Original Research Article

Spermatotoxic effects of some medicinal plants (*Carica papaya, Hibiscus rosa-sinensis* and *Ipomoea batatas*) on sperm quality and testicular weight in male African catfish (*Clarias gariepinus*)

ABSTRACT

This study was designed to evaluate the effects of pawpaw (*Carica papaya*) seeds, hibiscus plant (*Hibiscus rosa-sinensis*) leaves, sweet potato (*Ipomoea batatas*) leaves on sperm quality (sperm motility, sperm density, semen volume) and weight of testes of male *Clarias gariepinus*. One hundred and twenty (120) juveniles of *C. gariepinus* were collected from the University of Calabar fish farm. The 120 fish were randomly divided into 12 experimental tanks measuring 80x80x80cm (L x W x H), with three tanks for each treatment, using a completely randomized design (CRD). Three grams (3g) of each test plant were incorporated into 1kg of Coppens feed (3g/kg) and reformulated into four experimental diets; Treatment A- Control, B- Pawpaw seed meal (PSM), C-Hibiscus leaf meal (HLM) and D- sweet potato leaf meal (SPLM). The experiment was done in three replications. The fish were fed twice daily for 6 months. Data obtained were analyzed using a one way analysis of variance (ANOVA). Results showed that fish fed with HLM had significantly ($p = .05$) higher testicular weight when compared with the control and other test plants. Moreover, sperm volume and density significantly ($p = .05$) reduced in fish samples treated with PSM and SPLM when compared with the control and fish fed with HLM. The highest mean sperm volume and density were obtained in fish samples fed with HLM. No significant difference was observed in the sperm motility of the fish in all the treatment groups. Conclusively, this study reveals the pro-fertility potential of *H. rosa-sinensis* in male *C. gariepinus* while *C. papaya* and *I. batatas* possess anti-fertility properties. Therefore, HLM can be utilized as feed additive to minimize the dependence on synthetic drugs as fertility enhancing agents.

KEYWORDS: *C. gariepinus,* medicinal plants, spermatotoxicity, sperm quality, testicular weight.

Introduction

The use of medicinal plants as fertility enhancer in aquaculture has now received much attention [1]. With the shift away from synthetic drugs, the use of plants for enhancing growth and reproductive performance in animals and fishes is becoming acceptable [2-5]**.** However, some plants have been shown to have deleterious impact on aquatic organisms [6–9].

Aquaculture is a fast growing sector in Nigeria contributing less than 5% of the total fish supply but at a growth rate of about 2% per year [10]. Among the culturable fishes in Nigeria, *C. gariepinus* is a major tropical aquaculture species and the most popular among fish farmers and consumers [2]. Fish farming has contributed greatly to the availability of food in Nigeria and the world over with products from fish farming widely exported and traded to earn income. Fisheries sector employs over 44.5 million people and a lot of them are from developing countries. Also, industries engaged in the marketing, supply and distribution of fish product create job opportunities for over 150 million individuals [2].

The African catfish (*Clarias gariepinus*) belongs to the family Clariidae and is the most cultivated fish in Nigeria, and highly demanded freshwater fish all over the world due to its resistant to stress, ability to tolerate a wide range of environmental conditions, high stocking densities under culture conditions and relatively fast growth [11–12]. They are found throughout Africa and the Middle East, and live in freshwater lakes, rivers, and swamps, as well as human-made habitats, such as oxidation ponds or even urban sewage systems. Due to the high demand of quality fish and fish dietary proteins, there have been an increase in various researches in different ways to improve fish fertility to meet the demand and target productivity in aquaculture, with a dramatic movement from synthetic drugs to medicinal plants of natural importance.

Sweet potato (*Ipomoea batatas*) is a member of the family convolvulaceae with almost 1650 predominately tropical species. The genus Ipomoea comprises the largest number of the convolvulaceae family. The family is characterized by climbing or twinning woody or herbaceous plants that usually have heart-shaped leaves and funnel-shaped flowers [13]. *I. batatas* is a tuberous-rooted perennial plant mainly grown annually. The roots are adventitious, mostly located within the top 25 cm of the soil. Some of the roots produce elongated starchy tubers. Tuber flesh colour can be white, yellow, orange and purple while skin colour can be red, purple, brown or white. Sweet potatoes and it derivatives are powerful antioxidant and may be potent in boosting the immune system and treating fever, asthma, bug bites, burns, catarrh, ciguatera, convalescence, diarrhoea, nausea, stomach distress, tumors and whitlows. It has also been reported to affect fertility [14–15, 16–17].

Hibiscus (Malvaceae) is a genus of herbs, shrubs and trees. Its 250 species are widely distributed in tropical and subtropical regions of the world and are reported to possess various medicinal properties viz; antitumor, antihypertensive, antioxidant, anti– ammonemic [18–22]. The flowers have been reported to possess anti-implantation and anti – spermatogenic activities [23–24]. The petroleum ether extracts of the leaves and flowers have been shown to potentiate hair growth in vivo and in vitro [25]. Leaves and flowers also possess hypoglycemic activity [26-27]. The mucilage of the leaf has anti – complementary activity [28].

Carica papaya is a soft–wooded perennial plant that has a life span of 5–10 years although commercial plantations are usually replanted [29]. It normally grows a single – stemmed tree with a crown of large palmate leaves emerging from the apex of the trunk but plant strands may become multi stemmed when damaged. The fruit, seeds, leaves contained novel biologically active compounds which are potent as therapeutics [30]. *C. papaya* seeds have been reported to contain glycosides and polyphenols in excess among other compounds such as alkaloids, saponins, flavonoids and quinnones [30].

Therefore, this study was aimed at investigating the effect of pawpaw (*Carica papaya*) seeds, hibiscus plant (*Hibiscus rosa-sinensis*) leaves, sweet potato (*Ipomoea batatas*) leaves on sperm quality and testicular weight in male African catfish (*Clarias gariepinus*).

Materials and methods

Duration and location of the study

The study was conducted for six months at the University of Calabar fish farm.

Collection and preparation of plant samples

The plant samples (seeds of *Carica papaya*, leaves of *Hibiscus rosa-sinensis* and *Ipomoea batatas*) were collected within the University of Calabar campus and authenticated in the herbarium unit of the Department of Botany, University of Calabar. The samples were washed with clean water, air dried for three weeks, ground using electric blender (Qlink-Q15L40) to get the powdery form and extracted using Soxhlet method with 70 percent ethanol as solvent. The filtrate was obtained using rotary evaporator at 45^0C, while the extract was reduced into pastes with hot-air oven at 60^0c. The pastes obtained were stored in plastic screw capped bottles, labeled and stored in refrigerator for use.

Collection of fish samples

One hundred and twenty (120) juveniles of *C. gariepinus* were purchased from the University of Calabar fish farm. An average initial body weight of 46.3g, and 17.7cm length were obtained using weighing balance (Scout-pro; 3000g), and a measurement meter, respectively at the time of stocking. The fish were acclimated for 7 days in tanks and the water parameters tested to be ideal, before feeding with commercial feed (Coppens) twice daily (morning and evening) throughout the period of the experiment.

Experimental design and procedure

Experimental design and procedure

Twelve experimental tanks measuring; 80cm x 80cm x 80cm (L x W x H) were constructed with an outlet and inlet pipe in the University of Calabar fish farm hatchery complex and each tank was filled with clean water. The 120 fish were randomly divided into four experimental groups using a completely randomized design (CRD) in three replicates with each treatment containing 10 female fish. Sex determination was done through visual examination of the gonad. Three grams (3g) of each plant extract were incorporated into 1 kilogram of commercial feed (3g/kg; Coppens). The plants extract made up 75% of each experimental diet. The extracts were dissolved in 5ml dimethylsulphoxide (DMSO) and made into solution with water, and mixed with fish feed homogeneously using a spreader and air dried for 48 hours. This procedure was repeated for each plant and the prepared diets stored in airtight containers, labeled as follows; Treatment A-Control, B- Pawpaw seed meal (PSM), C-Hibiscus leaf meal (HLM) and D-Sweet potato leaf meal (SPLM). The physico–chemical parameters of the water were measured using the APHA [31] method of water quality assessment.

Evaluation of sperm quality

At the end of the feeding trial, 3 male fish at table size, randomly selected per dietary treatment were sacrificed under chloroform anesthesia and the testes were removed to determine the following sperm quality indices: sperm volume, motility duration, percentage motility and spermatozoa count.

Sperm volume

Small incision was made into the lobes of the testes, the sperm squeezed out into a Petri dish. This was measured with plastic syringe in mL.

Percentage motility

Each sample was estimated using light microscope at 400x magnification immediately after addition of 20 µL distilled water as an activating solution. During spermatozoa activation, Immotile Sperm Cell (ISC) was counted and when the activation stopped, Whole Sperm Cells (WSC) were counted [32]. The Motile Sperm Cells (MC) were calculated as:

$$MC = WSC - ISC$$

$$\% \, MC = \frac{MC}{WSC} \times 100$$

Spermatozoa count

Sperm count was determined by counting the number of spermatozoa in sample dilute with distilled water (100x) in a Burker haemocytometer, under 400x magnification [33]

Statistical analysis

All data collected on the weight of testes, sperm volume; sperm count and sperm motility were subjected to analysis of variance (ANOVA) using predictive analysis software (PASW), version 18.0. Significant means were separated using the Least Significant Difference (LSD) at 5% probability level.

Results

Effect of *Carica papaya*, *Hibiscus rosa-sinensis* and *Ipomea batatas* on some reproductive parameters in male catfish

Testes weight

Results obtained on the effects of the different treatments are presented in Table 1. Results revealed that fish treated with extract of *H. rosa-sinensis* had significantly higher (p= .05) testicular weight (11.22g) when compared with the control (7.22g) and other treatment groups (6.11 and 7.56g for *C. papaya* and *I. batatas*, respectively) as shown in figure 1.

Semen volume

Results presented in Table 1 also showed that sperm volume was significantly lower (p=.05) in animals treated with extracts of *C. papaya* and *I. batatas* (1.79 and 1.60mL, respectively) when compared with the control and *H. rosa-sinensis* groups. The highest volume of sperm was obtained in group of fish treated with *H. rosa-sinensis* (2.18mL) while the control had mean sperm volume of 1.9mL (figure 1).

Semen density

There was significant reduction (p= .05) in the density of semen obtained from the male catfish treated with extracts of *C. papaya* and *I. batatas* (2.22 and 2.22 x10^{9}sperm/ml, respectively) when compared with the control and *H. rosa-sinensis* treated fish samples (Table 1, Figure 1). Sperm density was highest in the catfish treated with extracts of *H. rosa-sinensis* (2.78^{a}x10^{9}sperm/ml) and closely followed by the control (2.66^{a} x10^{9}sperm/ml). Sperm density of the control and *H. rosa-sinensis* groups were statistically similar.

Semen motility

No significant difference (p= .05) was observed in the sperm motility of fish in all the experimental groups. However, numerically, *H. rosa-sinensis* had the highest percentage of motile sperm cells (1.20%) followed by the control animals (1.08%). *C. papaya* and *I. batatas* treated animals had 1.02 and 0.98%, respectively (Table 1; figure 1).

TABLE 1

Effects of *C. papaya*, *H. rosa-sinensis* and *I. batatas* on weight of testes and some sperm parameters in male catfish (*Clarias gariepinus*)

Sperm parameters	Control	*C. papaya*	*H. rosa-sinensis*	*I. batatas*
Weight of testes (g)	$7.22^{b} \pm 0.67$	$6.11^{b} \pm 0.59$	$11.22^{a} \pm 0.22$	$7.56^{b} \pm 0.48$
Sperm volume (mL)	$1.91^{a} \pm 0.15$	$1.79^{b} \pm 0.87$	$2.18^{a} \pm 0.19$	$1.60^{b} \pm 0.07$

sperm density (x10^9sperm/ml)	$2.66^a \pm 0.11$	$2.22^b \pm 0.11$	$2.78^a \pm 0.20$	$2.22^b \pm 0.11$
Semen motility (%)	$1.08.^a \pm 0.00$	$1.02^a \pm 0.00$	$1.20^a \pm 0.00$	$0.98^a \pm 0.00$

**Means with different superscript letters along each horizontal array differ significantly (p= .05)*

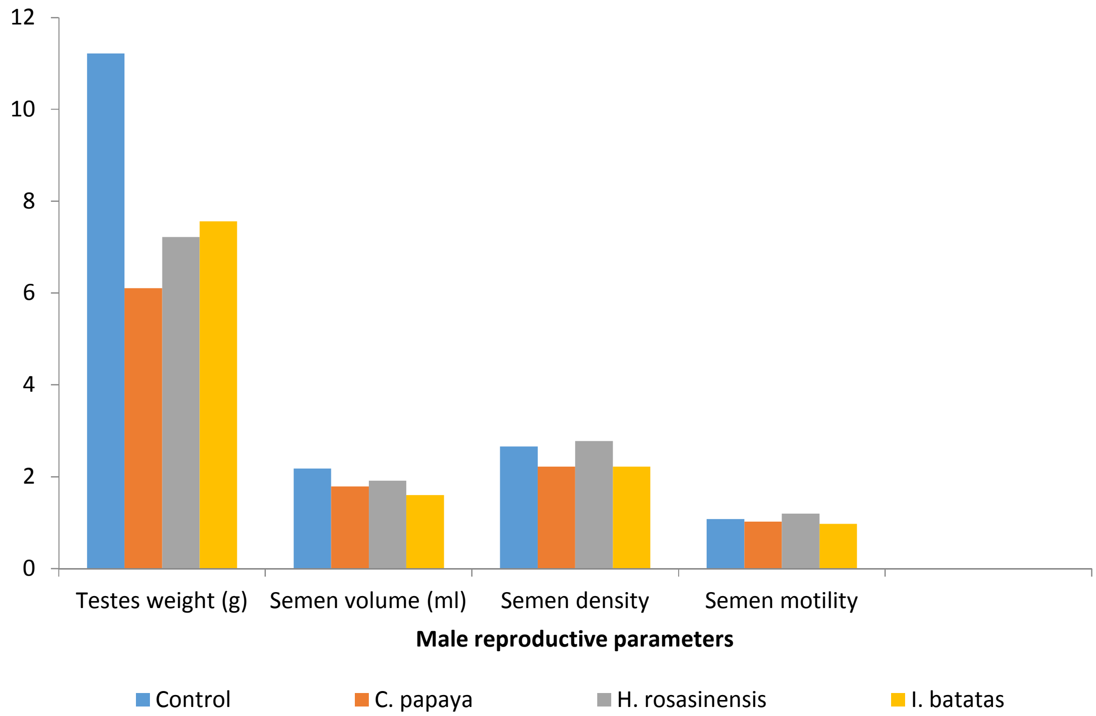

FIGURE 1: Reproductive parameters in male catfish treated with extracts of some medicinal plants compared to the control

Discussion

Viable sperm is an essential component of any successful animal production operation and the success of reproduction process is dependent on a supply of high quality gametes [34].

Result of the present study showed a significant reduction in the sperm parameters of fish samples treated with *C. papaya* and *I. batatas* when compared with the control and *Hibiscus rosa-sinensis* treated fish which agree with Ekpo et al. [8], Ikpeme *et al*. [35] and Jegede [36]. Sperm volume and density significantly decreased (p= .05) in animals treated with *C. papaya* and *I. batatas* suggesting the presence phytochemicals such as saponins, alkaloids, terpenoids, flavonoids, etc. [35], that might have altered the spermatogenic processes and pathways. This assertion is corroborated by Elham *et al.* [37], Ayotunde *et al*. [38], Udoh and Kehinde [39], Uno *et al*. [40] and Ekpo *et al*. [8,41] who reported anti-fertility properties of *C. papaya* and *I. batatas*, respectively. In human, mammals and fish, the length of time and intensity of spermatozoa motility, the percentage motile sperm and sperm density are all parameters that have been measured in an attempt to assess sperm quality [42].

On the other hand, sperm count and density significantly increased in animals treated with extract of *H. rosa-sinensis* which indicate the pro-fertility potential of the plant. This disagrees with the findings of Jegede [36] who reported reproduction inhibitory potential of *H. rosa-sinensis*. This suggests that the plant contains inherent phytochemicals that might have enhanced the hormonal milieu and/or spermatogenic processes in the fish. Hormonal balance and bioavailability have been shown to play very important role in spermatogenesis [43-44].

The Results obtained also showed that testicular weight significantly reduced ($p= .05$) in *C. papaya* and *I. batatas* treated animals which suggest distortions in the testicular integrity of the affected fish samples. The decrease in the testicular weight of *C. papaya* and *I. batatas* treated animals support the concomitant decrease observed in the sperm volume and density of the same fish.

Moreover, there was no significant difference in the sperm count of the fish in all the treatment groups. This suggests that the plant did not significantly alter the vigor of the sperm cells when compared with the control. However, numerically, animals treated with *H. rosa-sinensis* had the highest sperm count while those treated *C. papaya* and *I. batatas* had the lowest which is also in line with result of other parameters studied. Terner [45] and Adeparusi *et al*. [2] reported that spermatozoa motility varies in rigor and duration not only among male but also within an individual male depending on the ripeness, age and time of sampling.

Conclusion

In conclusion, this study reveals the pro-fertility potential of Hibiscus leaf meal in male *C. gariepinus* while *C. papaya* and *I. batatas* possess anti-fertility properties. Therefore, future studies should focus on the enhancement of seedling production strategies for different fishes using *H. rosa-sinensis* since the main objective of fish farming is to improve fish production and this plant has promising pro-fertility property which can be harnessed in aquaculture.

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