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# THE EFFECT OF SELENIUM, IRON, AND SILVER NANOPARTICLES ON OVARIAN HISTOPATHOLOGIC CHANGES IN TREATED RATS WITH ISONIAZID

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## ABSTRACT

Introduction: Isoniazid medicine is a strong toxic agent to various organs. Today, use of nanotechnology is very common to reduce complications from drugs and to treat diseases; therefore, this study is aimed to investigate the effect of selenium, iron, and silver nanoparticles to prevent destruction of ovarian tissue in rats treated with isoniazid.

Materials and methods: Three types of iron (150 and 250 mg/kg/dose), silver (0.2 and 0.5 mg/kg/dose), and selenium (0.1 and 0.3 mg/kg/dose) nanoparticles, isoniazid drug, and Wistar rats in 9 groups of 8 each were used in this study. The rats orally received isoniazid on the basis of 50 mg per kg of body weight. Nanoparticles were intraperitoneally injected during 12 consequent days. After 24 hours, the nanoparticles in combination with isoniazid at a dose of 50 mg per kg of body weight were injected again to the rats. On the 15$^{th}$ day, all groupswere killed, dissected, and their ovaries removed for microscopic studies.

Findings: Results of this study reveal reduced number of primordial, primary (single- and multi-layer(s)), and secondary follicles, graffian follicles and corpus luteum in the group receiving isoniazid drug. This shows the negative effect of isoniazid on the number of ovarian follicles. Selenium, iron, and silver nanoparticles partly prevent the destructive effect caused by isoniazid due to their antioxidant activity.

Conclusion: according to the above, by induction of oxidative stress and lipid peroxidation, production of reactive oxygen and damage to ovarian tissue, isoniazid reduces ovarian follicles.

It is also concluded that ironnanoparticles have more antioxidant properties thanselenium and silver nanoparticles to reduce isoniazid side effects on ovarian tissue.

**Keywords: Selenium Nanoparticles, Iron Nanoparticles, Silver Nanoparticles, Isoniazid, Ovarian Follicles, Rats**

## INTRODUCTION

An estimated 1.7 million people still die of tuberculosis annually [1]. Tuberculosis affects about 15 million people and approximately 9 million new cases are added each year. Tuberculosis mostly arises in developing countries and isoniazid as an effective medicine which is normally used to treat the disease, is considered to be a strong toxic agent for other organs [2]. It has been revealed that endogenous lipid peroxidation is the main factor in cytotoxicity of isoniazid. Oxidative damages caused by isoniazid are attributed to formation of highly reactive oxygen species that lead to lipid peroxidation and damage and destruction of lipid membranes [3]. Thus, given the above by consumption of the medicine, cell and tissue abnormalities arise; many new researches investigate various antioxidants to reduce the drug side effects and contradictory results have been obtained. Today, nanotechnology is a part of science which is associated with rapid economic growth and high technology. It is a base in natural nanoparticles and man-made or engineered nanoparticles (ENPS) [4]. More medical studies on the effect of nanoparticles on body cells and tissues have shown that they can enter body organs through the respiratory pathways. Among important nanoparticles selenium, iron, and silver nanoparticles can be mentioned [5].

Usually, available selenium is in two forms of selenate and selenite. Selenium deficiency has been recognized in human with more than 40 diseases. It has also been shown to be effective and useful against cancer [6]. Evidence suggests that selenium is a cancer-preventive agent [7]. It is essential for sperm motility and can reduce abortions. Nano-selenium has recently received a lot of attention due to high biological stability, low toxicity, and antioxidant activity [8].

Iron is an essential metal for living organisms and plays an important role in some biological functions. This material is critical for growth, development, and natural functioning of the central nervous system. It has various roles in neurotransmitter system and metabolism of nervous system, some of which include positioning on oxidoreductase enzymes, classification, uptake and transfer of neurotransmitters, myelin formation and brain energy metabolism [9]. According to performed studies, selenium nanoparticles have numerous applications in medicine; so the aim of this study is to investigate selenium nanoparticles on ovarian histopathological changes in rats treated with isoniazid.

Silver nanoparticles are one of the most common types of nanoparticles used in all daily life and laboratory researches. They have unique properties that help in molecular diagnoses andtreatment, as well as in devices that are used in several medical methods. Although silver nanoparticles are common and are used in many medical methods and devices and in various biological fields, they have drawbacks associated with cell toxicity [10]. It was stated in studies that consumption of low doses of silver nanoparticles will be more effective than higher doses of them [11].

However, it is also expressed that widespread use of these particles in different areas produces environmental pollution due to the high permeability of a large amount of them entering the body. It is possible that undesirable effects appear with these particles, which so far have not been adequately addressed. Therefore, in this study according to the importance of reproductive system in survival-generation and reproduction, and given the significant progress of nanoscience in other fields of medicine, an attempt is made to study the effects of selenium, iron, and silver nanoparticles on ovarian tissue in rats treated with isoniazid.

Methods:

A total number of 72 adult female Wistar rats with 110 to 120 days of age and weighing approximately 180 to 200 g were prepared from Islamic Azad University, Jahrom Branch. Rats were kept in the condition of 12 hours of light and 12 hours of darkness, ambient temperature was 22±1 and animals were fed with special rat food. Then, they were randomly divided in 9 groups of 8 each, including:

Control group: Contained 8 rats. They received sufficient water and food without receiving any medicine treatment.

Blank group: Contained 8 adult rats which received the isoniazidsolvent medicine (saline) by gavage.

Experimental 1 group: Contained 8 adult rats which received 50 mg/kg of body weight isoniazid solution (in saline) by gavage.

Experimental 2 groups: Contained 8 adult rats. In addition to receiving 50 mg/kg of body weight isoniazid solution (in saline) by gavage, they received 0.1 mg/kg seleniumoxide nanoparticlesintraperitoneally.

Experimental 3 groups: Contained 8 adult rats. In addition to receiving 50 mg/kg of body weight isoniazid solution (in saline) by gavage, they received 0.2 mg/kg seleniumoxide nanoparticlesintraperitoneally.

Experimental 4 groups: Contained 8 adult rats. In addition to receiving 50 mg/kg of body weight isoniazid solution (in saline) by gavage, they received 150 mg/kg iron nanoparticlesintraperitoneally.

Experimental 5 groups: Contained 8 adult rats. In addition to receiving 50 mg/kg of body weight isoniazid solution (in saline) by gavage, they received 250 mg/kg iron nanoparticlesintraperitoneally.

Experimental 6 group: It contained 8 adult rats. In addition to receiving 50 mg/kg of body weight isoniazid solution (in saline) by gavage, they received 0.2 mg/kg silver nanoparticlesintraperitoneally.

Experimental 7 groups: Contained 8 adult rats. In addition to receiving 50 mg/kg of body weight isoniazid solution (in saline) by gavage, they received 0.5 mg/kg silver nanoparticles intraperitoneally.

After 14 days and 24 hours from the last treatment, all rats were anesthetized by diethyl ether in desiccators. Then ovarian tissues were separated for microscopic studies of ovaries, and were fixed in 10% formalin and sent to laboratory for preparation of tissue sections. Finally, the prepared tissues were studied by optical microscopy in terms of the number ofprimordial, primary (single- and multi-layer(s)), and secondary follicles, graffian follicles and corpus luteum.

The obtained raw numbers were statistically analyzed by counting the number of follicles in the samples of various groups. Data were analyzed on the basis of SPSS software version 16 using one-way ANOVA and statistical Duncan tests were used for comparison between the groups. P≤0.05 was considered significant level and the obtained results of the tests with relevant statistical calculations were expressed as mean and mean error $(\overline{X} \pm SEM)$.

**RESULTS**:

Charts 1 to 6 showsignificant reduction in the number of primordial, single- and multi-layer(s)), and secondary follicles, graffian follicles and corpus luteum in the experimental 1 group receiving isoniazid only compared to the control group. The number of follicles in the experimental 2 and 3 groups has significant reduction at 5% level compared to the control group. According to the Chart (1) it is found that there is a significant reduction at 5% level in the number of primordial follicles of the experimental 6 and 7 groups compared to the control group. The number of primordial follicles in the experimental 4, 5 and 7 groups has significant increase at 5% level compared to the experimental 1 group. According to Chart (2) there is a significant increase at 5% level in the number of single layer primary follicles in the experimental 5, 6, and 7 groups compared to the experimental 1 group. Chart (4) indicates that there is a significant reduction at 5% level in the number of secondary follicles in the experimental 6 and 7 groups compared to the control group, and this amount has a significant increase at 5% level in the experimental 4, 5, 6 and 7 groups compared to the experimental 1 group. According to Chart (5) there is a significant reduction at 5% level in the number of graffian follicles in the experimental 6 and 7 groups compared to the control group. Also, this amount in the experimental 4 and 5, and 6 and 7 groups shows significant increase at 5% level compared to the experimental 1 group. Chart (6) shows there is a significant reduction at 5% level in the number of corpus luteum in the experimental groups 4 and 5, and 6 and 7 groups compared to the control group.

(Charts 1-6)

According to photomicrograph, ovarian tissues in the control group (rats were kept without receiving any material) are healthy and no necrosis and cell damage are observed (Image 1).

Based on the photomicrograph, it is found that ovarian tissues in the blank group compared to the control group are healthy and no cell damage is observed. Corpus luteum as well as graffian and multi-layer primary follicles, which are normal compared to the control group, are shown in the following image (Image 2).

It is found from the ovarian follicles and corpus luteum counting charts that tissue damage and loss of ovarian follicles is very high in the experimental 1 group which received isoniazid alone compared to the control group. It is also observed that isoniazid causes cell necrosis, cell disruption, as well as creation of follicle atresia in ovarian tissues (Image 3).

The results in the experimental 2 group which received isoniazid and minimum dose of selenium oxide nanoparticles show that ovarian follicles have improved slightly compared to the other isoniazid receiving groups and this difference is not significant (Image 4).

Histology results in the experimental 3 group, which received isoniazid and maximum dose of selenium oxide nanoparticles, show a decline in the number of follicles as well as small tissue damage compared to the control group (Image 5).

Photomicrograph (6) showed that damage and destruction of ovarian follicles as well as depletion of corpus luteumin the experimental 4 group receiving isoniazid with minimum dose of iron oxidenanoparticles is very slight compared to the control group, but improved cell wall and reduced cell damage is observed in this group compared to the experimental 1 group, as well as much less tissue damage compared to the experimental 2 and 3 groups. This photomicrograph does not show much difference in terms of the number of follicles in the experimental 4 group compared to the control group.

Photomicrograph (7) showed that in the experimental 5 group which received isoniazid and maximum dose of iron oxidenanoparticles, slight necrosis in the cell walls and tissue destruction occur and less than the other experimental groups. There is no significant difference in follicle count in this group with the control group.

Photomicrograph (8) shows ovarian follicles as well as tissue destruction in the experimental 6 group receiving isoniazid and minimum dose of silver oxidenanoparticles compared to the control group. Less tissue damage is observed in this group compared to the experimental 1 group, but tissue hyperemia is still slightly visible.

Photomicrograph (9) shows reduced ovarian follicles in the experimental 7group receiving isoniazid and maximum dose of silver oxidenanoparticles compared to the control group. This group shows improved necrosis and tissue destruction compared to the experimental 1 group.

**Chart 1- Comparison of investigated groups in terms of primordial follicles**

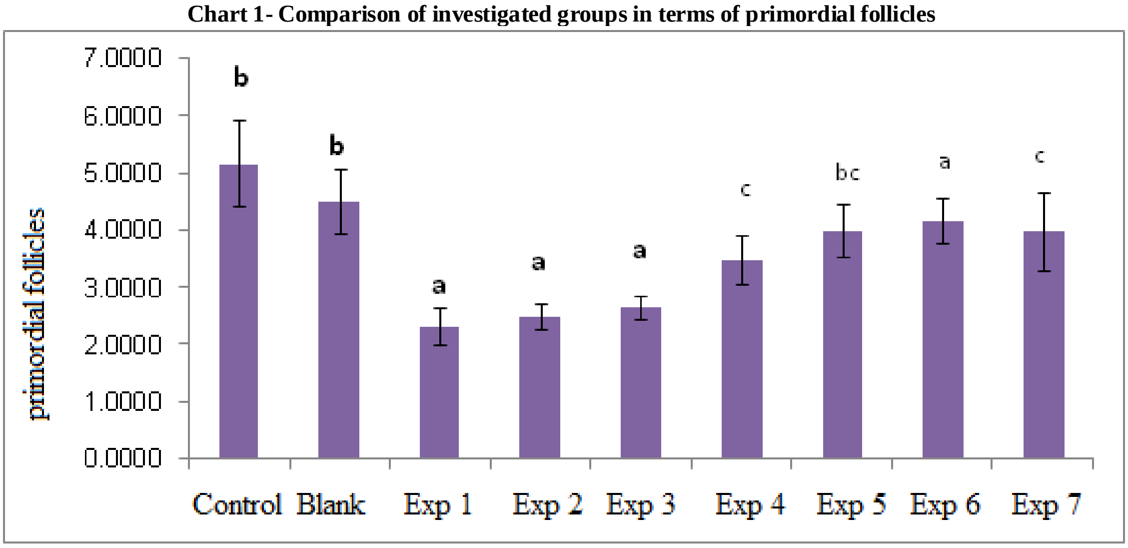



**Chart 2- Comparison of investigated groups in terms of single-layer primary follicles**

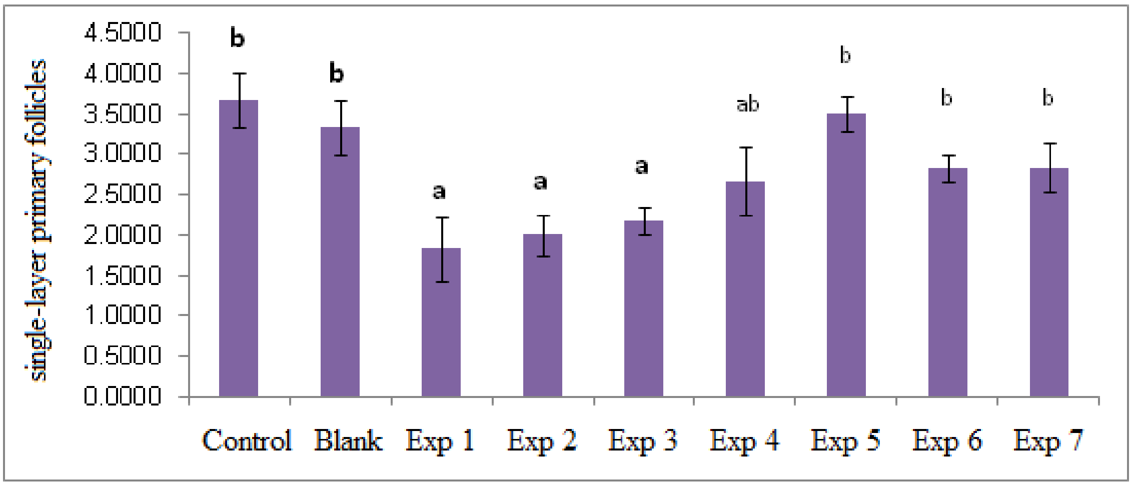



**Chart 3- Comparison of investigated groups in terms of multi-layer primary follicles**

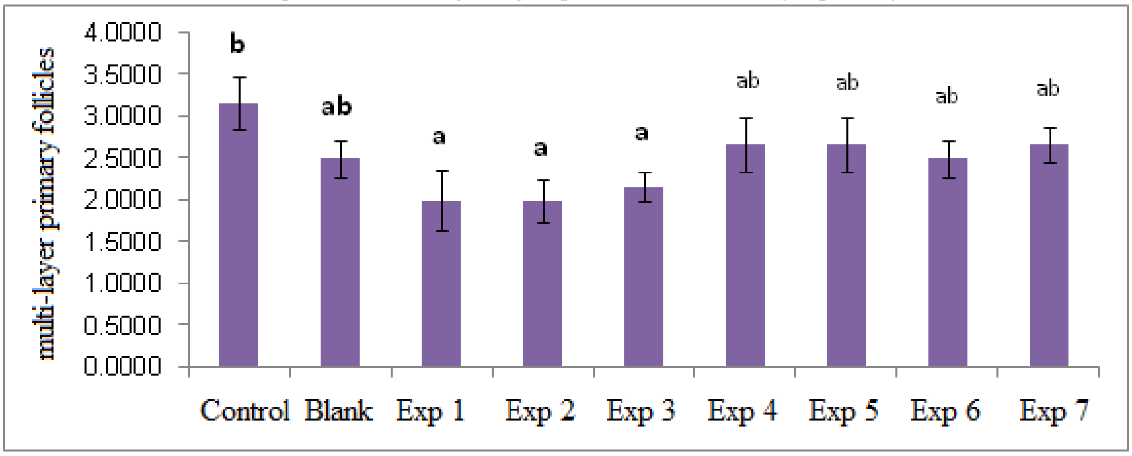



**Chart 4- Comparison of investigated groups in the terms of secondary follicles**

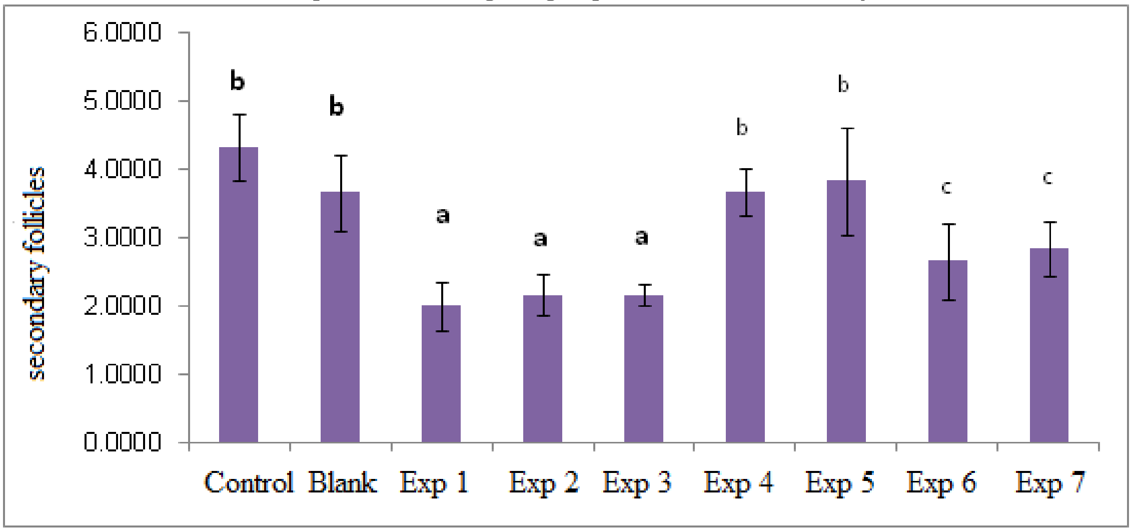



**Chart 5- Comparison of investigated groups in the terms of graffian follicles**

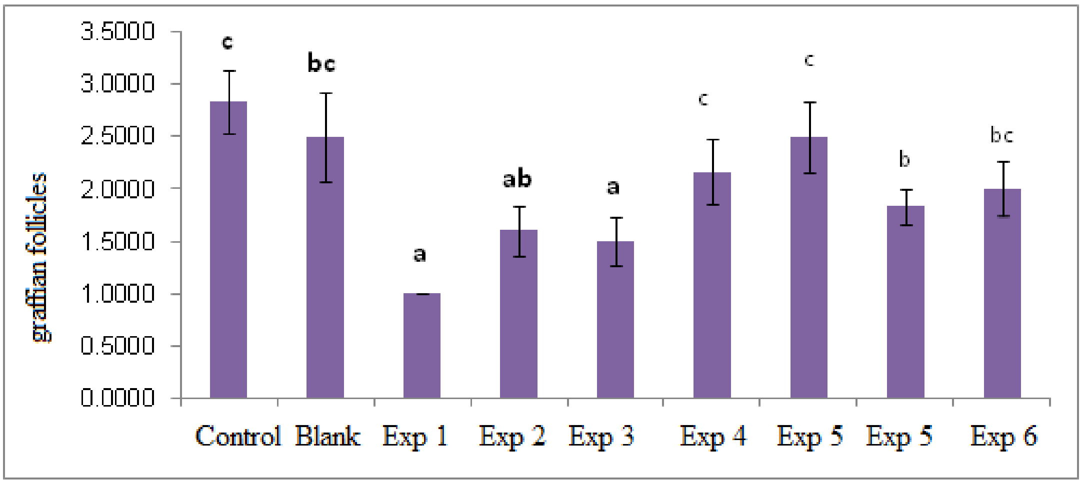



**Chart 6- Comparison of investigated groups in terms of corpus luteum**

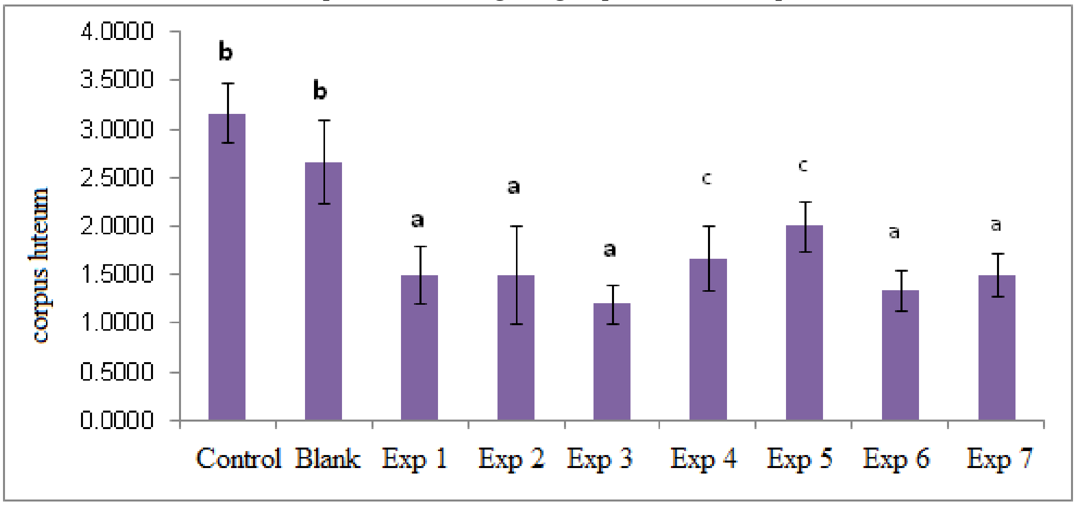



**Image 1- Photomicrograph of ovarian tissue in the control group, magnification X100, Hematoxylin-Eosin (H&E) stained. 1: primary follicles; 2: secondary follicles; 3- graffian follicles; 4- corpus luteum**

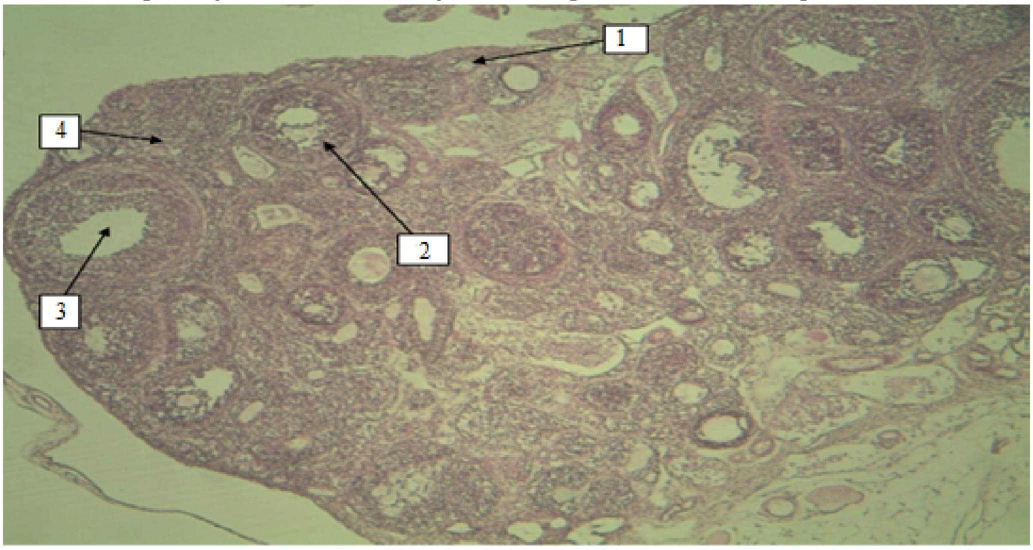



**Image 2- Photomicrograph of ovarian tissue in the blank group; magnification X100; Hematoxylin-Eosin (H&E) stained. 1: graffian follicles; 2: multi-layer primary follicles; 3- corpus luteum**

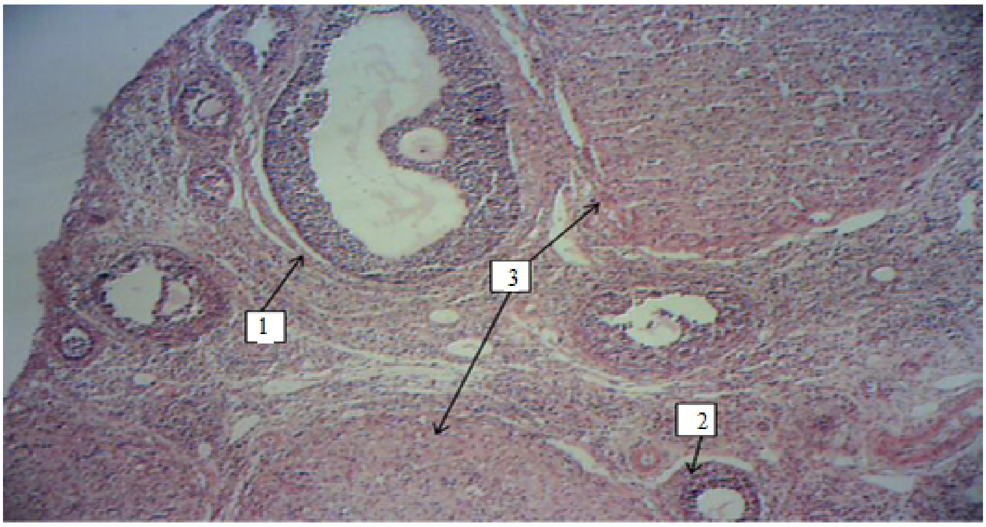



**Image 3- Photomicrograph of ovarian tissue in the experimental 1 group; magnification X100; Hematoxylin-Eosin (H&E) stained. 1: secondary follicles; 2: atrophied corpus luteum; 3- atretic follicles; 4- hyperemia**

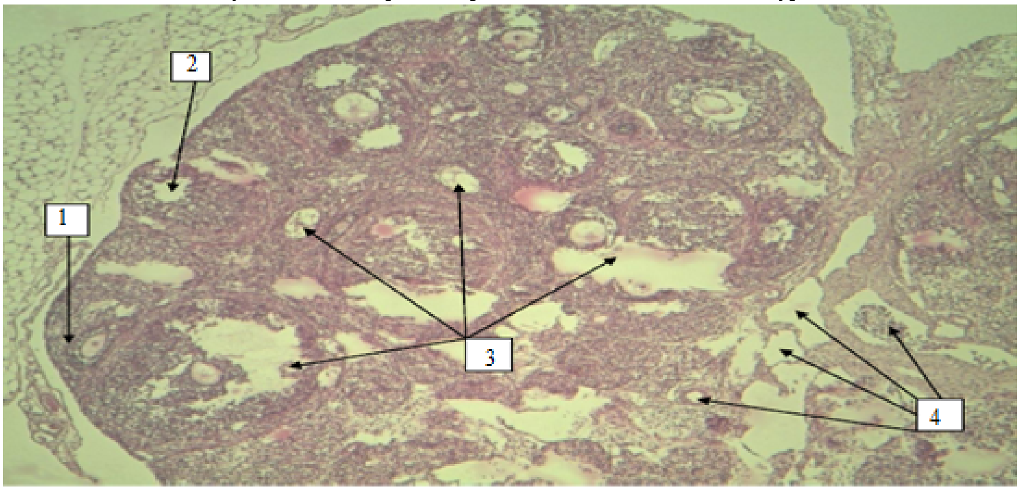



**Image 4- Photomicrograph of ovarian tissue in the experimental 2group; magnification X100; Hematoxylin-Eosin (H&E) stained. 1: blood vessels growth; 2: follicle atresia**

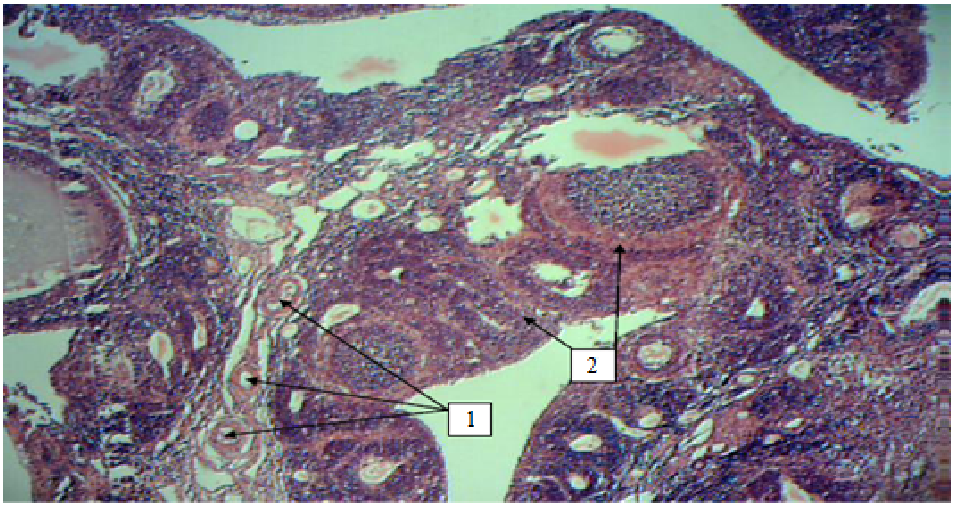



**Image 5- Photomicrograph of ovarian tissue in the experimental 3group; magnification X100; Hematoxylin-Eosin (H&E) stained. Atretic ovarian folliclesare shown by arrows**

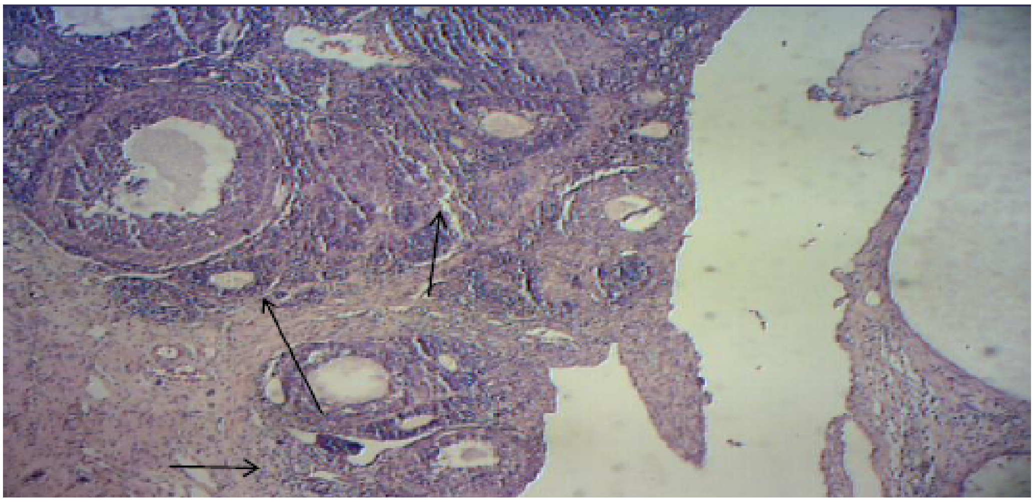


Image 6- Photomicrograph of ovarian tissue in the experimental 4group; magnification X100; Hematoxylin-Eosin (H&E) stained. 1: Atretic ovarian follicles; 2- graffianfollicles; 3- blood vessels

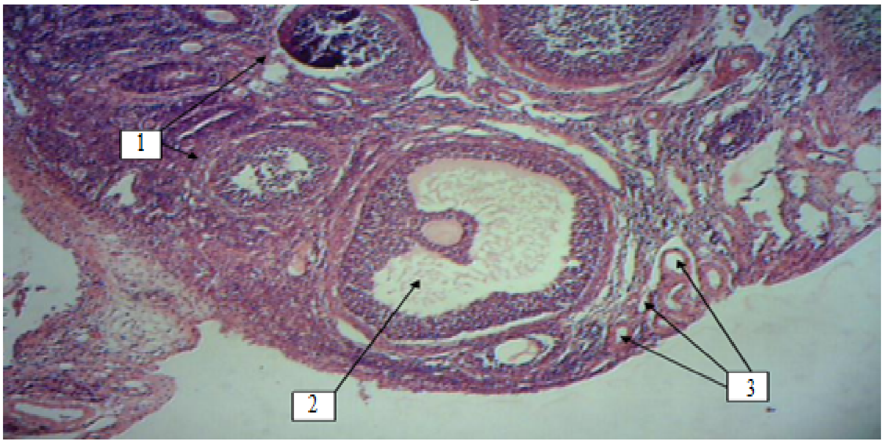



Image 7- Photomicrograph of ovarian tissue in the experimental 5group; magnification X400; Hematoxylin-Eosin (H&E) stained. 1: multi-layer primary follicles; 2- depleted corpus luteum; 3- primary follicles; 4- aretic ovarian follicles

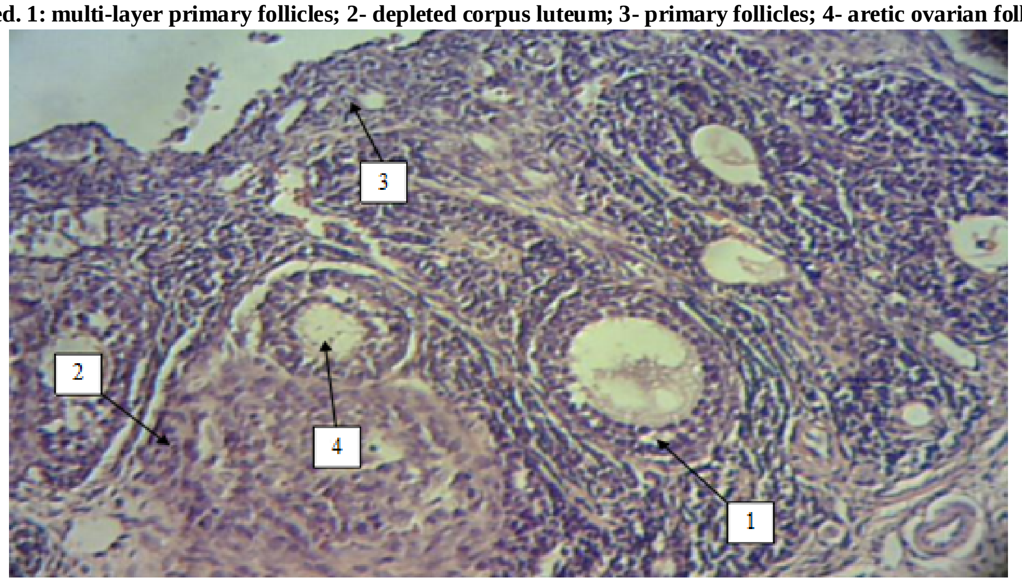



Image 8- Photomicrograph of ovarian tissue in the experimental 6group; magnification X100; Hematoxylin-Eosin (H&E) stained. 1: graffian follicles; 2- atretic ovarian follicles

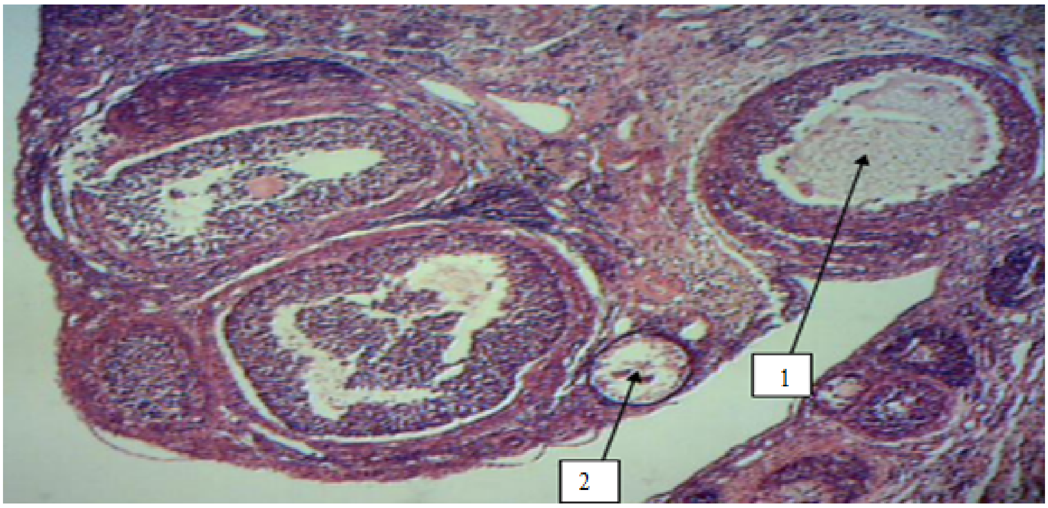
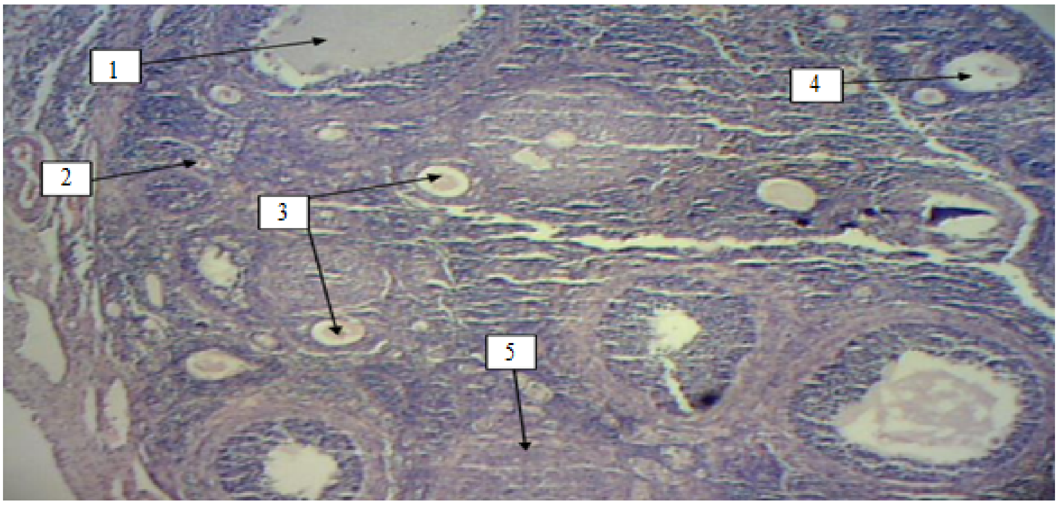



**Image 9- Photomicrograph of ovarian tissue in the experimental 7group; magnification X100; Hematoxylin-Eosin (H&E) stained. 1: graffian follicles; 2- primordial follicles; 3- multi-layers primary follicles, 4- secondary follicles, 5- corpus luteum**




## DISCUSSION

Results showed significant reduction in the number of primordial, primary (single- and multi-layer(s)), and secondary follicles, graffian follicles and corpus luteum in the isoniazid groups compared to the control group.

It was stated in performed studies on the effect of isoniazid on spermatogenesis process in mice that taking isoniazid at doses of 25 and 125 mg/kg of body weight causes abnormal chromosomes and as a result non-fertile sperm in mouse and hamster. It also reduces spermatogenesis and causes abnormal sperms during 12 weeks of treatment with isoniazid. It is further shown that these isoniazid doses in male mice are higher than normal limits [12]. Studies on the effect of isoniazid on oogenesis in laboratory mice showed that this medicine causes ovarian Germ-cells mutations; it also reduces fertilization and stops ovum at oocytes stage II. This shows adverse effect of isoniazid on ovarian tissue and as a result on oogenesis [13] which agrees with the results of the present study and shows negative effects of isoniazid on ovarian tissue in patients taking this medicine. Previous studies on DNA damage in male rabbits germ cells treated with isoniazid and procarbazin showed that higher dose isoniazid causes DNA damage of germ cells and it has fewer mutagenic effects in these cells compared to procarbazin [14]. It is probable in this study that isoniazid by creation of mutation in DNA causes reduced germ cells and thus reduced ovarian follicles. On the other hand, it was stated that angiogenesis is necessary for the growth of ovarian follicles and angiogenesis disorders lead to follicular atresia [15]. Studies revealed that reactive oxygen species as signal converterscan provide angiogenic responses [16] and increase or decrease of reactive oxygen species causes disruption in angiogenesis [17]. As stated before isoniazid causes disruption in production of reactive oxygen species [18]. In this study isoniazid is likely to impair angiogenesis via production of reactive oxygen species and thereby reduce ovarian follicles in the experimental groups and cell necrosis.

On the other hand, increased number of follicles in the groups receiving selenium is observed compared to the group receiving isoniazid alone. This shows antioxidant properties of selenium. Previous studies on investigation of selenium nanoparticles effects on reproductive tissues in male mice showed that these nanoparticles improve sperm parameters during spermatogenesis period [19]. Kaur et al. reported that taking 8 ppm selenium which is 4 times greater than the needed amount for six weeks reduces reproductive organs weight [20].

It was also stated that selenium is a rare and essential element that plays an important role in many metabolic functions of body and its role has been shown in prevention of many diseases. Selenium is a key component of some functional selenoproteins and it has positive interference in the body’s natural functions [21]. The best known usage of this element is its role in the structure of antioxidant enzymes such as glutathione peroxidase whose function is removal of free radicals and reactive oxygen species [22].

Some studies reported that selenium results in improved reproductive performance in animals such as mice, Indian pigs, and sheep [23]. Selenium was also indicated as an antioxidant whose presence is essential for normal sexual functions, and this element as a cofactor of antioxidant enzymes results in reduced free radicals and increased fertility [24]. According to the results, selenium nanoparticles as an antioxidant can reduce the risk of toxicity [25, 26].

Conducted research on the effect of selenium on sex changes in male mice revealed that this element has positive effect on sperm parameters and due to its antioxidant properties increased mobility, and live sperms percentage is observed [27, 28]. This probability is also applied related to ovaries.

According to the previous studies, increased selenium content in organisms which are faced with a shortage of this element will help to improve fertility [29, 30]. Macpherson et al.'s studies showed an improvement in sperm motility in infertile persons who have received sufficient amount of selenium [30]. The results of current study are consistent with the result of previous researches which show improved effect of selenium nanoparticleson ovarian tissue in the groups that received isoniazid.

The results showed significant increase in the number of corpus luteum, primordial and secondary follicles, and graffian follicles in the experimental 4 group and significant increase was observed in the number of primordial, primary (single-layer) and secondary follicles, and graffian follicles in the experimental 5 group compared to the experimental 1 group. In performed studies on the effects of 5, 15, and 45 mg/kg of body weight of iron nanoparticles on ovarian tissue and fertility revealed that these nanoparticles accumulate in all parts of ovarian tissue. Stress oxidative indexes are also increased in this condition and have a direct correlation with the amount of iron accumulation. Iron nanoparticles through interference in cellular oxidative routes probably reduce the number of follicles and corpus luteum due to the formation of oxygen free radicals, microfilaments and intermediate filaments destruction, and damage to mitochondria followed by iron overload increase the process of cell apoptosis and therefore the number of atretic follicles. This can create a negative impact on the fertility of female mice [31]. Probably the difference in ovarian follicles in the experimental 2 to 7 groups compared to the experimental 1 group that received isoniazid only and previous studies will be due to iron oxide nanoparticles consuming dose, their prescription methods and duration, and lack of isoniazid prescription in other studies. As stated above, long-term consumption of nanoparticles causes negative effects on various tissues and in this case its effect on ovarian tissue reduces in minimum dose. This could be the probable reason for the difference between the results of this study and previous studies results. On the other hand, it was stated in other studies that iron nanoparticles have many biomedical applications such as tissue regeneration through increased number of cells, safety assessment, detoxification of biological fluids, heat therapy of cancer cells, etc [32]. Despite the abundant use of these nanoparticles their effects on human health are not yet fully understood [32]. It is likely that increased number of follicles in the mentioned groups compared to the experimental 1 group is due to iron nanoparticles properties in tissue regeneration and cell numbers.

The number of primordial and graffian follicles in the experimental 7 group that received 0.5 silver oxide nanoparticles with isoniazid has significant increase compared to the experimental 1 group, and the number of primary single-layer follicles in the experimental 6 and 7 groups has significant increase compared to the experimental 1 group. Some studies suggested that silver oxide nanoparticles show their toxic effects with increased cell divisions [33-35]. It is likely that in the present study, an increase in ovarian follicles compared to the experimental 1 group does not exclusively reflect the positive effects of silver nanoparticles.

It is stated according to performed studies on impact of silver oxide nanoparticles on ovarian tissue that long-term consumption of these nanoparticles with higher dose has toxic effect on ovarian tissue and chronic exposure to silver nanoparticles may damage and weaken the function of reproductive system in women [36]. Studies showed that intraperitoneal injection of 1 and 10 ppm of silver nanoparticles has toxic effect on ovarian tissue [37]. This is not consistent with the results of the present study which is probably due to laboratory conditions as well as prescription methods and consumed dose of silver nanoparticles, age, and type of laboratory animals. Low dose silver nanoparticles consumption, according to studies, has more positive impact and these nanoparticles in this condition will have suitable results [38].

Silver nanoparticles, on the other hand, are introduced in studies as one of the most powerful anti-bacterial and anti-fungal materials. However, concern has always existed that excessive use of these nanoparticles has potential threat for ecosystem, human life and other living things. So that mitochondria activity is reduced after long-term consumption of silver nanoparticles and lactate dehydrogenase function is significantly increased. This increase indicates cell death [39, 40]. Studies also indicated that silver nanoparticles are accumulated in various tissues such as liver, kidney, spleen, lung, and brain [41]. Another reason for the difference in the results of this study with other studies' results is duration of silver nanoparticles use. It is likely in this study that non-antioxidant properties of silver nanoparticles as well as their accumulation in ovarian tissue appear less than isoniazid, due to the short-time use of these nanoparticles.

Conclusion

With respect to the above, induction of oxidative stress and lipids peroxidation, production of reactive oxygen species and damage to ovarian tissue reduce the number of ovarian follicles and iron nanoparticles have more antioxidant activity in reduced complications of isoniazid tissue. Supplemental examinations are recommended for possible effects of selenium and silver nanoparticles. Some parameters such as time, nanoparticles method of administration, duration of experiment, sex and age of laboratory animal probably impact on related results in reduced isoniazid complications on ovarian tissue.

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