Comparison of the effect of olanzapine and pimozide on development and changes of oocytes , endocrine marker and aromatase enzyme in *Trichogaster trichoptrus*

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Abstract

In this survey the effects of olanzapine and pimozide on endocrine hormones and aromatase enzyme in Trichogaster trichopterus were analyzed. In this reason 120 mature female fishes were divided into 8 treatments and two control groups the fishes in one control group received ethanol 70% while the other control group was intact.All injections were done in muscles between dorsal fin and lateral line (0/02 mL per injection). Fishes were killed 7 days after the injection and their ovaries were dissected for histologic studies the fishes ovaries were fixed in formaline 10%. After tissue passage and cutting and Hand E staining the ovary tissues in all treatment groups were studied by optical microscope and were compared with control groups. Then, endocrine hormone and aromatase levels in all treatment groups were compared with fishes in control groups by SPSS software and DUNCAN and one way of ANOVA exams.the differences between averages in different treatment fishes were determined with p-value≤0/05. The results of comparison between control and treatment groups of 1, 2, 3 and 4 receiving 1, 3, 6, and 10 mg/kgb.w of olanzapine relatively and treatment groups of 5, 6, 7 and 8 receiving 1,3,6 and 10 mg/kgb.w of pimozide relatively showed the effects of dopamine antagonists on endocrine hormones and aromatase enzyme which decreases aromatase and testosterone and estradiol levels and thus had a reasonable difference with control fishes. Studying the oocytes histologic samples by photo microscopy in control group showed that cells were in vitellogenesis and maturation stages while in pimozide and olanzapine treatments by increasing the dosages despite presence of cells in cortical stage the cell number in prenuclear stage increase steadily. The results of this study showed that dopamine antagonists effect of oocyte maturation.

Keywords: Olanzapine, Pimozide, Endocrine hormones, aromatase, *Trichogaster trichopterus*

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Introduction

By now the dopamine receptore antagonist are the second line of drug therapy for schizophrenia and other psychotic disorder. Because of their instate relifing effect. They are the first line drugs for psychose acute episodes. Therapiutic effects and their endocrine and mental side effects are the result of blocking dopamine receptors. Some dopamine receptore antagonists block noradrenergic, histaminergic and cholinergic receptors (Laduron and Leysen, 1979; Woolverton, 1986). Olanzapine acts as a dopamine (D_2) and serotonine ($HT_2A/2C_5$) receptore antagonis and it also has blocking effects on adrenergic-cholinergic and histaminergic receptors.Its absorbed instantly and effectively in GI system. 40 percent of drug is metabolise in its first pass. Half times of drug is 30 hours and its bindy rate to protein is 93%. It's mainly metabolised in liver. Drug secretion in urine is 7% unchanged and 57% as metabolite and in feces is 30% (Wagner *et al*., 2005; De Hert, 2011). Pimozide is another drug acting as dopamine D_2 receptore antagonist in central nervous system.Its used in treating vocal and gestural tics.this drug also increase dopamine turnover, voltage dependent calcium channel and opined receptor blocking and prolactin concentration (Bertolesi *et al*., 2002; Shapiro *et al*.,1987).

In a study by Josefa on Asian catfish, a dose of 1 mg of Pimoside per kg (1 µg of pimoside per gram of fish weight) was used as an induction factor for oocytes, which also increased the ovum diameter (Josefa, 2018). Although the inhibitory role of dopamine in determining the prolactin induced antipsychotics side effects is not fully understood, but prolactin addition by blocking dopamine indirectly decreases endocrine hormones and blocking sexual maturation in some species of bony fishes. In a study by Hahn *et al*. (2013) which was conducted to investigate the effect of olanzapine monotherapy on endocrine metabolism and inflammatory markers, olanzapine significantly increased fasting glucose and serum prolactin levels. Serum cortisol levels and FFAs also decrease (Hahn *et al*., 2013).

Regulating puberty and reproductive practices in the normal state requires the integrity and precise coordination of hormonal regulation at the levels of the hypothalamic-pituitary gonad (Baumann, 1990). The Gurami fish is three clones from the Anabantidae family, which is used as an ideal model in reproductive studies due to the similarity of the pituitary-hypothalamus-gonad axis to humans (Govind, 2011; Khiabani, 2015; Madsen, 2015).

Materials and methods

After decolerizing the aquariums, 150 fishes were devided into10 groups and in each group15 fishes were placed in glass aquariums containing 40 liter of water (aquarium size: 30×40×60). This survey was done during April, 2018 to July, 2018 in fish lab of Islamic Azad University Drug Faculty. Fishes were

kept in lab conditions. Temperature 22-28℃, pH6-8 and water hardness5-35 (mg/liter $caco_3$). After adaptation of fishes for48 hours, experiments were done in10 group (Table 1).

Table1: Treatment and injection concentration in *Trichogaster trichoptrus.*

	Treatment	Dose of drug	Number
1	Olanzapine (Treatment1)	1mg per kg fish	15
2	Olanzapine (Treatment2)	3mg per kg fish	15
3	Olanzapine (Treatment3)	6 mg per kg fish	15
4	Olanzapine (Treatment4)	10 mg per kg fish	15
5	Pimozide (Treatment1)	1 mg per kg fish	15
6	Pimozide (Treatment2)	3 mg per kg fish	15
7	Pimozide (Treatment3)	6 mg per kg fish	15
8	Pimozide (Treatment4)	10 mg per kg fish	15
9	(Control) Ethanol70		15
10	No injection		15

After completation of the injection (as every other day for 20 times injections were done in muscles between dorsal fin and lateral line.) fishes were killed and studied. Ovaries were dissected and their weights was analyzed. In order to measure steroid, levels of estradiol and testosterone and aromatase enzyme, because of small size of fishes and the inability to bleed, all the tissues except limbs and head in each treatment were separately squeezed and tissue liquids were centrifuged in 3000 rpm for 5 minutes ovaries were kept in formalin 10% as fixative. After tissue passage tissue section were taken by microtome (diameter 5µ) and staining was done by Eosine and Hematoxiline under photo microscopy.

Statistical method

The general design of this study was (randomized design completely) and all of registered information during the study was done by one way Anova test analyses. All the procedures were analyzed by Spss. In order to compare averages, the multi range Duncan exam was used and difference between averages in different treatments was determined in *p*-value of ≤0/05.

Results

The results gonadal index is shown in Fig 1. In which there is no significant difference between gonadal weight and fish weight, while difference was seen in dose of 6 mg/kg olanzapine which in comparison to intact it decreases is not dose dependent (*p*=0/235). The results of studies on testosterone was shown in Fig. 2 in which after 20 days of injections testosterone level has decreased competing with control group. This decrease in treatments 1 and 2 and also 5 and 6 (dose of 1 mg/kg and 3mg/kg of olanzapine and pimozide) was more obvious.

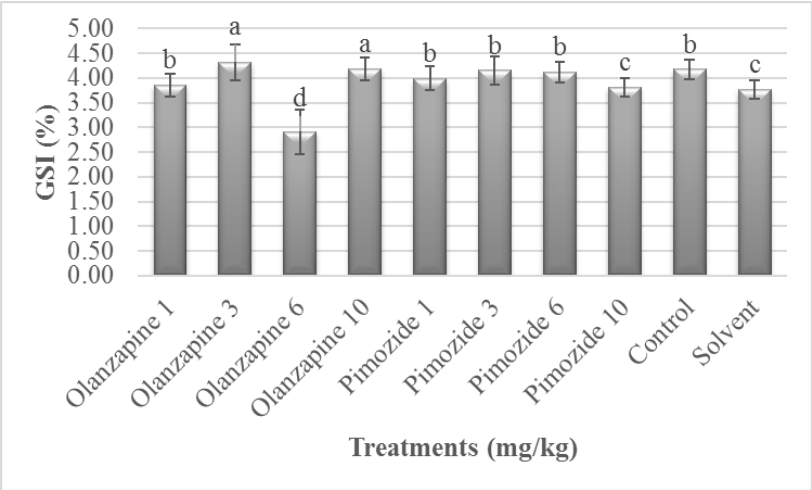

Figure 1: Comparison of gonadal index percentage between different groups of *Trichogaster Trichoptrus.*

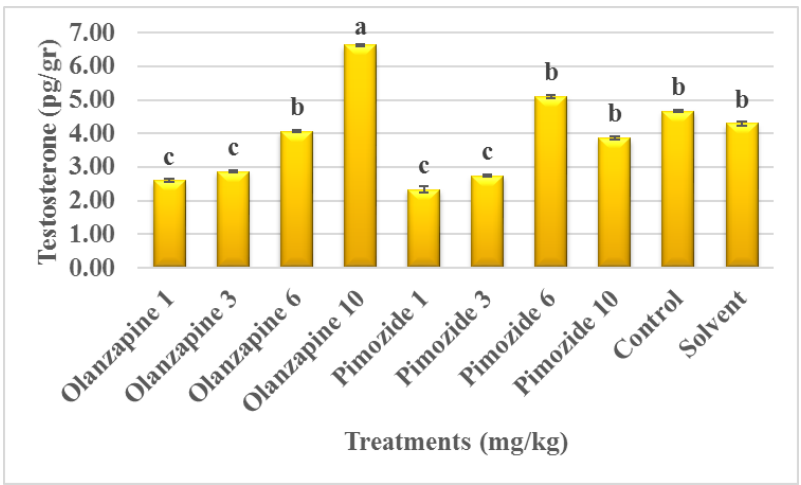

Figure 2: Comparison of testestron levels between different groups of *Trichogaster trichoptrus.*

Comparing average by Duncan multi range exam showed that significant difference was between treatments based on testestron levels.

Results of estradiol analysis was shown in Fig. 3 that after 20 days of injection, estradiol level in comparison with control and intact group showed a comparatively decrease in smaller dose based on p=0/00 and also comparing averages by Duncan multi range method showed that there was a significant difference between treatments based on estradiol. Results from analyzing aromatase enzyme was shown in Fig. 4. During treatment injections decreased in aromatase level comparing with control and intact group was decrease and this was more obvious in pimozide group and showed a direct relationship between dose addition and aromatase level reduction. also comparing averages by Duncan multi range method showed that there was a significant difference between all treatments based on aromatase level (Figs 5 to 8) (Table 2).

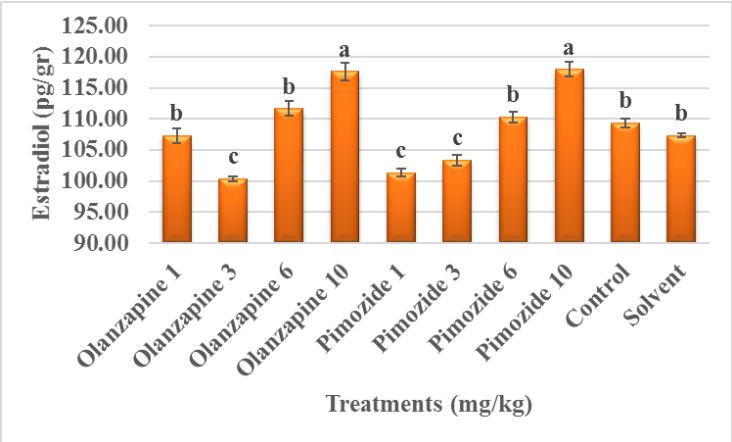

Figure 3: Comparison of estradiol levels between different group of *Trichogaster trichoptrus.*

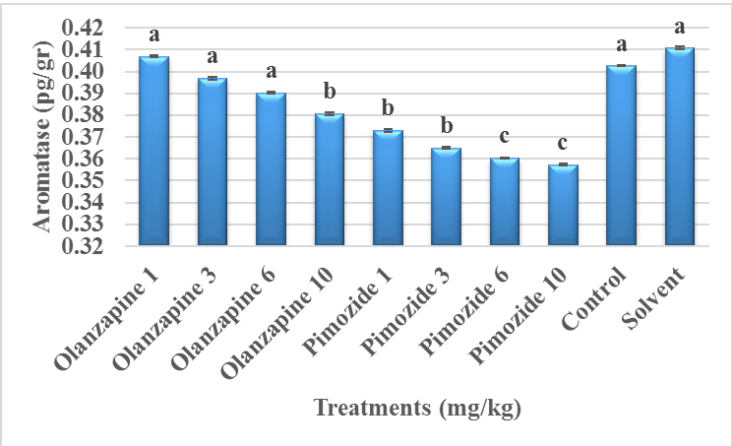

Figure 4: Comparison of aromatase enzyme levels between different group of *Trichogaster trichoptrus.*

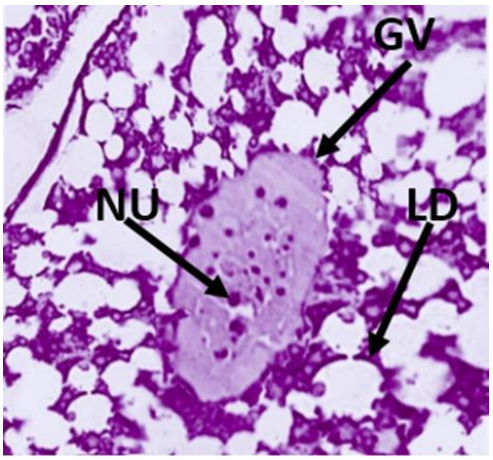

Figure 5: In studying the histologic samples of oocyte in fishes from witness group, the oocyte tissue containing lipid droplets (LD) as dispersed in ovoplasm and the beginning of the movement of germinal vesicle was seen.

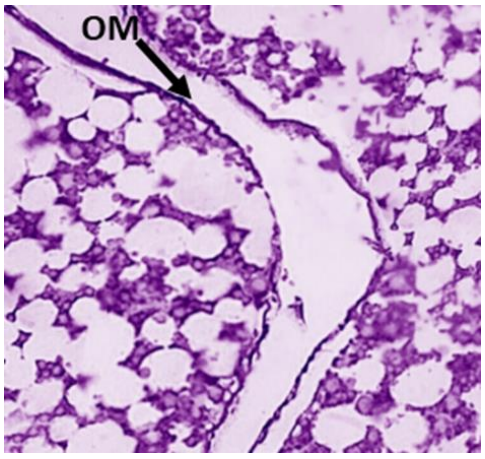

Figure 6: In comparing the histologic samples from control group presence of oocytes at beginning stages of vitellogenesis was seen. The oocyte membrane was also seen.

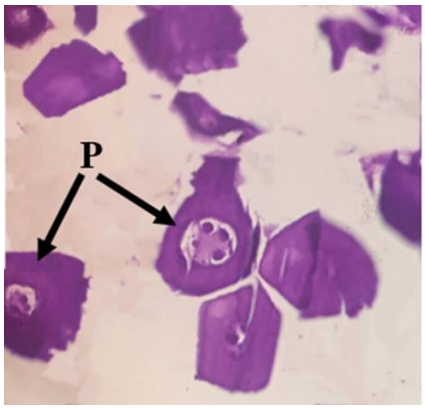

Figure 7: In histologic study by photomicroscopy of oocyte in treatment by Olanzapine 10mg/kg. Most of oocyte were in primary prenuclear stage. This was a confirmation for blocking oocyte maturation.

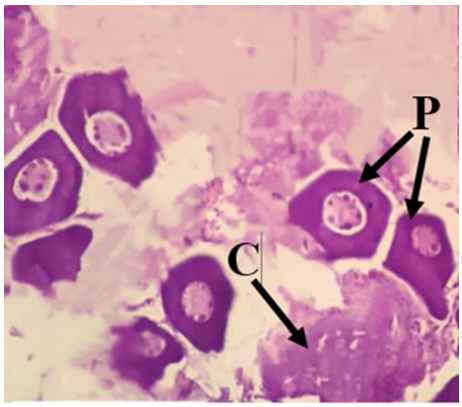

Figure 8: In histologic study of oocyte by photomicroscopy in treatment by Pimozide 10mg/kg. Most of oocyte were in primary prenuclear stage and some others were in cortical stage. This was a confirmation for blocking oocyte maturation.

Tablet 2: adding up of relationship between aromatase, testestrone and estradiol.

		Aromatase	Estradiol	Testosterone
Aromatase	Pearson Correlation	1	-0.248	-0.061
	Sig. (2-tailed)		0.187	0.751
	N	30	30	30
Estradiol	Pearson Correlation	-0.248	1	.721**
	Sig. (2-tailed)	0.187		0.000
	N	30	30	30
Testosterone	Pearson Correlation	-0.061	.721**	1
	Sig. (2-tailed)	0.751	0.000	
	N	30	30	30

**. Correlation is significant at the 0.01 level (2-tailed).

Histological finding of olanzapine treatment in this study showed that the oocyte maturation process has been blocked and in olanzapine doses compared to intact there had been no maturation and ovogenesis stages. In lower doses of olanzapine there was some cells in Prenuclear and cortical stages which by increasing doses the number of prenuclear cells increased while cortical cells decreased as in 10 mg/kg of doses of olanzapine all of the cells were in pre nuclear stages and no cell was seen in cortical stage.

The histological photomicroscopy of pimozide treatment showed that a fewer number of mature cell compared to intact and control group was seen and a number of cell were in prenucleard some other were in cortical stages.in doses of 10 mg/kg of pimozide (Fig. 8) most of the cells were in prenuclear stage and a fewer numbers cells were in cortical stage.

Discussion

Results from comparing control and intact groups in mature fishes with treatments1, 2, 3, 4 which received 1, 3, 6, 10 mg/kg of olanzapine relatively and treatments 5, 6, 7, 8 which received 1, 3, 6, 10 mg/kg of pimozide relatively showed the effects of dopamine antagonist drugs on endocrine hormones and aromatase levels which decreased aromatase, estradiol and testestrone levels and showed a significant difference with control group ($p \leq 0/05$).

This decrease in the level of endocrine hormones and aromatase can be due to the decrease in the level of dopamine and the increase in prolactin level due to the administration of both drugs (dopamine receptor antagonists), also the difference in effect of this drug in different doses and species indicates the complex behavior of this drugs (dopamine receptor antagonists) is shown (Jeong *et al*., 2012).

Analysis GSI group in fishes of control and intact showed, no significant difference and this showed that the used solvent had no effect on GSI level. In pimozide treatment group there was no difference in study GSI comparing to control and intact group. Also between treatments with different doses of pimozide no difference was seen in GSI. In olanzapine treatment group, at doses of 6 mg/kg there was a reduction at GSI in control and intact group and on the whole between olanzapine doses, there was a significant difference in GSI level which was not dose dependent (Fig. 1).

As in Simone study, weight loss in endocrine hormones organs was also observed in Olanzapine-treated treatments. In this study, the weight loss of gonads in fish treated with a dose of 6 mg/kg of olanzapine was considered to be attributed to the effect of olanzapine on a number of dopamine receptors and, consequently, prolactin levels (Simone, 2013). In a study by Crawford *et al*. (1997) with the aim of comparing the long-term effects of olanzapine, haloperidol, and placebo on serum levels of prolactin, it was shown that haloperidol affects and increases serum prolactin levels less than olanzapine (Crawford, 1997).

Although the inhibitory role of dopamine in determining the prolactin induced antipsychotics side effects is not fully understood, but prolactin addition by blocking dopamine indirectly decreases endocrine hormones and blocking sexual maturation in some species of bony fishes (Claude Fabre *et al*., 2003; Suzuki, 2013). Studying testestrone level group between control and intact fishes there was no significant difference and this shown that the used solvent was no effect on testestrone levels.In two pimozide and olanzapine treatments there was a significant difference compared with control and intact group and the decreasing behavior was not dose dependent. In dosed 1 and 3 mg/kg of both drugs, decreasion testestrone level was seen while irregular increaseIn doses of 6mg/kg and 10mg/kg of both drugs might be a result of drug toxicity (Fig. 2)

Studying estradiol level graphs in fishes of intact and control group showed that the used solvent had no effect on estradiol level in two Pimozide and Olanzapine treatments there was a significant difference compared with intact and control group and this decreasing behavior was not dose dependent in dose 3 mg/kg of olanzapine and 3 mg/kg and 1 mg/kg of pimozide there was a decrease in estradiol level while irregular increase in doses 6 mg/kg and 10 mg/kg of both drugs might be a result of drug toxicity (Fig. 3). Studying aromatase level graphs in fishes of control and intact group showed no significant decrease and this means that the used solvent had no effect on aromatase level in treatment groups with olanzapine and pimozide there was a remarkable difference compared with control and intact group and this decreasing behavior was dose dependent which means in higher doses aromatase decreased more significant. In this graph Pimozide showed a more powerful blocking effect compared with olanzapine (Fig.4).

In a study by Sharaf *et al* aimed at investigating the effect of GnRHa, pimozide and Ovaprim on the level of sex steroid hormones in African catfish, it was found that ovulation in the Ovaprim group was further increased, as well as ovulation in The pimozide + GnRHa group was greater than GnRHa alone and the Ovaprim group. There was no significant relationship between these drugs and fertility (Sharaf, 2014). Also, in another study, the effects of pimozide on fertility and ovulation in whitefish by Ahmadinejad and colleagues, showed that the combination of pimozide and LHRH agonist had the most positive effect on egg production and quality indices (Ahmadinejad, 2013).

In spite of the findings of the two studies, the positive effect of pimozide in combination with gonadotropins on ovulation, in the present study, decreased levels of endocrine hormones and aromatase enzymes in pimozide doses in Gurami triplets, Studies show more pimozide behaviors in doses and different species. Also, in the histological examination, the increase in the number of cells in the pre-nucleus phase was confirmed by the inhibitory effect of pimozide in this study. Comparing the pictures of two intact and control group showed no significant difference and cells in treatment group were also in vitellogenesis stage. Studying fig of olanzapine in lower doses showed that despite the presence of cells in cortical phase the number of cells in prenuclear stage increased. Also the number of cells in prenuclear phase increased by increasing Doses (stage 3 of oocyte maturation) which in 10 mg/kg of Olanzapine most of the cells where in prenuclear stage and oocyte maturation blockage was seen.as shown by Smith *et al*. (2002) research; treatment whit antipsychotic drugs induced significant disturbances in sexual maturation. Thus in this study we showed that oocyte maturation was affected by 2nd generation of antipsychotics (Smith, 2002). Studying oocyte histologic

samples of pimozide treatments showed that the cells in prenuclear and cortical stages are increased compared with control and witness group and cells in vitellogenesis and maturation stages decreased. The cell number in prenuclear stage was much more than Olanzapine treatments.

In treatments with high doses of Pomozide most of the cells were in prenuclear stages. Bargiota *et al*. (2013) studied the effects of antipsychotic drugs on fertility and showed that mensural periods and ovulation had been disturbed. In this study we too showed irregularity in maturation of oocyte and blockage of ovulation (Bargiota, 2013)

There was also a significant correlation in this statistic study which was between aromatase and testosterone (p:0/751) in which research showed aromatase decrease in high doses while testosterone increased at the same doses and this was because of aromatase function in converting testosterone into estradiol (Table 2).

Conclusions

Based on analysis and results, two drugs of Olanzapine and Pimozide affected oocyte maturation. According to remarkable decrease in testosterone, aromatase and estradiol level compared with control group, it seems that Pimozide has a more powerful blocking effects on oocyte maturation.

Based on results from hormone levels and pictures of photo-microscopy it seems that two drugs of Pimozide and Olanzapine affect the oocyte maturation procedure.

Resources

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