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Effect of anastrozole on masculinization in ornamental fish, dwarf gourami, *Trichogaster lalius* (Hamilton, 1822)

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In ornamental fishery, for commercialization and efficient propagation of fish species in demand, controlling their sex, male or female, plays an important role as it influences reproduction, growth and product quality. In one such common aquarium fish, dwarf gourami, *Trichogaster lalius* (Hamilton, 1822), males look more attractive and fetch better market price encouraging masculinization of this species. In the present study, we evaluated the effect of anastrozole on masculinization potential in *T. lalius*. The experiment consisted of two trials, in trial 1; anastrozole was incorporated into feed at 50, 100, 150 and 200 mg/kg and fed to first feeding fry for 50 days. In trial 2, immersion treatment of anastrozole was carried out at different doses of 250, 500, 750 and 1,000 µg/L for 3 h each on 3rd, 5th and 8th day of post-hatching. Oral administration of anastrozole resulted in 90.32% masculinization at 200 ppm and the immersion treatment of anastrozole produced 100% masculinization at 1000 µg/L. Histological sections indicated that the anastrozole treatments suppressed ovarian development, leading to the atretic oocytes. However, the testicular development was unaffected. Testosterone level increased whereas estradiol level decreased with increased dose of the chemical. Cortisol level also showed a significant increase in male with increased doses of anastrozole, indicating stress which in turn helped to synthesize the testosterone. The results show that the immersion treatment of anastrozole is more effective than oral administration in inducing masculinization in *T. lalius*.

Keywords: Aromatase inhibitor, Gonadosomatic index, Hormonal profiling, Ovarian development

For commercialization and efficient propagation of fish species, sex control is one of the key tools as it can have an influence on reproduction, growth and product quality. Therefore, research is needed to understand sex control mechanism in ornamental fishes. Earlier studies show that androgens and estrogens could be used for induction of functional sex in fish[1,2]. Efficacy of non-steroidal aromatase inhibitors in reversing the sex of genotypic female (genotype: XX) to phenotypic male (genotype: XX) completely has been proved successfully.

Aromatase inhibitors, used in the treatment of ovarian and breast cancer in postmenopausal women[3], are known to induce masculinization in fish through inhibition of estrogen induced ovarian differentiation[4]. In fish sex differentiation, the cytochrome P450 aromatase enzyme converts the androgens into estrogens. This is a crucial step for aromatase inhibitors application which in turn induces the masculinization because the estrogens are actively involved in natural female sexual differentiation whereas natural androgens do not participate in sex differentiation. Therefore, commercially available synthetic aromatase inhibitors *viz*., anastrozole, letrozole, fadrozole, exemestane, etc. are used in the production of an all-male population. Aromatase inhibitors are able to induce masculinization in fish through inhibition of estrogen induced ovarian differentiation[4]. Several studies have been conducted in the past to produce monosex population in *Betta splendens*[5], *Carassius auratus*[6], *Zebra* fario[7], *Lebistes reticulates*[8], *Xiphophorus helleri*[9], *Halichoeres trimaculatus*[10] and *Petenia splendida*[11]. The monosex fish is intrinsically desirable in case of a variety of fish species in a range of ornamental production systems. The male dwarf gourami [*Trichogaster lalius* (Hamilton, 1822)] has extended anal and dorsal fins and looks more colorful than female. Males are in greater demand and fetch better

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price than females. The current price of one piece of dwarf gourami ranges from Rs. 120-150/- for male in Mumbai retail market. In this context, all male production in *T. lalius* will be profitable. Therefore, here, we have made an attempt to produce monosex population of *T. lalius* with the aromatase inhibitor anastrozole. The present study was conducted in Aquaculture Wet lab, ICAR-Central Institute of Fisheries Education Mumbai, India.

Materials and Methods

Diet preparation

The first bite fry feed (Hikari, Japan) was used for administration of anastrozole. The required quantity (100 g) of finely powdered feed was taken in a plastic tray. Anastrozole, was dissolved at 50, 100, 150 and 200 mg/kg (ppm) diet in 50 ml of 95% ethanol and sprayed on the feed and dried at room temperature of about 26 to 29°C. The control diet was prepared in the same manner using ethanol only[12].

Animals

Healthy stock of *T. lalius* was obtained from local aquarists to raise brood fish by feeding them daily twice with mosquito larvae and zooplankton. The obtained stock of *T. lalius* was authenticated by consulting finfish taxonomist of CIFE, Mumbai. The gravid female and male were stocked in an aquarium (2×1×1.5 ft) for breeding. The resultant fry obtained were used for anastrozole treatment. Glass aquaria and plastic jars were used for oral and immersion treatment, respectively. Glass aquaria and plastic jars were disinfected with a solution of 5 ppm potassium permanganate, cleaned, dried and filled with fresh filtered water. Gentle aeration was maintained during the treatment period.

Anastrozole administration

Dietary administration of anastrozole was carried out for 50 days. Three-day-old fry were randomly distributed into five groups of 80 each for 0, 50, 100, 150 and 200 ppm anastrozole, respectively in triplicate aquarium tanks and fed *ad libitum* thrice daily for 50 days. For immersion treatment, a stock solution of anastrozole (1 mg in 1 mL) was prepared using absolute alcohol. Four groups of 40 fry each were subjected to discrete immersion of the chemical at 250, 500, 750 and 1000 µg/L water. Discrete immersion treatment was given to fry for 3 h daily on 3rd, 5th and 8th day after hatching, in triplicates. A control group was maintained without anastrozole.

Fry rearing

After treatment, the fry were reared in plastic crates (250 L) for 30 days and then transferred into outdoor FRP tanks (500 L) for a period of 130 days, and were fed with sieved zooplankton. On termination of the experiment, the phenotypic sex of the fishes was determined based on secondary sexual characters, such as the shape of the belly and coloration.

Gonadosomatic index

The gonadosomatic index (GSI) of matured fish was determined using the formula

$$\text{Gonado somatic index}(\%) = \frac{\text{Gonad weight (g)}}{\text{Body weight (g)}} \times 100$$

Hormonal and Cortisol assays

On termination of the post rearing period, the fishes were sacrificed aseptically. The gonads and liver tissues were collected, weighed, homogenized with chilled sucrose solution (0.25 M) in a glass tube using tissue homogenizer. During homogenization, the glass tube was continuously kept in ice bath. The homogenate samples were centrifuged at 5000 rpm for 20 min at 4ºC in a cooling centrifuge and the final collected supernatant sample was kept frozen at –20ºC till further analysis. A 5% homogenate was prepared for all the tissues. The tissue testosterone and estradiol (E_2) level was determined using EIA kit procured from Cayman Chemicals, USA.

Tissue cortisol level was determined by enzyme-linked immunosorbent assay (ELISA)[13]. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Histological examination

The gonads of experimental animal for both control and treatment groups were collected, preserved, fixed, embedded, sectioned, stained and slides were observed under a microscope[14].

Statistical analysis

All data are expressed as mean ± SE. One way analysis of variance (ANOVA) was used for statistical analysis of male percentage, GSI and hormonal levels (SPSS 18).

Results

Sex conversion

The results of the oral administration of anstrozole are presented in Table 1. The survival ranged between

57.91 (control) and 45.41% (150 ppm) during anastrozole treatment period, whereas during post treatment rearing period, the survival was between 82.78 and 91.95%. Among the anastrozole treated groups, significantly higher (<0.05) percentage of males was observed in the group treated with anastrozole at 200 ppm (90.32%). On the other hand, the lowest percentage of males (51.35%) was found in 50 ppm group where the females accounted for 48.64% (Table 1). The control group had 47.77 females and 52.22% males. At 100 and 150 ppm, 58.79 and 72.64% males and 41.20 and 27.35% females were observed respectively. The percentage of male and female (with the exception at 50 ppm) was significantly (P <0.05) altered in the anastrozole-treated groups, compared to control.

The results of the immersion treatment of anastrozole are presented in Table 2. The survival of fry during treatment period ranged between 82.50 and 70% while during post treatment rearing period, it was between 91.69 and 88.12%. Among the anastrozole treated groups, the highest percentage of males 100% (P <0.05) was obtained in the group treated with anastrozole at 1000 µg/L, whereas the other groups had 49.95, 64.16 and 86.45% males respectively at 250, 500 and 750 µg/L. The percentage of male and female (with the exception of males at 250 µg/L) significantly (P <0.05) altered compared to control (Table 2).

Gonadosomatic index

The results of the effect of oral and immersion treatment of anastrozole on gonodosomatic index of male and female are shown in Fig. 1A. In the oral group, no significant (P >0.05) difference was observed in male GSI between the treated and control groups. The female GSI of treated groups was significantly (P <0.05) lower than that of control which is an indication of suppression of ovarian development. In the immersion treatment, the highest and lowest GSI of males was observed at 1000 µg/L anastrozole and control groups, respectively. The GSI of female was higher in control than the treated groups, which indicate that the ovarian development was slightly suppressed in the treated groups, with significantly lower value at 1000 µg/L. The GSI of both male and female was altered significantly (P <0.05) compared to control.

Cortisol

The cortisol levels of fish of oral and immersion treatment are presented in Fig. 1B. In the oral trial, the male cortisol value ranged between 149.00 pg/mL in control and 217.33 pg/mL in 200 ppm which indicated high significant difference (P <0.05) whereas the other groups had 167.67, 180.00 and 192.33 pg/mL males at 50, 100 and 150 ppm, respectively. Female cortisol value was 130.00, 141.00, 140.00, 136.67, 141.33 pg/mL at 0, 50, 100, 150 and 200 ppm which were not significantly

Table 1 — Number of fry stocked, recovered, sex composition and survival of *T. lalius* with oral treatment of anastrozole

Treatment (mg/kg)	Treatment Period			Post- treatment period					
	No. of fry (Initial)	No. of fry (Final)*	Survival (%)*	No. of fish recovered*	Male*	Female*	Male (%)*	Female (%)*	Survival (%)*
Control	80	46.33±1.20^{c}	57.91±1.50^{c}	38.33±0.88^{c}	20.00±0.00^{a}	18.33±0.88^{d}	52.22±1.18^{a}	47.77±1.18^{d}	82.78±1.83^{a}
50	80	40.33±0.88^{b}	50.41±1.10^{b}	36.33±0.88^{bc}	18.66±0.66^{a}	17.66±0.33^{d}	51.35±0.76^{a}	48.64±0.76^{d}	90.07±0.21^{b}
100	80	38.33±0.88^{ab}	47.91±1.10^{ab}	34.00±0.57^{ab}	20.00±1.15^{a}	14.00±1.00^{c}	58.79±3.04^{b}	41.20±3.04^{c}	88.74±1.58^{b}
150	80	36.33±0.88^{a}	45.41±1.10^{a}	33.00±0.57^{a}	24.00±1.1^{b}	9.00±0.57^{b}	72.64±2.22^{c}	27.35±2.22^{b}	90.85±0.69^{b}
200	80	37.33±0.88^{ab}	46.66±1.10^{ab}	34.33±0.88^{ab}	31.00±0.57^{c}	3.33±0.33^{a}	90.32±0.72^{d}	9.67±0.72^{a}	91.95±0.18^{b}
P value	-	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

[Data expressed as Mean ± SE. *Mean values in the same column with different superscript differ significantly (P <0.05). **Statistically significant (P <0.05) from the expected 1:1 sex ratio. M: Male; and F: Female]

Table 2 — Number of fry stocked, recovered, sex composition and survival of *T. lalius* with immersion treatment of anastrozole

Treatment (µg/l)	Treatment Period			Post- treatment period					
	No. of fry (Initial)	No. of fry (Final)*	Survival (%)*	No. of fish recovered*	Male*	Female*	Male (%)*	Female (%)*	Survival (%)*
Control	80	33.00±0.57^{b}	82.50±1.44^{b}	29.33±0.66^{b}	13.66±1.20^{a}	15.66±0.88^{d}	46.50±3.41^{a}	53.49±3.41^{c}	88.88±1.03
50	80	30.00±0.57^{a}	75.00±1.44^{a}	26.66±1.20^{ab}	13.33±0.88^{a}	13.33±0.66^{c}	49.95±1.90^{a}	50.04±1.90^{c}	88.80±2.37
100	80	28.33±0.88^{a}	70.83±2.20^{a}	26.00±1.15^{a}	16.66±0.66^{b}	9.33±0.66^{b}	64.16±1.48^{b}	35.83±1.48^{b}	91.69±1.40
150	80	30.00±0.57^{a}	75.00±1.44^{a}	27.00±0.57^{ab}	23.33±0.33^{c}	3.66±0.33^{a}	86.45±1.01^{c}	13.54±1.01^{a}	89.99±0.19
200	80	28.00±0.57^{a}	70.00±1.44^{a}	24.66±0.33^{a}	24.66±0.33^{c}	-	100^{d}	-	88.12±0.96
P value	-	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.448

[Data expressed as Mean ± SE. *Mean values in the same column with different superscript differ significantly (P <0.05). **Statistically significant (P <0.05) from the expected 1:1 sex ratio. M: Male; and F: Female]

(P >0.05) different from each other. In immersion trial, the cortisol level ranged between 135.00 and 197.67 pg/mL in case of male. Among the anastrozole treated groups, the highest level of 197.67 pg/mL was observed at 1000 µg/L. The lowest level (135.00 and 151.00 pg/mL) was found in control and 250 µg/L group, respectively whereas at 500 and 750 µg/L, the cortisol levels were 155.33 and 179.00 pg/mL . The cortisol level at 500, 750 and 1000 µg/L was statistically significant (<0.05) from control and 250 µg/L group. Female cortisol level was similar (P >0.05) compared to control.

Testosterone

Data on testosterone level recorded in the oral as well as immersion trials are presented in Fig. 1C. In oral trial, male showed testosterone level between 161.33 pg/mL in control and 256.67 pg/mL at 200 ppm of anastrozole. In the other groups the testosterone level was 206.33, 226.67 and 251.33 pg/mL at 50, 100 and 150 ppm, respectively, which clearly indicates significant difference (<0.05) between control and treated groups. In female testosterone level was 99.33, 128.33, 135.33, 143.33 and 144.33 pg/mL at 0, 50, 100, 150 and 200 ppm, respectively which showed a significant (<0.05) variation between treatments and control. In the immersion trial, the male testosterone was 166.67, 213.67, 231.33, 256.00 and 268.33 pg/mL at doses of 0, 250, 500, 750 and 1000 µg/L, respectively which shows that the treated groups significantly (<0.05) differ from the control. The highest level of female testosterone found was 138.00 pg/mL at 750 µg/L, whereas lowest level was observed (96.33 pg/mL) in the control group. The other treatments had 118.67 and 132.67 pg/mL (250 and 500 µg/L, respectively). The female testosterone level of treated group altered significantly (<0.05) compared to control.

Estradiol (E_2)

Results of E_2 level of the oral and immersion trials are presented in Fig. 1D. In the orally administered group, the highest male E_2 level was 146.67 pg/mL (control) and the lowest level was 101.00 (200 ppm), whereas the other groups recorded 133, 129 and 125.33 pg/mL E_2 at 50, 100 and 150 ppm, respectively. In female, the E_2 level was 615.33 pg/mL (control) which has significantly (<0.05) higher than that of all other treatments. In other groups, E_2 l level was 511.33, 430.67, 411.67 and 382 pg/mL at 50, 100, 150 and 200 ppm, respectively. In the immersion trial, the highest and lowest level of E_2 was observed in control (143.67 pg/mL) and 1000 µg/L (76.33 pg/mL), whereas the other groups had 139.33, 126.33 and 122.33 pg/mL E_2 at 250, 500 and 750 µg/L, respectively, which indicated that the E_2 level significantly (<0.05) decreased as the doses increased. Similarly, the female E_2 was maximum in control group, i.e. 715.33 pg/mL which was significantly (<0.05) higher than that of the other treatments *viz*. 612.00, 523.00, 509.67 pg/mL at 250, 500 and 750 µg/L.

Histological observations

The results of histological examination of ovary of *T. lalius* of control group and those fed orally with a nastrozole are presented in Fig. 2 A and B,

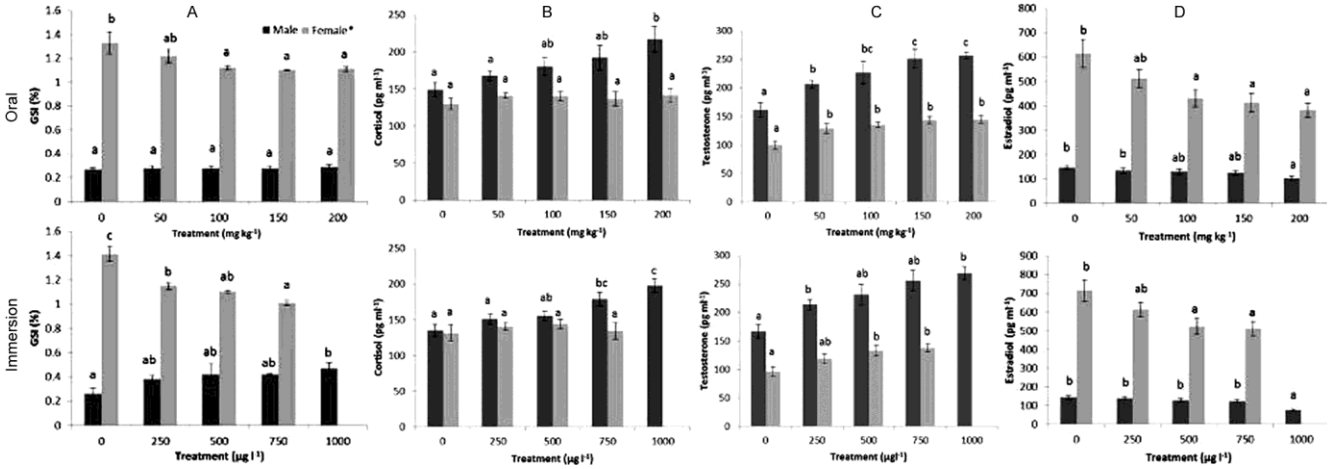

Fig. 1 — Effects of oral and immersion treatment for anastrozole on (A) Gonadosomatic index; (B)Level of cortisol; (C) Level of testosterone; and (D) Level of estradiol in *T. lalius* expressed as Mean ± SE (n=3). [*Mean values in the same bar with different superscript differ significantly (P <0.05)]

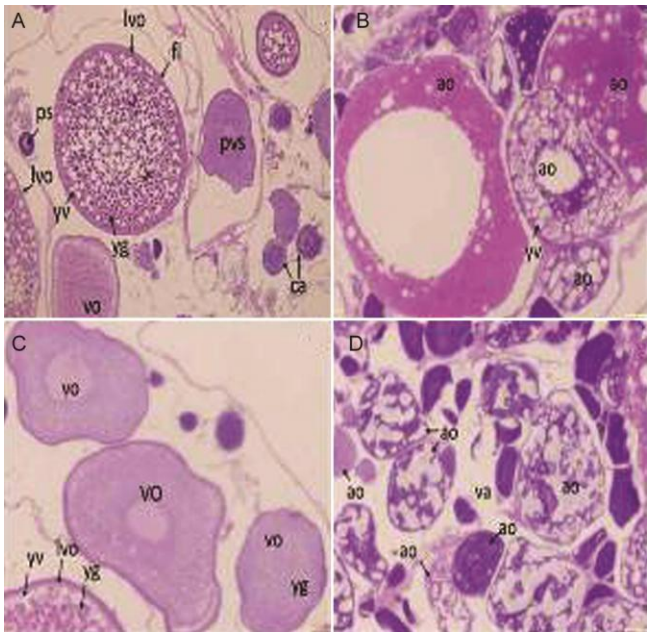

Fig. 2 — Ovary photomicrograph (X10) of fishes after (A & B) oral; and (C & D) immersion treatments of anastrozole. (A) Control fish in oral treatment showing different stages of oogenesis; perinucleolar stage (ps), yolk vesicle (yv), yolk granules (yg), vitellogenic oocyte (vo), follicular layer (fl), late vitellogenic oocyte (lvo), pre-vitellogenic stage (pvs), cortical alveoli (ca); (B) anastrozole oral treated showing degenrated atretic oocytes (ao); (C) Control fish of immersion treatment showing different stages of oogenesis; yolk vesicle (yv), yolk granules (yg), vitellogenic oocyte (vo), late vitellogenic oocyte (lvo); and (D) anastrozole immersion treated showing degenrated atretic oocytes (ao), and vacuolar area (va). [Scale bar: 64 µm]

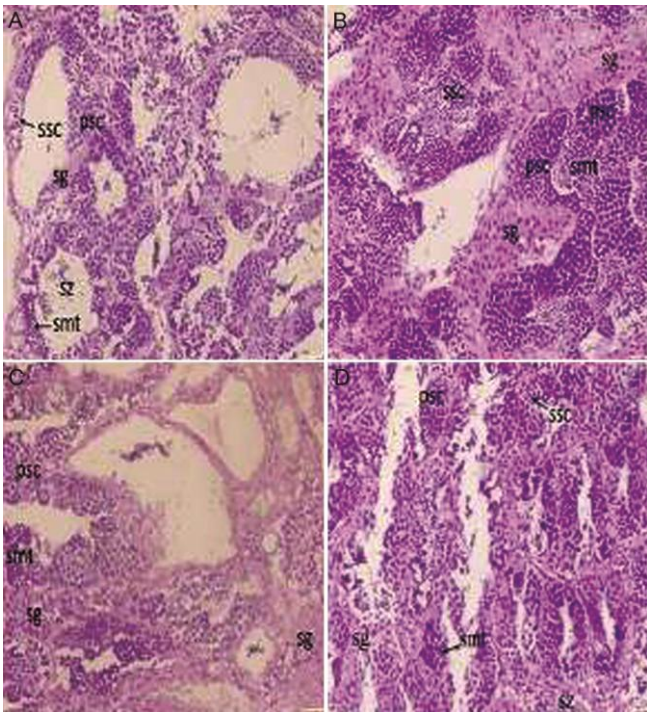

Fig. 3 — Testis photomicrograph (X20) of fishes after (A & B) oral; and (C & D) immersion treatment of anastrozole. (A) Control and (B) anastrozole (orally) treated fish showing different stages of spermatogonia (sg), primary spermatocytes (psc), secondary spermatocytes (ssc), spermatids (smt) and spermatozoa. (C) Control and (D) anastrozole (immersion) treated fish showing different stages of spermatogonia (sg), primary spermatocytes (psc), secondary spermatocytes (ssc), spermatids (smt) and spermatozoa (sz). [Scale bar: 32 µm]

respectively. The ovaries of control fish showed different stages of oogenesis like cortical alveoli, vitellogenic oocyte, perinucleolar stage, primary vitellogenic stage and late vitellogenic stage, etc. (Fig. 2A). On the other hand, the histology of treated fish showed suppressed ovary with degenerating atretic oocytes (Fig. 2B).

The histological observations of ovary of immersion treatment of control and treated group are presented in Fig. 2 C and D, respectively. The ovaries of control fish showed different stages of oogenesis like vitellogenic oocyt, late vitellogenic oocyte, yolk vesicles and yolk granules, etc. (Fig. 2C). On the other hand, the histology of treated fish showed suppressed ovary with degenerating atretic oocytes (Fig. 2D).

The histological section of testis of *T. lalius* treated (both oral and immersion) with anastrozole are presented in Fig. 3 (A-D). No significant difference was found in the spermatogenesis of both control and treated fish. The histology of treated fish revealed higher density of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa than that of the control.

Discussion

The anastrozole oral treatment for 50 days produced a maximum of 51.35, 58.79, 72.64 and 90.32% male population in *T. lalius* at a dose of 50, 100, 150 and 200 ppm, respectively. The control group had 47.77% females and 52.22% males. In general, a higher percentage of males were observed in all the treated groups across all the treatment, including the control except 50 ppm. Likewise, at 50 mg/kg of 17α-methyltestosterone (MT) induced 75% males in the Ussuri catfish, *Pyrus ussuriensis*, whereas even high dose of letrozole 100 mg/kg produced only 66% masculinization[15]. In the present study also, 50 and 100 mg/kg of anastrozole induced only 51.35 and 58.79% males, respectively. All the gynogenetic ship sturgeon (*Acipenser nudiventris*)

fed by 50 mg MT/kg feed for 7 months had been sex reversed to male[16]. Fadrozole-PLGA nanoparticles at 50 ppm induced 88.96% masculinization in the Nile tilapia *Oreochromis niloticus*[17].

Similarly, letrozole (0.2 mg/kg) has the potential with combination MT (5.0 mg/kg) to produce 100% sex-reversed male in greasy grouper, *Epinephelus tauvina*[18]. All the letrozole treated (from 25 to 85 days post hatch) blue drum, *Nibea mitsukurii* (0, 1, 10, 100 mg/kg) and gynogenetic yellow drum, *N. albiflora* (0, 10, 100 mg/kg) fishes turned into phenotypic males[19]. The minimum dose of letrozole (75 mg/kg diet enough for masculinization in guppy is, to be fed to female brooders for 12 days and the resultant fry for 30 days in the guppy, *Poecilia reticulata*[20]. The oral administration of letrozole at 20, 50, and 100 mg/kg diet produced a dominant male progeny 75.5%, 83.3%, and 75.0%, respectively in yellow catfish, *Pelteobagrus fulvidraco*[21]. The 11 day-post-hatched genetically female fry of *O. niloticus* treated with fadrozole (250 mg/kg feed) for 28 days yielded 100% masculinization[22]. The MT treatment at 50 mg/kg and 100 mg/kg to 10 days post hatch undifferentiated mandarin fish (*Siniperca chuatsi*) were successfully produced all-male stocks[23].

In the present study, both oral and immersion treatment of anastrozole induced the dominant male progenies but in oral treatment survival was significantly less compared to the immersion treatment including control probably because the prepared artificial feed negatively may affect the physiology of the fish after few days of hatching. The hormone administration through diet has few limitations like difference in distribution of hormone into the diet, differential intake, and degradation of the hormone in the digestive system[24]. Similarly in the present study also, oral treatment did not induce 100% masculinization possibly due to the differential intake and degradation of the hormone in the digestive tract.

Anastrozole treatment for 3 h daily on 3rd, 5th and 8th day after hatching produced 100% males at 1000 µg/L. In other groups, immersion treatment resulted in 49.95, 64.16 and 86.45 % male population at 250, 500 and 750 µg/L, respectively. Fadrozole treated *Betta splendens* at 50 µg/L developed as 91% males[25]. Letrozole in aquatic water at concentrations of 0, 5, 25, 125 or 625 µg/L induced 49, 53, 56, 64 and 73% masculinization in *Cyprinus carpio*, respectively[26]. The XX *O. niloticus* females after letrozole treatments of 0.5, 1.5, 4.5, 13.5 and 40.5 µg/L for 12 days induced 44.58, 58.33, 66.25, 77.08 and 84.17% masculinization, respectively[27]. In gynogenetic yellow drum, 0.2 µg/L MT is reported to be the efficient concentration for immersion treatment to produce neo-males[28]. Crossing of gynogenetic sex-reversed males with normal females produced 100% females[28].

Similar to the present study, norethindrone treatment to *B. splendens* through immersion for 3 h each on the 2nd, 5th and 8th days post hatch produced only 92% males at 1000 µg/L[29]. The MT treatment through 4 h immersions (0, 100, 200, 400 or 800 µg MT/L) and 50 days feed treatments (30, 60, 120, 150 or 250 mg MT/kg feed) induced paradoxical feminization in *T. lalius*[30]. This clearly indicated that the non-steroidal anastrozole used in the present study is more potent even at high doses to induce masculinization in *T. lalius*. The 87.23% masculinization was observed in 0-day-old larvae of the convict cichlid, *Cichlasoma nigrofasciatum* by the immersion of *Tribulus terrestris* extract at dose of 0.30 g/L once weekly for two months[31].

In the present study, anastrozole treatment at 500, 750 and 1000 µg/L yielded 64.16, 86.45 and 100% males, respectively but the lower dose 250 µg/L produced only 49.95% males which indicated that as the doses of anastrozole are increased the masculinization also increased. It may be because of instead of 24 h, in the present study the immersion treatment was carried out for only 3 h daily on 3rd, 5th and 8th day after hatching. Unlike the present study, letrozole treatment successfully produced dominant male progeny in the bluegill sunfish through oral as well as immersion treatment[32]. The swordtail *Xiphophorus cortezi* fry were treated with letrozole-loaded PLGA nanoparticles produced 100 % males for both 50 ppm and 100 ppm for 30 days treatment[33].

In the present study, the gonadosomatic index of male fish did not vary significantly between different groups in the oral treatment. Anastrozole treatment (50 days) produced lower male GSI compared to immersion treatment. In this trial, while the GSI of males was more or less same in the treated and control groups and slightly stimulated testicular development was observed only at a dose of 200 ppm, while in females, suppression of ovarian development was observed in all the treatment, compared to control. In anastrozole immersion treated groups, the

highest GSI of males was found in 1000 µg/L and lowest GSI was observed in control which revealed that as dose of anastrozole increased, it promoted the testicular development while female GSI was higher in control. Suppression of GSI was observed in all treated groups as the doses of anastrozole increased. Similarly, suppression of GSI reported in MT treated females of *T. lalius*[34]. In the anastrozole immersion and oral treated groups, slightly suppressed ovarian development were observed. The significantly higher gonadosomatic index was observed in the fadrozole group in the protandrous fish, black porgy[35].

The level of cortisol significantly differed (P <0.05) in male population but not in female, and the level of cortisol increased with 50 to 200 ppm in oral treatment and 250 to 1000 (µg/L) in immersed treatment, indicating that the drug may impose slight stress in male and it increased with increasing dose of anastrozole which helped in testosterone synthesis. Cortisol is an indicator of stress and has a direct and indirect relation with gonad maturing hormones, testosterone and E_2. This is because as the cortisol level is increased the testosterone level is also increased and E_2 level decreased. Similarly, in the present study, cortisol and testosterone levels were upregulated. In another study of cortisol treatments had elevated 11-ketotestoterone (11KT) and testosterone in Pejerrey[36]. The male testosterone level of oral as well immersion treatment stated that as the doses of anastrozole increased the testosterone level also increased, similar observation were also found in case of female testosterone level. The mean 11-KT plasma levels in fadrozole treated Atlantic halibut fry fish were significantly higher than the control group[37].

The oral administration or pellet implantation of aromatase inhibitor and MT in *Epinephelus* exhibits increased in plasma 11-KT, decreased E_2) levels[38]. The anastrozole fed male groups showed highest and lowest E_2 levels in (146.67 pg/mL) control and (101.00 pg/mL) 200 ppm, respectively. Same trend has been observed for immersion treatment also the highest and lowest level of anastrozole observed in control (143.67 pg/mL) and 1000 µg/L (76.33 pg/mL), respectively. The female E_2 level in both the case was high and reduced as the dose increased which claimed as doses increased testosterone increases and E_2 decreases which in turn increased the male percentage. Similarly, the use of exemestane, a steroidal aromatase inhibitor at 1000 µ/g of feed from 70 days after hatching through 100 days after hatching significantly suppressed plasma E_2 level and increased level of 11-KT in the Nile tilapia[39]. The E_2 levels in fadrozole treated yellowtail clownfish *Amphiprion clarkii* were significantly lower than those in the control group[40].

Aromatase inhibitor treatment inhibit the E_2 synthesis which promote masculinization or protogynous sex change that has been shown in a large number of gonochoristic and hermaphroditic fish, respectively[41,42].

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 anastrozole treated fish showed suppression with degenerating oocytes. Histological examination of testis showed that there were no significant differences in the testicular development between the control and anastrozole treated fish. Exemestane exposed females exhibited early histological signs of sex change and significantly higher rates of ovarian atresia relative to control females in black sea bass (*Centropristis striata*) whereas; exemestane treatment did not impact spermatogenesis or testicular gene expression[43].

Conclusion

The present study produced a 100% male population when anastrozole was administered @1000 µg/L through discrete immersion for varying periods with third, fifth and eight days of post hatching in the dwarf gourami, *Trichogaster lalius*. The results of the oral treatment of anastrozole were inconsistent produced maximum 90.32% male population at a dose of 200 mg/kg. With the results observed in the study, it can be concluded that the immersion treatment of anastrozole is more effective than oral administration in inducing masculinization in *T. lalius*. The available literature suggest that this could be the first report on the manipulation of the sex in *T. lalius* by anastrozole, an aromatase inhibitor administered through oral and immersion method. More research is required to induce 100% masculinization through oral treatment either by increasing the dosage and/or duration of administration.

Conflict of Interest

Authors declare no competing interests.

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