

## Effect of Garlic Hydro-Alcoholic Extract on Pituitary-Gonad Axis in Female Mice

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**Abstract:** Garlic (*Allium sativum*) is belonging to Alleacea family. This plant has anti microbial, anti cancer properties because of its organosulfuric compounds. In this research, effect of hydro-alcoholic extract of garlic was studied on reproduction system of female mice (Balb/C). At first, samples were divided in 5 groups with 10 members: 1 group as control, 3 groups received treatments and 1 group received placebo. Samples were kept in same places. For synchronizing estrous cycles of rats, cloprostenol and progesterone were injected in their peritoneum with a 3 days interval. Hydro-alcoholic extract of garlic was prepared in 3 doses (50,100 and 200 mg kg<sup>-1</sup>) and was injected in peritoneum for 20 days. Control group was not injected and placebo group was injected using physiological serum. After 10 injections, samples bloods were analyzed for FSH, LH, estrogen and progesterone using RIA method. Results showed significant reduction in FSH and LH levels and significant increase in estrogen and progesterone levels. According to results of this study, hydro-alcoholic extract of garlic has a negative effect on female reproduction system in all various doses (50,100 and 200 mg kg<sup>-1</sup>).

**Key words:** Garlic, reproductive system, female mice, peritoneum, blood samples

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### INTRODUCTION

Traditional medicine has a long time history. This division of medicine is a complex of knowledge, experiences and functions according to theories, believes, indigenous experiences and various cultures which is used in presentation, diagnosis and treatment of physical or mental disease. Complementary, replacement or non-official medicine phrases have been used in some countries in place of traditional medicine (Ardakani and Farjadmand, 2005). Traditional medicine includes using medicinal plants, parts of animal bodies and minerals. Medicinal plant is a plant with definite effective matter which is used in prevention or treatment diseases and is known by one of international valid Pharmacopoeia (Modaresi and Gholchobian, 2012). Effective matters are the same components of medicinal plants with therapy effects. Those pharmaceutical plants which their effective matters are known must be standardized with appropriate analysis methods to have definite amount of effective matters (Modaresi, 2012). If it is not possible to recognize effective matters whole plant can be considered as effective matter.

Among medicinal plants, garlic has many medicinal effects on various organs of body and many researchers have been conducted on it. It has a lot of antioxidant and

anti cancer properties but there are not many researches about its effect on reproduction system then the effect of garlic on reproduction system was the main goal of the study. Allium is a big genus with many biennial and perennial species. Many of these plants are aromatic and some are not (Ghahraman, 2009; Hosseini and Emami, 2007). Garlic (*Allium sativum*) is from Tulip family. It is called saum in Arabic, theriquedes pourres allicaulitre in French, knoblauch in Spanish and Aglio in Italic. Garlic bulbs have allicin, oleanolic acid, adenosine, ferulic acid, endolysin linoleic acid and phytoestrogen (Ayduke, 2007). Allicin is the strongest compound of garlic which has the most effect on healthiness. Plus antibiotic specifics, it is an organic anti fungi. One of the most important compounds of garlic which amplifies anti oxidant effect of garlic is allicin, the natural compound of garlic (Banerjee and Maulik, 2002).

Today, the public interest in the use of herbs and the aim of this study is the identification of the garlic effect on pituitary-gonad axis in female mice and identification of the most effective dose for the probable changes.

### MATERIALS AND METHODS

Female mice (Balb/C) were prepared from animal division of Isfahan University in range of 25-40 g. Samples

were divided to 5 groups (with 10 rats in each group) including one control group, 3 treatment groups and placebo group and were kept in separate cages. All samples were kept in natural radiation, water and food at 25-30°C for 2 weeks to environment adaption. This situation was continued in injection period too. Garlic's extract was injected in peritoneum in four levels: group 1: 50 mg kg<sup>-1</sup> of body weight, group 2: 100 mg kg<sup>-1</sup> of body weight and group 3: 200 mg kg<sup>-1</sup> of body weight. Placebo group received only 9% salt solution and control group did not receive any injection. All injections were done for 20 days, every other day from 8 a.m. to 1 p.m.

The estrous cycle of all female rats must be synchronized for evaluating drug effect. To this, half micro gram of cloprostenol was injected in peritoneum at first. The 3 days after, three microgram of progesterone was injected under the skin of all samples and then they became synchronized. The 1 day after progesterone injection, extract injection was started. After the last injection, blood samples were prepared.

## RESULTS AND DISCUSSION

Comparing FSH hormone (mLU/mL) of blood serum using Duncan test ( $p < 0.05$ ) showed significant reduction in experimental groups, in proportion to control group. Figure 1 shows these differences.

Studying the LH amount (mLU/mL) in blood serum of experimental and control rats and comparing them at 5% level using Duncan test, showed significant reduction in experimental groups in proportion to control group. The results are shown in Fig. 2.

Studying the estrogen amount in blood serum of experimental and control groups and comparing them at 5% probability level, showed significant increase in experimental groups in proportion to control group. These results are shown in Fig. 3.

Studying the amount of progesterone hormone (mg/mL) in blood serum of experimental and control groups and comparing them at 5% probability level, using Duncan test showed significant increase in group 1 (50 mg kg<sup>-1</sup>), group 2 (100 mg kg<sup>-1</sup>) and group 3 (200 mg kg<sup>-1</sup>) in proportion to control group. These results are presented in Fig. 4.

Garlic extract did not destroy tissues in this study. This is in agreement with Omotoso *et al.* (2010)'s study which testis tissue of rats was not destroyed by using garlic aquatic extract.

FSH reduction increases the level of IGFBP 4.5 (which is an ovarian growth factor) and protease function will be prevented then FSH antagonists will be increased and

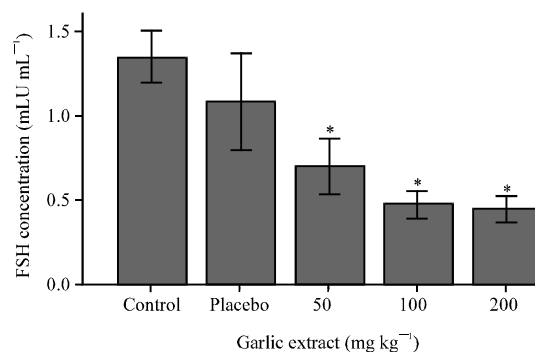


Fig. 1: FSH amount in control, placebo and experimental groups

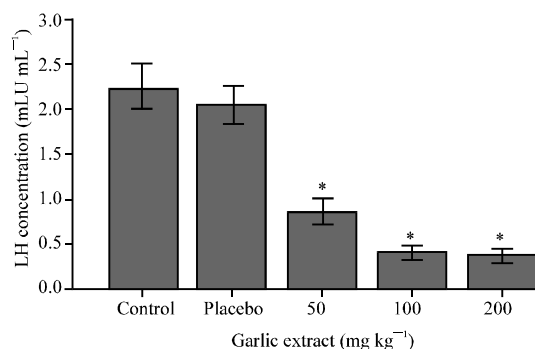


Fig. 2: LH amount of experimental, placebo and control groups

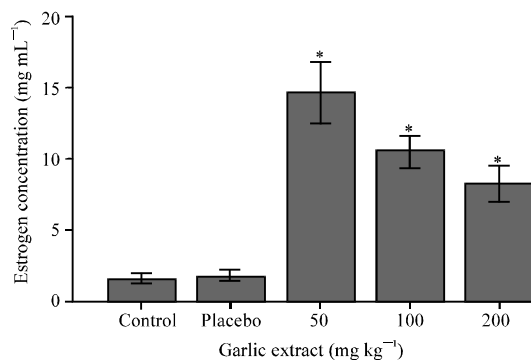


Fig. 3: Estrogen amount of experimental, control and placebo groups

follicles will be involved with atresia (Sun and Ku, 2006). Then, FSH level inside follicle plays an important role in selection phenomenon or atresia. Also, GnRH stimulates production of IGFBP 4.5 in granulosa cells in follicle and meanwhile reduces IGFBP proteases then causes follicle atresia. So, GnRH can control granulosa cells and cause pyknosis appearance. Also some other factors are effective in apoptosis process of granulosa cells (EGF, IGF and BGTF) then researchers can say

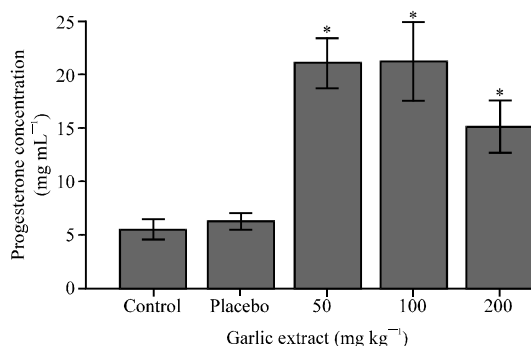


Fig. 4: Progesterone amount of control, placebo and experimental groups

that regulating follicles atresia is affected by growth factors and some other factors like FSH and steroids (Shafieiha, 2003).

The amount of LH hormone was reduced significantly in all three experimental groups. LH is regulating by negative feedback mechanism and controlling secreting sexual hormones via pituitary-gonad system. Low amounts of estrogen controls LH production highly. Also when progesterone is extant, control effect of estrogen will be increased several times. This will reduce secreting LH from front pituitary and LH will be reduced (Zamiri, 2006). Results show that FSH was reduced in all 3 groups. Increase in estrogen secreting from dominant follicle at half follicular phase reduces FSH secreting via negative feedback. Also, high progesterone is one of the other reasons of FSH reduction which prevents FSH secreting and prevents new ovulation. Estrogen increasing was occurred more in group 1 (dose 50 mg kg<sup>-1</sup>) which has been because of more graffian follicles in this dose. Granulosa cells of follicle are estrogen producers in ovary (Zamiri, 2006).

At first, many small antral follicles start to growth and make stradiols. Some of these follicles will be involved with atresia. Dominant follicle will be selected from the rest of follicles. The highest amount of estrogen is being secret in this stage and garlic extract will increase the time of pro stress cycle and because this phase is together with estrogen hormone secreting, researchers can say that this is the reason of increase in estrogen.

### CONCLUSION

Considering the obtained results it seem that garlic reduced graffian follicle of every three doses and stopped

folliculogenesis via affecting ovary and increase in corpus leuteum is because of previous yellow bodied. Folliculogenesis has been stopped after injecting garlic extract and then increase in corpus luteum has been stopped consequently. Also, this extract causes estrogen secreting which cause itself negative feedback on pituitary-gonad system and reduced FSH and LH. Considering obtained results, garlic plant affects secondary sexual characteristics and increases them but it has anti fertility effect because of folliculogenesis reduction. According to results, 50 mg kg<sup>-1</sup> can be the most effective dose.

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