World Journal of Zoology 8 (4): 425-431, 2013
ISSN 1817-3098

DOI: 10.5829/idosi.wjz.2013.8.4.81243

Antioxidant Effects of Ginseng Root Extract Against Side Effects of Azathioprine on the Rat Ovary

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Abstract: *Introduction* Azathioprine, as an immunosuppressive drug used in the treatment of many diseases. However, by creating oxidative stress can cause side effects on the body's tissues. Ginseng is a medicinal plant, which is common in traditional medicine in Asian countries. The purpose of this study, was to find out the effect of ginseng root extract against the adverse effects of azathioprine on the ovaries of female rats. *Materials and methods* : In this study, 60 healthy female Wistar rats with an average weight of about 200 ± 25 g were used. All animals had free access to water and food. Animals room was adjusted at the temperature of (21±2°C). All cages were selected the same and animals were divided into 6 groups of 10, including control group , sham group and 4 experimental groups. for 14 consecutive days, the sham group received distilled water and control group did not receive any treatment other than food and water. Experimental group 1 received ginseng with dose of 300 mg per kg of body weight, Experimental group 2 received azathioprine witha dose of 50 mg per kg of body weight, Experimental group 3 received azathioprine with the dose of 50 mg per kg of body weight and simultaneously with ginseng with the dose of 300 mg per kg of body weight, Experimental group 4 : ginseng with the dose of 300 mg per kg of body weight in 14 days and in the next 14 days the ginseng with the rate of 300 mg per kg of body weight simultaneously with azathioprine of 50 mg per kg of body weight that injected intraperitoneally. In each of the six groups, ovarian tissue in term of number primordial follicles, number of primary and secondary follicles, Grafian follicles, the number of atretic follicles and corpora lutea. Blood plasma was used for estimation progesterone, LH and FSH levels. Results revealed that in: Ginseng extract increases mean serum parameters of progesterone, FSH and also reduces the number of corpus luteum in the group injected with ginseng, compared to the group treated with azathioprine.In conclusions,Ginseng extract can be used to reduce the risks of azathioprine on ovarian tissue.

Key words: Ginseng · Azathioprine · Ovary · LH · FSH · Progesterone

INTRODUCTION

Azathioprine is Immunosuppressive drug with brand name of Azasan, that is a derivative of mercaptopurine-6. Structurally, this drug is considered part of purine analogues and in the body after conversion to Mercaptopurine, it would block DNA producing enzymes, so it would affect cells with high dividing such as lymphocytes T and B (which has no recycle for purine production) [1, 2]. Azathioprine for prevent transplant rejection [3], autoimmune diseases, as well as to treat diseases such as leukemia [1] and many other diseases can be used. Side effects of this drug, anemia, weakened bones and repeated infections have been reported [4]. For the metabolism of the drug s-methyl transferase enzyme is needed [4]. Lack of this enzyme in some people genetically, causes excessive weakened of bone marrow and severe anemia [5]. Ginseng is a medicinal plant that is involved native plants of eastern Asia and North America. First by a botanist named Collinson in England

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in 1740, was presented to the scientific community [6]. Ginseng plant in laboratory rats leads to lower blood glucose and reduce the complications of diabetes, such as blood cholesterol. Also in diabetic rats causes decrease of the BUN renal markers [7]. Hyperlipidemic effect of ginseng plant has been investigated [8]. Medicinal properties of this plant is from its root, which contains a steroidal glycoside called jinsonoid and scientific name of Saponins Tri pterpenoid [9]. Ginseng also contains phytoestrogens compounds and with effects on the pituitary gland causes increase secretion of gonadotropins (FSH, LH) [10-12]. In this study, the effect of azathioprine on tissue and ovarian function has been examined. The purpose of this research was to study the effect of ginseng root extract against side effects of azathioprine on rat ovarian tissue with regard to number of primordial follicles, primary and secondary follicles, the Graphian follicles, number of corpus luteum and number of atretic follicles, also considers the impact of the plant on ovarian functions of the progesterone, LH and FSH levels. With this study we can say by Ginseng root consumption simultaneously with the Azathioprine drug, side effects of this drug on the ovaries can be avoided.

MATERIALS AND METHODS

In this study, 60 healthy female rats (Wistar race) with an average weight of about 200 ± 25 g were procured from the Animal House of Medical Sciences of Shiraz University. All animals had free access to water and food. Rats food was like compressed food or pellete (which were purchased from the animal food industry). Animals room was adjusted at the temperature of (21± 2 °C). All cages were selected the same. In this study, the ginseng root has been used which was purchased from Shafa attari in the Shiraz city. Rats after initial weight were divided into 6 groups and they were placed into 6 separate cages. Injection was intraperitoneally and six experimental groups received target materials every day at 10 am.

Control Group: Ten female Wistar rats were used throughout the study, that only received food and water and no treatment in this group was done.

Sham Group: Ten female rats were given the same volume of material to animals, distilled water was as a solvent and extract for 14 days were received.

Experimental Group1: Ten female rats were injected Ginseng root extract with the rate of 300 mg per kg of body weight for 14 days.

Experimental Group2: Ten female rats were injected azathioprine drug with dose of 50 mg per kg of body weight for 14 days.

Experimental Group 3: Ten female rats in this group were injected azathioprine drugs with dose of 50 mg per kg of body weight and Ginseng root extract with dose of 300 mg per kg, while it was administered Simultaneously for 14 days.

Experimental Group4: In this group that was ten female rats, at the first 14 days only Ginseng Root Extract with dose of 300 mg/kg was given. Then, simultaneously azathioprine drug with dose of 50 mg/kg and ginseng root extract with dose of 300 mg/kg was given for 14 consecutive days. Finally blood samples were collected from rats heart and both ovaries (right and left) were removed with the abdominal cavity in estrus phase.

The ovaries of rats from all groups were examined for the presence of follicles (Primordial, Primary, Secondary, Graphian follicles), atretic follicles and corpora lutea. Follicles of were counted with a Nikon light microscope at ×400.

Blood plasma levels of progesterone (MonoBind, Cat n. 4925-300), LH (Pishtas Teb, Lot n. LH0313) and FSH (Pishtas Teb, Lot n. Fs0313) were investigated by Elisa method.

Methods of Statistical Analysis : Results based on SPSS software and Duncan test in significant level of $P<0.05$ were evaluated and following, charts of each parameters were drawn based on Excel.

RESULTS

Results from laboratory animals weighs in Figure 1 shows that only experimental group 2 shows significant decrease compared with the control group.

Results from the right ovary weighs in Figure 2 shows that only experimental group 4 has significant increase compared to control group.

Results of measurements of left ovary weight in Figure 3 shows that the experimental group 4 has significant increase compared to control group and other experimental groups did not show significant changes. Counting number of Primitive follicles in the ovaries in

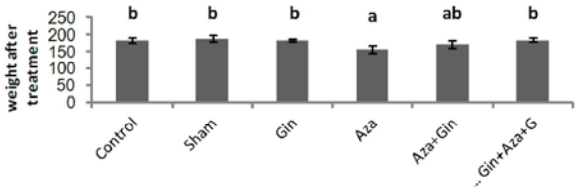

Fig. 1: Weights of control and treated rats (g).

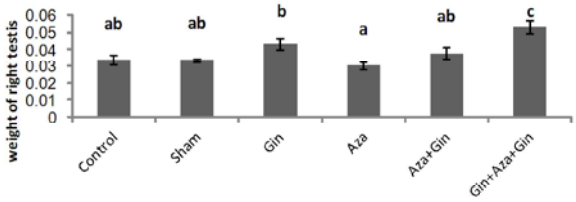

Fig. 2: Comparison mean weight of right testis after material effect in different groups

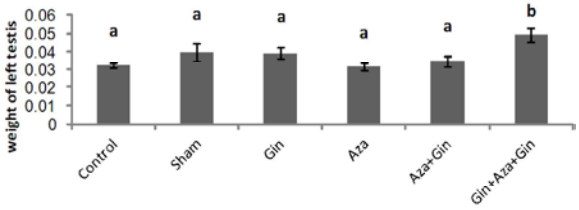

Fig. 3: Comparison mean weight of left testis after material effect in different groups

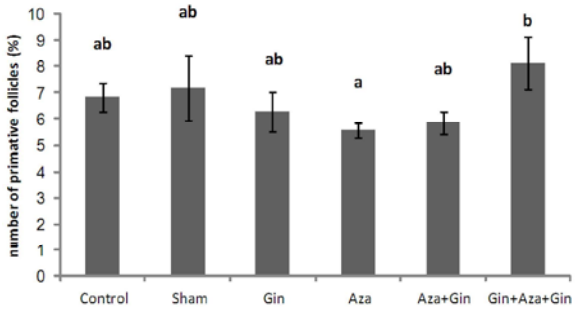

Fig. 4: Comparison mean number of primitive follicle after material effect in different groups

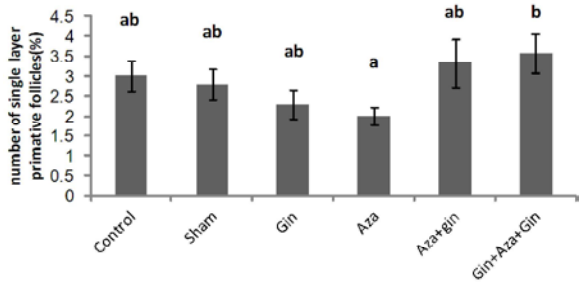

Fig. 5: Comparison of groups in term of primary follicles (single layer)

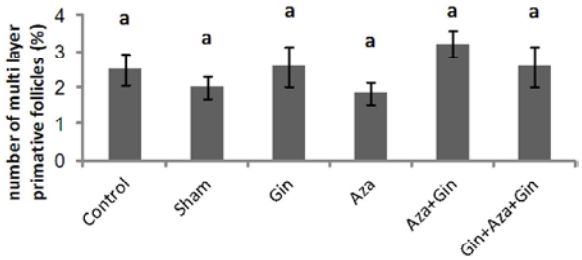

Fig. 6: Comparison of groups in term of primary follicles (multi-layer)

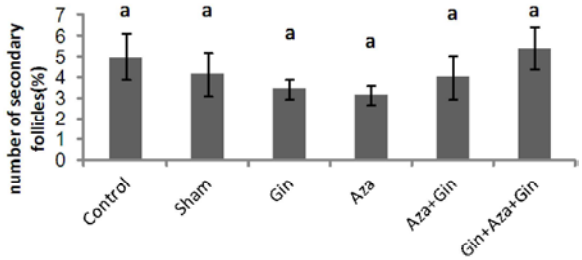

Fig. 7: Comparison groups in term of number of secondary follicle

Figure 4 shows that the experimental group 4 has significant increase compared to experimental group 2.

Counting number of single layer Primitive follicles in Figure 5 shows that the experimental group 4 has significant increase compared to the experimental group 2.

Counting the number of multilayer primary follicles in Figure 6 shows that, all experimental groups compared to the control group, did not show any significant changes.

The results of counting the number of the secondary follicles of the ovaries in Figure 7 suggests that all experimental groups did not show significant changes compared to control group.

Counting the number of Graphian follicles of ovarian in Figure 8 shows that, all experimental groups compared to the control group showed no significant changes.

The results of counting the number of corpus luteum in Figure 9 shows that the experimental group 4 has significant increase compared to the control group and experimental groups 1 and 2.

Counting the number of atretic follicles in Figure 10 shows that the experimental group 2 had a sigsnificant increase compared to the sham group and other experimental groups did not show significant changes.

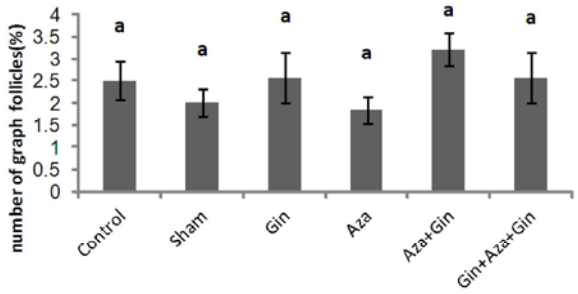

Fig. 8: Comparison mean number of graph follicles after material effect in different groups

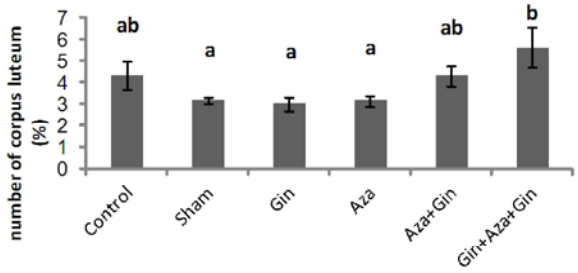

Fig. 9: Comparison groups in term of number of corpus luteum

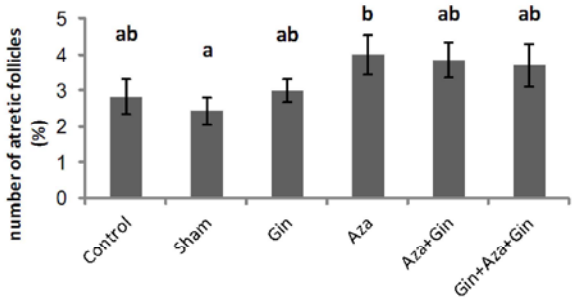

Fig. 10: Comparison groups in term of number of atretic follicle

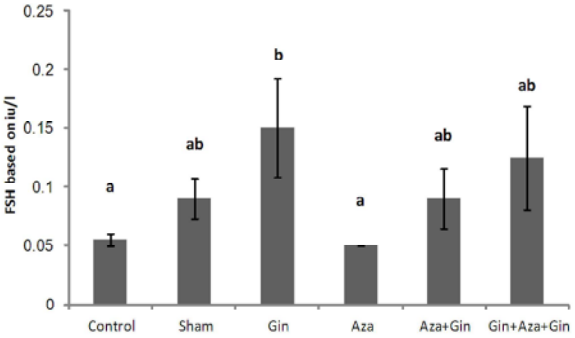

Fig. 11: Comparison groups in term of FSH levels

The results of the measurement of hormone FSH in Figure 11 shows that only experimental group 1 has s ignificant increase compared to the control group.

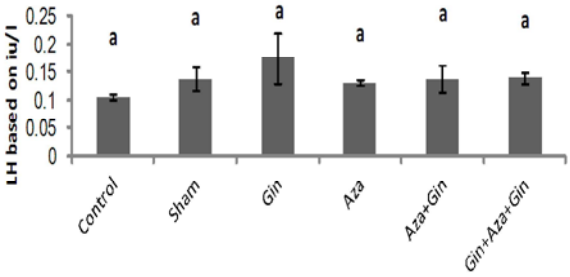

Fig. 12: Comparison groups in term of LH levels

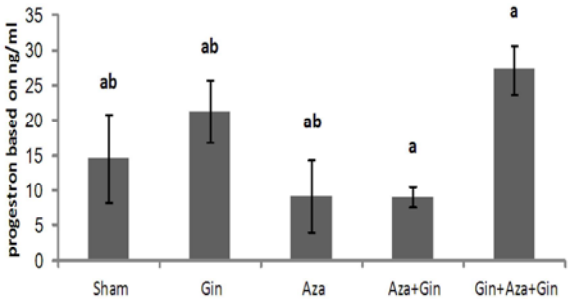

Fig. 13: Comparison groups in term of progestron level In the all of Figure : Aza = Azathioprin, Gin = Ginseng, Aza+Gin = Azathioprin+Ginseng, Gin+Aza+Gin = Ginseng+ Azathioprin+Ginseng

Results of measurements of hormone LH in Figure 12 shows that all experimental groups did not show significant changes compared to the control group.

Results of measurement of progesterone hormone in figure 13 shows that the experimental group 4 has significant increase compared to experimental groups 2 and 3 and other experimental groups, did not show significant changes.

DISCUSSION

Ginseng is an aromatic medicinal plant which takes its name from a Greek word means treating all diseases (Pana kos). The antioxidant properties of ginseng is, by stimulating the activity of antioxidant enzymes such as Glutathione Peroxidase and Superoxide dismutase, also by inhibition activity of hydroxyl radicals and anions prevents lipid peroxidation in cell membranes [13-15]. Azathioprine drug, has been used since 1960 to weaken the immune system. But like many other drugs have side effects on the body tissues. Toxicity of Azathioprine drug is due to production of free radicals in the body and organs of the patient [16,17]. According to Figure 1, the weight of rats in the experimental group 2, shows significant decrease compared to control group.

In research that was done on Azathioprine and hepatic side effects stated that azathioprine reduces the weight of laboratory animals [18] which is consistent with this research. The cause of this weight loss can be due to free radicals and so damage to various organs tissues. According to the research done in the past, stated that Ginseng use in reducing the risks of chemotherapy drugs such as cyclophosphamide in rats has been effective. So that weight loss of testes were observed in groups without antioxidants and the groups that used ginseng antioxidants weight gain of testes has been observed [19]. According to the charts [2, 3] weight of the right and left ovarian in the experimental group 4, has significant increase compared to the control group. According to chart 4, the number of primary follicles in the experimental group 4, has a significant increase compared to the experimental group 2. Also according to the chart number [5], the number of single layer primary follicles in the experimental group 4 has significant increase compared to the experimental group 2. Number of luteinizing according to chart [9] in experimental group 4, has a significant increase compared to sham group and also experimental groups 1 and 2. According to Figure 10, number of atresia follicles in experimental group 2, has a significant increase compared to the sham group. It is known that, azathioprine causes inhibition of synthesis DNA in various organs such as the liver, pancreas, duodenum, lymph nodes and kidneys and it causes oxidative stress [20]. Also, it has been demonstrated that oral use of azathioprine causes cell necrosis and the rough endoplasmic reticulum and mitochondrial proliferation [21]. Thus, a significant reduction in follicle weight in experimental group 2, compared to the experimental group 4, it is entirely reasonable. According to Figure 11, the experimental group 1 has a significant increase of FSH concentrations compared to the control group. In research done in the past stated that the use of ginseng extract causes increased FSH and LH levels in the experimental groups [10-12], that is consistent with this research. According to Figure 12, no significant changes in LH in the present study have been observed. According to research conducted stated that, azathioprine drug reduces the nitric oxide in rats that its effect is dose-dependent manner [22]. This active molecule (nitric oxide) increases the secretion of gonadotropins and hormone LH and also increase sperm motility [23]. Nitric oxide in turn stimulates LHRH and increase secretion of LH [24]. Nitric oxide is a free radical gas with a short-term effect that arise From L-arginine and it is the major mediator in many body function like immune system [25]. According to various studies, the ovaries are capable of synthesizing nitric oxide [26]. Probably this article in ovarian steroidogenesis, ovulation and loss of corpus luteum plays an important role [27 -29]. Ginseng by effect on the anterior pituitary, causing the secretion of nitric oxide, which this effect is due to jin Sinusoidal compounds (saponins)[27]. Thereby reducing the corpus luteum in the experimental group 1 compared to experimental group 4 is reasonable ; Because increase of nitric oxide causes decrease of corpus luteum. But as expected that it should be the same in the experimental group 2 but as mentioned, azathioprine produces oxidative stress that has a more damage. ISO enzymes that make Nitric oxide are active in different parts of the ovary in which this activity is controlled by gonadotropins [30]. Except ovaries, the enzymes producing Nitric oxide can be found in the hypothalamus - pituitary-gonadal [25]. Nitric oxide in the ovaries affected by gonadotropin, but in hypothalamus effect on them [28]. Place of neurons producing Nitric oxide is near by neurons GnRH in the hypothalamic [30]. As mentioned, ginseng increases nitric oxide release. Thus, FSH increased in the experimental group 1 is quite logical. According to Figure 13, the concentration of progesterone hormone in experimental groups 2 and 3 showed a significant decrease compared to the experimental group 4. In This study as described azathioprine induces changes in ovarian follicles and this action may generate free radicals and prevent the synthesis of purine bases and impaired replication [31]. As is known, after puberty, from the anterior pituitary large amounts of hormones LH and FSH secreted that can cause the growth of ovarian and some follicles. Gonadotropin-releasing hormone (GnRH) from the hypothalamus is released and causes the secretion of LH (luteinizing hormone-producing) and FSH (follicle stimulating hormone) from the anterior pituitary and these two hormones cause ovarian secretion of estrogen and progesterone from ovaries. So by destruction of ovarian tissue, dysfunction in the secretion of these hormones is caused and decrease progesterone in experimental group 2 can be attributed to this issue. It also stated that female sex hormones are influenced by the negative feedback [32]. So since in this research concentration of hormones LH and FSH have increased in experimental group 3 (also it is not significant) it causes change in secretion of estrogen in experimental group 3.

According to the above it can be stated that the azathioprine drug has adverse effects on tissues and function of female reproductive organs and Ginseng extract for reducing the risks of taking this medicine is recommended.

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