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Original Article

Propranolol and Prazosin Have a Positive Effect on the Sexual Performance of Female Three-Spot Gourami

Marzieh Monemi[1], Tahereh Naji[1], Afshin Kheradmand[2*]

[1]Department of Basic Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[2]Department of Pharmacology and Toxicology , School of Pharmacy, Iran University of Medical Sciences, Tehran, Iran

***Corresponding Author:** Afshin Kheradmand, Email: Kheradmand.a@iums.ac.ir

Abstract

Background: Sexual function is one of the most important aspects of life and is likely to be affected by the side effects of antihypertensive drugs. This study aimed to evaluate the effect of propranolol and prazosin on the sexual performance of female three-spot gourami.

Methods: In this study, 84 female three-spot gourami were randomly divided into seven groups, including a control and six experimental groups. The experimental groups were injected with prazosin or propranolol at doses of 1, 2, and 4 mg/kg for twenty days. Finally, the histological morphology of the ovaries and sex hormone levels in the experimental groups were examined.

Results: Our experiments showed changes in the oocyte stages, progressing to the cortical and vitellogenesis stages in the treated groups. Additionally, sex hormone levels increased in the groups exposed to that of propranolol and prazosin compared to the controls. The gonadosomatic index (GSI) exhibited a trend similar to the hormonal changes. The weight and length of the fish remained unchanged across the different groups.

Conclusion: Our findings indicated that propranolol and prazosin, at the mentioned doses and treatment duration, had a positive effect on the sexual function of female three-spot gourami.

Keywords: Propranolol, Prazosin, Three-spot gourami, Sexual hormones, Gonadosomatic index

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Introduction

The adrenergic system is dysregulated in cardiovascular disease, hypertension, and other diseases. Moreover, this system is the target of front-line therapeutics for these diseases. However, most of the work aimed at elucidating the adrenergic signaling response has been conducted in adult males. Consequently, mechanisms of adrenergic signaling in females remain unclear (1).

Due to the high prevalence of hypertension and cardiovascular disease (2,3), millions of people worldwide are currently being treated with antihypertensives, including adrenergic drugs. However, this widespread use has raised concerns about drug-related side effects. The prevalence of the side effects of antihypertensive drugs is estimated to range between 20% and 97% (4,5). Based on studies, these side effects are the main reason for patients' low adherence to medication (6-8).

Sexual function is a fundamental aspect of life and is often affected by the side effects of antihypertensive drugs, such as adrenergic antagonists. Preliminary studies in men have suggested that adrenergic antagonists may significantly influence sexual function (9), with most effects reported during the use of beta-blockers and diuretics (9-11). Furthermore, beyond the effects of these drugs, high blood pressure itself is linked to female sexual dysfunction. As much evidence has demonstrated a link between hypertension and female sex hormones, the relationship between antihypertensive drugs and female sexual function is complicated (12,13).

Sexual function in women is complex because it is influenced by various physiological and biological factors (14). No clear mechanism or specific route is known to be responsible for the effects of adrenergic antagonists on female sexual function; however, one of the most common effects of adrenergic antagonist agents is relaxing the smooth muscle of the lining membrane of blood vessels. This effect can lead to venous dilation, which results in increased blood flow to the vaginal tissues during arousal as a result of relaxation of the smooth muscles (15). Beta-blockers reduce serum testosterone levels in men (16), while their effects on serum testosterone in women are unknown. Several studies have investigated the serum

levels of testosterone in women with sexual dysfunction; however, this is still a matter of debate (17,18). The female sex hormones progesterone and estradiol play crucial roles in coordinating the timing of female sexual acceptance with ovulation in many mammalian species. The findings suggest that estradiol and progesterone play a significant role in regulating synaptic connections, including those involving norepinephrine, a catecholamine, within the hypothalamus. Additionally, estrogen can modulate beta-adrenergic and α1-adrenoceptor signaling. These alterations influence hormone functions via hypothalamic responses to norepinephrine and lead to reproductive success by coordinating the release schedule of gonadotropins before ovulation with the behavioral acceptance period (19,20). Interestingly, some widely used adrenergic antagonists, including propranolol and prazosin, are adrenoceptor blockers, which selectively block α1- and β-adrenoceptors, respectively, and could influence the mentioned signaling.

Fish were first used as laboratory models in genetic research for genome studies and gene manipulation, oncology, and toxicology (21). The endocrine system of fish is known for its simplicity and, in many ways, resembles that of vertebrates such as humans. For this reason, it has the potential to be used as an animal model to evaluate the effects of drugs on other vertebrates and humans (22-25). The hypothalamic-pituitary-ovarian axis controls reproductive function in fish, and the chaining feature of this mechanism allows the testing of different substances at several levels to evaluate different parameters (26).

This study compared the effects of two common alpha and beta-adrenergic antagonist drugs, propranolol and prazosin, on sexual activity in female fish. In addition, we assessed the effect of propranolol and prazosin at different doses on ovule maturation, the levels of 17-beta estradiol and testosterone, and the amount of vitellogenin in female three-spotted gourami fish.

Methods

Animals

The present study used 84 three-spot gourami with an average weight of 2–3 g. The fish were housed in aquariums in dimensions of 30 × 40 × 60 in controlled conditions (12–12 hours of light-darkness; temperature: 22 to 25 °C). Physicochemical properties of water, including temperature, hardness, and pH, were monitored every 24 hours throughout the experimental period. The fish were fed sufficient standard gourami food every other day. Also, the aquarium water filters were cleaned every other day. The experiments were reviewed and approved by the Islamic Azad University Ethics Committee, Tehran, Iran (IR.IUMS.AEC.1402.086).

Experiment design

The fish were divided into seven groups, including a control group (receiving deionized water injection) and six experimental groups injected with prazosin or propranolol at 1, 2, and 4 mg/kg (dissolved in water) (27-29). Each group consisted of 12 fish.

Drug administration

An intramuscular injection was administered between the fish's dorsal fin and lateral line in all the treatment groups. After the injection, the fish were placed in chlorinated water equilibrated at room temperature since the day before. Resuscitation was performed with an oxygen hose, and then all fish were transferred to the group aquariums. Each fish in the treatment and control group was injected 10 times during 20 days (30).

Tissue sampling

At the end of the twentieth day, six fish in each group were anesthetized and fixed on the dissection tray. Then, the ovaries were removed carefully and placed in a pre-prepared 10% formalin solution. As blood sampling was impractical due to the small size of the fish, tissue homogenization was used to measure hormones in the other six fish of each group. To do this, after separating the internal viscera and their head, tail, and fins, the rest of the tissues were homogenized (31,32).

Hematoxylin and eosin staining

The formalin-fixed fish ovaries were dehydrated by graded alcohol and embedded in paraffin wax. The 5 μm-thick paraffin sections were then cut into slices from these paraffin-embedded tissue blocks. The tissue sections were deparaffinized by immersion in xylene and were then rehydrated. All slices were stained with hematoxylin and eosin (H&E) dyes and then rinsed with water. Each slide was dehydrated through graded alcohol. Ovary sections were finally soaked in xylene twice. Photomicrographs were obtained using a Nikon microscope (Nikon, Tokyo, Japan).

Measurement of estradiol, testosterone and vitellogenin

Estradiol and testosterone were assessed using an enzyme-linked immunosorbent assay (ELISA) and a Monobind kit (Monobind Inc, USA) following the photometric method according to the manufacturer's instructions. Moreover, vitellogenin was evaluated using a vitellogenin ELISA kit as a sensitive sandwich enzyme-linked immunosorbent assay to measure vitellogenin (DiaPharma, USA). Finally, each sample's optical density (OD) in each assay was measured at 450 nm wavelength, and calculation was done according to the standard curve linear regression equation.

Determination of gonadosomatic index

The gonadosomatic index (GSI) is widely used as a simple tool for measuring reproductive capacity. This index is

obtained by equating the ratio of fish gonad weight (GW) to fish body weight (W) multiplied by 100 (33).

$$GSI = \frac{WG}{W} \times 100$$

Statistical analysis

All data were presented as the mean ± SEM and processed using GraphPad Prism 6 software. Fish weight and height before and after drug injection were performed by two-way ANOVA, and estradiol, testosterone, and vitellogenin were compared using one-way ANOVA. Statistical significance was considered to be significant when $P < 0.05$ was achieved.

Results

Ovary histology

Five successive stages have been identified during the development of germ cells in fish based on egg morphology and the nucleus-to-cytoplasm ratio: young cells, previtellogenic oocytes (perinucleolar), cortical-alveoli oocytes, vitellogenin oocytes, and mature oocytes (34).

The histological evaluation of fish ovary indicated that most oocytes were in the perinucleolar stage; no egg was in the cortical growth or vitellogenin stages in the control group (Figure 1A).

Treatment with propranolol and prazosin altered the germ cell stage at different doses and groups. As shown in Figure 1B, some oocytes were observed in the perinucleolar stage and some in the cortical stage after treatment with 1 mg/kg propranolol. Treatment with a 2 mg/kg dose increased the number of eggs in cortical stages and initiated the vitellogenic stage. In addition, the majority of oocytes were in the vitellogenesis stage after administration of 4 mg/kg propranolol (Figure 1B). In prazosin-treated fish (1 mg/kg), the number of perinucleolar cells decreased compared to the control group, while cortical cells increased in proportion. Most germ cells in the group receiving 2 mg/kg of prazosin were observed in the cortical stage. Also, treatment with 4 mg/kg prazosin changed the oocyte stage to vitellogenesis (Figure 1B).

Sex hormones and vitellogenin levels

During the female reproductive lifespan, 17-beta estradiol, a steroid hormone, is produced primarily by the ovaries and is responsible for the development of secondary sexual characteristics and maintenance of the female reproductive tract (35). Fish treated with 2 and 4 mg/kg propranolol showed a significant increase in 17-beta estradiol levels ($P = 0.0454$ and $P = 0.001$,

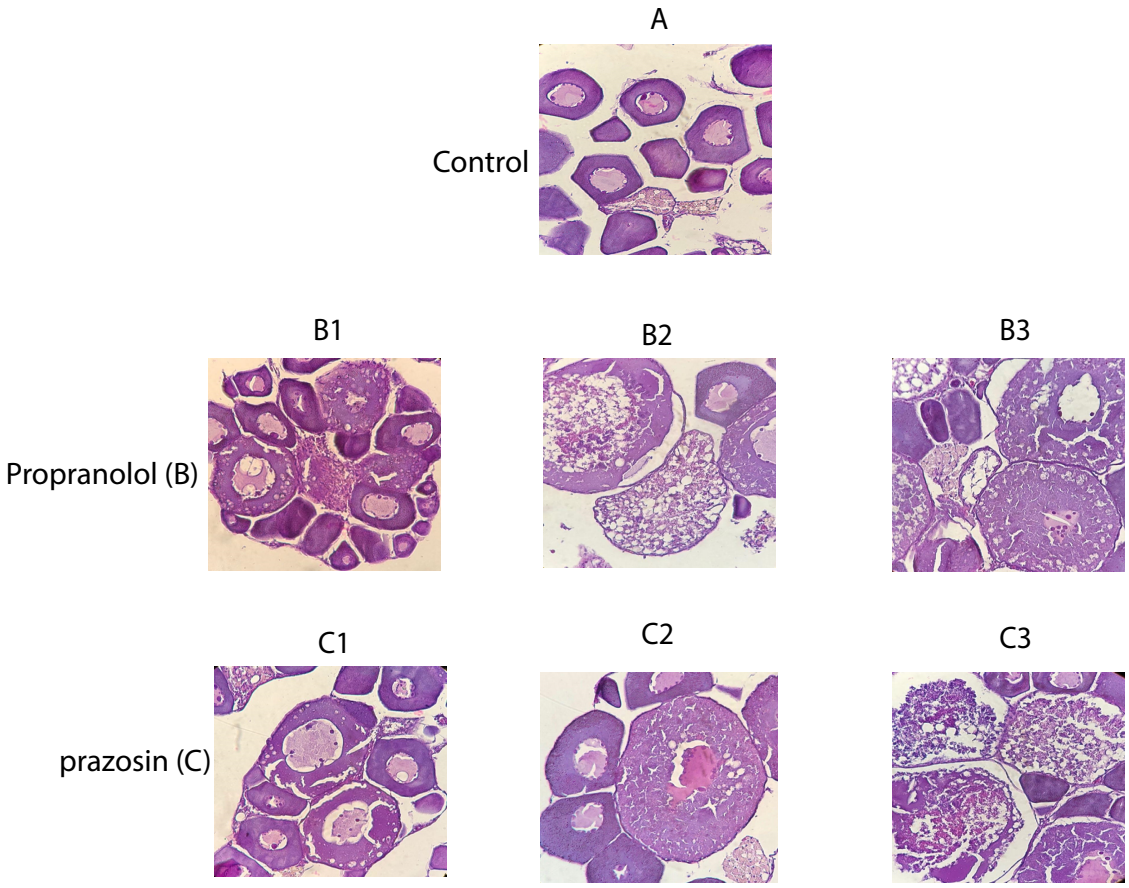

Figure 1. Histological analysis of the three-spot gourami fish ovary using hematoxylin and eosin (H&E) staining. (A) Control group displaying ovarian morphology with oocytes predominantly in the perinucleolar stage. (B) Ovarian morphology after exposure to propranolol at doses of 1 mg/kg (B1), 2 mg/kg (B2), and 4 mg/kg (B3). (C) Ovarian morphology following prazosin treatment at doses of 1 mg/kg (C1), 2 mg/kg (C2), and 4 mg/kg (C3).

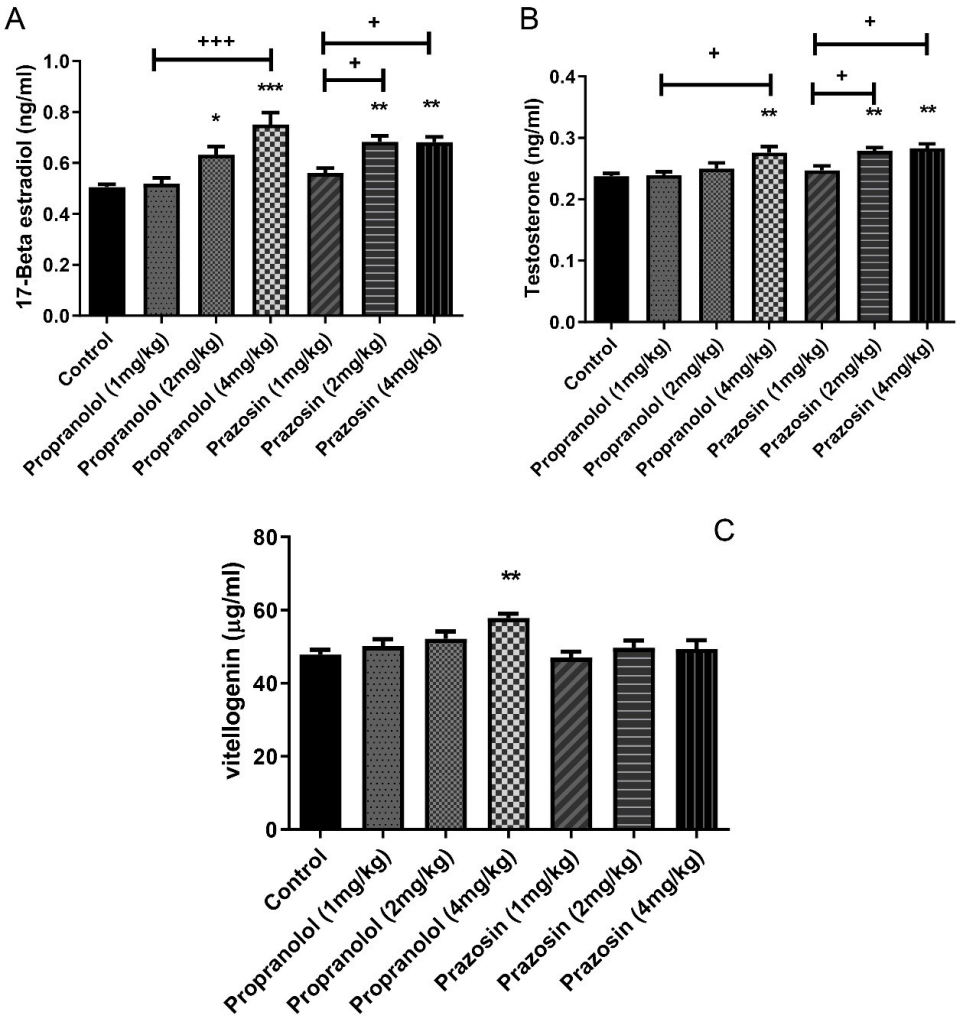

Figure 2. Different doses (1, 2, and 4 mg/kg) of propranolol and prazosin influence the level of 17-beta estradiol (A), testosterone (B), and vitellogenin (C) in the three-spot gourami fish. All data values are expressed as mean ± SEM (one-way ANOVA, *$P<0.05$, **$P<0.01$ and ***$P<0.001$ comparisons with the control group, +$P<0.05$, and +++$P<0.001$ comparison between different doses).

respectively), as did those receiving prazosin ($P=0.0015$ for both) compared to the control group. However, the hormone level showed no change after 1 mg/kg treatment with either of the drugs ($P=0.9999$ and $P=0.8005$, respectively). Statistical analysis between different dose groups revealed a significant elevation in 4 mg/kg propranolol compared to the 1 mg/kg dose ($P=0.001$). Furthermore, the group treated with 1 mg/kg prazosin showed significant differences in 17-beta estradiol levels compared to the groups receiving 2 mg/kg ($P=0.0478$) and 4 mg/kg ($P=0.0495$) prazosin (Figure 2A).

Testosterone is a hormone found in humans and other animals. The testicles primarily make testosterone in men, whereas women's ovaries make testosterone in much smaller amounts as well. Our results indicated that treatment with 4 mg/kg propranolol increased the level of testosterone when compared with control and 1 mg/kg groups ($P=0.0083$ and $P=0.0228$, respectively). A notable distinction was observed between the groups administered 2 mg/kg and 4 mg/kg of prazosin in comparison to the control group ($P=0.0035$ and $P=0.0014$, respectively), as

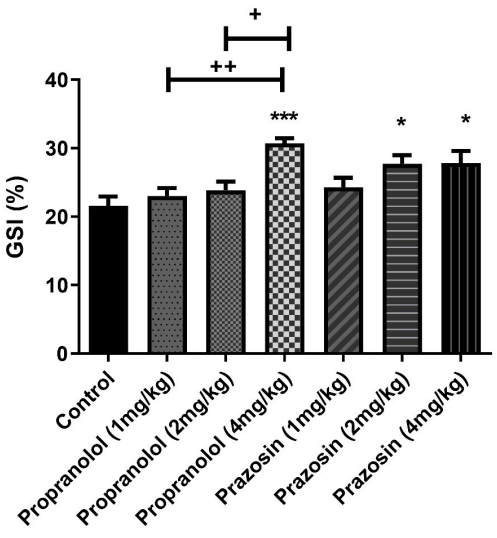

Figure 3. Different doses (1, 2, and 4 mg/kg) of propranolol and prazosin affect the gonadosomatic index of the three-spot gourami fish. All data values are expressed as mean ± SEM (one-way ANOVA, *$P<0.05$ and ***$P<0.001$ comparisons with the control group, +$P<0.05$ and ++$P<0.01$ comparison between different doses).

well as when compared to the 1 mg/kg group ($P=0.0497$ and $P=0.0212$, respectively) (Figure 2B).

As the egg yolk precursor protein, vitellogenin is normally produced by females in response to normal cycles of estradiol during oogenesis. It has been considered a biomarker for measuring the exposure of oviparous animals to estrogen or its mimics. Based on Figure 2C, the assessment of vitellogenin in different groups did not reveal any significant changes ($P>0.05$), except in the 4 mg/kg propranolol group compared to control fish ($P=0.0084$) (Figure 2C).

Gonadosomatic index

One of the major characteristics of oocyte maturation is the GSI index. GSI expresses gonad weight as a proportion of total or somatic weight. The result analysis showed that fish receiving 4 mg/kg propranolol had significantly higher GSI when compared with control and other doses of propranolol ($P=0.0003$, $P=0.0029$, and $P=0.0108$, respectively). Also, prazosin at 2 and 4 mg/kg doses increased this index compared to the control group ($P=0.0301$ and $P=0.0247$, respectively) (Figure 3).

Weight and length of fish before and after injection of prazosin and propranolol

Fish weight and length were measured in all groups before and after injections. Statistical analysis of our results didn't show any significant difference in these parameters. In addition, there were no significant changes between groups ($P>0.05$) (Figure 4).

Discussion

Concerning the effects of antihypertensive drugs on female sexual function, our results demonstrated that following the injection of propranolol and prazosin, oocytes developed closer to maturity in a dose-dependent manner. Also, the levels of sexual hormones, vitellogenesis, and GSI were influenced by antihypertensive drugs; however, propranolol had a greater impact on the mentioned markers.

A study carried out by Polidoro et al in 1973 on Dutch female rabbits showed that propranolol (dose of 7 mg/kg) induced maturity in the rabbit ovum (36). In addition, an evaluation of propranolol's effects on fish ovaries showed that this drug causes ovarian contractions and strengthens the growth process of oocytes in fish ovaries (37). Prazosin has also been reported to affect ovary contractions in the literature (38). In line with the mentioned study, our examination of ovarian sections showed that treatment with both propranolol and prazosin-induced cortical stages eventually led to the vitellogenin stage. Most of the cells were in the vitellogenesis stage after receiving 4 mg/kg of drugs. The observed effect was probably due to the influence of propranolol and prazosin on the oocyte cell receptor, which elevated the level of 17-beta estradiol hormone, thereby causing the development of oocytes to the vitellogenesis stage.

As pituitary gonadotropin secretion is a prerequisite for oocyte maturation and ovulation and this is done with the help of steroid hormones, increasing the level of 17-beta estradiol hormone could cause the growth of oocytes in the ovary (39,40). According to studies, the injection of 17-beta estradiol, as an important factor in controlling sex differentiation, induces the growth of oocytes in the ovaries of fish (40,41). Moreover, ovarian steroid hormones, progesterone, and estradiol act in many species to coordinate the timing of female sexual acceptance with ovulation (20,42). The findings of this study indicated a notable disparity in 17-beta estradiol levels when comparing the control group to those receiving treatment with prazosin and propranolol. Consequently, it appears that the treatments may have enhanced follicular activity and elevated estradiol secretion, particularly at higher doses of both medications.

It should be noted that propranolol had more impact on this hormonal change. In 2002, Huggett et al. examined

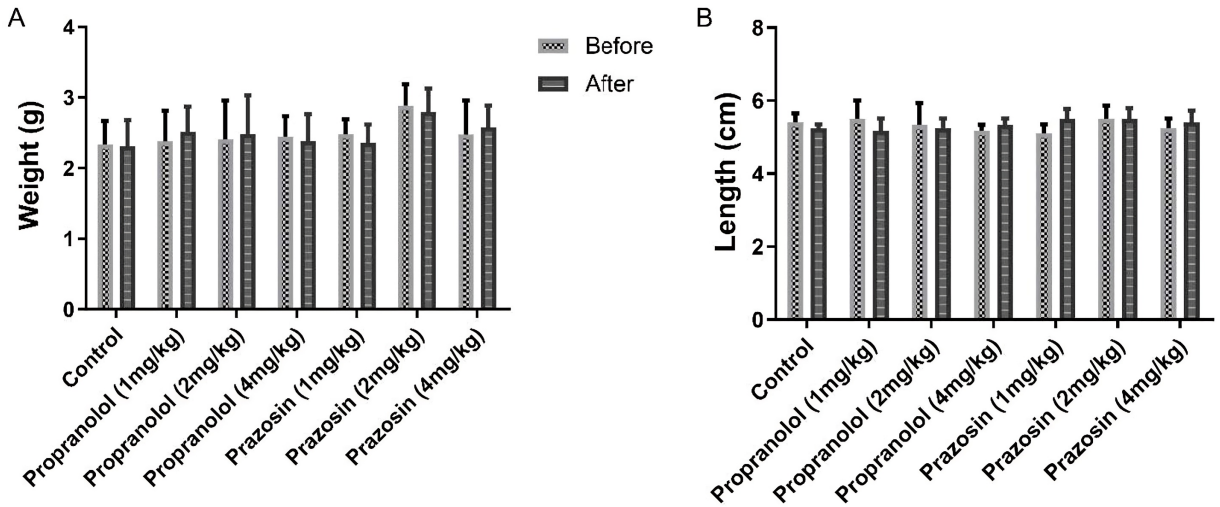

Figure 4. The effect of different doses (1, 2, and 4 mg/kg) of propranolol and prazosin on the weight (A) and length (B) of the three-spot gourami fish before and after experimental treatments. All data values are expressed as mean ± SEM.

the effects of beta-blockers, including propranolol. They showed a significant change in the plasma level of estradiol compared with the control group in aquatic organisms; however, they did not observe any change in the average number of ova produced (43). Besides, another investigation, including a wide range of hormonal measurements in men and women, showed that beta-blockers and alpha-blockers led to different sexual responses in men in comparison with women. This might be due to the complexity of women's sexual function, which is influenced by various physiological factors (14).

Testosterone in bony fish plasma is a precursor to 17-beta estradiol. Studies have shown that testosterone secretion is also affected after adjusting for LH concentration. Testosterone concentrations increase with the onset of meiotic division in male fish and ovum growth in female fish and remain high during gonadal maturation (44,45). In the current study, testosterone level changes in the treated groups were aligned with 17-beta estradiol alternations. Both propranolol and prazosin had a significant impact on testosterone levels at a dose of 4 mg/kg. However, in contrast with 17-beta estradiol results, 2 mg/kg of propranolol did not influence the testosterone level significantly.

Oocyte growth depends on the uptake of glycolipophosphoproteins or vitellogenin, which is the precursor of the yolk. Vitellogenin is synthesized in the liver in response to the 17-beta estradiol hormone. It binds to vitlogin receptors on the surface of oocytes with a high affinity and enters the cell through endocytosis. It has been observed that as the follicle diameter increases, vitellogenin uptake also increases (46). Interestingly, the administration of propranolol and prazosin had different effects on the vitellogenin status. Propranolol administration at varying dosages resulted in an increase in vitellogenin levels, whereas only the highest dosage of prazosin produced a notable effect. Conversely, no significant differences were observed between the control group and the treatment group. Research conducted by Naida and associates has demonstrated that vitellogenin levels are influenced by estrogen and can also be modulated by adrenergic signaling (47).

Besides the hormonal changes, we assessed GSI as one of the essential macroscopic indicators in the study of fish evolution (48). Our investigation on three-spot gourami fish indicated that even though both 2 and 4 mg/kg of prazosin increased the GSI levels, the effect of 4 mg/kg propranolol was much greater than that of the same dose of prazosin. This observation was consistent with the vitellogenin results.

Adrenergic neurotransmitters are the important transmitters that regulate LH secretion. Studies have indicated that they both have inhibitory and stimulatory effects on gonadotropin-releasing hormone (GnRH) neuron activity and LH secretion levels (49). It seems that the highest dose of adrenergic blockers could influence the pituitary-gonad axis by increasing hormone secretion using administrated drugs, and this may be followed by ovarian development and weight gain. In contrast with our findings, one study focused on the GSI of female fathead minnows reported a significant decrease in the GSI of fish in response to high doses of propranolol due to increased spawning rate. However, after exposure to propranolol, the number of ova increased significantly, and the size of the ova increased dramatically, increasing the fertility rate (50).

Finally, we assessed the length and weight of the animals, and our results showed that neither was affected by propranolol and prazosin treatments. In a 2016 experiment on the life cycle of fathead minnows treated with propranolol, Parrott and Balakrishnan found no significant difference in fish weight gain and length despite long-term exposure to the different doses of propranolol (50).

Conclusion

The present study demonstrated that treatment with propranolol and prazosin, antihypertensive drugs that act through adrenergic blockade, significantly improved the sexual function of female three-spot gourami fish under the specified doses and treatment duration. This beneficial effect appears to result from modulations in the endocrine system and an increase in sexual hormone levels. However, further research is required to clarify the long-term effects of antihypertensive drugs on female sexual performance. Additionally, the next phase of this research should focus on evaluating these adrenergic blockers in a hypertensive animal model to obtain more precise and comprehensive insights.

Authors' Contribution

Conceptualization: Afshin Kheradmand.
Formal analysis: Marzieh Monemi.
Investigation: Afshin Kheradmand, Marzieh Monemi.
Methodology: Tahereh Naji, Afshin Kheradmand.
Project administration: Afshin Kheradmand.
Supervision: Afshin Kheradmand.
Visualization: Afshin Kheradmand.
Writing–original draft: Marzieh Monemi.
Writing–review & editing: Tahereh Naji, Afshin Kheradmand.

Competing Interests

The authors declared no conflict of interest.

Ethical Approval

The protocol of this study was reviewed and approved by the Ethics Committee of the Islamic Azad University, Tehran, Iran (code: IR.IUMS.AEC.1402.086).

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