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EVALUATING THE EFFECT OF CHAMOMILE EXTRACT IN COMPARISON WITH AMPICILLIN ON REPRODUCTIVE HORMONES IN MALE MICE TREATED WITH *E. COLI* LIPOPOLYSACCHARIDE

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Abstract:

The objective of this study was to evaluate the effects of chamomile extract in comparison with ampicillin on parameters of reproductive system in male mice treated with E. coli LPS. In this study, 65 adult male mice were divided into 6 groups. Control, LPS, LPS+ampicillin, LPS+chamomile extract 50 mg/kg, LPS+chamomile extract 100 mg/kg and LPS+chamomile extract 200 mg/kg. IP injection were performed for 20 days and the fertility of each group was evaluated. The results of hormonal tests of each group were compared with the control group. The results showed no significant difference between the mean FSH level of the treatment groups and that of control group ($p<0.05$). The mean LH level of LPS group decreased significantly compared to that of control group ($P\leq0.05$). The mean level of testosterone in chamomile extract group 200 mg/kg and ampicillin group was significantly higher than that in control group ($p\leq0.05$). Given these results, while LPS can reduce male fertility potential, treatment of samples with chamomile extract in all three doses of 50, 100 and 200 can reduce the effects of LPS and increase the fertility potential.

Keywords: *Chamomile, ampicillin, lipopolysaccharide, reproductive system, mice*

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INTRODUCTION:

Based on the reports, out of all four couples, one couple is infertile, which about 55% of these infertilities are related to the male gender, and the rest of them are related to the female gender for unknown reasons [1]. One of the main causes of male infertility is semen infections and reproductive organs. A wide range of bacteria in varying degrees are involved in male infertility [2, 3]. LPS lipopolysaccharide in gram-negative bacteria, as a biologically active part damages endotoxin, is one of the major building blocks of host immune system. It is located in the outer membrane of the cell wall, leading to infection [4]. Infection indirectly causes abnormal reduction in number of spermatozoids in semen, reduced motility and changes in sperm morphology, leading to reduced fertility.

Treatment of bacterial diseases in this group is primarily an antibiotic treatment, which is appropriate for pathogenic agent. While infectious diseases and their treatment have always been considered, and in this regard throughout human history and many attempts have made to eradicate the factors involved in development of these diseases, changing the behavior of microorganisms has caused that these microbial agents are not eradicated completely and successfully, and the range of these diseases in new forms has increased with emergence and prevalence of new strains [4].

Plants of the chicory family Astraceae-Compositae are herbaceous plants with diverse leaves and forms that are rarely found in the form of shrubs. One of the most valuable species of this family is chamomile with scientific name of *Matricaria chamomilla.L* and the English name of *German camomolla* [5]. It is an annual and short plant with durable aromatic odor [6]. Given the scientific studies conducted, chamomile extract is composed of 120 different chemical compounds [7]. One of the most important compounds of this plant include flavonoid, Chamazulene, alpha-bisabolol , farnesene, as well as its Cis and trans-isomers, are di-cyclotour and terpeneides of alpha-bisabolol and its oxides. In addition, it contains pyrazolone, luteolin and coumarin derivatives. Its flavonoids primarily belong to flavonoids and flannels classes, and they are found in either free or glycoside forms, and florets of this plant contain rutin, apigenin, and also free quercetin [8]. In addition, 11 bioactive compounds such as herniarin, umbelliferone (coumarin), chlorogenic acid and caffeic acid, phenylpropanoid, apigenin, epigenin-7-O-glucoside, luteolin and luteolin-7-O-glucoside (flavones), quercetin and rutin (flavonol) and naringenin (flavono) are found in chamomile extract. German chamomile is the natural source of essential oil. Chamomile flowers and branches are main organs in producing essential oil. The main components of essential oil extracts from the flowers include Farnesene -β- (E)(8.1-4.9%), terpine alcohol (farnesol), Chamazulene (2.3-10.9%), α-bisabolol (4.8% - 11.3%), and α-bisabolol oxide A (28.7-25.5%) and α-bisabolol oxide B (12.2-30.9%), with anti-inflammatory, antiseptic, antiphlogistic and spasmolytic and antifungal properties. It has been reported that, among the various compounds of chamomile, α-bisabolol (antimicrobial and antiseptic properties) and Chamazulene (antiseptic) are more beneficial than other compounds [6].

The compounds contained in the chamomile extract have anti-inflammatory, anti-bacterial and antioxidant activity [9], which its anti-inflammatory and anti-allergic properties are due to having α-bisabolol and Chamazulene [10]. Studies conducted on chamomile chemical compounds have shown that this plant contains high levels of antioxidant [11]. The chamomile is rich in flavonoids, which are effective antioxidants to neutralize oxygenated radicals [12]. Free oxygen species can peroxidase sperm membrane lipids, which these effects is associated with reduced motility and damage to sperm membrane portions. Antioxidants are compounds, which prevent the formation of free radicals and peroxidation of lipids, protect the sperm cell damage by free radicals and improve the quality of sperm and fertility parameters [13].

Thus, chamomile plant was selected due to its anti-inflammatory, anti-bacterial, antifungal and antioxidant properties, wide use of this herbal plant in the treatment of many diseases and the lack of evaluation of the effect of this extract on *E. coli* lipopolysaccharide in In-Vivo conditions so that its effects, in comparison to ampicillin, on hormonal in male mice treated with *E. coli* lipopolysaccharide to be examined in order to show if a given dose of chamomile extract can be an appropriate alternative to ampicillin antibiotic for bacterial infections.

MATERIALS AND METHODS:

This experimental study was conducted in June 2016 at Postgraduate Education Center of Payam-e Noor

University of Esfahan, Iran.

- ***Experimental animals***

Sixty-five adult male Balb/C mice weighing 30±5 gr along with their dietary requirements were obtained from Isfahan Royan Biotechnology Research Center.

- ***Conditions to keep the samples***

In this study, male and female mice in separate cages were randomly divided into groups (each contained 10 mice). The condition of keeping the mice was in the natural circadian cycle. While receiving adequate food and water, the temperature of the environment was controlled from 26 to 28 degrees Celsius. Two weeks were considered necessary to adapt the animal to the environment and humans. All points of guidelines to handle the animals were observed in this study.

- ***Grouping experimental samples***

The classification of groups is as follows:
1) Control group
2) LPS *E.coli* group
3) LPS + ampicillin group
4) LPS + chamomile extract with dose of 50 mg/kg
5) LPS + Chamomile extract with dose of 100 mg/kg
6) LPS + Chamomile extract with dose of 200 mg/kg

Grouping the male mice at the end of experiment for mating and fertility. One week before the end of the 30-day injection period (to adapt the male mice to the new environment, where they are supposed to mate), four male mice were selected from each of the 6 experimental groups randomly (a total of 24 male mice) and they placed individually in 24 separate cages. It should be noted that the ambient temperature, access to water and food, and especially their injections, were performed based on previous procedure.

- ***Preparation of ampicillin antibiotic***

Ten vials of ampicillin (ampixil; 1 mg) were prepared. The vials were kept in a dry and cold refrigerated standard temperature of laboratory.

- ***Preparation of bacterial Lipopolysaccharide (LPS E.coli)***

The commercial lipopolysaccharide *E.coli* 10 mg was prepared from Sigma Code Company (L2880) by Yekta Gostar Vision Company. Nine mg of LPS lyophilized vial were measured in sterile conditions by digital scales. Then, 150 cc of the injectable serum was added and regenerated. Then, the obtained lipopolysaccharide solution was kept at a temperature of 2-8 $^{\circ C}$ in the laboratory refrigerator, to be used in the next steps of the experiment.

- ***The method of injections***

Two weeks before implementing the main steps of the experiment (during a period specified for male mice adaptation to the environment) on the 5 male mice, considered for LPS test, intravenous injection of 1 mg/kg LPS was performed in two replicates with interval of five days to ensure the survival of mice in this dose.

- ***Main steps of injections***

The duration of the experiment lasted 30 days, which is as follows:

1- The control group received only daily water and food.
2- Intravenous injection of 1 mg/kg LPS every 5 days in 5 treatment groups LPS, ampicillin, chamomile with dose of 50, 100 and 200.
3- Intravenous injection of the hydro alcoholic extracts of chamomile at dose of 50 mg/kg, 100 mg/kg, and 200 mg/kg to three groups of chamomile every two days.
4- Daily intravenous injection of ampicillin at dose of 100 mg/kg to the ampicillin treatment group

- ***Blood sampling and explaining***

Twenty-four hours after the last injections, blood sampling was taken from 6 mice in each group. Then, the testis tissue of each group without tissue trauma was removed from the body and placed in glass containers containing 10% formalin for fixation of the samples to be used for the preparation of tissue slides.

- ***Hormone measurement experiments***

After blood sampling, blood serum of mice was isolated by centrifugal machine and prepared for hormonal testing. The Levels of FSH, LH, Testosterone hormones in this experiment were determined by using enzyme immunoassays (EIA) method and ELISA reader device.

Statistical analysis

In this study, to compare the mean of the data obtained from the experiment result in order to examine the existence or absence of significant differences between the groups, one-way ANOVA analysis, Tukey (HSD) test, and SPSS software were used. $p<0.05$ was considered as a significance level. The Minimum Standards of Reporting Checklist

contains details of the experimental design, and statistics, and resources used in this study.

RESULTS AND DISCUSSION:

- ***Examining the FSH hormone level***

Comparing the mean FSH hormone level showed no significant difference in the serum blood of mice in 5 treatment groups ($p < 0.05$).

- ***Examining the LH hormone level***

Comparing the mean level of LH in LPS treatment group with mean level of control group showed significant difference ($p \leq 0.05$). Other treatment groups of ampicillin and chamomile in three doses of 50, 100 and 200 mg/kg did not show a significant difference with control group ($p > 0.05$). (Figure 1).

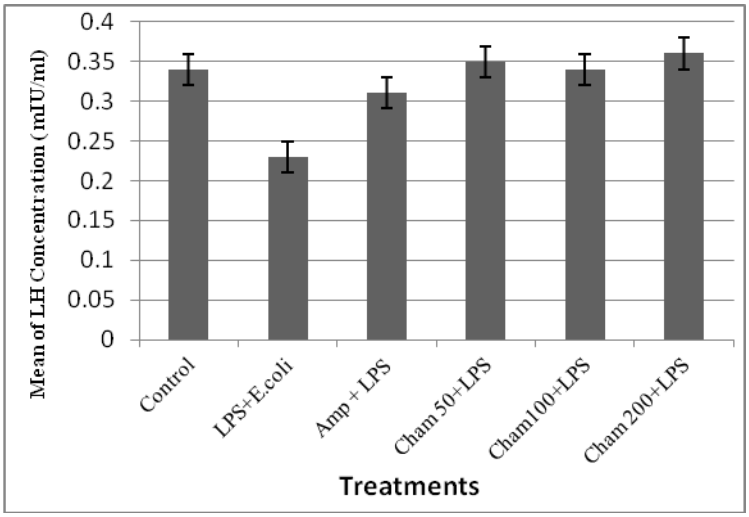

Figure 1: Changes in LH concentration in control and treatment samples

- ***Examining the Testosterone hormone level***

The comparison of the mean testosterone level in the chamomile 200 mg/kg extract group and the ampicillin treatment with that of control group showed a significant increase ($p \leq 0.05$). The mean testosterone level in the LPS group decreased compared to the mean level in the control group, but this reduction was not significant ($p < 0.05$), and the mean testosterone hormone level in chamomile 50 and 100 mg/kg treatment group did not show significant difference with that of control group ($p < 0.05$) (Figure 2).

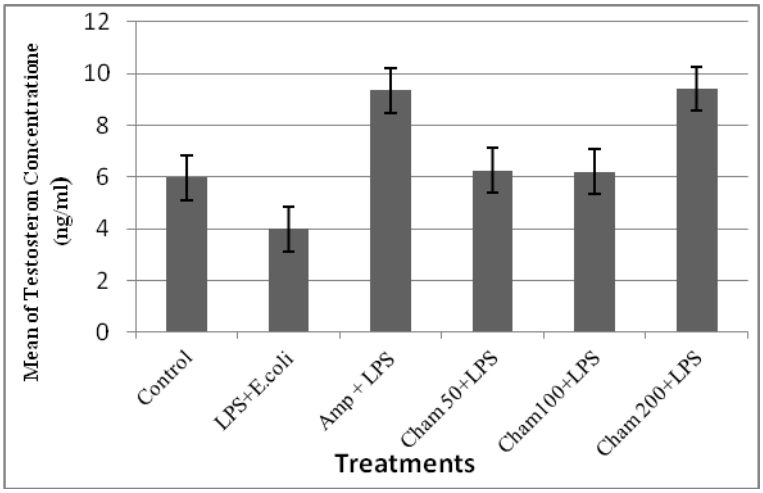

Figure 2: Changes in Testosterone concentration in control and treatment samples

The results of this study suggest that FSH levels in the 5 treatment groups did not show any significant difference compared to control group. It could be stated that the infection caused by LPS 1 mg/kg LPS did not affect the level of FSH, and the ampicillin antibiotic property was not induced due to non-involving the microbe and the infection caused by it with level of the FSH hormone, so this hormonal parameter did not change in other groups. The FSH hormone is secreted from the anterior pituitary and directly affects sertoli cells and plays an important role at the beginning of the sperm production process [14]. Investigations suggest that FSH secretion is independent of GnRH [15], and probably factor other than the LH regulator factor is involved in the release of FSH. Moreover, results of other studies suggest that many mutations occur in LH receptors in Leydig cells, but these mutations rarely occur in FSH receptors related to sertoli cells [16].

Thus, it can be considered as one of the reasons to justify why the infection has not been able to change the level of the hormone, while further studies are finding more reasons are required in this regard. The mean LH level in the LPS treatment group was significantly lower than that in the control group. Other treatment groups, including ampicillin and chamomile groups in three doses of 50, 100 and 200 mg/kg did not show a significant difference with control group. In this regard, it can be stated that infection caused by LPS 1 mg/kg led to reduced level of LH hormone in treatment group of LPS by leaving an effect on GnRH hypothalamic-pituitary axis. However, in the ampicillin treatment group, the level of LH increased compared to the treatment group of LPS due to inducing the ampicillin antibiotic effect. In other words, the reduction was compensated, which lack of significant differences in the level of LH in this group compared to the control group is an evidence for this claim. The mean of LH level in the treatment group of chamomile extract with doses of 50-100-200 mg/kg, increased compared to the LPS group, due to treatment of this group with chamomile extract and inducing its antimicrobial activity. In other words, the reduction was compensated, which lack of significant difference in the level of LH in this group compared with the control group is an evidence for this claim. The mean of testosterone level showed a significant increase in the treatment group of chamomile extract with dose of 200 mg/kg and ampicillin treatment group compared to that of control group. The mean of testosterone level in the LPS group decreased compared to that in the control group, but this reduction was not significant.

There was no significant difference between the mean testosterone level in the chamomile treatment groups (with doses of 50 and 100 mg/kg) and that of control group. It can be stated that the infection caused by LPS 1 mg/kg led into a significant reduction in the level of LH in the LPS group and naturally the results of this reduction affect the level of testosterone in this group and led to reduced level of the hormone, while this reduction was not significant. In the ampicillin treatment group, due to the induction of antibiotic effect of ampicillin, not only the reduction in testosterone level was compensated, but also its level increased significantly compared to that in control group. The mean testosterone level in the treatment group of chamomile extract with doses of 50 and 100 mg/kg increased compared to treatment group LPS, due to treatment with chamomile extract and inducing antimicrobial activity. In other words, this reduction was compensated, which lack of significant difference between testosterone level in this group and that of control group is evidence for this claim. Increasing the mean testosterone level in chamomile extract treatment group with dose of 200 mg/kg compared to the control group, due to the treatment of this group with chamomile extract and the induction of antibacterial activity of chamomile extract, not only reduced level of testosterone was compensated, but also its level increased significantly compared to control group. In this study, in addition to the antimicrobial effects of chamomile extract, the level of testosterone hormone 200 mg/kg showed significant increase compared with the control group. As stated before, FSH secretion is independent of GnRH [15], and possibly a factor other than the LH regulator factor is involved in the release of FSH. Moreover, based on the results of other studies, there are many mutations in LH receptors in Leydig cells, but these mutations rarely occur in FSH receptors related to sertoli cells [16]. Therefore, it can be considered as one of the reasons to justify why chamomile has not been able to change the level of the hormone. As testosterone is an androgenic hormone produced in response to stimulation of LH secreted from pituitary gland by testis leydig cells, it is possible that the mechanism in which the testosterone levels increased after chamomile injection was due to the direct effect of this drug on luteotropic cells in the anterior part of pituitary and increased LH [17]. In addition, testosterone hormone

controls the LH secretion from the anterior pituitary through the negative feedback mechanism and chamomile may indirectly increase the secretion of the gonadotropin-stimulating hormones from the hypothalamus, and thus, increase secretion of LH from the anterior pituitary and increase the level of Testosterone hormone. However, that the negative feedback mechanism of testis pituitary might require more time, which more studies are required in this regard [18]. The research conducted by Karbalaei Dust et al in 2010 showed that chamomile hydro alcoholic extract in dose of 400 mg / kg reduced sperm count, motility and length of sperm tail and decreased serum testosterone levels and increased estradiol levels in adult male mice. The study conducted by Hatami (2012) showed that chamomile extract in dose of 100 mg/kg increased pituitary-testis axis activity and spermatogenesis in male rats [19]. The results of this study showed that chamomile extract had an androgenic property, increased the androgen-dependent parameters and caused polyspermy and weight gain of genital organs, which is consistent with results the present study [20].

The study conducted by Johari (2014) showed that chamomile extract in doses of 10, 20 and 40 mg/kg per body weight in the form of intravenous injection reduced testosterone secretion in male rats, which is not in line with result of the present study [21]. It should be noted that in the studies mentioned above, the effect of chamomile extract on sex hormones and their level change was studied in a condition where the level of hormones was normal and this extract was used to evaluate the decrease or increase in sex hormones, and the effect of chamomile extract on the change in level of male sex hormones was evaluated under conditions of infection and inflammation. Given the use of chamomile extract in this study and its different compounds, accurate determination of regulatory pathways as well as effective compounds on the increase of some hormones and its antibacterial activity in in-vivo conditions requires further studies. Therefore, based on the results of this study and the studies conducted on antibacterial and anti-oxidative and anti-inflammatory effects of chamomile, the extract of this plant can be used as an antibacterial agent in medical sciences, pharmacy and infertility with bacterial origin.

CONCLUSION:

Finally, it seems that the use of dose-dependent chamomile extract, as an anti-bacterial, anti-inflammatory and anti-oxidant agent, to be effective in improving infection compared to ampicillin antibiotic and in increasing male sex hormones. We hope that the results obtained from further studies to be useful in medical sciences, pharmacy and solving the infertility, especially antibiotic resistance created at current century.

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CONFLICT OF INTEREST

The authors contributing to the present study and to this very manuscript have no conflict of interests to declare.

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