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Effects of the Aqueous Seed Extract of *Tribulus terrestris* on Sex Reversal of Nile Tilapia, *Oreochromis niloticus*

KEYWORDS: Aqueous extract, *Tribulus terrestris*, *Orochromis niloticus*, Sex reversal

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ABSTRACT *Three days old mixed sex juveniles of Nile tilapia (mean weight 0.025 ± 0.009 g; mean length 1.25 ± 0.012 cm) were treated by immersion method with aqueous seed extract of Tribulus terrestris (ASET) at the concentration of 0.0 (control), 0.05, 0.10 and 0.15 g/l for four weeks. The highest survival percentage (87.5±2.5) was observed in control group, but there was no significant difference (P>0.05) in survival percentage among the different treatment groups. The highest percentage of males (81.4±0.5) was observed in 0.15 g/l group and it was significantly higher (P<0.05) compared to all other treatment categories. Immersion treatment with ASET caused significant increase (P<0.05) in percentage of males compared to that in untreated control. The extract showed presence of phytochemicals such as tannins, saponins, steroids and alkaloids, which might be associated with its androgenic property.*

Introduction

The Nile tilapia, *Oreochromis niloticus* (Linnaeus) is a well-studied, fast-growing and widely cultured fish species that is currently ranked second only to carps in global production and is likely to be the most important cultured fish in the 21st century (Ridha, 2006). Rapid growth rates, high tolerance to low water quality, efficient food conversion, resistance to disease, good consumer acceptance and ease of spawning make tilapia a suitable fish for culture (El-Saidy & Gaber, 2005). Female organism of tilapine species have a high fecundity, generally reproducing at a small size and exhibiting stunted somatic growth at higher densities, while male tilapias exhibit faster growth rates and are often the preferred gender for monosex aquaculture (Hines & Watts, 1995). Synthetic steroids are commonly used to induce sex reversal in tilapia but because of the potential hazards of such steroids; the use of new chemicals is a potential alternative to be explored (Papoulias, Noltie, & Tillitt, 2000). Plant extracts containing diverse bioactive principles such as alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils have been reported to promote various activities like antistress, growth promotion, appetite stimulation, tonic and immunostimulation, and antimicrobial properties in fish culture (Citarasu, 2010; Chakraborty & Hancz. 2011). Phytochemicals are also reported to block biosynthesis as well as action of estrogen by acting as aromatase inhibitors and antagonists to nuclear estrogen receptor in gonad germ cells (Rempel & Schlenk, 2008) and hence may be considered as potential mean for inducing sex reversal in fish. However, there are significant variations regarding the efficacy of different phytochemicals for production of all-male fish population and the potential anabolizing and virilizing effects of such plant extracts needs to be clearly documented. The herb, *Tribulus terrestris* has been reported to raise testosterone levels (Bucci, 2000) and to induce sex reversal in fish while administered through immersion technique (Çek, Turan, & Atik, 2007a; Çek, Turan, & Atik, 2007b). The plant extract has also been found to stimulate growth in fish (Çek et al., 2007a; Çek et al., 2007b). However, no studies have been reported related to its in vivo effect on sex reversal, growth and immunostimulation of tilapia. Considering these aspects, the objective of the present study was to investigate the potential effect of the aqueous seed extract of *T. terrestris* (ASET) on the masculinisation of *O. niloticus* by immersion technique.

Materials And Methods

Collection of fish seed

Just hatched juveniles of mixed-sex Nile tilapia was collected from the Fish Hatchery of West Bengal Government, oxygen packed and transported to the laboratory.

Plant extracts preparations

T. terrestris seeds were procured from the local plant market, washed in sterile distilled water, air-dried in shade and powdered. The aqueous seed extract of *T. terrestris* (ASET) was prepared by boiling 18 g powder in 1500 ml distilled water for 30 minutes and then filtering it with a Whatman filter paper twice (Çek et al., 2007b). The solution was prepared weekly.

Immersion treatment of fish with plant extracts

Three days old mixed sex juveniles of Nile tilapia (mean weight 0.025 ± 0.009 g; mean length 1.25 ± 0.012 cm) were randomly assigned in 12 glass aquaria to four different treatment groups (0.0 or control, 0.05, 0.1 and 0.15 g/l). The treatment was conducted for 30 days and the fish were exposed to the ASET 4 times (once weekly) during this period. The aquaria were continuously aerated and maintained in heated (T = 27±2^{0}C) static systems. Water in all aquaria was replaced manually and the fish was kept under similar photoperiod (14 L: 10 D). Each aquarium was stocked with 40 fish. The fish was fed finely ground (< 500-1000 mm) artificial diet containing 30% crude protein (Tokyu, Japan) at a rate of 20% body weight / day. The experiment was conducted simultaneously in triplicate.

Sexing of fish

Sexing of the juvenile fish was done by the standard acetocarmine squash technique of gonads (Guerrero & Shelton, 1974). Histological studies of the gonads were also performed.

Statistical analysis

All data are expressed in terms of mean ± standard error (SE). Treatment effects on different parameters were analyzed by one-way analysis of variance (ANOVA) after checking normality by Shapiro-Wilk's test. Where significant differences were found, a Tukey's test was performed for

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separating treatment means. All statistical analysis was performed using the SPSS version 11.5 for Windows.

Qualitative phytochemical studies

Qualitative phytochemical analysis of the ASET was carried out using standard procedures (Malpani, Rajput, Mane, & and Dhabe, 2011; Kumar & Bhardwaj, 2012; Ray, Chatterjee, & Chakrabarti, 2013).

Results and Discussion

Survival percentage in control was similar to those observed in the ASET treated groups, where no significant dose-related inter-group differences were noted (P>0.05) (Table 1). The highest survival percentage (87.5±2.5) was observed in control fish while the lowest survival percentage was observed in treatment with ASET at the concentration of 0.1 g/l (80.8±2.2) (Table 1). The result indicates that immersion treatment with ASET has no adverse effects on general fish health. Similar results were obtained in other studies with *Poecilia reticulata* and *P. latipinna* as well where immersion treatment with *T. terrestris* extract showed no significant difference in survival of fish compared to that of untreated control (Çek et al., 2007b; Kavitha & Subramanian, 2011).

All the treatment categories showed significantly higher (P<0.05) percentage of males compared to the control. The highest percentage of males (81.4±0.5) was observed in treatment with ASET at the concentration of 0.15 g/l, which is significantly higher (P<0.05) compared to all other groups (Table 1). Interestingly, all the treatment categories except the control group showed intersex with both male and female gonadal tissue. The highest percentage of intersex (7.2±1.1) was observed in ASET 0.15 g/l treatment group (Table 1). Dietary inclusion of commercially available *T. terrestris* extract at a concentration of 2.5 g/kg basal diet have resulted in 84% male population in *O. niloticus* (Omitoyin, Ajani, & Sadiq, 2013). In another experiment, 97% masculinisation was achieved in *P. latipinna* by immersing 0-day-old fry for 60 days in water containing 50 ppm *T. terrestris* extracted in 70% ethanol (Kavitha & Subramanian, 2011). Results emanating from this study indicate a dose dependent masculinisation effect of *T. terrestris* extract on Nile tilapia. This result corroborates with other studies, where percentage of males increased with increase in the *T. terrestris* concentration in *P. latipinna, P. reticulata, Cichlasoma nigrofasciatum* and *Clarias gariepinus* (Kavitha & Subramanian, 2011; Kavitha, Ramesh, & Subramanian, 2012; Çek et al., 2007b; Çek et al., 2007a; Turan & Çek, 2007). As the highest treatment concentration of 0.15 g/l produced the maximum percentage of males among the different treatment categories in this study (Table 1), further analysis with increased concentration might be required to achieve 100% sex reversal with ASET.

Table 1: Percentage of survival, male, female and intersex during immersion treatment with ASET at different concentrations. Different superscripts mark significant differences in means within columns.

Treatment category	% Survival	% of male	% of female	% of intersex
Control	87.5±2.5[a]	45.75±1.35[a]	54.25±1.35[c]	0.0±0.0[a]
Tribulus 0.05 g / l	83.3±1.7[a]	71.5±2.1[b]	21.3±3.2[ab]	7.2±1.1[a]
Tribulus 0.1 g / l	80.8±2.2[a]	72.0±0.8[b]	23.0±0.5[b]	5.0±1.0[a]
Tribulus 0.15 g / l	81.7±1.7[a]	81.4±0.5[c]	13.4±0.7[a]	5.2±1.2[a]

T. terrestris has been reported to be used in traditional medicine to treat sexual asthenia and infertility in man (Adaikan, Gauthaman, & Prasad, 2001). Oral treatment with *T. terrestris* extract was found to significantly increase body weight, intracavernous pressure, mount and intromission frequencies while to decrease mount latency and postejaculatory interval in Sprague-Dawley rat (Gauthaman, Ganesan, & Prasad, 2003). *T. terrestris* extract was reported to contain steroid saponin protodioscin and found to increase concentration of some of the sex hormones in rat (Gauthaman, Adaikan, & Prasad, 2002; Gauthaman & Ganesan, 2008). However, Neychev and Mitev (2005) observed that the steroid saponins in *T. terrestris* possess neither direct nor indirect androgen-increasing properties in young men. Although the present work indicates that immersion treatment with ASET could induce high rate of masculinisation in tilapia, whether this potency is caused by increase in androgen level cannot be deduced as the serum testosterone level was not measured during the study. Thus, extensive studies would be required to clarify the probable mode of action of *T. terrestris* steroid saponin in fish. Qualitative analysis for phytochemicals revealed the presence of tannins, saponins, steroids and alkaloids in ASET, while flavonoids, glycosides and carbohydrates are not present in the extract. These phytoconstituents might render the androgenic activity of the extract. A variety of pathways have been postulated to be associated with functional mechanisms of phyto-compounds causing both masculinisation and feminization at different concentrations (Chakraborty, Horn, & Hancz, 2014).

The results emanating from this study indicates that ASET might be used as an alternative method to produce all-male tilapia population in an environment-friendly manner using a natural product. However, the highest percentage of males produced by immersion in ASET is 81%, which is well below the ideal requirement of 100% male population. Thus, further studies would be required to establish an ideal treatment regime for production of all-male tilapia population using ASET and to provide conclusive evidence regarding its efficacy to be used as a sex-reversal agent in tilapia culture.

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