The Effects of *Origanum vulgare* Extract on Ovary Morphology and Histology in Immature *Trichogaster trichopterus*

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***Abstract*---** Phytoesterogen is a Greek term consisting of *phyto* meaning plant, *estrus* meaning sexual desire, and *gene* meaning production or bearing child. *Origanum vulgare* is a plant of the mint family containing phytoestrogens. In this study the effects of *Origanum vulgare* on ovary morphology and histology in immature *Trichogaster trichopterus* were examined. For this purpose, ten fish were divided into seven groups: saline, placebo, ethanol receiving and four experimental groups. Experimental groups received four doses (10, 20.30 and 50 µg/kg body weight) of *Origanum vulgare*. Injection was carried out intramuscularly as one dose/day for a period of 1 week. Oogenesis in all groups was examined by light microscope. Our results showed that *Origanum vulgare* plant extracts caused significant changes in oocyte diameter and average percentage gonadosomatic index. The results also showed that *Origanum vulgare* can accelerate oocyte maturation in *Trichogaster trichopterus*. Histological studies indicated that *Origanum vulgare* increased growth and maturation of oocytes. Based on this study, *Origanum vulgare* extract can influence fertility of the *Trichogaster trichopterus*.

***Keywords*---**Origanum vulgare, Trichogaster trichopterus, ovary, Morphology, Histology.

I. Introduction

PHYTOESTROGENS are compounds occuring in plants, they are not identical to human estrogen. They are similar in structure and thereby elicit an estrogenic response by reacting with estrogen receptors. In all vertebrates, including fish, pituitary axis - the hypothalamus - gonadal (HPG) plays an important role in reproduction (Okubo et al, 2008). *Trichogaster trichopterus* is a fish of anabantidae familiy and provides ideal model for studying pituitary - hypothalamus - gonadal (Degani et al, 1995). Oocytes maturation *in vitro* conditions can increase the beta subunit of gonadotropin expression in gonadotroph cells (Degani et al, 2004). Oocyte maturation is occurred wit help of a luteinizing hormone-releasing analogs and 17beta-estradiol (Anathy et al, 2003). In *Trichogaster trichopterus* oocyte maturation there are 6 phases:

1) oogonia
2) primary oocyte with chromatin- nucleoluse
3) perinucleolar
4) cortical alveolar
5) vitellogenic
6) maturation of oocytes (Degani et al, 1994).

Phytoestrogens are xenoestrogens plant-derived that can mimic sex hormones (estrogens) (Yildiz, 2005). *Origanum vulgare* of the mint family (Novak et al, 2008) contains four phytoestrogens: apigenin, biochanin A, quercetin and luteolin (Van Meeuwen et al, 2007). In Northern Peru, leaves and stems, fresh or dried, of oregano are used as traditional remedies for menstrual cramps, menstruation and lower stomach cramps related to premenstrual stages (Bussmann & Glenn 2010). Studies show that the leaves of *Origanum vulgare* increase lactation in cows (Tekippe et al, 2011).

This study was exerted to determine the effects of hydroalcoholic extract of the aerial branches of *origanum vulgare* on ovary morphology and histology in immature *Trichogaster trichopterus.*

II. Material And Methods

70 immature *Trichogaster trichopterus* were obtained from *Mahyran* Company (Iran,Tehran). The fish were divided into seven group, three control groups: saline, placebo, ethanol receiving and four experimental groups. Experimental groups treated with four doses (10, 20.30 and 50 µg/kg body weight) of *Origanum vulgare*. Fish were incubated in standard aquarium with free access to food in 22°-25 °C and pH = 7±1. Average length and weight of fish in each group was 5 ± 1 cm and 2.8 ± 1 mg, respectively.

To prepare the extract *Origanum vulgare*, aerial branches of plant were collected from *Giah Essence* farm in Iran in April 2013. The plant was identified by a taxonomist (Herbarium number AUPF1162). Aerial branches of the plant were cleaned and dried in the shade. The ethanol extract of the aerial branches of the plant was percolated (Ahmed et al, 2002). The powdered material was mixed with ethanol in a plate and extraction process was carried out by percolation method at room temperature. Extracts were weighted and dissolved in ethanol. Solutions were injected intramuscularly. as one dose/day for a period of 1 week. Finally, fish were anaesthetized

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with clove oil (0.1ml/l). The body cavity was opened with a semi-circle incision running anteriorly from the cloaca to the pectoral fin. The organs were dissected out and carefully separated. In order to determine the macroscopic oogenesis, the gonads were fixed in Bouin's solution for 24-72h (according to the size of the gonad), followed by washing with 70% alcohol solution to remove picric acid. Then tissues dehydrated with alcohol 70%, 90%, 90%, and 96% step by step. The samples were treated by Xylene for 8 hours and then exposed to paraffin. Tissue sections (thickness of 5-10 microns) were obtained using microtme (HM 325, Thermo Fischer Scientific Inc., and Germany). The sections were stained with ***Hematoxylin - Eosin*** and examined by Nikon optical microscope (Eclipse E100) (Huang et al. 2002). Oocyte diameter was determined using Axiovision Rel Applications (H.Murua et al, 2003). Gonadosomatic index (GSI) was used to measure reproductive capacity (Yoneda et al, 2013). Average percentage gonadosomatic index was calculated (Gonad weight / body weight x 100) (Yiwang et al, 2001). The data were analyzed using One-way analysis (ANOVA) with SPSS 18. Duncan test was used as post hoc test. $p \leq 0.05$ was considered as significant difference between groups.

III. Results

There was no significant difference in gonadosomatic index between control groups 1(intact), 2(normal saline) and 3(ethanol), however, there was significant difference between *origanum vulgare receving* and control groups ($P \leq 0.05$). There was no significant difference in gonadosomatic index between groups receiving 10, 20 and 30 µg/kg of *Origanum vulgare* , however, there was significant higher gonadosomatic index in *origanum vulgare receving* 50µg/kg than groups receiving 10, 20 and 30 µg/kg of *Origanum vulgare* (Figure I).

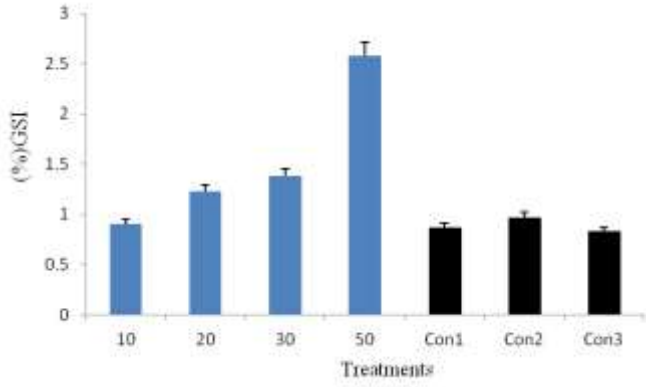

Fig. 1 Gonadosomatic index in *Origanum vulgare* receiving and control (con) groups.

Table I shows the oocyte diameter in different groups (Table I). Statistical analysis of the average oocyte diameter in controls 1, 2 and 3 did not show a significant difference in oogonia phase. There was no significant difference in oocyte diameter between groups receiving 10 and 20 µg/kg of *Origanum vulgare*. The maximum average diameter of the oocyte was observed in animals receiving 50µg/kg of *Origanum vulgare* (FigII).

TableI. Average oocyte diameter and GSI% after injection in 7 groups

GSI average %	Average oocyte diameter after injection(µm)	Group	Number
1.10	44.37	*Origanum vulgare* 10µg/kg	1
1.39	67.50	*Origanum vulgare* 20µg/kg	2
1.46	104.97	*Origanum vulgare* 30µg/kg	3
3.48	200.23	*Origanum vulgare* 50µg/kg	4
0.87	33.52	Control 1(placebo)	5
0.97	38.46	Control 2(normal saline)	6
0.82	38.14	Control 3(ethanol)	7

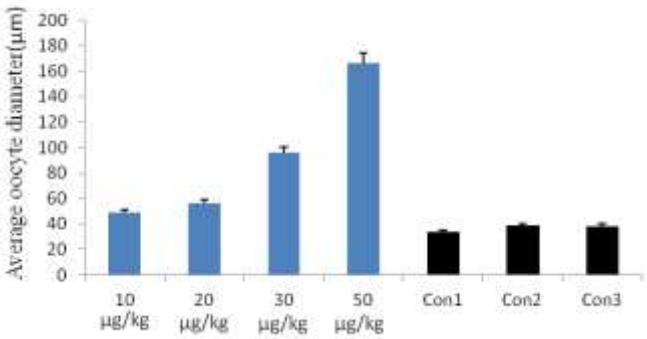

Fig. 2 Average oocyte diameter(µm) in different treatments

A. Gonad Histology

Oogenesis in fish has 6 phases: oogonia, primary oocyte with chromatin-nucleoluse, perinucleolar, cortical alveolar, vitellogenic and mature oocyte. Control treatments are often immature oocytes and in perinucleolar phase (Fig IIIa). With increasing doses from 10 to 50µg/kg, oocytes progressed from perinucleolar phase to vitellogenic phase. Oocytes in group receiving 50µg/kg of plant extract showed maximum growth and nucleus or germinal vesicle (GV) to migrate towards the periphery and immediate forming of coagulated lipid particles and globule yolk. Also, break down of germinal vesicle (GVBD) was observed (Fig IIIb). In groups receving *Origanum vulgare* extract, the nucleus moving to the periphery oocyte (Fig IIIc) and the accumulation of lipid particles were also observed and oocyte in perinucleolar phase were also observed (FigIII d).

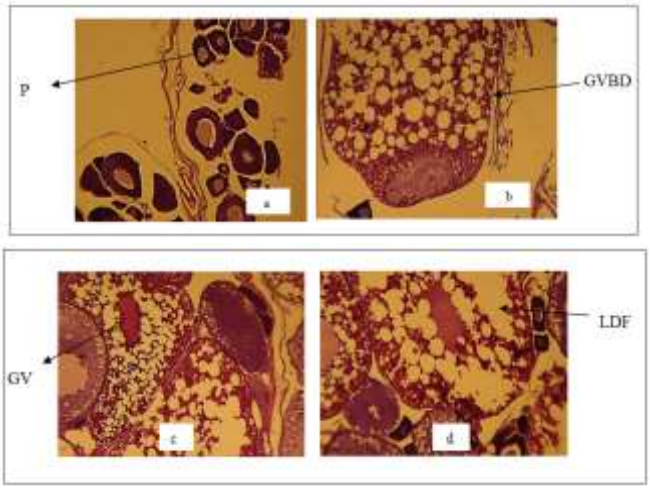

Fig. 3 Sections of ovarian tissue in different groups: a. control group. Dominant phase oocytes contain perinucleolar (p). b. treated by Origanum vulgare at dose of 20µg/kg, germinal vesicle break down (GVBD) are observed, c. treated by Origanum vulgare at dose of 30µg/kg, germinal vesicle (GV), coagulation lipid particles (LDF) are apparent. D. treated by Origanum vulgare at dose of 50µg/kg, germinal vesicle (GV), coagulation lipid particles (LDF) are observed (×400, H&E).

IV. Discussion

Our findings in line with other studies showed that normal saline and ethanol did not affect on oocyte maturation (Ahmadnezhad et al, 2013). Results of this study showed that Gonadosomatic index was higher in some groups of *Origanum vulgare* extract receiving groups than control group. The results also indicated that all injections of *Origanum vulgare* increased growth and maturation of oocytes which there was also dose response correlation. Our findings also indicated that *Origanum vulgare* extract at a dose of 50µg/kg can increase gonadosomatic index. Studies show that 17 beta-estradiol plays an important role in oocyte maturation of *Trichogaster trichopterus* (Degani et al, 2004). Four phytoestrogens apigenin, biochanin A, quercetin and luteolin have been found in this plant (Shan et al. 2005; Yanishlieva et al. 2006; USDA 2007) .These phytoestrogens are nonsteroidal that can be used as the primary sex hormones (estrogens) (Yildiz, 2005,Kraak et al, 1984). In studies that examined the effect of LHRH-A_2 on rainbow trout and on *Trichogaster trichopterus* (Degani et al, 1995), it has been shown that the substance stimulates gonadotroph cells and influences oocytesn growth. During oocyte development, oocyte diameter in each phase is greater than the previous phase (Anathy et al, 2003). Oocytes treated with *Origanum vulgare* had diameter of 200/23 mm in the phase between vitilogenic and maturation.

Phases of maturation in *Trichogaster trichopterus* is similar to other teleosts (Degani et al, 1992). Final oocyte maturation is determined by GVBD (Patino et al, 1990a, b). The results of this study showed that fish oocytes in the control group were in perinucleolar phase of maturity.The study by Degani et al (2004) on *Trichogaster trichopterus* indicated that LHRH analogues can influence maturation of oocytes to vitilogenic. In fish treated with 30µg/kg *Origanum vulgare* extarct, oocyte development were between vitilogenic phase and final maturity. Britt, et al (2002) study indicated that, phytoestrogens could also like estrogens play an important role in the reproductive process. In fish oocyte treated with 50µg/kg *Origanum vulgare*, germinal vesicle located beside periphery oocyte and GVBD also appears. Final oocyte maturation and GVBD appears with 17-beta-estradiol supplement in immature monkeys (Zheng et al, 2003) and in *Trichogaster trichopterus* has been also demonstrated (Degani et al, 1992).Gonadotrophin releasing hormone (GnRH) controls secretion of gonadotropin from pituitary gland by affecting on gonadotroph cell (Sherwood et al, 1989; Swanson, 1991). Stimulation of gonadotroph cells cause the secretion of small granules with electron density and large globules of semi-transparent (Leunissen et al, 1982). Cook et al (1987) in studies on *goldfish* showed that LHRH analogues can cause degranulation of the gonadotroph cells. Olivereau et al (1986), according to studies carried on *silver eel*, concluded that 17 beta-estradiol can lead to the development of gonadotroph cell and increase secretory granules in the cells. Gonadotrophin releasing hormone neurons is regulated by gamma-aminobutyric acid (GABA). Studies show that apigenin affects on gamma-aminobutyric acid receptor (Medina et al, 1989) and the ability to bind to estrogen receptor beta (Jarry et al, 2006) stimulates gonadotroph cells. Biochanin A is other phytoestrogen in *Origanum vulgare*. Studies show that this compound has the ability to bind to estrogen receptor alpha in ovary and estrogen receptor beta in the brain (Beck et al, 2003). It seems that the effects of *Origanum vulgare* on gonadotroph cells comes from its phytoestrogens.

V. Conclusion

We have shown that *Origanum vulgare* extract can influence fertility of the Trichogaster trichopterus.

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