolic extracts. This growth differential observed between the control group and the group treated with H. rosa sinensis leaves and flowers extracts could be due to the presence of tannins in the various hydroethanolic extracts. These compound, which is an anti-nutritional factor, could have had a repulsive effect on the various experimental feeds based on H. rosa sinensis leaves and flowers hydroethanolic extracts which could have affected the food intake of the fishes from the various group treated with the extracts. Analysis of the growth parameters of the fishes from the different group treated with H. rosa sinensis leaves and flowers hydroethanolic extracts revealed that the group treated with a dose of 100mg/kg of H. rosa sinensis leaves hydroethanolic extract obtained better growth performances compared with the other treated batches in terms of AWG, ADG, SGR, FCR and K (Condition factor), with respective averages of 12.4±1.13g; 0.31±0.02g/d; 2.439±0.003%/d; 1.09±0.089; 1.88±0.023. These different growth parameter values are lower than those obtained by Jebede [27] in O. niloticus juveniles fed with a diet supplemented with H. rosa sinensis powder, with respective averages of 25.80 g and 0.82 % /day for AWG and SGR. This difference could be associated with the type of treatment applied, which could have a differential effect on the growth performance of the offspring. However, Jebede's work also revealed a significantly greater effect of control group on growth performance compared with group treated with H. rosa sinensis powder. These results indicate an inhibitory effect of H. rosa sinensis on feed intake and hence on growth performance. An analysis of the gonado- somatic index at 45 and 60 days post-treatment of males from group treated with different doses of H. rosa sinensis leaves and flowers hydroethanolic extracts compared 11 with control group showed a significant difference ($p < 0.05$) between treatments. At 45 days posttreatment, males treated with different doses of H. rosa sinensis leaves and flowers hydroethanolic extracts showed significantly ($p < 0.001$) lower gonad weight/mass ratios compared with males from control group (with an estimated average of 0.415±0.031%). This lower ratio in fishes from group treated with extracts reflects an inhibition of gonadal development in male individuals. In addition, a decrease in the gonado somatic index was observed as the dose of extract increased. These results indicate a dose-dependent effect of the treatment and the gonad weight/mass ratio. Although these results are inferior to those obtained by Jegede [27] in O. niloticus juveniles fed with a diet supplemented with H. rosa sinensis powder (with a mean Gonado - somatic Index values ranging from 0.4 to 1.7%), they are in agreement with his hypothesis that as the extract dose increases, the gonado - somatic Index value decreases. This difference could be associated with the type of treatments applied, which acted differently on the gonad/mass weight ratio. However, at 60 days post-treatment, the results obtained did not show a decrease in the Gonado somatic Index as a function of the increase in the dose of extract. In fact, the males from the control group and those treated with 100 mg/kg of flowers and leaves extract recorded a higher Gonado-somatic Index than the other treatments, with mean values of 0.415 ± 0.022 (Control), 0.431±0.044% (FL'100) and 0.387±0.016% (LE'100) respectively. The LE'150, LE'200 and FL'150, FL200 treatments had a similar effect. These results observed in satellite males could reflect a possible reversibility of gonads development in males treated with 100mg/kg of H. rosa sinensis flowers and leaves hydroethanol extracts. Analysis of the Gonado-somatic Index of females at 60 days post-treatment also revealed a significant difference between the treatments. Females treated with 200mg/kg of flowers extract recorded a higher Gonadosomatic Index than the other treatments, with an average value of 1.34±0.37%. However, group fed with 100, 150 and 200mg/kg of H. rosa sinensis leaves hydroethanolic extract and group fed with 100 and 150mg/kg of H. rosa sinensis flowers hydroethanolic extract had a similar effect. These results observed in satellite females could also reflect a possible reversibility of gonads development in females from group treated with 200mg/kg of H. Rosa sinensis flowers hydroethanolic extract. These results show that the reversibility of gonad development in males and females depends on the dose and type of extract. Observation of the histological sections of the male and female gonads from the different group treated with H. rosa sinensis leaves and flowers hydroethanolic extracts compared with the offspring from the control group at 45 days post-treatment reveals a differentiation of the gonadal structures in both males and females. Analysis of the testicular structures of males from Control group revealed a normal microstructure of the testis, with seminiferous tubules (showing germline cells at different stages of development and organised centripetally) and conjunctivo-vascular tissue (containing clusters of Leydig cells). The testicular structures of males from group treated with H. rosa sinensis leaves and flowers hydroethanolic extracts showed a dose-dependent alteration in spermatogenesis, marked by destruction of the spermatozoa. These

alterations observed in the testicular structures of males treated with different doses of H. rosa sinensis leaves and flowers hydroethanolic extracts reflect an inhibition of gonads development, which justifies the low gonado-somatic Index values observed in these different treated group. These observations are in line with those reported by Farnsworth [51]. According to this author, treatment with H. rosa sinensis flowers and leaves hydroethanolic extracts led to a reduction in testicular spermatogenic elements and epididymal spermatozoa. However, the changes observed in our experiments were less significant than those obtained by Ekanem et al. In fact, the work of these authors revealed greater alterations of gonadal structures in male O. niloticus treated with a feed supplemented with different doses of papaya seed powder (at doses of 4.9 and 9.8g/kg of feed respectively). This difference in terms of alteration could be correlated with the difference in treatment applied, which would act differently on the gonadal structures of the fishes. However, in satellite males (at 60 days post-treatment) from group treated with 100mg/kg of H. rosa sinensis leaves and flowers hydroethanolic extracts, spermatozoa were restored. These results are in line with the higher value of the gonado -somatic index obtained in this treated group compared with the values obtained from the 12 males from the other treatments. Observations of the histological sections of the female gonads at 60 days post-treatment revealed, in the females from the control group, a healthy ovarian structure with oocyte cells at different stages of development. On the other hand, those from group treated with H. rosa sinensis leaves and flowers hydroethanolic extracts underwent changes in ovarian structures (damaged oocytes). These changes were more marked in females treated with 150 and 200mg/kg. However, the integrity of ovarian tissue was only restored in females treated with 100mg/kg of H. rosa sinensis leaves and flowers hydroethanolic extracts. These observations indicate a reversible effect on gonadal development in females treated with 100mg/kg of H. rosa sinensis leaves and flowers hydroethanol extracts. These results are consistent with the higher Gonado-somatic index in females from this treated group compared to those from group treated with H. rosa sinensis leaves and flowers hydroethanolic extracts.

Conclusion

The aim of the present study was to evaluate the effect of H. rosa sinensis leaves and flowers hydroethanolic extracts on survival, growth performance and gonads development of O. niloticus . This work showed that the dose of extract did not significantly affect juvenile mortality. Analysis of the growth parameters of fishes from the different group treated with H. rosa sinensis leaves and flowers hydroethanolic extracts revealed that the group treated with a dose of 100mg/kg of H. rosa sinensis leaves hydroethanolic extract obtained better growth performances than the other treated group in terms of AWG, ADG, SGR, FCR and K (Condition factor), with respective averages of 12.4±1.13g; 0.31±0.02g/d; 2.439±0.003% /d; 1.09±0.089; 1.88±0.023. With regard to the Gonado somatic-Index, it's noted that at 45 days post-treatment, male treated with different doses of H. rosa sinensis leaves and flowers hydroethanolic extracts showed significantly ($p < 0.001$) lower gonad weight/mass ratios compared with subjects from control group (with an estimated mean of 0.415±0.031%). However, at 60 days post-treatment, satellite males from Control group and those from group treated with 100 mg/kg of flower and leaves extracts recorded a higher Gonadosomatic Index than the other treatments, with mean values of 0.415±0.022% (Control); 0.431±0.044% (FL'100) and 0.387±0.016% (FE'100) respectively. Observation of the testicular structures of males from group treated with H. rosa sinensis leaves and flowers hydroethanolic extracts at 45 days post-treatment showed a dose-dependent alteration in spermatogenesis marked by destruction of the spermatozoa. These alterations reflect an inhibition of gonadal development, which justifies the low gonado – somatic Index values observed in these different treated groups. However, satellite males from group treated with 100mg/kg of H. rosa sinensis leaves and flowers hydroethanolic extracts showed a restoration of spermatozoa. These results are in line with the higher value of the Gonado-somatic index obtained in this treated group compared with the values obtained from the males from the other treatments. Analysis of the ovarian structures of satellite females from group treated with H. rosa sinensis leaves and flowers hydroethanolic extracts revealed changes in ovarian structures (damaged oocytes). These changes were more marked in females treated at a dose of 150 and 200mg/kg. However, the integrity of ovarian tissue was only restored in females treated with 100mg/kg of H. rosa sinensis leaves and flowers. hydroethanolic extract These results confirm the hypothesis that H. rosa sinensis leaves and flowers hydroethanolic extracts have an effect on survival, growth performance and inhibition of gonad development in Oreochromis niloticus.

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