**ABSTRACT**

Introduction: Diabetes is an endocrine and metabolic disorder, a variety of effects on the structure and function of many body systems leaves. Reproductive system, the rule is different. The aim of this study was to investigate the effects of the herb Artemisia extracts of ovarian tissue is improved due to diabetes complications.

Materials and Methods: 50 rats, adult female aged approximately 10 weeks of Wistar rats randomly divided into 5 groups: control, diabetic (streptozotocin 50 mg/kg), diabetic + extract of Artemisia (200 & 300 mg/kg) and a positive control (metformin 250mg/kg) were included. The streptozotocin was administered intraperitoneally, and other materials as gavage. Treated mice continued for 8 weeks. At the beginning and end of the experiment, mice were weighed and blood glucose was measured, so the mice were dissected and the right ovary weighed after fixation in 3% formaldehyde and cutting and preparing for special studies were conducted respectively. Mason with three more chrome slides, were stained with hematoxylin eosin and periodic acid Schiff. Few studies include estimates of mean ovarian volume and follicle determines the quality and histopathological studies were performed. The results indicate an increase in blood sugar levels, ovarian volume, the percentage of atretic follicles quantitatively and cause congestion, hyperemia, and increased thickness of the germinal epithelium and fibrosis in diabetic rats was qualitatively, in the other groups, these parameters showed a decreasing trend (p ≤ 0.05). Conclusion: This study suggests that as plant extract could significantly reduce the complications of diabetes in female reproductive system changes. Key words: diabetes, ovarian, moose, mountain sagebrush, stereology, histopathology

**Key words:**

- diabetes
- ovarian
- moose
- mountain sagebrush
- stereology
- histopathology

**Introduction**

Infertility related problems as one of the major problems in the couple's life is known [3]. According to the statistics 35% of men and 25% of infertile couples, infertility is related to the second pair [16,11]. Because diabetes is a disease associated with sexual activity in men and women is about 90% of patients deficient in sexual activity in reduced libido and reduced fertility are included [6]. This causes ovarian dysfunction, including changes in estrous behavior, follicular growth, oocyte maturation and ovulation is reduced or not [5,21], this shifts the focus from disrupting the hypothalamic - pituitary - gonadal axis is [5] FSH and LH hormone levels, which in effect reduced then the ability of the ovaries to produce the hormones estrogen and progesterone are decreased [5]. In addition, histopathological changes in the ovary, including diabetes, polycystic ovary syndrome, ovarian enlargement [2,9] and atretic follicles have also been reported [2,21], although the exact mechanism is well known to have diabetes mellitus but construction has not increased free radical damage is the major mechanism [23] of antioxidants such as vitamins, flavonoids in the diet can have protective effects in diabetic patients [17]. Free radicals are reduced flavonoid [20] recently for the treatment of diabetes blood glucose lowering effects of herbs that are used and expected to have similar effects, but drugs have less side effects [18,4] Such as Artemisia plants are [1] that recent research has demonstrated its ability to reduce blood sugar [10,1]. Metformin is a combination - Govandi lowering of blood glucose that is used in the
treatment of diabetes. The drug works by a series of reactions in various organs and tissues can be obtained as follows: Reduction of hepatic gluconeogenesis in hepatocytes and Gelicozhenz and Govanidi increase, decrease glucose uptake by muscle and fat cells. Oral administration of intravenous administration of metformin has better performance \[8,24\]. Since stereological and histopathological study on the effect of hydroalcoholic extract of Artemisia compared with metformin on ovarian structures on diabetic rats has been reported, this is exactly the outcome sought to do.

**Materials and Methods**

In this study, 50 adult female Wistar rats, approximately 10 weeks of age, education and care of laboratory animals were purchased from Shiraz Medical School. Initially, rats were randomly divided into 5 groups: control, diabetic, diabetic + extract (200 & 300 mg/kg) and diabetic + metformin (250 mg / kg) into the chamber Animals Branch arranged at a temperature of 24 °C and 12 h light and 12 h dark photoperiod and free access to enough food and water, were kept. To induce diabetes in rats injected intraperitoneally dissolved streptozotocin as a single dose or dose 50mg / kg on 90% Nacl, 100 mM sodium citrate buffer PH = 4/5 solution were used \[3\]. After 4 days of STZ passed blood sugar with glucometer in All Departments Czech made (prior to glucose for 12 hours feeding of rats was used) after diabetic They (300mg / dl) were treated for 12 weeks starting at the end of the experiment, blood glucose Czech animals were anesthetized by ether and autopsied. Right ovaries of all animals completely separated and washed with normal Saylin the formalin 3% for 3 days were performed after fixation adequate tissue passage was cut by 5 mm with three painted a chrome Mason (for diagnosis of fibrosis), periodic acid Schiff (glycogen deposition in tissue diagnosis) and H & E preparations. Stereological studies: Cavalieri’s method was used to study the ovarian volume. Cavalieri showed \[14\] that uses a uniform random sections and parallel and the distance, the sample size can be cut to size and the distance between them can be calculated. Different stages of the procedure are as follows: The right to be selected. Samples were cut parallel to the interval (t) so in cases where the preparation can be difficult to cut with the same average thickness (t) can be used for cutting. Should be identical to the first accident cut subsequent random and uniform will be provided. Cut random number m of area A1, A2... Am, and the distance (t) are prepared and the formula for calculating volume is estimated non Bias \[14\].

\[\text{estV} = t \left( A_1 + A_2 + \ldots + A_m \right)\]

To calculate the area (A) from a network point that is randomly placed on the cut surface is used.

First, should the range or scope of any point \((a / p)\) can be computed in the network. Section area (A) multiplied by the total points of the network which has the cutting surface, is obtained \[14\].

\[\text{est} A = \sum P_i, a(p) \cdot t\]

The area of the section thickness \((t)\) and thus multiplying the volume (V) is calculated by the following equation:

\[V = \sum_{i=1}^{m} P_i, a(p) \cdot t\]

After preparing the slides and put them on the micro projector and after dropping the image on the display of the cross-shaped probe is quite clear that the scope of each point (Fig. 1). (Point lattice probe) On transparent paper copy is accidentally unleashed on the image and then counting the number of points that have to deal with image size of the following formula is easily obtained.

\[V = \sum_{i=1}^{m} P_i, a(p) \cdot t\]

In the distance, v the volume, \(\Sigma P\) is the total number of points counted in the area or anywhere in the frame and t is the thickness of the initial slides. Meanwhile, \(a / p\) or scope of any point can be obtained by the formula where \(\Delta y\) and \(\Delta X\) linear dimensions of each point and M is the magnification Linear Micro Devices Projectors \[14\].

**Data analysis:**

Results using SPSS version 15 software and ANOVA tests (one way ANOVA) and Duncan's test, were analyzed \((p \leq 0.05)\). Mean and standard deviation were calculated for the data and the graphs were plotted by Excell software.

**Blood Sugar:**

Secondary blood sugar in diabetic rats showed a significant increase compared to the other groups \((p <0.0001)\) that the amount of glucose in the treated group showed a significant decrease. It should be noted that the extract significantly increased mean ovarian weight in the diabetic group than the control group, metformin, diabetes + Artemisia is 200mg/kg. It should be noted that the ovarian weight in diabetic rats compared to diabetic + extract of Artemisia 300mg/kg soon was reduced but this reduction was not significant \((p \leq 0.05)\) (Table 1). Ovarian volume in diabetic rats increased significantly compared to the control group from other groups of diabetic + metformin, diabetes + Artemisia 200 and 300 (mg / kg) significantly decreased in size compared to the diabetic group show \((p \leq 0.05)\) (table 1).
Image 1: Probe or cross network used to determine ovarian volume. Histopathological studies: The histopathological studies, in terms of congestion and ovarian vasculature, fibrosis, vacuolization of cells of ovarian tissue, follicular atresia, the thickness of the germinal epithelium were studied.

**Pre-antral follicles:**

Average pre-antral follicles was significantly lower in the diabetic group compared to other groups show (p ≤ 0.05) (Table 2).

**Antral follicles:**

Average antral follicles in the diabetic and diabetic + metformin with other groups shows a significant decrease (p ≤ 0.05) (Table 2).

The number of luteinizing

The results of counting the number of CL among different groups suggests that increased significantly between diabetic + metformin and diabetes + Artemisia 200mg / kg and 300 to the control group but are different in other groups together seen is not (p ≤ 0.05) (table 2).

**Atretic follicles:**

Average atretic follicles significantly increased in diabetic rats compared to other groups show (p ≤ 0.05) (Table 2).

Results related to oocyte diameter, antral follicles, corpus luteum and the germinal epithelium. Average oocyte diameter decreased significantly in diabetic rats as compared to diabetic + extract of Artemisia 300mg/kg show. But other groups have no significant difference in the diameter of antral follicles in groups of no exist, the mean diameter of the corpus luteum in the diabetic + metformin group decreased significantly as compared to the diabetic group show (p ≤ 0.05). The mean diameter of the germinal epithelium significantly increased in the diabetic group compared to the control group. Side diameter of germinal epithelium in diabetic rats compared to other groups declined, but this decline was not significant.

**Histopathological changes:**

Histopathological changes in the ovaries of rats in different groups, there is congestion and vasculature and fibrosis were observed in the diabetic group.

**Table 1:** Comparison of primary and secondary blood glucose testing at the beginning and end of the comparison groups examined ovary weight and volume.

<table>
<thead>
<tr>
<th>Ovarian volume (mm³)</th>
<th>Ovarian weight (g)</th>
<th>Secondary blood glucose (mg / dl)</th>
<th>Initial blood glucose (mg / dl)</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.40 ± 3/67</td>
<td>0.0029 ± 0.032</td>
<td>0/77 ± 96/6</td>
<td>1/44 ± 92</td>
<td>Control</td>
</tr>
<tr>
<td>0.59 ± 5/40</td>
<td>0.0091 ± 0/053</td>
<td>25/12 ± 469/6</td>
<td>20/70 ± 467/4</td>
<td>Diabetic</td>
</tr>
<tr>
<td>0.34 ± 2/88</td>
<td>0.0052 ± 0.031</td>
<td>31/17 ± 207/6</td>
<td>13/18 ± 472/2</td>
<td>Diabetic + metformin</td>
</tr>
<tr>
<td>0.51 ± 3/79</td>
<td>0.014 ± 0.031</td>
<td>45/49 ± 195/4</td>
<td>19/14 ± 465</td>
<td>Diabetes + Artemisia 200mg/kg</td>
</tr>
<tr>
<td>0.79 ± 3/82</td>
<td>0.022 ± 0.038</td>
<td>18/45 ± 247/2</td>
<td>13/73 ± 496/2</td>
<td>Diabetes + Artemisia 300mg/kg</td>
</tr>
</tbody>
</table>

Marked * showed no significant difference before and after treatment is. Letters indicate significant differences at the end.
Table 2: Comparison of mean number of follicles in groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Atretic follicles</th>
<th>CL diameter (μm)</th>
<th>Antral follicles</th>
<th>Pre-antral follicles</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>1/17 ± 4/38</td>
<td>4/05 ± 18/85</td>
<td>3/23 ± 19/47</td>
<td>4/36 ± 57/13</td>
<td>Control</td>
</tr>
<tr>
<td>Diabetes + Artemisia</td>
<td>2/11 ± 23/36</td>
<td>2/07 ± 52/88</td>
<td>1/14 ± 78/34</td>
<td>5/05 ± 36/78</td>
<td>Diabetes + metformin</td>
</tr>
<tr>
<td>Diabetes + metformin</td>
<td>2/46 ± 4/65</td>
<td>6/26 ± 34/45</td>
<td>1/44 ± 7/78</td>
<td>5/05 ± 56/78</td>
<td>Diabetes + Artemisia 200mg/kg</td>
</tr>
<tr>
<td>Diabetes + Artemisia</td>
<td>2/07 ± 21/16</td>
<td>4/99 ± 34/98</td>
<td>1/35 ± 17/25</td>
<td>4/49 ± 54</td>
<td>Diabetes + Artemisia 300mg/kg</td>
</tr>
</tbody>
</table>

Table 3: Comparison of mean CL diameter, germinal epithelium, antral follicles and oocytes in groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Oocyte diameter (μm)</th>
<th>Antral follicle diameter (μm)</th>
<th>Atretic follicle diameter (μm)</th>
<th>CL diameter (μm)</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2/13 ± 73/04</td>
<td>3/97 ± 388/96</td>
<td>0/38 ± 5/20</td>
<td>64/144 ± 92</td>
<td>Control</td>
</tr>
<tr>
<td>Diabetic</td>
<td>2/07 ± 65/5</td>
<td>3/22 ± 370/88</td>
<td>0/3 ± 12/12</td>
<td>58/55 ± 92/56</td>
<td>Diabetic</td>
</tr>
<tr>
<td>Diabetes + Artemisia</td>
<td>4/28 ± 72/96</td>
<td>2/22 ± 391/04</td>
<td>0/62 ± 6/64</td>
<td>32/180 ± 758</td>
<td>Diabetes + Artemisia 200mg/kg</td>
</tr>
<tr>
<td>Diabetes + Artemisia</td>
<td>3/18 ± 74/8</td>
<td>1/62 ± 408</td>
<td>0/56 ± 8/03</td>
<td>75/016 ± 872/8</td>
<td>Diabetes + Artemisia 300mg/kg</td>
</tr>
<tr>
<td>Diabetes + metformin</td>
<td>4/09 ± 78/4</td>
<td>2/22 ± 390/56</td>
<td>0/44 ± 8/16</td>
<td>60/56 ± 870/8</td>
<td>Diabetes + Artemisia 300mg/kg</td>
</tr>
</tbody>
</table>

Discussion:

In this study, a significant decrease in blood glucose levels in the treated group compared to the diabetic group was soon, the results Tastekin [10] and colleagues in 2006, Joud [13] and colleagues in 2001, Eddouks [19] And colleagues in 2002 and Eidi [29] and colleagues in 1386 is consistent with most species of the genus Artemisia contains flavonoids seskoe terpene lactones, especially derivatives 11 and Persian date 13 Di Hydrolyseskoee terpenes, are 1 [1] the current use of medicinal plants containing flavonoids, due to their pharmacological properties and therapeutic for the treatment and prevention of diabetic complications, researchers have noted [29]. Flavonoid, polyphenols are a diverse biological activities such as nitric oxide and reduced performance of low-density lipoprotein and an increase in plasma antioxidant body [27]. So the above can be said that the mechanism of the plant Artemisia extract on blood glucose levels of insulin in the diabetic group receiving the extract is another mechanism that can be considered for these extracts are the reabsorption of glucose from the intestine, which may reduce or inhibit the uptake of glucose by the kidney tubules or by decreasing glucagon lowers blood glucose [10]. Another possible mechanism of increased sensitivity to insulin receptors and can also be considered such a mechanism similar to that of second-generation sulfonylureas such a mechanism has been shown from the plant Artemisia pallens [29]. Although the mechanism of action of metformin reduces glucose production in the liver by inhibiting gluconeogenesis No direct effect on insulin resistance or reduced insulin effectively reduce serum glucose levels [8,24]. In the present study the mean ovarian weight in diabetic rats compared to diabetic + plant extract (200 and 300 mg kg body weight), and diabetic + metformin significant increase that could possibly be due to the increased number of stromal cells , and the remaining follicles in the polycystic ovary syndrome or be diabetic [28]. Koudiner studies and colleagues (2006) Histopathological changes including polycystic ovary syndrome, and enlargement of the ovaries are ovaries in women with type 1 diabetes have been reported [9]. In a study in 1388 by Soleimani et Mehranjani diabetic mice was performed on a total volume of ovarian cortex and medulla increased significantly in the diabetic group than in the other groups [28], which results We are in agreement. In the present study a significant increase in the thickness of the germinal epithelium was observed that likely cause of persistent follicles and subsequent ovulation and ovarian volume is another reason is the increase in size of the ovary and Sekoulareh and creates congestion and cortical vasculature [28]. Artemisia plants with antioxidant compounds such as flavonoids types can reduce the adverse effects of free radicals. So that a significant reduction in ovarian volume in the metformin group receiving juice 200 and 300 mg / kg can be observed as compared to the diabetic group. The study Moghetti and colleagues in 2000 increased significantly after metformin ovulation has been reported [22]. Thus, metformin and Artemisia plant extracts can prevent the accumulation of follicles and ovulation increases ovarian volume is reduced. Grace and colleagues (1982) in a study to reduce the number of secondary follicles and increases the number of follicle atresia were reported in diabetic, Significant differences between the control group and the diabetic soon saw the number of corpus luteum in the ovary [12]. The results of the present study is similar in this study, a significant increase in the mean CL in the group treated diabetic as...
compared to controls was observed indicates that the process of ovulation in these groups is increasing. In this study, the significant reduction in the average primary follicles was observed in diabetic rats compared to other groups that Grace & Associates, which is consistent with the research of the Balstr Research (2007) can be said that serum LH and FSH levels in the diabetic animal models [5] and thus reduce estrogen and eventually slow the proliferation of granulose cells and unravel the layers. The change in ovarian function and pathological changes such as increasing and becoming Sekoulareh ovarian granulose cell necrosis, fibrosis and vacuolization so they are one of the causes of atretic follicles and pathological changes of diabetes based on the hypotalamus - pituitary - gonadal that low levels of gonadotropins and ovarian cell sensitivity to these hormones is [5]. The plant extract and metformin have a lower blood sugar and decrease lipid peroxidation and oxidation of DNA and proteins to prevent the primary follicle atresia [12]. In a study conducted in 2005 by Chanj and colleagues smaller diameter and size of the oocyte and ovarian follicle development in rats has been reported in diabetic rats [7]. The present study is similar, in this study, a significant difference in diameter antral follicles were observed in the groups tested with other studies, Grace and colleagues (1982) show the follicles which are more evolved or at a higher level of growth is more likely to stay healthy and continue their growth [12]. In this study, a reduction in the diameter of the corpus luteum in the diabetic + metformin was compared with the other groups Luteal cells is probably due to decreased lipid reserves because metformin addition to the above-mentioned effects and plasma leptin levels, reduce fat stores [25,26].

Conclusions:

Because of the complications of diabetes appear on the ovarian tissue is a cause of reduced fertility in females so the disease can be controlled with the reproductive capacity of women said. As the survey results indicate Hydroalcoholic extract of Artemisia could be somewhat similar to metformin significantly reduced the blood glucose and other complications of diabetes on female reproductive system created and can be used as a traditional medicine with fewer complications useful in improving diabetes.

References