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# THE EFFECT OF LINSEED HYDRO ALCOHOLIC EXTRACT (*LINUM USITATISSIMUM*) ON THE SEX HORMONES AND FOLLICULAR CELLS IN ADULT FEMALE MICE

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## ABSTRACT

**Background and objective:** Linseeds are a rich source of trace elements, fiber, magnesium, vitamin B1 and alpha linolenic acid or ALA which is also known as omega-3. The aim of the present study is to evaluate the effect of linseeds extract on the sex hormones and cells in adult female mice.

**Materials and methods:** A total of 30 adult female mice using in this research were divided into 3 main groups including control, experimental 1 and experimental 2 groups. The experimental groups were received 150 and 300 mg/kgbw of the extract, daily. The hydro alcoholic linseed extract was fed by gavage. All tested mice were anesthetized at the end of day 30 and blood samples were taken from the left ventricle after anesthesia, ovarian tissues were removed out and estrogen, testosterone and progesterone amounts and the number of the sex cells were examined. The obtained results were analyzed by SPSS software version 17 and t-test and one-way ANOVA were used ($P \leq 0.05$).

**Results:** The results of this study show that the use of linseed hydro alcoholic extract causes an increase in the amounts of estrogen and progesterone hormones in the mice of experimental groups, while it does not have a significant effect on testosterone hormone. Also, using the linseed hydro alcoholic extract causes an increase in primary and secondary and antral follicles counts.

**Conclusion:** The results of this study showed that linseed hydro alcoholic extract affects on the sex cells and hormones in adult female mice and therefore causes the increased fertility by increased in estrogen, progesterone and sex cells counts.

**Key words: Linseed hydro alcoholic extract, mice, sex hormones, female.**

## INTRODUCTION

In the recent years, infertility and its problems are known as one of the important issues in the life of couples (Devine PJ, 2000). According to World Health Organization's (WHO) definition, infertility is the lack of pregnancy in a couple after one year of sexual intercourse without the use of contraceptive methods which is seen in ten to fifteen percent of couples and fifty percent of cases are seen in women and one of the causes of infertility is disruption in folliculogenesis. During the different stages of follicular growth, most of them have been destructed and within this process, follicular cells and oocyst degenerated and phagocytized by phagocytic cells (John queria et al, 2005) and since a small percentage of oocysts release, it can be concluded that most follicles became regressed and atresia (Wood et al, 2001). This situation has caused a lot of research on morphology and morphometry on ovarian follicles are conducted (Ariyaratna et al, 1997).

Today, medicinal herbs are important and valuable in providing the communities health both in terms of treatment and prevention. We also have this trend to medicinal herbs in Iran which is one of the seventh Asian countries with the most medicinal herbs (Madrasi and Tavanaei, 2013).

*Linum usitatissimum* characteristics: Linen with the scientific name of *Linum usitatissimum* and the English name of flax (flax fiber) and linseed (linseed oil), is an annual plant of the family linen (Tadesse et al, 2009). Growth period of the linen in suitable areas and spring planting is 90 to 150 days and reaches to 250 days in autumn planting. In general, linen is a long-day plant and flowering in this plant is 10 to 15 days and completed when the day length is 14 to 18 hours (Omidbeigi et al, 2001; Bernath 1993; Khajepour 2000). In the recent years, the useful effect of linen oil on human health has been considered (Green, 2000). Today, particular attention is dragged to the use of omega3 oil in the world. This material is found in some plant species like linseed as well as aquatics. Omega 3 does not have the harmful effects of animals' fats (Thompson et al, 1996) and causes a decrease in low

density lipoprotein (LDL) and blood triglycerides (Paschos et al, 2007). Reduction of cancers and prevention of breast cancer are the other useful effects (Denis et al, 1999; Chen et al, 2006; Silva 2005). Finally, the use of flaxseed (linseed oil) produced from the seeds is a valuable drug and using linseed inhuman nutrition can be considered as a nutrient (Wang et al, 2007).

According to the conducted studies regarding the effect of some medicinal herbs on the fertility rate, no more research on linseed has been done. The purpose of this research is to evaluate the effects of the extract of this plant on the fertility rate in female mice.

**Method:**

**The present research is an experimental study which was conducted in the Faculty of the Basic Sciences, Azad Islamic University, Kazerun branch in 2013.**

**-The animals under experiment and their maintaining condition**

A total of 30 adult female mice weighing approximately 30-35 g were prepared from the Laboratory Animal Production Center of Pasteur Institute of Iran. Mice were maintained under the new condition three days before the start of experiment to be acclimated to the new environment. The ambient temperature during the test was 20-25ºC. The animals were maintained in the time cycle of 12 hours of light and 12 hours of darkness. All mice accessed to the urban drinking water and unlimited food. The protocol of this study was based on the international laws in laboratory animals and all are approved in the professional ethics committee of the University.

**Grouping of the tested animals**

A total of 30 adult female mice in this research were divided into three main ten-individual groups including control, experimental 1 and experimental 2. Details of grouping and drug administration are as follows. Control group: they received no drugs. Experimental 1 group: 150 mg/kgbw of linseed hydro alcoholic extract per day and the experimental 2 group received 300 mg/kgbw of linseed hydro alcoholic extract per day.

To prepare the linseed extract, percolation method was used. In this method, after making 40 g of linseed powder, it is poured to the percolation and 350 ml of Alcohol 96% was added and incubated for 72 hours in the room temperature. Then, open the tap of the device to have the extract passed through the separating funnel drop by drop and extracted. During this process, the solvent alcohol was added drop by drop until the solution containing extract became colorless. Then, the obtained extract was placed in bain

marie at 50ºC to have the alcohol vaporized and finally it was completely dried by Rotary operator.

Linseed hydro alcoholic extract with doses of 150 and 300 mg/kgbw were fed to mice of the experimental groups 1 and 2 respectively, between the hours of 8-9 am every day. It was while mice in the control group received no treatment. At the end of day 30, mice were anesthetized using a cotton soaked to Ether in the anesthesia jar and after anesthesia, complete blood sampling was done from the left ventricle. The taken blood was poured slowly into the test tubes and kept in the laboratory temperature until the clot formation. Then, the clot was removed by a swab and centrifuged at 3000 rpm for 20 minutes to separate the serum. The obtained sera were stored at -20ºC to the time of examination. Along with blood sampling, ovarian tissue was removed out of the body to use to evaluate the sex cells counts. Finally, the results were analyzed by t-test and one-way ANOVA ($P \leq 0.05$) and SPSS software version 17.

**RESULTS**

**The results related to the linseed hydro alcoholic extract on the testosterone amount in adult female mice:**

The results of one-way ANOVA associated with Tukey follow-up test show that it is not observed a significant difference ($P<0.05$) between the average amounts of testosterone in experimental groups compared to the control group.

**The results related to linseed hydro alcoholic extract on testosterone amount in adult female mice:**

As it is observed in table 2, a significant difference between the experimental groups 1 and 2 and the control group ($P<0.05$),so that testosterone amount has a dose-dependent increase in the experimental groups 1 and 2 compared to the control group.

**The results related to the effect of linseed hydro alcoholic extract on estrogen amount in adult female mice:**

In table 3, X±SEM of estrogen amount in adult female mice in different groups has been provided. The results of one-way ANOVA associated with Tukey follow up test show that a significant difference ($P<0.05$) is observed between the average amounts of estrogen hormone in the experimental groups 1 and 2 compared to the control group. So that, estrogen amount has had a dose-dependent increase in the experimental groups 1 and 2 compared to the control group. On the other hand, using the linseed hydro alcoholic extract causes an increase in the amount of estrogen hormone in the experimental groups 1 & 2.

**Results related to the effect of linseed hydro alcoholic extract on the primary follicles counts in adult female mice:**

In table 4, X±SEM of the primary follicles of ovary in adult female mice in different groups has been provided. The results of one-way ANOVA associated with Tukey follow-up test show that a significant difference (P<0.05) between the primary follicles counts of ovary is seen in the experimental groups compared to the control group. So that the primary follicles counts of the ovary have had a dose-dependent increase in the experimental groups compared to the control group. On the other hand, the use of hydro alcoholic extract of Linum usitatissimum has caused an increase in the primary follicles counts of the ovary in the mice of experimental groups.

**Results related to the effect of linseed hydro alcoholic extract on the secondary follicles counts in adult female mice:**

The results of this research show that a significant difference (P<0.05) is observed between the secondary follicles in experimental groups compared to the control group, so that the secondary follicles counts of the ovary have had a dose-dependent increase in the experimental groups compared to the control group. On the other hand, using this has caused an increase in the secondary follicles counts of the ovary in the mice of experimental groups.

**Results related to the effect of linseed hydro alcoholic extract on the antral follicles counts in adult female mice:**

In table 6, X±SEM of the antral follicles of ovary in adult female mice in different groups has been provided. The results of this research show that a significant difference (P<0.05) is observed between the antral follicles in experimental groups compared to the control group, so that the antral follicles counts have had a dose-dependent increase in the experimental groups compared to the control group. On the other hand, using the linseed hydro alcoholic extract has caused an increase in the antral follicles counts in the mice of experimental groups.

**Table 1- X±SEM of testosterone amount in adult female mice in different groups**

| Different groups | X±SEM (ng/ml) |
|---|---|
| Control (without treatment) | 0.18±0.01 |
| Experimental 1 (linseed extract 150 mg/kgbw) | 0.19±0.01 |
| Experimental 2 (linseed extract 300 mg/kgbw) | 0.20±0.02 |

**Table 2- X±SEM of progesterone amount in adult female mice in different groups**

| Different groups | X±SEM (ng/ml) |
|---|---|
| Control (without treatment) | 5.17±0.32 |
| Experimental 1 (linseed extract 150 mg/kgbw) | 8.84±0.40* |
| Experimental 2 (linseed extract 300 mg/kgbw) | 9.56±0.56* |

***significant at the level of P<0.05 with the control group**

**Table 3- X±SEM of estrogen amount in adult female mice in different groups**

| Different groups | X±SEM (ng/ml) |
|---|---|
| Control (without treatment) | 21.66±1.33 |
| Experimental 1 (linseed extract 150 mg/kgbw) | 29.49±2.03* |
| Experimental 2 (linseed extract 300 mg/kgbw) | 31.82±1.45* |

***significant at the level of P<0.05 with the control group**

**Table 4- X±SEM of primary follicles counts of adult female mice in different groups**

| Different groups | X±SEM (ng/ml) |
|---|---|
| Control (without treatment) | 9.44±0.13 |
| Experimental 1 (linseed extract 150 mg/kgBW) | 12.32±0.15* |
| Experimental 2 (linseed extract 300 mg/kgBW) | 12.80±0.37* |

***significant at the level of P<0.05 with the control group**

**Table 5- X±SEM of secondary follicles counts of adult female mice in different groups**

| Different groups | X±SEM (ng/ml) |
|---|---|
| Control (without treatment) | 3.28±0.03 |
| Experimental 1 (linseed extract 150 mg/kgBW) | 4.75±0.04* |
| Experimental 2 (linseed extract 300 mg/kgBW) | 5.46±0.05* |

***significant at the level of P<0.05 with the control group**

**Table 6- X±SEM of antral follicles counts in adult female mice in different groups**

| Different groups | X±SEM (ng/ml) |
|---|---|
| Control (without treatment) | 2.26±0.04 |
| Experimental 1 (linseed extract 150 mg/kgBW) | 2.96±0.08* |
| Experimental 2 (linseed extract 300 mg/kgBW) | 3.11±0.08* |

***significant at the level of P<0.05 with the control group**

## DISCUSSION

### Discussion and conclusion regarding the effect of using linseed hydro alcoholic extract on the sex hormones in adult female mice

The results of this research showed that linseed hydro alcoholic extract causes a significant increase in sex hormones and graph and antral follicles counts in the adult female mice. The obtained results indicated that the amounts of estrogen and progesterone hormones in the experimental 1 and 2 groups has had a dose-dependent increase compared to the control group. It was while using this extract had no effect on the amount of testosterone hormone in the female mice in experimental group. Since, the use of linseed hydro alcoholic extract has caused an increase in the progesterone and estrogen levels, improvement of ovarian function in mice of experimental groups are justified. It can be due to the flavonoids and steroidal compounds found in linseed that stimulates the secretion of endrogens and LH and thereby increasing the amount of progesterone hormone. In addition, this extract prevents the degradation of this hormone and thus reduced estrogen amount

by inhibiting the cytochrome P450 gene which is involved in the metabolism of estrogen and this action caused a significant increase in this hormone amount in the studied mice. The results of this study are in consistent with the study results of Hosseini et al, Mokhtar et al, Pour Ahmadi et al and Divisty et al.

Phytoestrogens are the natural compounds derived from the plants which have a similar structure to the estrogen in terms of function. Phytoestrogens have estrogenic and anti-estrogenic effects and impress the brain-pituitary-gonad axis and the environmental genital organ (Zhao et al). The number of follicles were also increasing in this study that it can be due to the increased LH hormone. Increased LH caused a decrease in inhibin hormone and so makes the secretion of FSH hormone, possible and therefore stimulated folliculogenesis and increased ovarian follicles in the estrous phase.

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