Hibiscus petals as sterility agent on Nile tilapia

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ABSTRACT

To determine if hibiscus petals can induce sterility in Nile tilapia and if the sterility so induced is reversible, matured Nile tilapia each 40g were treated for 30 days with a low dose (5.6g/kg/day) and high dose (11.2g/kg/day) of powdered hibiscus petals incorporated into their feed. Fish of similar sizes in the control experiment were fed with feed that did not contain hibiscus petals. Both the high and the low dose treatment induced irreversible sterility in male and female Nile tilapia. Fish in the control experiment spawned two weeks into the experiment and again in the fifth week but fish treated with hibiscus petals did not spawn after the treatment had been discontinued, not even when they were mated with untreated fish. High dose treatment caused a significant reduction in fish weight ($P<0.05$). The study showed that low dose of hibiscus petals, given through feed, could be used to control prolific breeding in Nile tilapia.

INTRODUCTION

A testimony to the importance of tilapia in aquaculture is the fact that Nile tilapia *Oreochromis niloticus* (Linnaeus) had been introduced into all continents of the world (Pullin, 1994). The reason for the widespread culture of this food fish is that Nile tilapia is hardy and exhibits most of the desirable qualities of a suitable culture species enumerated by Huet (1972). Fitzsimmons (2000) indicated that tilapia is poised to become the biggest aquaculture crop of the 21st century. Tilapia culture is however hampered by problems of prolific breeding, over-population and stunting. Nile tilapia matures and starts to breed at a size as small as 20g (Mair and Little, 1991). Uncontrolled reproduction of this species in ponds led to the harvest of stunted tilapia of low commercial value (Beardmore, 1996).

Mair and Little (1991) enumerated various methods for the control of prolific breeding in tilapia but each method has its shortcoming. Masculinisation and production of mono-sex tilapia through hormonal sex reversal has become the standard method and the best available technique for checking of prolific breeding and for improvement of tilapia culture. Also there is concern that introduction (transfer) of genetically improved farm tilapia (GIFT) might contaminate the wild tilapia germplasm in African countries, the home of tilapia (Gupta and Acosta, 2004). In Nigeria and indeed in other developing countries, the use of this technique is hampered by the lack of facilities and the high cost of hormones. Such situation warrants the search for a cheaper method to control tilapia reproduction. As the search for a better solution of the problem continues, medicinal plant offers an alternative method. Different plants used for inducing sterility include Neem plant (*Azadinachata indica* A. Juss) which Austin and Short (1972) found to reduce pregnancy in mice. African cucumber fruit (*Monordica charantia* Linn.) was reported to disorganize seminiferous tubules in male albino rats (*Rattus norvegicus* Dawley) leading to a decrease in sperm production (Oliver, 1960). Papaya seed (*Carica papaya* Linn.) had been used successfully to induce sterility or reduce litter size in laboratory animals and in Nile tilapia (Gary and Garg, 1971; Bodharker *et al*. 1974; Das, 1980; Udoh and Kehinde, 1999; Ekanem and Bassey, 2003). Mucuna bean seed (*Mucuna urens* Linn), administered orally to matured male guinea pig (*Cavia porcellus* Dunkin), caused complete degeneration of sperm in testicular tubules and rendered the animal sterile (Udoh and Ekpenyong, 2001). Antifertility properties of *Hibiscus rosa-sinensis* Linn. (Vern Java) were highlighted by Kholkute and Udupa (1974) while Kholkute *et al* (1977) reported on the effect of hibiscus on reproductive organs of rats. There is no literature on the use of hibiscus as sterilizing agent on fish.

The plant, *Hibiscus rosa-sinesis*, is commonly known as "Shoe Flower" because in emergency situation the petals of this flower can be used in cleaning shoes. Another common name for this plant is hibiscus. Hibiscus produces flower of various colours ranging from white to different shades of orange to dark red (Greensill 1964). *Hibiscus rosa-sinensis* belongs to the plant family Malvaceae. It is found in warm temperate, subtropical and tropical regions of the world. In China, India, Indonesia, New Guinea and Peru various uses are made of the flowers, leaves and roots of this plant. Newly sprouting hibiscus leaves are used as vegetable for soups in eastern states of Nigeria. In most tropical countries hibiscus is used as hedge. Hibiscetin is an active ingredient in hibiscus petals with anti-fertility activity (Kholkute and Udupa, 1978). Hibiscetin is a flavone with molecular formula $C_{15}H_{10}O_5$.

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Flavones are compounds which occur naturally in higher plants.

They are insoluble in water and fluoresce violet in concentrated sulphuric acid; they are responsible for ivory, yellow and red colouration in plants, flowers and fruits.

The use of hibiscus flower petals for this study was prompted by the report of its contraceptive activity by Batta and Santhakumari (1971). The study was designed to find out if hibiscus petal can act as sterility agent on Nile tilapia, to determine its mode of action and to verify if the effect was reversible or not.

MATERIALS AND METHODS

Experimental fish, feed formulation and treatment

Matured Nile tilapia used in this study were obtained from the University of Calabar Institute of Oceanography pond and acclimated in aquaria inside the Institute's hatchery where the study was conducted. The Institute maintains a stock of native *O. niloticus* which is used for research and breeding. Acclimation to aquaria conditions lasted for two weeks. During this period the fish were fed a diet composed of 40.4% wheat bran, 20.2% groundnut cake, 20.2% palm kernel cake, 6.2% bone meal, 6.0% blood meal, 6.0% palm oil and 1% vitamin premix. This diet is the same as that offered on regular basis to fish in the Institute's hatchery and ponds, it is used as the control feed in this study. To each kilogram of the control feed components 70g of powdered hibiscus petals was added to prepare the low dose treatment while 140g of powdered hibiscus petals was added to the same quantity of the control feed components to get the high dose treatment. Red hibiscus flower petals used in this study were collected within the University of Calabar environ where it is used to beautify the university. The petals so collected were dried in the oven at 50^{o}C before being ground into powder which was added to the feed. After hibiscus petal additions, nutrient imbalance was corrected by adding 140g of sawdust to each kilogram of the control feed and 70g of sawdust to each kilogram of the low dose treatment. Sawdust, an inert substance with negligible energy content, was introduced to make up for the increase in weight which accompanied additions of hibiscus petals powder. Each feed so obtained contained 30% protein, 10% fat and 18% carbohydrate which were determined using square method (New, 1987). One litre of water was added to a kilogram of the dry ingredients of each feed and mixed properly. The dough so prepared was dried in the sun and stored in closed plastic container in the refrigerator. This was used to feed the fish for two weeks before a new batch of feed was prepared.

Experimental set-up

After acclimation, five male and five female Nile tilapia were stocked in each of nine aquaria. Dimensions of each aquarium used in this experiment were 95cm x 50cm x 30cm and was filled to half its capacity with well-aerated fresh water. The study was conducted in water of temperature 27^{o}C $\forall$ 1^{o}C, pH 7, alkalinity of 30ppm and 6.4 – 6.7mg/l dissolved oxygen under continuous aeration. Each fish used in the study weighed 40g. Ohius beam balance was used for all weighings. Feeding of the fish commenced a day after stocking and lasted for 30 days. The fish were fed 8% of their initial body weight daily for 30 days. This quantity of feed was divided into two equal parts and fed to the fish in two installments at 1000 and 1500 hours. At that feeding rate, the hibiscus petal dosage was 5.6g and 11.2g per kilogram of fish per day for the low and high dose treatments respectively. Three aquaria formed the control experiment while three aquaria constituted the low dose treatment and the remaining three aquaria made up the high dose treatment.

Eight-centimeter diameter PVC pipes 30cm long (two in number) were placed in each aquarium to provide shade for the fish. Each aquarium was observed and cleaned daily by siphoning out accumulated wastes. At the end of the 30 days treatment period the fish were weighed, and the weights were compared using analysis of variance. A male fish and a female fish from each aquarium of the treatment and the control were sacrificed and the gonads were removed for sectioning and histological examination while the liver, spleen and kidney were examined for physical changes, possibly due to damage by the drug. The remaining fish in each treatment and the control were all fed at the same rate as before with control feed for another 30 days to see if reproduction will take place in any treatment. The 30 days post treatment period was the same duration it took similar sizes of Nile tilapia to recover and start reproduction after treatment with pawpaw seed (Ekanem and Bassey, 2003). At the end of this trial, male fish from the different treatments were stocked with untreated ripe females while female fish from the different treatments were stocked with untreated ripe males. At this stage, stocking rate was two male fish and four female fish per aquarium. These and the control were set up in triplicate and fed with control feed. The set-up were observed for another 30 days at the end of which the experiment was finally terminated.

Tissue preparation and microphotographs

The gonads removed for sectioning were fixed for 24 hours in formal-saline solution made of equal volumes of 10% formalin and 0.9% sodium chloride solution. Standard method was followed in pretreatment of the gonads for microtome sections (Baker and Silverton, 1985). The microtome sections of the gonads were made at 8m: thickness and each section was attached to a glass slide using egg albumen. Staining was done with haematoxylin and eosin following the method of Tseng and Chan (1982). The sections were dehydrated by subjecting them to ascending grades of ethanol (50%, 70% and 90%) and also to three changes of absolute ethanol before they were cleared in Canada balsam. Each slide of tissue so prepared was

mounted on a microscope and microphotographed at 40x magnification. One of the triplicate microphotographs prepared for each sex in each treatment and the control are presented in the result because all three were similar.

RESULTS

One hundred percent survival of fish was recorded in all stages of the experiment. Spawning did not occur in any of the treated aquaria during the 30 days treatment period whereas spawning was observed in the control experiment two weeks into the experiment but was limited to one female per aquarium. Thirty days after the treatments had been discontinued and 30 days after the fish were stocked with untreated mates, there was still no spawning in any aquarium which contained previously treated male or female fish, but spawning reoccurred in the control aquaria five weeks into the experiment. Weights of the fish at the end of the 30 days treatments were used for analysis of variance on Table 1.

Analysis of variance test found a significant difference in fish growth (weight) among treatments at 5% level (F = 9.07, 2 and 87df). Duncan's multiple Range test showed that growth in high dose treatment was different from others. The kidneys, spleens and livers of fish in the control and low dose treatments were not physically affected by the drug but the livers of fish which received high dose treatment were pale in colour. Also there were reductions in size of testes of the fish which received high dose treatment.

Histological section of Nile tilapia testis from the control experiment in Fig. 1 illustrates normal and even distribution of sperm cells. Fig. 2 is a picture of testis from tilapia, which received low dose treatment of hibiscus petals. There was deformation of cells, cell erosion at some point and clumping of cell at other points. The high dose treatment caused greater clumping of sperm cells and greater erosion of sperm cells as shown in Figure 3. Sperm cells in this experiment lost cellular integrity and deformation of nuclei were also observed.

Ovary section of Nile tilapia from the control experiment in Fig. 4 gives a picture of normal egg cells evenly distributed. Microphotograph of histological section of ovary from fish that received low dose treatment of hibiscus petals, presented in Fig. 5, show proliferation of dead cells. Fig. 6 is a picture of histological section of ovary from Nile tilapia, which received high dose hibiscus petal treatment. It showed depigmentation of the cell and hypertrophy.

Table 1. Analysis of variance table for fish weights in the control, low dose and high dose hibiscus petals treatments.

One Way Analysis of Variance: Summary

Groups	***Count***	***Sum***	***Mean***
Control	30	1293	43.10 ± 0.64
Low Dose	30	1299	43.10 ± 0.63
High Dose	30	1274	42.47 ± 0.60

Source of Variation

	SS	***df***	***MS***	***F***	***F-crit***
Between Groups	11.36	2	5.68	9.07*	3.10
Within Groups	54.47	87	0.63		
Total	65.82	89			

* Significant difference (P < 0.05)

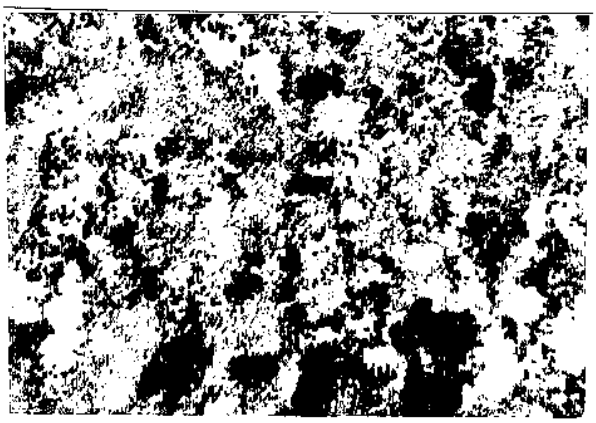

Fig. 1. Histological section of Nile tilapia testis (x40) from control experiment showing normal sperm distribution.

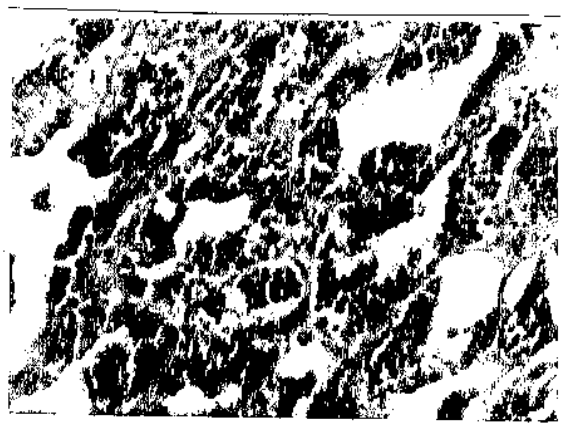

Fig. 2. Histological section of Nile tilapia testis (x40) from low dose hibiscus petals treatment showing cell erosion, deformation and cell clumping.

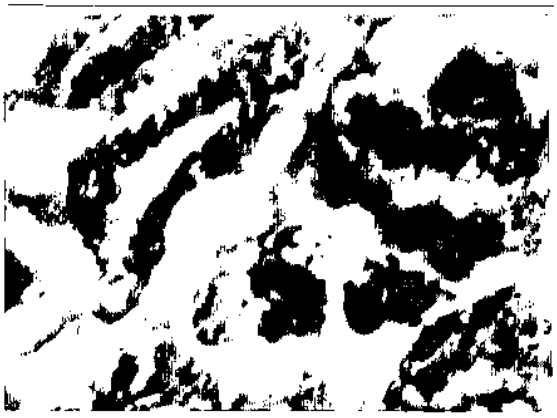

Fig. 3. Histological section of Nile tilapia testis (x40) from high dose treatment showing sperm cells with deformed nuclei, cell erosion and clumping.

Fig. 4. Histological section of Nile tilapia (x40) from the control experiment showing normal egg cells.

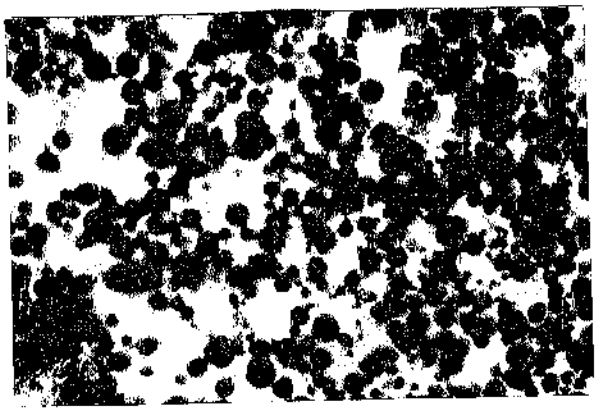

Fig. 5. Histological section of Nile tilapia (x40) from low dose hibiscus petals treatment showing proliferation of dead cell.

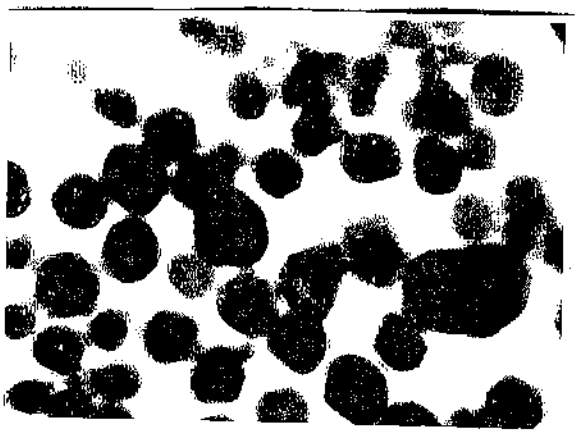

Fig. 6. Histological section of Nile tilapia (x40) from high dose hibiscus petals treatment showing hypertrophy and depigmented eggs cells.

DISCUSSION

One hundred percent survival of the fish recorded in all stages of the experiment could be attributed to adequate feeding which reduced aggressive behaviour. The inclusion of PVC pipes to provide hiding place might have contributed also.

The inability to spawn by fish that received low and high doses hibiscus treatments during the 30 days treatment period showed that hibiscus petal is an effective sterility agent on both male and female Nile tilapia. The irreversible effect of the treatment even at low dose was evident from the fact that 30 days after the fish were taken off the treatment they still did not spawn. This was further strengthened by lack of spawning when the treated fish were mated with matured untreated fish. However, further studies with extended recovery period may be necessary to confirm irreversibility of the sterility so induced. The irreversibility found in this study is a sharp contrast to the finding by Angwafor (1996) in which rats that received low dose treatments of hibiscus petals were able to reproduce after they were taken off the treatment. The reason is that the low treatment used on the rat was very low as it was measured in milligrams. The dosage used in this study was meant to ensure adequate intake of the drug after some might have been washed off the feed by water. Also there was no previous work on fish or under water environment to provide guiding information on the dosage to use; this study now provided such data. The study on rat by Angwafor (1996) points to the fact that further studies can produce the dosage of this drug which could be used reversibly to control prolific breeding in Nile tilapia.

Spawning of fish in the control experiment, which was limited to a single female fish in each aquarium, could be attributed to limiting carrying capacity of the aquarium. The carrying capacity of the aquarium must have been filled immediately one female fish spawned and that could have suppressed the urge for further spawning by other female tilapia as discussed by Mair and Little (1991).

In the testes of Nile tilapia, the drug combined deformation, clumping and erosion of sperm cells to achieve sterility. These actions got intensified with increasing concentration of the drug. Whereas in the ovaries, low concentration of the drug caused disintegration of the cells and proliferation of dead cells which could not get fertilized. Depigmented dead cells, even though enlarged, could not get fertilized in fish which received high dose of hibiscus petals. Enlargement of egg cells is a deformity created by high dose treatment to cause sterility.

The significant difference ($P<0.05$) in weight detected by analysis of variance must have come from the reduction in sizes of testes in fish which received high dose hibiscus petal treatment. It could also have resulted from the toxic effect of the drug, which gave a pale colour to the liver and probably interfered with the metabolic processes of the fish that received high dose of hibiscus petals. Kidneys and spleens, which were not affected by high concentration of the drug, were adjudged to be less sensitive to the drug. It appeared that low dose hibiscus petal treatment focused its action only on the gonads and had no deleterious effect on other organs, which makes it an ideal sterility agent as pointed out by Kholkute *et al* (1977).

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