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ORIGINAL ARTICLE

The Histological Effects of *Cucurbita pepo, Silybum marianum, Linum usitatissmum, Vitex agnus_castus* 17β estradiol on ovarian tissue in three Spot Gorami (*Trichogaster trichopterus)*

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ABSTRACT
Sexual disorders and related issues has been a problem in the scientific and medical community. These disorders can be both physically and psychologically affect a person's life. Medicinal plants, dedicated to solving this problem have been used in traditional medicine of different nations.Infertility is one of the most common and important problems among women.The aim of this study was to compare the reproductive effect of extracts of four phytoestrogenic plants (Cucurbita pepo, Silybum marianum, Linum usitatissmum and Vitex agnus_castus) with 17β estradiol (E_2) on ovarian tissue of immature female three spot gorami. For this purpose,220 immature female gourami fish with an average weight of 2.5±0.5g were analyzed in 22 treated groups. The experiment conducted based on a completely randomized design in with 4 groups treated with different doses of plant extracts (10-20-30-50 mg/kg bw) were compared with 4 groups treated with E_2.The ovarian tissue was removed, weighed, processed and stained through hematoxylin- eosin method. The histological structure of the ovaries and the average percentage of gonadal index of treated fish were compared between treated groups, using spss 18 software package anova test. The results showed that the diameter of the oocytes in E_2 treated group was significantly different from Linum Usitatissimum, Vitex agnus_castus and Cucurbita Pepo treated groups as the injected dose increases up to 50 mg/kg. ($p \leq 0.05$). Additionally, GSI of E_2 treated group was significantly different with all other groups, except Silybum Marianum treated group ($p \leq 0.05$). the average percentage of oocyte growth for E_2 and Silybum Marianum treated groups, were the most identical. Therefore, this study demonstrates that the extract of Silybum marianum plant is the most effective extract among other 3 investigated plants in fertility enhancement of immature female three spot gorami fish.
***Keywords:**, 17β Estradiol, Trichogaster trichopterus ,ovary, Cucurbita pepo, Silybum marianum, Linum usitatissmum, Vitex agnus_castus*

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INTRODUCTION

Phytoesterogen is a Greek term consisting of *phyto* meaning plant, *estrus* meaning sexual desire, and *gene* meaning production or bearing child. Phytoesterogens are similar to 17 β-estradiol in terms of the structure and function or effects [1 – 2].

Phytoestrogens include several groups of compound, namely, lignans, isoflavones (daidzein & genistein), coumestans, andresorcylic acid lactones. Isoflavones are very weak agonists of estrogen and bind to estrogen receptors with a binding affinity less than that of estradiol. When the amount of estradiol in the body is too low to compete for binding to the receptor, isoflavones present more agonistic properties. Moreover, their antiestrogenic properties depend on relative concentrations of internal estrogens and phytoestrogens. Phytoestrogens may release estradiol from their receptors when the internal estrogens are abundant (3).

Isoflavones are plant estrogens that, similar to female estrogen hormones, bind to cell estrogen receptors in different organs of the body. However, plant estrogen binding affinity is less than that of human estrogens. Recent studies have shown that cells have two type of estrogen receptors, α and β, and human estrogens have much affinity for binding to α receptor, while, isoflavones have an affinity for binding to β receptor (4).Furthermore, isoflavones have antioxidant, anti-bacterial, and anti-inflammatory properties

(5). Results of a study showed that isoflavones and their functions had positive effects on body compounds and factors causing menopause in women (6). As most natural substances containing estrogen have a low level of activity in nature, they cannot satisfy people's physiological need completely (7). Recent studies confirmed the beneficial effects of such phytoestrogens on reducing menopausal complications, such as hot flashes, sleep disturbances, vaginal mucus changes, memory impairment, and risk of cardiovascular diseases and cancers (8- 9).

And osteoporosis which is an age, sex and hormaonal changes associated disorder (1- 10).

Pumpkins with the scientific name *Cucurbita pepo* belong to Cucurbitaceae family. Studies on being conducted Pumpkin seeds in different parts of the world showed their high levels of protein and fat (11-13). They were also introduced as an important source for medicines in the recent decade. Moreover, studies showed that the high percentage of two significant unsaturated fatty acids necessary for the body, oleic acid and linoleic acid, especially α-linoleic acid, and also phytosterols, omega-3 fatty acids, and tocopherols in its seed oil were effective in treatment of intestinal helminthes, benign prostate hypertrophy, gastritis, and atherosclerosis; reduction of LDL levels; preventing cardiac arrhythmias; and reducing the risk of bladder and kidney stones (11 -16).Milk thistle, scientifically known as *Silybum marianum,* belongs to Compositae or Aster family .This plant is grown all over the world and is also native to Iran. It is of special importance in European, Chinese, and Indian traditional medicine. Seeds of this plant contain numerous compounds, including all types of flavones, and their antioxidant and free radical elimination properties in varieties of metabolic disorders were examined previously (17).

Flax, scientifically named *Linum usitatissimum,* belongs to Linaceae family. Linseeds or flaxes are favorable sources of omega-3 essential fatty acids, such as α-linoleic acid, so that, they have show protective effects against many diseases including atherosclerosis and similar heart diseases, and reduce blood cholesterol (18). Moreover, flaxseeds are good sources of lignans that are phytoestrogenic components with protective effects against diseases such as breast cancer and osteoporosis (19,20). They are also sources of fibers, proteins, minerals, and vitamins (18 -21).

Vitex, scientifically known as *Vitex agnus castus,* belongs to Verbenaceae family (22). All parts of this plant have a special kind of essence consisting of cineol, sabinene, pinene, and sesquiterpene. Essential oils are other ingredients of this plant (23,24). Vitex is one of the important plants used in herbal medicine for treating women's hormonal diseases and adjusting hormones (25). It is largely used to treat breast disorders, menstrual irregularities, and uterine bleeding (26). Other properties mentioned in Iranian traditional medicine for Vitex include anti-inflammatory, nutritious, diuretic, anaphrodisiac, appetizing, relaxing, carminative, and anti-flatulence (14). This study was conducted to compare the effect of the above-mentioned phytoestrogens with that of 17 β-estradiol on fertility.

MATERIALS AND METHODS

This study was done in Aquaculture Laboratory in Pharmaceutical Sciences Branch of Islamic Azad University. In total, 220 immature three-spot gourami with mean weight of 2.5±0.5 g were supplied from an ornamental fish hatchery in Astaneh Ashrafieh , and the hormone E_2 (purity 80%) was supplied from Aboureihan Pharmaceutical Company. The aquariums were filled with water and left for 48 hours to be dechlorinated before placing fishes into them. The gourami were put into the aquariums for 2 days at 20-23°C and 12 hour light/dark cycle for the better adaption to the environment. The fish were fed with the standard feed for gourami, and the filters were cleaned every other day.

This study was to compare the reproductive effect of extracts of four phytoestrogenic plants (*Cucurbita pepo, Silybum marianum, Linum usitatissmum* and *Vitex agnus_castus*) with 17β estradiol (E_2) on ovarian tissue of immature female three spot gorami. For this purpose,220 immature female gourami fish with an average weight of 2.5±0.5g were analyzed in 22 treated groups. The experiment conducted based on a completely randomized design in with 4 groups treated with different doses of plant extracts (10-20-30-50 mg/kg bw) were compared with 4 groups treated with E_2.we had also two control groups. one group was intact. The water physicochemical factors, including the temperature, hardness, and pH, were measured before, during, and after the experiment. Before and after the injections, biometry of fish, including their weight and length was also examined. The tests were conducted for the 22 groups. The applied dose of the studied substances was determined 2.5±0.5 g /kg of fish weight. The prepared extracts were injected to the muscle below the dorsal fin 10 times within 20 days. The dose of injection for each fish was 20 µl. Once the injections terminated, Then, they were weighed using a digital scale with 0.1 g precision. The ovaries of the fish were removed carefully and weighed. Each group's ovaries were placed in a container of 10% formalin. After the tissue processing, preparing sections of the same parts of fish's ovary, and staining with hematoxyline-eosin, the sections were histologically examined using an optical microscope. Finally, the treatments were compared in terms of the histological structure of the

ovaries, mean percentage of gonadosomatic index (GSI), oocyte diameter, and mean percentage of oocyte formation in gourami. The GSI for each fish in each treatment was calculated using the following formula:
$GSI = W_G/W \times 100$
where, W_G is the weight (g) of gonad and W is the total weight (g) of the body.
To measure the oocyte diameter using Axiovision software, the slides prepared from the ovaries in each treatment were photographed. The analysis of data and significance of differences was performed through SPSS18 and Excel software; the SPSS software for analysis of data and Excel for drawing figures. The data were compared using one-way ANOVA.

RESULTS

Figure 1 showed the results for GSI. The GSI increased with an increase in dose of Pumpkin, Flax, and Silymarin. However, such an increase was not observed about Vitex.

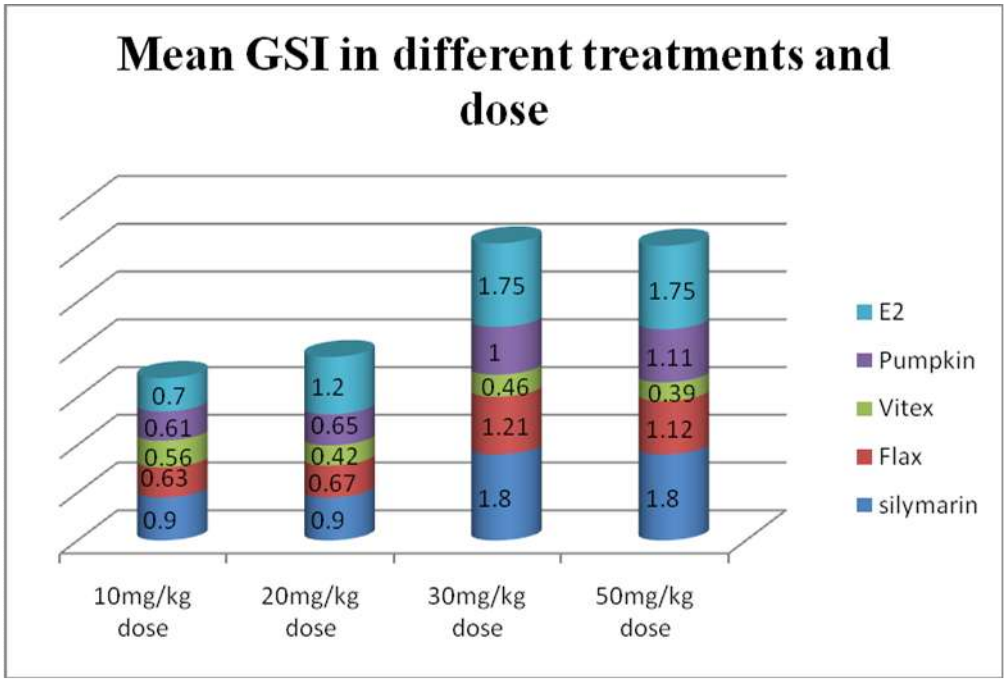

Fig 1. Comparison of treatments with alcohol extracts of the studied plants and 17 β-estradiol in terms of GSI (%) after injections to the three-spot gourami

In 10 and 20 mg/kg dose, treatments with E_2 had a significant difference with other treatments in terms of GSI.
In 30 and 50 mg/kg dose, treatments with E_2 had a significant difference with other treatments, except Silymarin, in terms of GSI.
The results for comparison of oocyte diameter showed in Fig 2. These results showed that the oocyte diameter increased with an increase in the dose. In 10 mg/kg dose, the treatment with E_2 had a significant difference with treatments with Silymarin and Flax in terms of oocyte diameter. In 20 mg/kg dose, the treatment with E_2 had a significant difference with the treatment with vitex in terms of oocyte diameter. In 30 mg/kg dose, the treatment with E_2 had a significant difference with the treatment with vitex in terms of oocyte diameter. In 50 mg/kg dose, the treatment with E_2 had a significant difference with treatments with Flax, Vitex, and Pumpkin in terms of oocyte diameter.

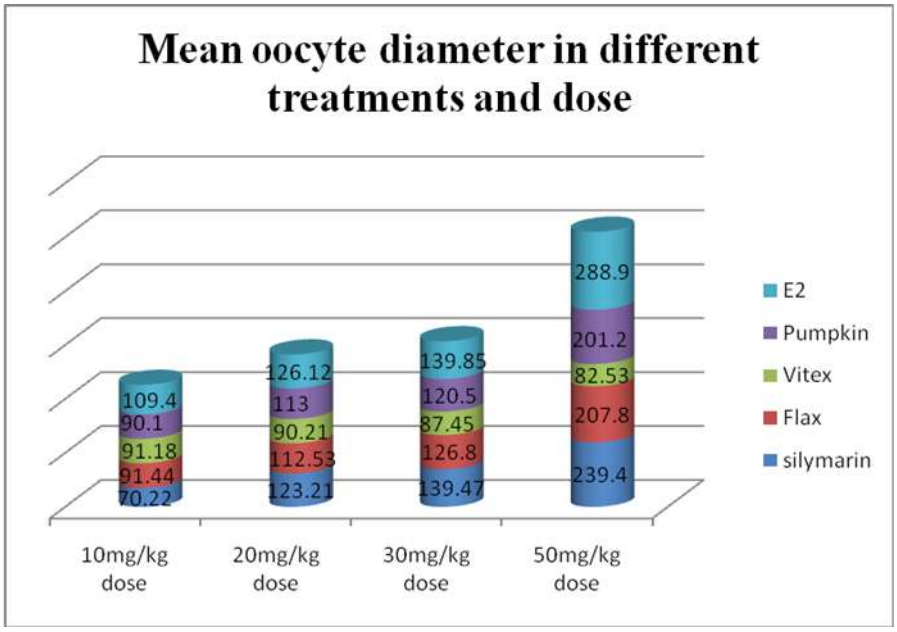

Fig 2. Comparison of treatments with alcohol extracts of the studied plants and 17 β-estradiol in terms of oocyte diameter after injections to the three-spot gourami

The comparison of the treatments in terms of oocyte formation is shown in Figure 3. The results showed that an increase in dose of Flax, Silymarin, Pumpkin, and E_2 resulted in increased number of oocytes. The increase was more significant at 50 mg/kg dose. Among the above plants,when the dose of Silymarin increased, resulted in reduction of oocytes in the first stage or chromatin nucleolus and increased of oocytes in the second and third stages.

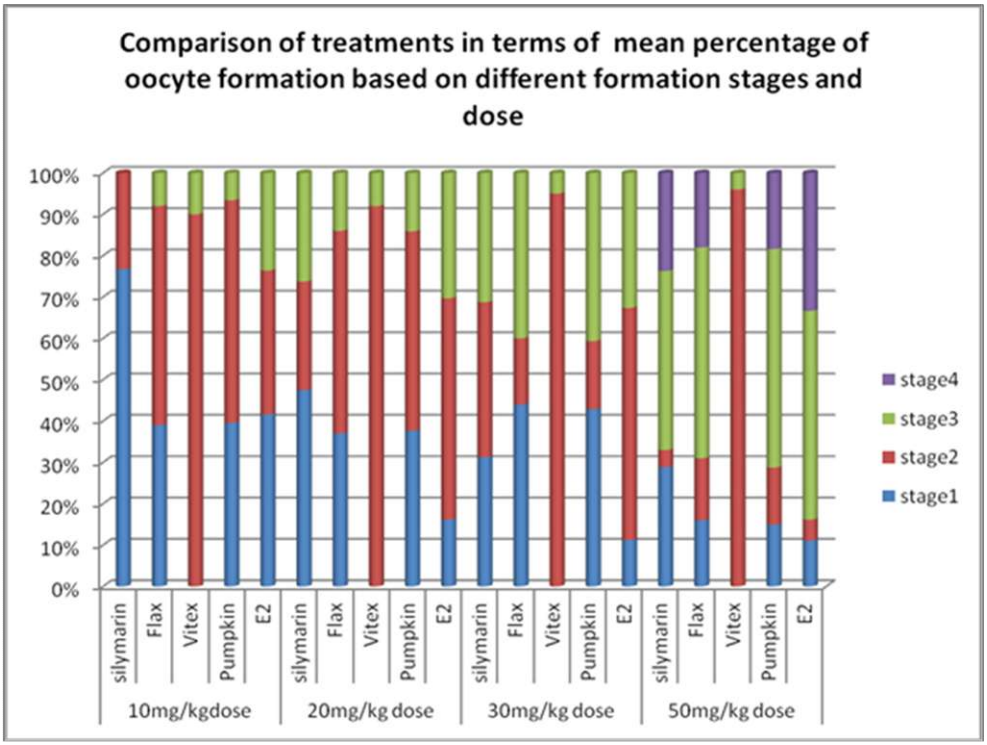

Figure 3. Comparison of treatments with alcohol extracts of the studied plants and 17 β-estradiol in terms of oocyte formation after injections to the three-spot gourami

RESULTS

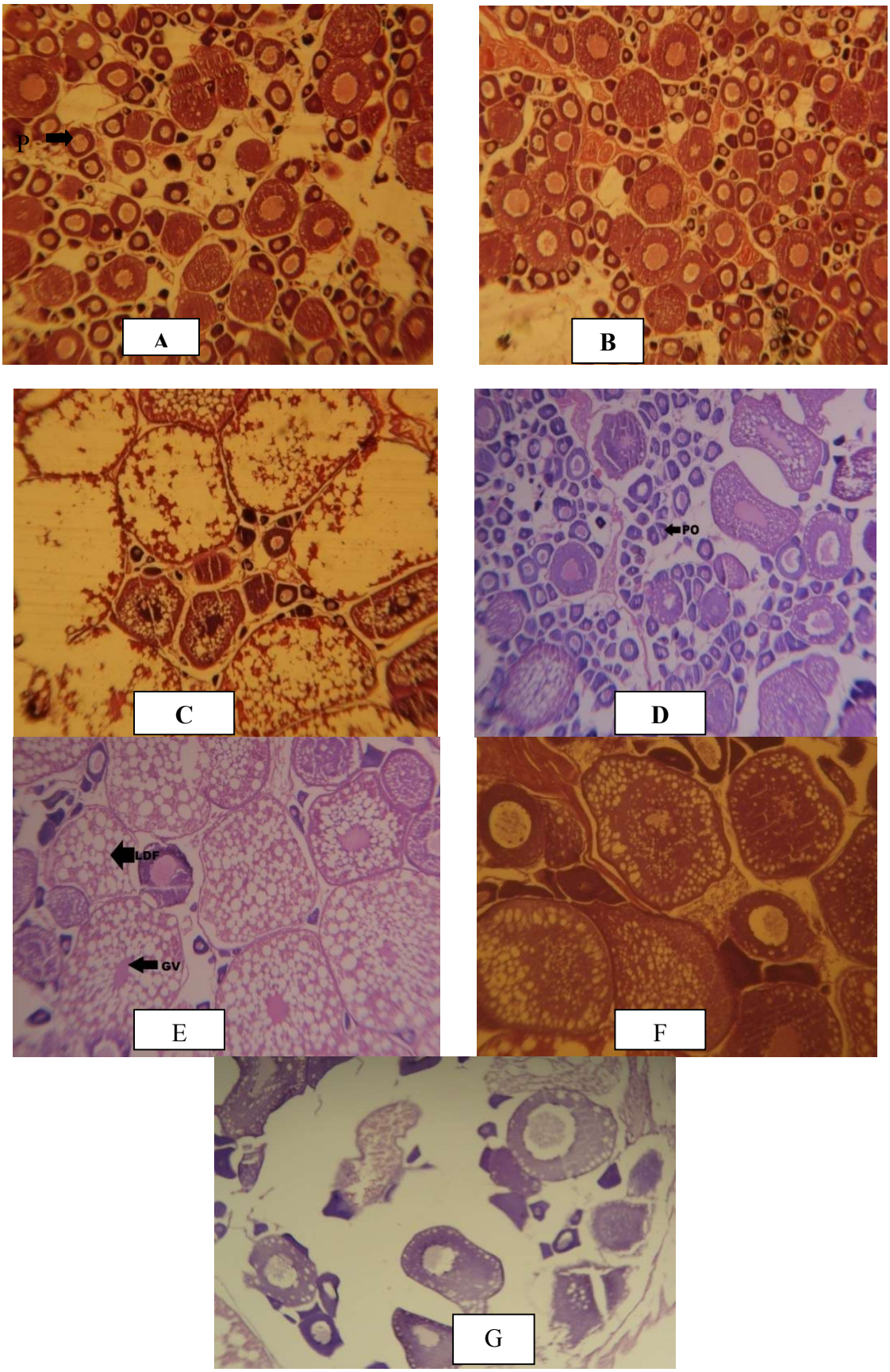

Fig 4. **A:** control group; most of oocytes at the perinucleolar stage (P), **B:** first treatment with Silymarin; most of oocytes at the perinucleolar stage (P), **C**: fourth treatment with Silymarin; germinal vesicle (GV)

moving toward the animal pole; Lipid drop fusion (LDF). **D**: first treatment with Flax; most of oocytes at the perinucleolar stage (PO); Lipid drop fusion (LDF). **E**: fourth treatment with Flax; germinal vesicle (GV) moving toward the animal pole; Lipid drop fusion (LDF). **F**: First treatment with pumpkin; most of oocytes at the perinucleolar stage (P); Lipid drop fussion (LDF);**G** : Fourth treatment with Pumpkin; germinal vesicle (GV) moving toward the animal pole; Lipid Drop Fussion (LDF) . (×400, H&E).

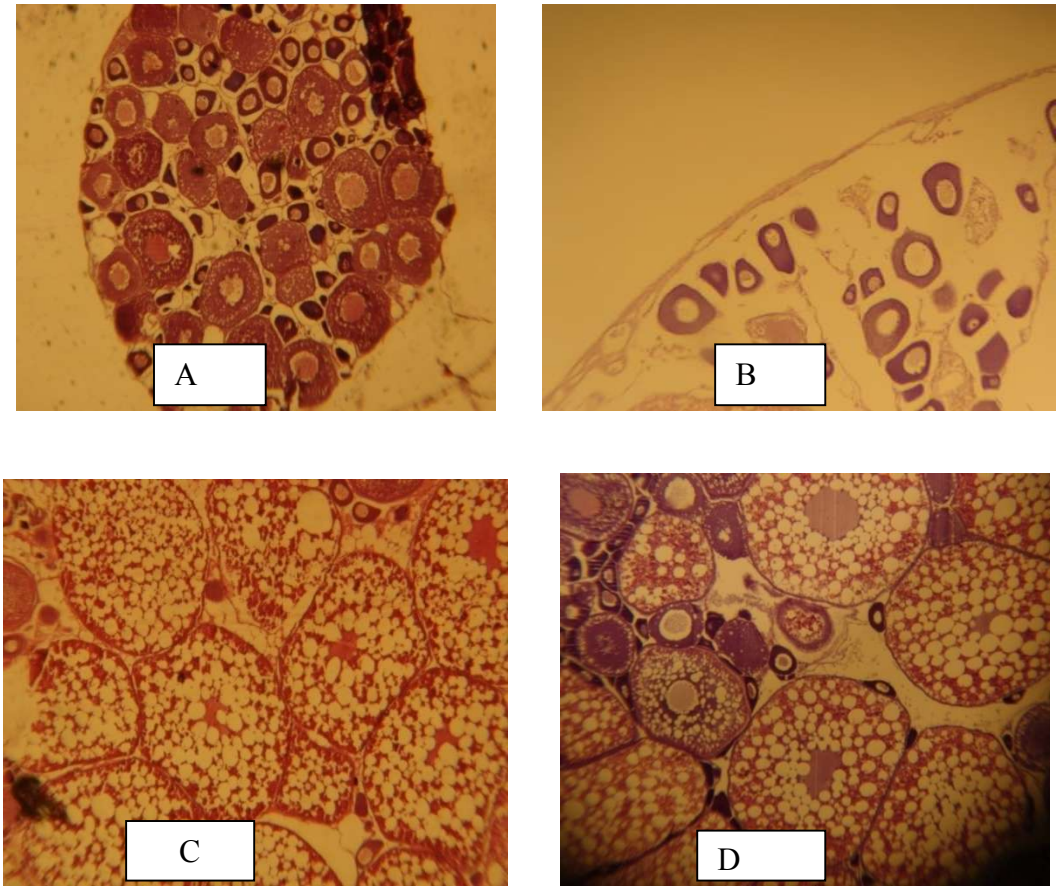

Fig 5: **A**: First treatment with vitex; at the perinucleolar stage (P); Lipid Drop Fussion.**B**: fourth treatment with Vitex; at the perinucleolar dominance stage (P) . **C**: First treatment with E_2; The presence of oocytes in the preliminary stages of vitellogenesis (v);.**D**: Fourth treatment with E_2; germinal vesicle (GV) moving toward the animal pole; Lipid Drop Fussion (LDF) (×400, H&E).

In the analysis of the histological sections of ovaries, germ cells mostly included oocytes at the perinucleolar stage. A few oocytes were at yolk production stage and preliminary stages of vitellogenesis (Fig4:B-F). The histological sections of the control group did not show any difference and the germ cells were mostly occytes at perinucleolar stage. Some oocytes were at yolk production stage and preliminary stages of vitellogenesis (Fig 4:A).

Similar results without any significant difference were obtained in comparing histological sections of the first treatment with Silymarin , Flax and Pumpkin, with those of the control group (Fig 4: B- D-F).

In comparison of the fourth treatment with Flax, Pumpkin, and Silymarin with the control group, oocytes at perinucleolar stage were much fewer than those of the control group, and most oocytes were at vitellogenesis stage. The onset of germinal vesicle (GV) moving toward the animal pole and the presence of lipid drop fusion (LDF) were of remarkable points in this treatment (Fig 4:C-E-G).

Moreover, histological changes resulting from various treatments in terms of the dominant developmental stage of oocytes in each group showed that the Vitex had an infertility effect on ovarian growth in immature three-spot gouramis (Fig5: A-B). An increase in the dose resulted in reduction of oocyte diameter. However, there was no significant difference between treatments with Vitex and control groups in terms of the oocyte diameter (Fig 2).

DISCUSSION AND CONCLUSION

Analysis of the results obtained from comparison of different treatments in each group in terms of the histological changes of the ovaries, mean GSI, mean oocyte diameter, and mean percentage of oocyte

formation showed that the extracts of Flax, Pumpkin, and Silymarin accelerated the final maturity of the three-spot gouramis, and this effect was more evident at higher doses. Comparison of the treatments and control groups in terms of the histological changes of the ovaries, mean GSI, mean oocyte diameter, and mean percentage of oocyte formation did not show any significant differences ($P>0.05$), and this revealed the inefficiency of the solvent used for accelerating the final maturity. The results indicated that the treatment with E_2 significantly differed from treatments with extracts of Flax, Vitex, and Pumpkin in terms of oocyte diameter ($P<0.05$). Also the weight increased at doses up to 50 mg/kg with Vitex extract ($P<0.05$). In terms of GSI, all treatments, except treatments with Silymarin, significantly differed from treatment with E_2 ($P<0.05$).

Regarding the mean percentage of oocyte formation, treatments with Silymarin was the closest to treatments with E_2. Scott et al.'s studies [27] showed that gonadotropin secretion by the pituitary was the prerequisite for maturity of oocytes and ovulation,gonadotropins function through esteroidal hormones . Lee, Yong et al. [28] revealed that the change in the level of 17 β-estradiol correlated with formation of oocytes in ovaries and increased GSI. The study of Shafiei Sabet et al. [29] stated that there was a direct correlation between oocyte formation index and injection of 17 β-estradiol in fish.

Based on this study, Silymarin extract was the most effective in fertility of the three-spot gouramis, and Vitex had a slight infertility effect.

REFERENCES

1. Knight DC, Eden JA. (1995). Phytoestrogens-a short review. Maturitas J. of the climacteric & postmenopause. 22 (3):167-174.
2. Varner Varner J, Bonner J. (1966). Plant Biochemistry. Academic press; 977-0-12-114856-0.
3. Davis SR,Mukies AL,Wilcox.G. (1998). Phytoestrogens in clinical practice. Integrative Medicine.,1(1):27-34.
4. Crouse JR, et al. (1998). Soy protein containing isoflavones reduces plasma concentration of lipids and lipoproteins. Circulation. 97: 816.
5. Verdrengh M. et al.(2004). Phytoestrogen genistein as an anti staphylococcal agent. Microbes Infect 2004; 6: 86-92.
6. Choquette S, et al. (2011). Effects of soya isoflavones and exercise on body composition and clinical risk factors of cardiovascular diseases in overweight postmenopausal women: a 6-month double-blind controlled trial. Br J Nurt ; 105:1199-209.
7. Mitchell JH, et al.(2001). Effect of a phytoestrogen food supplement on reproductive health in normal males. Clin Sci (Lond) ; 99: 613-8.
8. Food and Administration. Food labeling: healyh claims; soy protein and coronary heart disease. Food and drug administration, HHS. Final rule. Fed Regist 1999; 64: 57600-33.
9. Raiten DJ, picciano MF, Coates PM. Dietary supplement use in women: current status and future directions. J Nutr 2003; 133:19574-605.
10. Carusi D. Phytoestrogens as hormone replacement therapy: An evidence based approach Prim. Care Update Ob/Gyns. 2000; 7 (6): 253-259.
11. Bombardelli E, Morazzoni P. Cucurbita pepo L. Fitoterapia;1997. 68 (4): 291-302..
12. Lyman, O. T. T. An introduction to statistical methods an data analysis. Duxbury press. Massachusetts, U. S. A1997; 273.
13. Makai S. Balatin j. Comparative examination of biologically active compounds of fatty oil of medical and alternative herbs. Separated by coldmoulding; 2000.34(3)
14. Zargari A. Medical plants. Tehran Univ pub;1996.p:716_18.
15. Franzens, K. The revival of the green-gold. Published in the scientific magazine UNI-ZEIT 4/97. 2000; 34: 22-27.
16. Khan S, Zaka M, Raie M. Fatty acids from indigenous soures for possible industrial applications. Part 8. Investigation of some special of the Compositae family. Pak. J. Sci. Ind. R. 1977; 28; 300-402.
17. David J, Jaree P, James H, Somkiat K, Kim D, xandra A. immunostimulation of striped snakehead Channa striata against epizootic ulcerative syndrome. Aquaculture. 2001; 195, 1-15.
18. Oomah. B, Mazza G. (1995).Fractionation of flaxseed with a batch dehuller. Industrial Crops and Products in International Journal. ; 9: 19-27.
19. Pass E, Kaal N, Pierce G. Flaxseed ،[on line]. 2002; Available at: www. sbrc. ca/ncarm/.
20. Anonymous. Linseed, [on line]. 2002; Available at: www. Ienica. net/crops/linseed.
21. Oomah B. (2001). Flaxseed as functional source. Journal of the Science of food and Agriculture. 81: 889-894.
22. Hobs C. Vitex: (2003). The Women's Herb. Healthy Living Publications; Summertown, TN:. 203-207.
23. Nasri S, Ebrahimi S. (2006). Medical effect of Vitex agnus-castus. J Babol Univ of Med Sci. 7: 49-53..
24. Nyiligira E, Viljoen AM, Van Heerden FR, Van Zyl RL, Van Vuuren SF, Steenkamp PA.(2008). Phytochemistry and vitro pharmacological activities of south Africa Vitex (Verbenaceae) species. Ethnopharm.119: 680-5.
25. Cossuta D, Simndi E, Vagi J, Hohmann A, Prech I. (2008). Supercritical fluid extraction of Vitex agnus castus fruit. J Supercritical Fluid. 2008; 47: 188-94.
26. Roemheld-Hamm B. Chasteberry. Am Fm Phys. 2005; 72 (5):821-4.
27. Scott AP, Sumpter JP, Hardiman PA. (1983). Hormone changes during ovalotion in the Rainbow trout salmo gairdneri. Gen Comp Endocrinol. Jan; 49 (1):128-134.

28. Lee WK, Yang SW. (2002). Relationship between ovarian development and serum levels of gonadal steroid. Fish Biol. 63: 14-29.
29. Shafeie S, Mohammad Reza A, Gorgin S. (2011). Study on level of 17 beta estradiol and testestrone in female kutum reutilus drisii kutum of sothern caspian sea world. Journal of Zoology. 6 (3). 220-226.

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