



Effect of letrozole on masculinization of Siamese fighting fish (*Betta splendens*)

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Abstract: The aim of this study was to evaluate the effect of letrozole (a non-steroidal aromatase inhibitor) on masculinization of Siamese fighting fish (*Betta splendens*). Different doses of letrozole 50, 100, 150 and 200 ppm (mg/kg feed) were incorporated into diet and fed for periods of 30, 40 or 50 days. Immersion treatment of letrozole at selected doses (250, 500, 750 and 1,000 µg/l) for 3 h each on third, fifth and eighth day-post-hatching (dph) (Trial 1) and fourth, sixth and eighth dph (Trial 2) was given. The oral administration of letrozole for 30, 40 or 50 days did not have any significant effect on the sex ratio of *B. splendens*. The immersion treatment of letrozole induced 100% masculinization at 500 and 1000 µg/l in trial 1, while it yielded only 66.66 and 90% male population at 500 and 1000 µg/l in Trial 2. The dietary administration of letrozole for 30 and 50 days caused tail deformities and/or rudimentary and absence of dorsal fin. The progeny testing of males from letrozole treated (both oral and immersion) groups indicate that the sex ratio of progenies of each of the males tested did not differ significantly from that of control, indicating that all those males carried XY genotype. Letrozole treatments suppressed ovarian development (atretic oocytes were common). However, the testicular development was unaffected. The study revealed that immersion treatment of letrozole was more effective in inducing masculinization of *B. splendens* than the oral administration of letrozole.

Keywords: Aromatase inhibitor, Deformity, Fighting fish, Masculinization, Histology

INTRODUCTION

The quantity of ornamental fish traded worldwide has increased steadily over the years. The Siamese fighting fish (*Betta splendens*), also known as the Betta and simply as the fighter, is a popular species of fresh water aquarium fish. It has been affectionately nicknamed “The Jewel of the orient” due to large flowing fins and a wide range of colors. The male *Betta* will flare his gills, twist his body and spread his fins during mating. Female is duller in color and smaller in size. Males build bubble nests of various sizes and thickness at the surface of water. Males are in greater demand and fetch a much better price than females. Functional sex of an animal is reversed by direct treatment with estrogens or androgens during the period of sex differentiation in the early stage of gonad genesis. Fish breeders are able to produce either males or females separately depending upon the demand for monosex fish.

Earlier studies were extended to *B. splendens* to produce a monosex male population (Jessy and Varghese, 1988; Pandian and Sheela, 1995; Kavumpurath and Pandian, 1994; Kirankumar and Pandian, 2002; Balsubramani and Pandian, 2008; Kipouros *et al.*,

2011). Feeding *B. splendens* with a diet containing 17 α -methyltestosterone (17 α -MT) at doses of 80-140 ppm resulted in the production of 80, 86.9, 87.5 and 91.3% males, respectively (Jessy and Varghese, 1988). A 100% masculinization was achieved when *Betta* was fed at doses 8, 15, 60 and 90 mg/kg 19-nor-ethyltestosterone, 17 α -MT, 11-ketotestosterone and androstenedione, respectively (Kavumpurath and Pandian, 1994).

In recent years, aromatase inhibitors find increasing application for sex control in fish. Aromatase is a cytochrome P450 enzyme complex that is required for biosynthesis of estrogens that converts testosterone to estradiol and androstenedione to estrone. Blocking P450arom activity using chemicals is common and they are known as aromatase inhibitors (AI), leading to a decrease in the production of estrogen (Steele *et al.*, 1987). AIs have been used in the treatment of breast and ovarian cancer in postmenopausal women (Howell *et al.*, 2005) and in studies of sex change in a wide range of teleost fish, e.g., tilapia (Kwon *et al.*, 2000; Afonso *et al.*, 2001; Das *et al.*, 2012), salmon (Piferrer *et al.*, 1994), Japanese flounder (Kitano *et al.*, 2000), zebrafish (Fenske and segner, 2004) and guppy (Basavaraja *et al.*, 2012). It was possible to masculinize genetic females (XX) to phenotypic males (XX)

through dietary administration of a common aromatase inhibitor, femura (chemical name: letrozole) to the java tilapia (*Oreochromis mossambicus*), wherein 100% male population could be produced by treating the fry at 100-200 ppm diet for 30 days (Das *et al.*, 2012), letrozole in combination with 17 α -MT also induced the production of 100% male tilapia population; letrozole administration did not indicate any adverse effect on growth, survival and proximate composition of the tilapia. Letrozole at 100 ppm produced 92, 93 and 97% male guppy when treated for 30, 40 and 50 days (Basavaraja *et al.*, 2012). Since the use of steroids evokes public concern, one alternative strategy would be the application of non-steroidal drugs like letrozole. Hence, the study was conducted to produce an all-male progeny using letrozole and also to test its effect on morphology and gonadal development in Siamese fighting fish, *B. splendens*.

MATERIALS AND METHODS

The present study was conducted at fish farm of the College of Fisheries, Mangalore.

Incorporation of letrozole: The commercially available fry feed (Nutri feed) of Godrej Agrovet Vijaywada, Andhra Pradesh was used as a basal diet for the administration of letrozole. Required quantity of feed (100 g) was weighed, powdered using a grinder and packed in air tight polythene bags and stored at 4°C. The letrozole tablets (common name: Femura, trade name: Letroz, Sun-Pharmaceutical, Gujarat, India), molecular formula (C₁₄H₁₃N₃.HCL) and chemical name 4-(5, 6, 7, 8-tetrahydroimidazole (1, 5-a) pyridin-yl benzonitrilemonohydrochloride) procured from a local dealer was used for incorporation into feed. The required quantity of the powdered basal feed (100 g) was taken in plastic tray and used as the letrozole carrier feed prepared using alcohol evaporation method (Guerrero, 1975). The required quantity of letrozole i.e. 50, 100, 150 and 200 mg (weighed accurately using an electrical balance) was dissolved in 50ml of 95% ethanol and the AI-dissolved ethanol was sprayed on feed uniformly using a chromatogram column sprayer. The feed was mixed thoroughly with a glass rod to ensure proper mixing of letrozole into the feed. The letrozole incorporated diet was dried at room temperature to allow the ethanol to evaporate. The control diet was prepared in the same manner using ethanol only. After drying the respective feeds were packed in air tight polythene bags and stored in a refrigerator at 4°C.

Development of brood-stock and fry production: *Betta* stocks obtained from local aquarists and maintained at the College farm were used to develop brood fish. The same was raised by feeding daily twice with mosquito larvae and zooplankton. The gravid female and male *Betta* (identified based on their external characteristics) were selected for fry production. The female and male brooders (1:1) were held in an aquarium (2×1×1.5 ft) for breeding. In about one or two days of their release in the aquarium, breeding took place wherein the eggs were released in

batches and fertilized. The resultant fry were used for experimentation. The aquaria were aerated using an air blower. Before stocking the fry, the aquaria (2×1×1.5 ft) were disinfected with a solution of potassium permanganate (KMnO₄) at 5 ppm, cleaned, dried and filled with fresh filtered water.

Letrozole treatment to fry: Three or four-day-old *Betta* fry were divided into five groups of 30 to 50 each and designated as T₀, T₁, T₂, T₃ and T₄ for 0, 50, 100, 150 and 200 ppm letrozole, respectively and fed *ad libitum* twice daily for periods 30, 40 or 50 days. The immersion treatment of letrozole was carried out at concentrations of 250, 500, 750 and 1000 μ g/l water. Randomly selected fry were subjected to discrete immersion treatment of letrozole for 3 h each on third, fifth and eighth days of post hatching (dph) or for 3 hours each on fourth, sixth and eighth dph. Stock solution of letrozole was prepared in absolute alcohol. A control group was maintained without letrozole. About 50% water was exchanged once every four days during treatment period to remove excreta, uneaten food and the accumulated metabolites of letrozole. All the tanks were aerated using an air blower.

Fry rearing: Cement tanks (2.5×2.5×3 ft) were used for post-treatment rearing of fry from respective groups and reared for a period of 90 days. Before stocking of fry, the cement tanks were drained, dried, filled with fresh water and manured with chicken manure at 200g/m². During post-treatment rearing, the fry were fed with sieved zooplankton till maturity.

Evaluation of letrozole treatment: On termination of the experiments, all the surviving fish were harvested and counted. The phenotypic sex of the treated and control fish was determined by the secondary sexual characters, such as the shape of the belly, body coloration and finnage.

Progeny testing : In order to confirm the efficiency of sex conversion, progeny testing was carried out, for which sex reversed males from treated group were mated with normal females in glass tanks (2×1×1.5 ft) at a ratio of 1:1 (M:F); the resultant progeny was reared in cement tank (2.5 ×2.5 × 3 ft) for three months and during this period the fry were fed with control feed at 50% of their body weight. Upon maturity, the fishes were sexed by means of secondary sexual characters like colour, size, shape of the belly and finnage.

Histology of gonads: The gonads of control and treatment groups were collected, preserved, fixed, embedded, section taken, stained (Gray, 1964) and sections were observed under a microscope (Olympus) and photographed.

Statistical analysis: The Chi-square test was employed to test the equality of sex ratio among different groups.

RESULTS

Sex ratio: The results of the oral administration of letrozole for 30, 40 or 50 days are presented in tables 1 -3. The survival ranged between 14 and 32.5% during

letrozole treatment period. Among the letrozole treated groups, the percentage of males remained unaffected, indicating that the letrozole administration (oral) does not cause masculinization at the tested doses (Tables 1-3). On the other hand, a higher percentage of females were found in majority of the groups treated with letrozole. The survival during post-treatment rearing varied between 41.66 and 100% (Tables 1-3).

Tail deformity and fin abnormality: Tail deformities and fin deformities were often observed in the groups treated with letrozole for 40 and 50 days and the details are presented in fig. 1 and table 4. Various patterns of skeletal deformities like bent vertebral column and tail were observed. The absence of dorsal fin or rudimentary dorsal fin was frequently observed in the letrozole-fed fish. It was observed that as the dose increased, the percentage of deformity and abnormality also increased. The highest deformity (100%) was observed in the group treated with letrozole at 200 ppm for 40 days. The control did not exhibit any deformities (Fig. 1 and Table 4).

Immersion treatment: The results of the immersion treatment of letrozole are presented in tables 5 and 6. The survival of fry during treatment period ranged

between 37.5 and 70%, while it varied between 27.8 and 62.5% during post-treatment period (Tables 5 and 6). Among treated groups, the highest percentage of males (100%) was obtained in the group treated with letrozole at 500 and 1000 µg/l (Trial 1), whereas the highest percentage of males (90%) was observed at 1000 µg/l (Trial 2).

Progeny testing of letrozole treated fish: Table 7 shows the results of progeny testing of males that received letrozole incorporated feed and immersion treatment of letrozole. The survival of progenies of the oral trial ranged between 37.68 and 40%. All the three males (from 150 ppm group) when mated with normal female at 1:1, produced almost normal sex ratios (42.30, 47.82, 50% males and 57.70, 52.18, 50% females, respectively), indicating all the males had XY-genotype. The Control group yielded 58.34% males and 41.66% females. The sex ratio was not significantly different ($P > 0.05$) in each of the progeny tested groups, when compared with Control (Table 7). The survival of different progenies of the immersion trial ranged between 21.73 and 41.53%. Progenies from 500 µg/l group had 62.07, 46.66 and 40% males and 37.93, 53.34 and 60% females, respec-

Table 1. Number of fry stocked and recovered, sex composition and survival of *B. splendens* fed with varying doses of letrozole for 30 days.

Treatment	Treatment period				Post- treatment period				
	No. of fry (Initial)	No. of fry (Final)	Survival (%)	No. of fish recovered	Male	Female	Survival (%)	Sex ratio (M:F)	Chi-square value (against 1:1 sex ratio)
Control	40	10	25	7	1 (14.29)	6 (85.71)	70	1:6	3.56
50 ppm	40	13	32.5	7	2 (28.58)	5 (71.42)	53.84	1:2.5	1.28
100 ppm	40	11	27.5	6	3 (50)	3 (50)	54.54	1:1	0
150 ppm	40	11	27.5	7	3 (42.85)	4 (57.15)	63.63	1:1.3	0.14
200 ppm	40	9	22.5	5	2 (40)	3 (60)	55.55	1:1.5	0.2

Sex composition figures given in parentheses are expressed as a percentage of total number of fish. Statistically not significant at 5% level from the expected 1:1 sex ratio; M: Male, F: Female

Table 2. Number of fry stocked and recovered, sex composition and survival of *B. splendens* fed with varying doses of letrozole for 40 days.

Treatment	Treatment period				Post- treatment period				
	No. of fry (Initial)	No. of fry (Final)	Survival (%)	No. of fish recovered	Male	Female	Survival (%)	Sex ratio (M:F)	Chi-square value (against 1:1 sex ratio)
Control	50	10	20	7	2 (28.58)	5 (71.42)	70	1:2.5	1.28
50 ppm	50	13	26	8	4 (50)	4 (50)	61.53	1:1	0
100 ppm	50	14	28	9	3 (33.34)	6 (66.66)	64.28	1:2	1
150 ppm	50	12	24	5	2 (40)	3 (60)	41.66	1:1.5	0.2
200 ppm	50	7	14	7	2 (28.58)	5 (71.42)	100	1:2.5	1.28

Sex composition figures given in parentheses are expressed as a percentage of total number of fish. Statistically not significant at 5% level from the expected 1:1 sex ratio; M: Male, F: Female

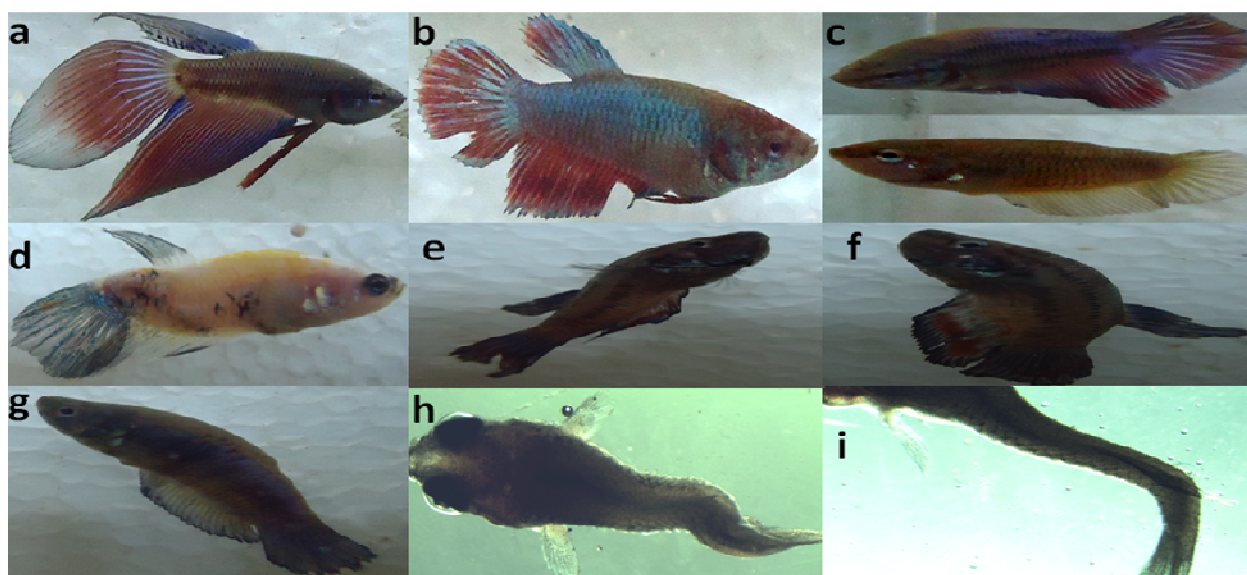


Fig. 1. Effect of untreated and letrozole-treated diet on the morphological characteristics of Betta. (a) Normal male, (b) Normal female, (c) Females without dorsal fin, (d) Male with a rudimentary dorsal fin and bent caudal region, (e) Male with rudimentary dorsal fin, anal fin and deformed body, (f) Male with a curved body, rudimentary dorsal fin, showing vertical movement, (g) Male without dorsal fin and abnormal tail, showing slanting movement, (h) A 40-day treated fry with an abnormal head region and a bent tail, (i) A 50-day letrozole treated fry showing an elongated and curved tail.

Table 3. Number of fry stocked and recovered, sex composition and survival of *B. splendens* fed varying doses of letrozole for 50 days.

Treatment	Treatment period				Post-treatment period				
	No. of fry (Initial)	No. of fry (Final)	Survival (%)	No. of fish re-covered	Male	Female	Survival (%)	Sex ratio (M:F)	Chi-square value (against 1:1 sex ratio)
Control	50	10	20	8	3 (37.50)	5 (62.50)	80	1:1.6	0.5
50 ppm	50	10	20	7	3 (42.85)	4 (57.15)	70	1:1.3	0.14
100 ppm	50	12	24	10	3 (30)	7 (70)	83.33	1:2.3	1.6
150 ppm	50	11	22	9	4 (44.44)	5 (55.55)	81.81	1:1.2	0.11
200 ppm	50	8	16	8	3 (37.50)	5 (62.50)	100	1:1.6	0.5

Sex composition figures given in parentheses are expressed as a percentage of total number of fish. Statistically not significant at 5% level from the expected 1:1 sex ratio; M: Male, F: Female

tively, while the progenies from 1000 µg/l group consisted of 64.29, 30.44 and 33.34% males and 35.71, 69.56 and 66.66% females, respectively. The two Control groups had 48.64 and 45.45% males and 51.36 and 54.55%

females, respectively. The sex ratio was not significantly ($P > 0.05$) different (Table 7).

Histology of gonads: The results of the histological examination of the ovary of fish fed with letrozole –incorporated diet are presented in fig. 2. The ovaries of Control fish showed different stages of oogenesis like perinucleolar stage, primary oocyte growth, early vitellogenesis etc. The histology of letrozole -treated fish showed a slight suppression of ovary with atretic or degenerating oocytes and increased

vacuolar area (Fig. 2). The results of the histological examination of the testis of fish fed with letrozole are presented in fig. 3. No significant difference was found in the spermatogenesis of both Control and letrozole treated fish. The histology of testis revealed the presence of normal spermatogonia, spermatocytes, spermatids and spermatozoa (Fig. 3).

DISCUSSION

This study reports the masculinization of *B. splendens* through a discrete immersion of letrozole. Letrozole treatment for 3 h each on third, fifth and eighth dph produced 100% males at both 500 and 1000 µg/l, whereas it yielded a maximum of 90% male population at 1000 µg/l when treatment was given on

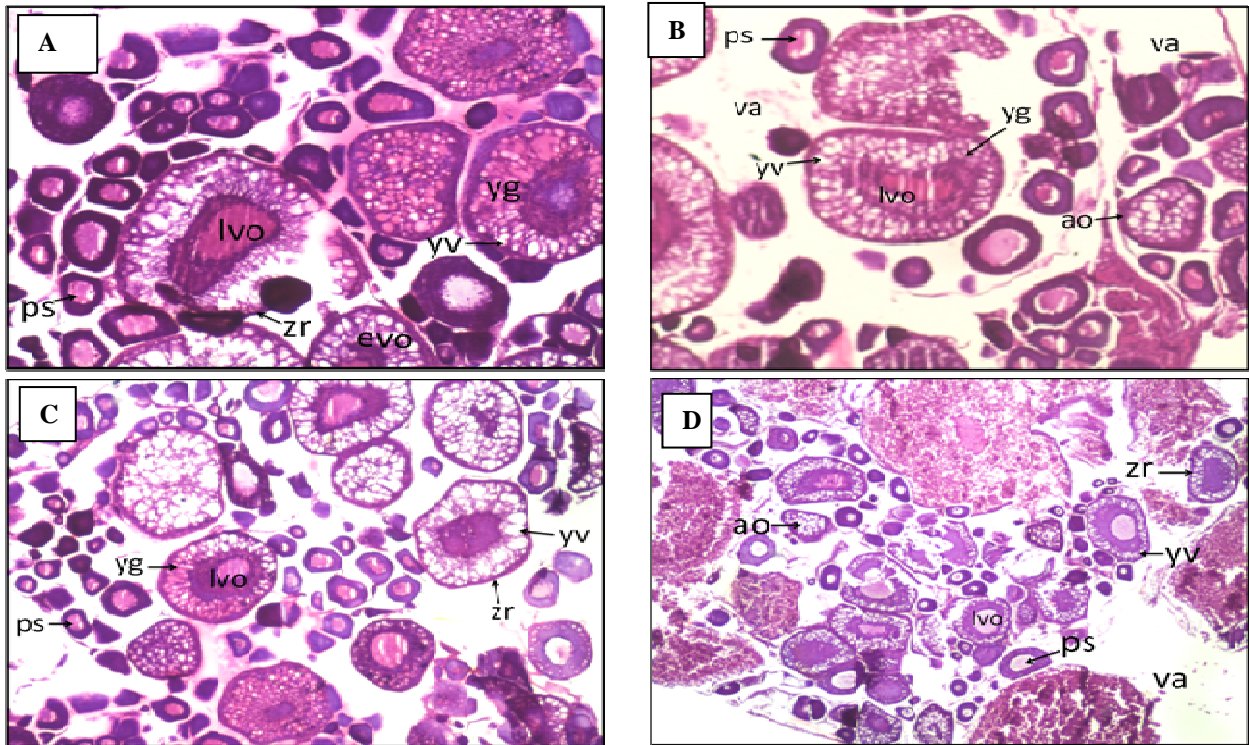


Fig. 2. Photomicrograph of ovary of control fish (A) and (B) fish that received letrozole diet; control fish (C) and (D) fish that received letrozole immersion treatment showing different stages of oogenesis; perinucleolar stage (ps), yolk vesicle (yv), yolk granules (yg), late vitellogenic oocyte (lvo), zona radiata (zr), nucleus (n), early vitellogenic oocyte (evo), atretic oocytes (ao), and vacuolar area (va) (H and E; 10).

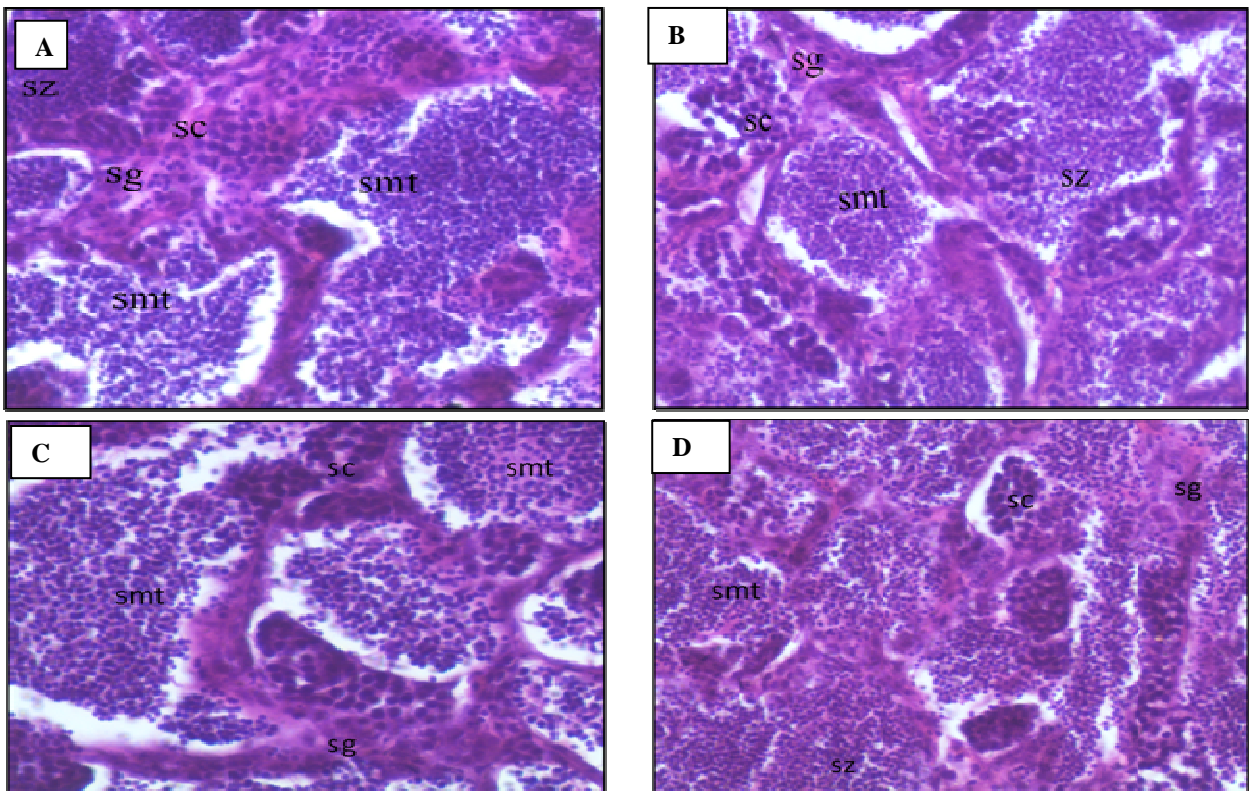


Fig. 3. Photomicrograph of testis of Control (A) and letrozole fed fish (B)(H & E; 40); control (C) and fish that received immersion treatment of letrozole (D) showing different stages of spermatogonia (sg), spermatocytes (sc), spermatids (smt) and spermatozoa (sz) (H & E; 40).

Table 4. Number of fry stocked and recovered and the proportion of deformed fish when fed varying doses of letrozole for 40 and 50 days

Treatment	Treatment period (40 days)				Treatment period (50 days)				Post-treatment period				
	No. of fry (Initial)	Total no. of abnormal fry	Deformity (%)	No. of fish recovered	Total no. of deformed fish	Deformity (%)	Treatment	No. of fry (Initial)	Total no. of abnormal fry	Deformity (%)	No. of fish recovered	Total no. of deformed fish	Deformity (%)
Control	10	0	0	7	0	0	Control	10	0	0	8	0	0
50 ppm	13	7	53.84	8	5	62.5	50 ppm	10	5	50	7	3	42.85
100 ppm	14	10	71.42	9	7	77.77	100 ppm	12	7	58.33	10	6	60
150 ppm	12	10	83.33	5	4	80	150 ppm	11	9	81.81	9	7	77.77
200 ppm	7	7	100	7	7	100	200 ppm	8	7	87.5	8	7	87.5

Table 5: Number of fry stocked and recovered, sex composition and survival of *B. splendens* given immersion treatment of letrozole (Trial 1).

Treatment	Treatment period				Post-treatment period				Chi-square value (against 1:1 sex ratio)
	No. of fry (Initial)	No. of fry (after 8 days)	Survival (%)	No. of fish re-covered	Male	Female	Survival (%)	Sex ratio M:F	
Control	30	21	70	6	3 (50)	3 (50)	28.57	1:1	0
250 µg/l	30	18	60	9	4 (44.44)	5 (55.55)	50	1:1.2	0.11
500 µg/l	30	18	60	5	5 (100)	0 (0)	27.77	1:0	5*
750 µg/l	30	16	53.33	6	5 (83.34)	1 (16.66)	37.5	1:0.2	2.66
1000 µg/l	30	14	46.66	5	5 (100)	0 (0)	35.71	1:0	5*

Sex composition figures given in parentheses are expressed as a percentage of total number of fish. *Statistically significant at 5% level from the expected 1:1 sex ratio; M: Male, F: Female

Table 6. Number of fry stocked and recovered, sex composition and survival of *B. splendens* given immersion treatment of letrozole (Trial 2).

Treatment	Treatment period			Post- treatment period					
	No. of fry (Initial)	No. of fry (after 8 days)	Survival (%)	No. of fish recovered	Male	Female	Survival (%)	Sex ratio M:F	Chi-square value (against 1:1 sex ratio)
Control	40	20	50	11	3 (27.28)	8 (72.72)	55	1:26	2.27
250 µg/l	40	16	40	10	5 (50)	5 (50)	62.5	1:1	0
500 µg/l	40	18	45	9	6 (66.66)	3 (33.34)	50	1:0.5	1
750 µg/l	40	15	37.5	9	4 (44.44)	5 (55.56)	60	1:1.25	0.1
1000 µg/l	40	17	42.5	10	9 (90)	1 (10)	58.82	1:0.11	6.4*

Sex composition figures given in parentheses are expressed as a percentage of total number of fish. *Statistically significant at 5% level from the expected 1:1 sex ratio; M: Male, F: Female

Table 7. Results of progeny testing.

Treatment	Dose	Replicate	No. of fry (Initial)	No. of fish recovered and sex	Survival (%)	Female	Male	Female (%)	Sex ratio (F:M)
Letrozole (oral)	150 ppm	Control	62	24	38.70	10 (41.66)	14 (58.34)	41.66	1:1.4
		Replicate 1	69	26	37.68	15 (57.70)	11 (42.30)	57.70	1:0.73
		Replicate 2	60	23	38.33	12 (52.18)	11 (47.82)	52.18	1:0.91
Letrozole (immersion)	Control	Replicate 3	70	28	40	14(50)	14(50)	50	1:1
		Replicate 1	90	37	41.11	19 (51.36)	18 (48.64)	51.36	1:0.94
		Replicate 2	75	22	29.33	12 (54.55)	10 (45.45)	54.55	1:0.83
	500µg/l	Replicate 1	70	29	41.42	11 (37.93)	18 (62.07)	37.93	1:1.63
		Replicate 2	69	15	21.73	8(53.34)	7(46.66)	53.34	1:0.87
		Replicate 3	80	25	31.25	15 (60.00)	10 (40.00)	60	1:0.66
	1000µg/l	Replicate 1	80	28	35	10 (35.71)	18 (64.29)	35.71	1:1.8
		Replicate 2	90	23	25	16 (69.56)	7(30.44)	69.56	1:0.43
		Replicate 3	65	27	41.53	18 (66.66)	9(33.34)	66.66	1:0.5

the production of an all-male population of *B. splendens* with letrozole. Kirankumar and Pandian (2002) reported the production of an all-male progeny of *Betta* through discrete immersion treatment of 17 α - MT at doses of 100, 200, 500, 700, 900 and 1,000 µg/l for a constant duration (3 h/day) on second, fifth and eighth dph; 17 α -MT at 900 µg/l produced 98% males. Balasubramani and Pandian (2008) also demonstrated the masculinization of the *B. splendens* through immersion treatment of norethindrone (NE) for 3 hours each on the second, fifth and eighth dph and obtained a maximum of 92% males at 1000 µg/l and observed normal growth and appearance of

secondary sexual characteristics. Letrozole induced masculinization in the *Lepomis macrochirus* through either oral administration or immersion treatment during the sexual differentiation period (Gao et al., 2010). In the present study, moderately high mortality was observed during the first 2-3 days of post-treatment rearing probably because of handling stress and transfer to a new rearing environment. Our study reveals the dietary administration of letrozole at 50, 100, 150 and 200 ppm to 3-4 days old fry for 30, 40 or 50 days did not have any significant effect on the sex ratio. In general, a higher percentage of females were observed in all the treated groups across all the

treatment duration, including the Control. Jessy and Varghese (1988) obtained 80-91% males by the oral administration of 17α -MT at 80-140 ppm for 30 days to 8 day-old-hatchlings in *B. splendens*. Similarly, the oral administration of 17α -MT at doses of 1, 2, 3 or 4 mg/kg diet for eight weeks induced sex reversal; the higher doses of 3 and 4 mg/kg resulted in 100% masculinization in *B. splendens* (Kipouros *et al.*, 2011). A 100% male progeny of *B. splendens* was obtained using NE at 8 mg/kg diet for 40 days from first day of feeding three times a day (Kavumpurath and Pandian, 1993,1994).

Das *et al.* (2012) produced 100% masculinized tilapia (*Oreochromis mossambicus*) progeny by treating the fry with letrozole at 100-200 mg/kg diet for 30 days. Similarly, letrozole has been found to induce masculinization in other fishes like *Poecilia reticulata*, *Lepomis macrochirus*, *Epinephelus akaara*, *Oryzias latipes* (Basavaraja *et al.*, 2012; Gao *et al.*, 2010; Li *et al.*, 2005; Sun *et al.*, 2007). In the present study, the oral administration of letrozole did not induce the production of all male progenies probably because of the less acceptance of the artificial feeds. Several authors point out that the use of artificial diets may give poor survival and low acceptance of feed by fish larvae (Sales and Janssens, 2003; Person *et al.*, 1993; Finn and Kapoor, 2007). This may be the main reason for inability of letrozole to alter the sex ratio when administered orally. High mortality of fry was observed among the treated groups including the control during initial treatment period might have affected the efficacy of letrozole, feeding letrozole only twice a day as against three times daily (Kavumpurath and Pandian, 1994, 1993).

Tail deformities like bent vertebral column and absence of dorsal fins/rudimentary fin were often observed in groups treated with letrozole for 40 and 50 days. Among the groups treated with letrozole for 40 and 50 days, the higher deformities (100 and 87.5%) were observed at 200 ppm compared to other treated groups, which indicate that as the dose increased the deformity and abnormality also increased. Although the reasons for the observed deformities could not be definitely determined, the same may be attributed to longer treatment period and higher doses of letrozole which might have caused deformity. Salam *et al.* (2001) observed 7-11% deformity in ovaries of large mouth bass when treated with diethylstilbestrol at 400mg/kg feed. Similarly, Vinod and Basavaraja (2010) observed 7.69, 13.69 and 20% abnormalities in testis/ovaries by the treatment of diethylstilbestrol at varying doses of 400, 500 and 600 ppm, respectively. Our study also reports the suppression of ovarian growth by letrozole.

Results of the progeny testing of males from letrozole treated (both oral and immersion) indicate that the sex ratio of progenies of each of the males tested did not

differ significantly from that of the Control. This shows that all the males carried XY genotype. Chandrashekhara (2011) reported that progeny testing of sex reversed males from 25 ppm letrozole treated group resulted in 30 fish, of which 27 were females and 3 were males and the percentage of females was 90%. The maximum percentage of females (96.90%) was found in 100 ppm group (out of 33 fish, 32 were females). The control group had 54.5% females and 45.5% males in *L. reticulata*.

The sex reversed male from androgen treatment when mated with normal female produced 100% monosex female progeny in *B. splendens* (Kavumpurath and Pandian,1994). Kirankumar and Pandian (2002) obtained 87.25% female population and only 12.75% males in *B. splendens* when sex-reversed males were mated with normal female indicating the possible role of autosomal genes in the sex determination mechanism of this species. Vasanthakumar *et al.* (2011) observed 88.9% female progeny in the *C. Carpio* by crossing normal females and males from 30 ppm NE-treated group. Moreover, males of 40 ppm NE treated group when mated with normal female, yielded 79.3, 72.2 and 76.2% female progeny.

In the present study, the histological examination of ovaries of control fish showed normal stages of oogenesis like perinucleolar stage, oocyte growth, vitellogenesis, etc., whereas the ovary of letrozole-treated fish showed suppressed ovary with degenerating oocytes. Similar results were reported for the letrozole treated and the control guppy *L. reticulata*. Histological examination of testis showed that there were no significant differences in the testicular development between the control and letrozole treated fish (Chandrashekhara, 2011). Similarly, Kirankumar and Pandian (2002) reported that histological examination of the testis of the 17α -MT treated male showed histology similar to that of the control male. In the ovary of NE-treated *Betta*, the no. of vitellogenic oocytes was reduced, while the vacuolar area was increased; in the testis of treated fish, the number of spermatogonia was increased whereas the vacuolar area was reduced (Balasubramani and Pandian, 2008). Vinod and Basavaraja (2010) noticed slightly suppressed testicular development and completely inhibited ovarian development in NE treated groups of common carp (*C. carpio*).

The present study failed to produce a 100% male population when letrozole was orally administered for varying periods in *B. splendens* but letrozole administration through diet has been found to produce deformities. Studies may be carried out to assess the toxicity of letrozole treatments. More research is required to induce 100% masculinization through letrozole treatment either by increasing the dosage and/or duration of administration.

Conclusion

It was concluded that the immersion treatment of letrozole was more effective than oral administration in inducing masculinization in *B. splendens*. The oral treatment of letrozole at the tested doses caused abnormalities and suppressed ovarian development of *B. Splendens*. The reasons for the observed deformities could not be definitely determined. Hence, this study indicates the scientific direction for future research to assess the toxicity of letrozole treatments in *B. Splendens*.

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