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ORIGINAL ARTICLE

Evaluation the Effect of Ginseng Plant Root Extract on Complications of Azathioprine on Ovarian Follicles and TSH, LH and Progesterone Hormones in Rats

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ABSTRACT

Background: The search for herbal remedies has gained significant attention due to chemical drugs' potentially harmful side effects. Finding plants that can mitigate these adverse effects is crucial for enhancing the well-being of individuals undergoing chemical drug treatments.

Aim: Numerous studies have demonstrated the potent antioxidant properties of ginseng. Azathioprine, a widely used drug, has been shown to induce detrimental side effects on various body tissues. Thus, this study aimed to assess the efficacy of ginseng in reducing the harmful effects of azathioprine on ovarian tissue.

Materials and Methods: In this study, mice were divided into groups and injected with ginseng root extract and azathioprine. Ovarian weight and histological analysis were conducted to evaluate the number of ovarian follicles and corpus luteum. Furthermore, the levels of FSH, LH, and progesterone in the blood of the study groups were assessed using ELISA.

Results: In treatment group 4 (ginseng extract and azathioprine), compared to treatment group 2 (azathioprine only), a significant increase in the weight of both left and right ovaries was observed. Treatment group 4 also exhibited a notable increase ($P<0.05$) in the number of primordial, primary, and atretic follicles. The concentration of progesterone significantly increased in treatment group 4 compared to treatment group 2 ($p<0.01$).

Conclusion: The findings of this study indicate that azathioprine can have destructive effects on ovarian tissues, while ginseng extract demonstrates the potential to reduce these detrimental side effects. Furthermore, ginseng extract appears to positively regulate FSH and progesterone hormones.

Keywords: Ginseng, Azathioprine, Ovarian, Progesterone.

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Introduction

Ginseng is an aromatic medicinal plant that belongs to the Panax genus and the Araliaceae family.[1] This plant is widely used in the traditional medicine of Asian countries.[1] Ginseng has been used for centuries to relieve the aging effects, strengthen the immune system, regulate the body's metabolism, prevent headaches, overcome insomnia and improve sleep quality, reduce menopausal complications, mitigate sexual problems, relieve fatigue, protect the cardiovascular system, regulate blood pressure, blood sugar regulation, and combat depression, enhance male sexual ability, improve memory function, and reduce stress and fatigue.[2–4] Studies have shown that triterpene saponins, alkaloids, aminoglycosides, polysaccharides, peptides, fatty acids, terpenes, carbohydrates, vitamins (A, B6, and D), and Phenolic compounds are the most important biological components of ginseng.[5] Different researches have shown that the most important element of this plant is saponins (ginsenosides), which leads to the most pharmacological activities of this plant.[6]

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Azathioprine is a 6-mercaptopurine-derived drug with an imidazole ring attached to a sulfur atom usually absorbed completely and rapidly from the gastrointestinal tract but does not cross the blood-brain barrier.[7] The maximum serum concentration occurs 2 hours after ingestion.[7] About 2% of the drug is excreted in the urine without any kind of modification.[7] Azathioprine is rapidly metabolized in the body, and the thiopurine methyltransferase (TPMT) and xanthine oxidase (XO) enzymes are required for the metabolization.[8]

Azathioprine is a purine antagonist that inhibits the S phase of cell division and exerts cytotoxic and immunosuppressive effects by degrading nucleic acids and disrupting the production of DNA, RNA, and proteins.[9,10] Because lymphocytes rely mainly on nucleic acids production by De Novo purine synthesis and have no other system, azathioprine impressively affects lymphocytes.[10]

Azathioprine has been used since 1960. Azathioprine has been used to treat leukemia, rheumatoid arthritis, acute lymphoblastic disease, inflammatory bowel disease, and ulcerative colitis.[10,11] It is used to affect the body's immune system in diseases of immunological origin, including active chronic hepatitis, biliary cirrhosis, lupus erythematosus, systemic lupus erythematosus, and glomerulonephritis.[11] Also, using this drug with corticosteroids is the best choice to prevent transplant rejection.[11] Azathioprine reduces pain and inflammation in rheumatoid arthritis and reduces joint damage.[11]

In addition to its therapeutic properties, azathioprine also has harmful side effects. This drug can lead to toxicity in the bone marrow and digestive system.[12] In addition, 10% of people using this drug suffer from diarrhea, nausea, vomiting, and abdominal pain, and 5% of them suffer from skin sensitivities and photosensitivity.[13] In addition to reducing the number of white blood cells in the body and increasing the susceptibility to infections, it can reduce the number of platelets and increase the body's susceptibility to bleeding.[14]

The harmful effects of different drugs on ovarian tissue and oogenesis have been proven, and because of the importance of the reproductive system and the extensive use of azathioprine, this study aimed to investigate the effect of ginseng extract on azathioprine side effects on ovarian tissue. Also, the effect of ginseng extract on changes of TSH, LH, and progesterone levels in blood was investigated under treatment with azathioprine.

Material and methods

In this study, 60 healthy female Wistar rats were used; these rats were prepared from the animal house of Shiraz University of Medical Sciences. The animals were 75 days old and weighed 190 ± 15 g. The animals were divided into six groups of 10, and each group was kept in two cages. For injection of ginseng extract and azathioprine according to the weight of animals, the required amount in doses of ginseng extract (300 mg/kg) and azathioprine (50 mg/kg) was injected by insulin syringes every day at 10 am (intraperitoneally for four weeks). Before the experiment, a vaginal smear was prepared from all rats to ensure they were all in the same estrous cycle phase.

Animal grouping

Control group: no injection was performed in this group during the study.

Non-Treatment (NT) group: This group of mice received only distilled water. This study used distilled water as the solvent for drug and ginseng extract.

Treatment group 1: They received azathioprine at a dose of 50 mg/kg.

Treatment group 2: Members of this group received 300 mg/kg of Hydroalcoholic extract of ginseng root.

Treatment group 3: This group simultaneously received azathioprine at a dose of 50 mg/kg and hydroalcoholic extract of ginseng root at a dose of 300 mg/kg.

Treatment group 4: At first, this group received only hydroalcoholic extract of ginseng root at a dose of 300 mg/kg for 14 days, and then azathioprine at a dose of 50 and 300 mg/kg of hydroalcoholic extract of ginseng root injected simultaneously for 14 days.

In all studied groups, ovarian tissue was carefully evaluated in terms of vascular congestion and hyperemia, ovarian tissue cells vacuolation, ovarian follicles (Primordial, Primary, secondary, Graafian, and atresia follicles), corpus luteum, and germinal thickness of the epithelium.

Prepare ginseng extract

Based on the study of Do et al. [15], ginseng root extract was prepared. But in summary, we first dried the Ginseng root and ground it, then put ginseng powder in ethanol for 24 to 48 hours to soak it. After that, placed in the percolation funnel to pour from the percolation funnel into the porcelain container placed under it, then the ethanol evaporates from the porcelain container, and the powder sticks to the wall of the container. After separating, water and ethanol evaporated by creating a temperature gradient from 80 to 100 °C, and then the extract enters the pot, which the extraction color was yellow.

Dissection and removal of the ovaries

Blood sampling

For blood samples, we insert the tip of the 5 cc syringe into the right ventricle of the animal and gently pull the syringe

plunger back, slowly moving the blood into the syringe. The desired serum was separated by centrifugation at 4000 rpm for ten minutes and stored at -20°C.

Preparation of microscopic slides from tissue samples

The desired tissue pieces were prepared using the automatic tissue processor device by fixing, dehydrating, clearing, and embedding. Then the 5 micron thick sections were prepared by a rotating microtome and prepared slides colored by Hematoxylin-eosin stains.

Hormonal measurement

The concentrations of FSH, LH, and progesterone (LOT89001, LOT88005, and LOT87011, respectively) in the serum of animals were evaluated by the ELISA method (Pishtaz Teb Iran). All steps were performed according to the provided instructions by the producer.

Statistical analysis

The hormone concentrations and the number of primordial, primary, secondary, atresia, and Graafian follicles in different groups were analyzed by GraphPadPrism 8.0.1 software through one-way ANOVA and Duncan test. The values used were the mean ± mean, standard deviation error (SEM), and the significant level was $p < 0.05$.

Results

Changes related to ovarian weight in the study groups

Ovarian weight was measured in all study groups, and the results showed that the weight of right and left ovaries in experimental group 4 showed a significant increase ($p \leq 0.01$) compared to the control group (Table 1). Changes related to ovarian weight were not significant in other study groups (Table 1).

Changes in ovarian follicles

Ovarian follicles were carefully identified and counted by evaluating tissue sections prepared from the study groups (Figure 1A-D). The results showed that the number of Primordial follicles in study group 4 with study group 2 was significantly different ($p < 0.05$) (Figure 2A). The difference in the number of Primordial follicles in other study groups was not significant (Figure 2A). As in primordial follicles, the number of primary follicles in study group 4 showed a significant increase ($p < 0.05$) compared to group 2, and in other study groups, there was no significant difference (Figure 2B). The number of secondary follicles and graphene in all study groups did not show a significant difference. However, we saw a decrease in both types of follicles in study group 2 (Figures 2C and D). The most significant change in the number of follicles was related to atresia follicles; the number of this type of follicles in the study groups 1, 2, and without treatment showed a decrease compared to other groups, while the number of this type of follicles in the study group was rejected. 4 increased. The number of follicles in study group 4 was significant with study groups 1, 2, and without treatment ($p < 0.05$) (Figure 2E). In addition, the number of corpus luteum in all study groups did not show a significant difference (Figure 2F).

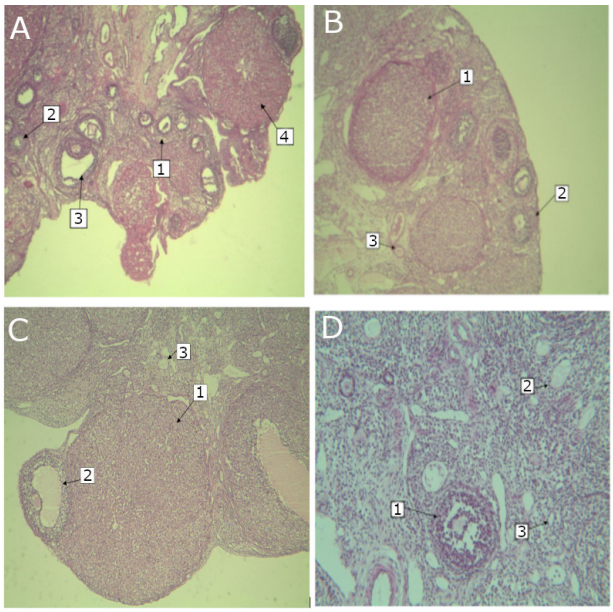

Figure 1: Pathological examination of ovarian tissues and ovarian follicles in the control group (A), Non treated group (B), Treatment group 1 (C), and Treatment group 4 (D). the points marked with the number 1 indicate the primary follicle, the number 2 indicates secondary follicles, and number 3 indicates Graafian follicles. Number 4 in A shows corpus luteum.

Table 1: Comparison of ovarian weight in each of the studied groups

	Control	*Non Treatment*	*Treat 1*	*Treat 2*	*Treat 3*	*Treat 4*
Right ovarian weight (g)	002/0 ± 034/0	001/0 ± 033/0	003/0 ± 043/0	002/0 ± 030/0	003/0 ± 038/0	003/0 ± 053/0
Left ovarian weight (g)	001/0 ± 032/0	004/0 ± 040/0	002/0 ± 039/0	001/0 ± 031/0	003/0 ± 034/0	003/0 ± 049/0

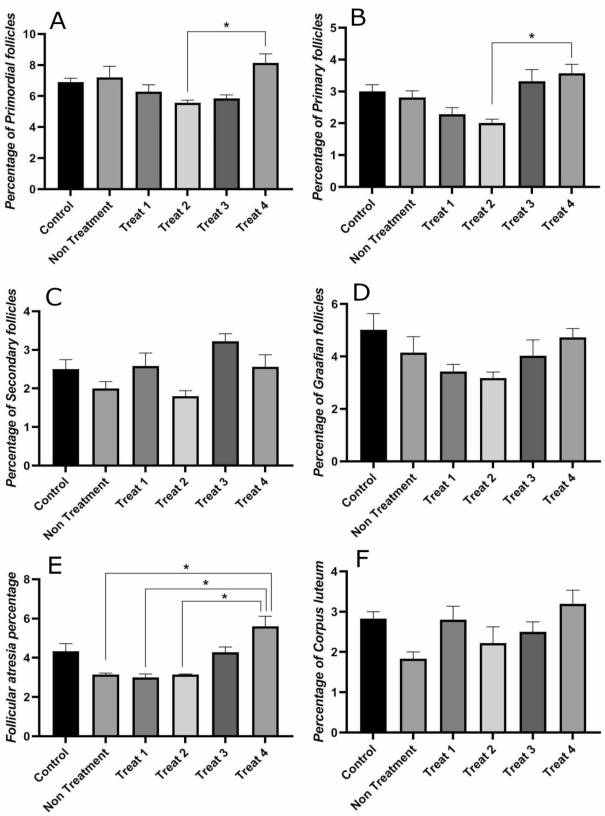

Figure 2: Changes related to the number of primordial follicles (A), primary follicles (B), secondary follicles (C), Graafian follicles (D), atresia follicles (E), and corpus luteum (F) in all groups of study. *; P<0.05, **; P≤0.01, ***; P≤0.001

Hormonal changes

The three hormones FSH, LH, and progesterone in the blood of all study groups were examined. Study group 1 showed an increase in FSH levels, and the hormone level of this group increased significantly with study group 2 (the only group in which hormone levels decreased compared to the control group) and the control group ($p \leq 0.01$). Figure 3A). LH hormone levels did not show a significant difference in all study groups (Figure 3B). However, like FSH, LH levels were higher in study group 1 than in other study groups (Figure 3B). Regarding progesterone, we saw a different pattern from the previous two hormones. Progesterone levels decreased in study groups 2 and 3 compared to other groups. In contrast, in study group 4, we had an increase in this hormone, and the difference between progesterone levels in study group 4 with both study groups 2 and 3 was significant ($p \leq 0.01$) was different (Figure 3C).

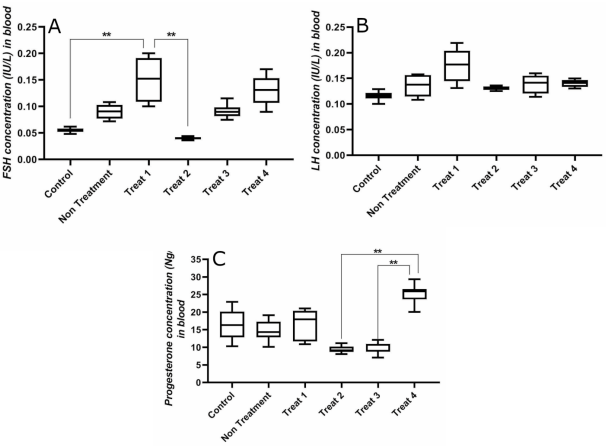

Figure 3: Concentrations of FSH; IU/L (A), LH; IU/L (B), and Progesterone; Ng/ml (C) in all groups of study. *; P<0.05, **; P≤0.01, ***; P≤0.001

Discussion

In addition to chemical drugs, herbal medicines are also used in treatment, and every day we see the publication of studies that evaluate the effects of plants on human health. Among chemical drugs, azathioprine is widely used to modulate the course of rheumatoid arthritis, which affects about 1% of the population.[16,17] Patients widely use azathioprine due to its easy access, appropriate therapeutic effects, reasonable price, and many other benefits.[11] Many studies have shown that ginseng can have very beneficial effects on human health.[1] Therefore, in this study, we examined the effect of ginseng and azathioprine on ovarian by examining the histology of the ovaries (assessing changes in the level of ovarian follicles) and assessing the FSH, LH, and Progesterone hormones in mice.

Azathioprine is an immunosuppressive drug.[10] This drug can cause toxicity to the bone marrow, gastrointestinal tract, and liver.[10,11] It has also caused an oxidative lesion in tissues.[11]

The use of ginseng in reducing the risks of chemotherapy drugs such as cyclophosphamide in male rats, testicular weight loss was observed in groups under chemotherapy drugs treatment while in groups that used ginseng, increased testicular weight Has been observed[18], which agrees with the present study results. The weight of right and left ovaries in experimental group 4 (treated mice by ginseng and azathioprine) had a significant increase compared to the control group, while in experimental group 2 (treated only by azathioprine), these changes were decreasing. These weight changes in the ovaries can also be related to reducing the number of ovarian follicles in most experimental groups.

In previous studies, azathioprine caused the production of free radicals in organs and tissues of the body, which is one of the most important causes of toxicity in organs.[19,20]

The female reproductive system, especially the ovaries, is susceptible to reactive oxygen species and free radicals and can lead to infertility if their levels exceed a certain level.[21,22] Studies have also shown that the use of azathioprine to measure the toxicity of this drug on rat ovarian follicles indicates that the drug does not significantly change the number of ovarian follicles.[23] Contrary to the present study, the cause can be related to the length of the trial period and the dose of the drug used in the previous study. A lower dose of azathioprine was used, so it may not have destructive effects. Research has also shown that ginseng has positive supportive and therapeutic effects against elliptical disorders caused by certain substances such as 8, 7, 3, 2-benzo-p-dioxin tetrachloride[24, 25], and Its antioxidant activity can eliminate superoxide disabilities and also inhibit lipid peroxidation in cell membranes by inhibiting the activity of hydroxyl radicals and anions.[25]

Therefore, considering that this plant has antioxidant compounds, the changes of the primordial and primary follicles of the monolayer, as well as the corpus luteum in experimental group 4, are quite logical, and the increase in atresia follicles in experimental group 2 compared to the control group is quite reasonable also. On the other hand, the increase in corpus luteum in experimental group 4 compared to experimental groups 2, 1, and control shows the beneficial effect of ginseng in reducing the harmful effects of azathioprine.

According to previous research, ginseng affects the anterior pituitary gland and causes the secretion of nitric oxide, which exerts this effect due to the presence of ginseng saponins.[26] Therefore, reducing corpus luteum in experimental group 1 compared to experimental group 4 is reasonable because the increase of nitric oxide reduces the corpus luteum. However, this is expected to happen in Experimental Group 2, but as mentioned, atropine produces oxidative stress, which has more destructive effects. In addition, nitric oxide stimulates TSH secretion[27]; the present study also shows that consumption of ginseng (followed by an increase in nitric oxide secretion), TSH levels increased. Like TSH, we should have seen an increase in LH, but the results did not show an increase that could be due to the effects of azathioprine.

The ovaries can secrete progesterone under the influence of the LH and TSH hormones[28]. Due to the fact that azathioprine has destructive effects on the ovaries, in study group 2, a decrease in progesterone levels was seen, and an increase in the level of this hormone in study group 4 indicates the protective effect of ginseng extract on the ovaries.

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Conflict of interest: We have no conflicts of interest to disclose.

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