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Investigating the Effects of Diabetes Mellitus on Several Biochemical Parameters and Histopathological Changes of Some Organs in Rats

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Abstract

One of the metabolic diseases that occurs frequently in elderly women is diabetes. In this study, the effects of diabetes on some biochemical parameters and histopathological changes in rats were investigated. In this experimental-interventional study, adult female rats were divided into four groups of 8. The first control group, the second ovariectomy group (for 55 weeks), the third induced diabetes group in the last 5 weeks of the study in mice with ovaries, and the fourth ovariectomy group (for 55 weeks) and induced diabetes in the last 5 weeks of the study. Pathological changes in lung, pancreas, kidney, and liver tissues and some serum biochemical indices were determined in each group at the end of the study. Diabetic and ovariectomized diabetic rats revealed an increase in blood glucose, alkaline phosphatase, alanine aminotransferase, gamma-glutamyl transferase, aspartate aminotransferase, urea, high-density lipoprotein, calcium, and triglyceride compared to the control group ($p < 0.05$). Ovariectomized rats showed an increase in cholesterol, low-density lipoprotein, high-density lipoprotein, lactate dehydrogenase, and triglyceride compared to the control group ($p < 0.05$). The findings revealed that the long-term reduction of estrogen in ovariectomized diabetic rats can have beneficial changes in reducing the serum levels of glucose, aspartate aminotransferase, alanine aminotransferase, urea, gamma-glutamyl transferase, very low-density lipoprotein, and triglyceride compared to non-ovariectomized diabetic rats.

Keywords: Diabetes mellitus, Biochemical parameters, Histopathological changes, Rats

Introduction

Diabetes Mellitus is one of the metabolic diseases in humans and animals that due to the decrease in insulin secretion from the beta cells of the Langerhans islets of the pancreas or due to the gradual development of insulin resistance, causes an increase in blood sugar (Alonso-Magdalena *et al*., 2008; Bakhshwin *et al*., 2022) and disturbances in carbohydrate, fat and protein metabolism (Banskota *et al*., 2006; Jiménez-Estrada *et al*., 2021). Estrogen is the main regulator of metabolic processes including body weight, glucose metabolism, fat tissue distribution, energy consumption, and fat absorption in women and men, and studies revealed that removing ovaries in rats can increase stress levels. Oxidative and acceleration of the aging process in different tissues (Behr *et al*., 2012). Estrogen and estrogen signals are among the most important regulators of blood glucose and play a role in maintaining insulin sensitivity. Fluctuations in estrogen levels below the physiological range as a result of menopause or ovariectomy may cause insulin resistance and facilitate type 2 diabetes (Banskota *et al*., 2006; Salehi *et al*., 2019).

Recently, it has been reported that estrogen receptors alpha and beta regulate glucose transport by glucose transporter 4 (GLUT4) and cause tissue sensitivity to insulin (Alonso-Magdalena *et al*., 2008; Sekido *et al*., 2019). Also, the short-term reduction of estrogen in ovariectomized rats causes changes in circulating insulin levels and sensitivity of insulin receptors, and this disorder in glucose homeostasis after eight weeks of ovariectomy in rats with significant changes in lipid profiles such as There was an increase in serum cholesterol, triglyceride, low-density lipoproteins and non-esterified fatty acids and a significant decrease in high-density lipoproteins, but it is not clear what effect postmenopausal estrogen has on carbohydrate metabolism (Zhang *et al*., 2002).

The role of ovarian hormones in glucose metabolism and insulin sensitivity is not well known (D'Eon *et al*., 2002; Liebmann *et al*., 2022). However, in a study, a decrease in insulin sensitivity was reported in the middle of the luteal phase compared to the middle of the follicular phase of the menstrual cycle and during the normal pregnancy period (Horton *et al*., 2002). Short-term use of estrogen replacement hormones in postmenopausal women with diabetes improved the level of glycosylated hemoglobin (Andersson *et al*., 1997). However, studies by Ferrara *et al*. did not show a difference between the levels of glycosylated hemoglobin in diabetic women treated with estrogen compared to untreated women (Ferrara *et al*., 2001; Tanaka *et al*., 2017).

Also, Godsland *et al*. (1993) studies showed that estrogen does not change fasting glucose levels, insulin levels, and increased insulin

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sensitivity in women after menopause. In a study, it was found that long-term use of estrogen in postmenopausal women may increase the risk of type 2 diabetes (Lau *et al*., 2021).

Okada *et al*. studies revealed that there is no significant difference in the level of glycosylated hemoglobin in Paleo women treated with replacement hormones compared to women who did not use replacement hormone therapy (Okada *et al*., 2003; Speksnijder *et al*., 2023). Due to the change in sedentary lifestyle, nutrition, genetics, etc., the incidence of diabetes has increased in society, and for women, diabetes may also occur with increasing age in addition to menopause. Despite the mentioned fact, little research has been done on the effect of diabetes on blood sugar, lipid profiles, and liver and kidney enzyme indices in long-term menopause. Therefore, the current study aimed to determine the impact of experimentally induced diabetes on blood sugar, lipid profiles, and enzyme indices of liver, kidney, and histopathological changes of pancreas, liver, and kidney in ovariectomized rats with long-term reduction of estrogen.

Materials and Methods

This experimental-interventional study was done on 32 female Wistar rats with an age range of 12 to 14 weeks and an average weight of 200 ± 200 grams over 55 weeks.

Study Groups, Ovariectomy, and Diabetes Induction in Rats

Female rats were randomly divided into 4 groups of eight (Dogru *et al*., 2012). Then, before surgery, female rats were anesthetized with a combination of xylazine and ketamine drugs at a dose of 80 and 7 mg per kilogram of body weight, respectively, intramuscularly, and after shaving the hair on the back surface of the back and cleaning with ethanol, shearing 20 mm length was created on the surface of the skin and 5 mm diagonal incisions were made on the muscles of the left and right testicles. Surgery to remove the ovaries was performed (Sadeghi *et al*., 2009). To induce experimental diabetes in ovariectomized and non-ovariectomized rats, a subcutaneous injection of alloxan (Sigma Aldrich, made in Germany) in the amount of 150 mg per kilogram of body weight in a physiological serum solution was performed. To make better conclusions and to deal with the competitive effect of glucose with alloxan, which might prevent the definitive effect of the drug on the beta cells of the pancreas, the intended injection was performed in each of the rats at 8:00 am and in the fasting state. After 72 hours of alloxan injection, a blood sample was provided from the tail vein of each rat, and their blood glucose levels were measured by a Glucocard made in Japan and their blood sugar was above 200 mg/dL. The criterion was their becoming diabetic (Unal *et al*., 2011).

In the first group, the surgical control group, the fat surrounding the ovary and the ovary was directed out and returned to its place, and the skin of the back surface of the waist was sutured with non-absorbable thread (Sadeghi *et al*., 2009) and for 55 weeks under standard conditions. In the second group, the ovariectomy group was kept for 55 weeks after surgery and removal of the ovary. The third group was the group of rats with ovaries that were kept for 55 weeks and became diabetic with alloxan in the last 5 weeks of the research period. The fourth group is the ovariectomy group and in the last 5 weeks of the research period, diabetics were treated with alloxan injection and kept under standard conditions.

Determination of Serum Enzymes

At the end of the 55th week, the mice were anesthetized with chloroform after fasting for 12 hours while they had access to water, and after opening the chest cavity, blood was taken from the heart. Blood was collected in tubes without anticoagulant and serum was separated by an H-11N centrifuge manufactured by KOKUSAN, Japan at 3000 rpm for 10 minutes. Serum blood sugar, AST (aspartate aminotransferase), ALT (alanine aminotransferase), creatinine, urea, CPK (creatine-phosphokinase), LDH (lactate-dehydrogenase) enzymes were measured by auto analyzer model 3000-BT manufactured by Biotecnica, Italy. Was performed. Serum concentrations of triglycerides, low-density lipoproteins, total cholesterol, and high-density lipoproteins were measured using recommended guidelines. Also, the amount of very low-density lipoproteins (VLDL) was determined using the Friedewald formula (Warnick *et al*., 1990) and plasma atherogenic index, cardiac risk, and atherogenic coefficient (Ikewuchi *et al*., 2014).

To study the tissue changes, immediately after blood collection, the tissues were placed in 10% buffered formalin for fixation, and according to the usual histological methods, the blocks of Paraffin were prepared and 5-micron thick sections were cut with a microtome and stained with hematoxylin and eosin in the common method.

Statistical Method

Based on the data collected using SPSS version 23 statistical software, the mean ± standard deviation was calculated. Data analysis was done with one-way analysis of variance and if there was a significant difference, the differences between the groups under study were compared using Tukey's test at a significant level of $P < 0.05$.

Results and Discussion

The amount of blood glucose in female diabetic rats was significantly higher than in diabetic ovariectomized rats ($P = 0.008$). The serum activity of gamma-glutamyltransferase, alanine aminotransferase, and aspartate aminotransferase was increased in female diabetic rats compared to rats in the surgical control group ($P < 0.05$). Also, serum alanine aminotransferase activity was increased in female diabetic rats compared to ovariectomized rats ($P = 0.036$). The amount of serum albumin in ovariectomized diabetic and diabetic rats was lower than that in rats of the surgical and ovariectomy control groups ($P = 0.05$). Alkaline phosphatase enzyme in female diabetic rats and ovariectomized diabetic rats showed a significant increase compared to the surgical control group ($P < 0.05$). Lactate dehydrogenase enzyme in ovariectomized and ovariectomized diabetic rats showed a significant increase compared to the surgical control group ($P = 0.001$) and the diabetic rat group ($P = 0.04$). Creatine phosphokinase enzyme in ovariectomized diabetic rats was more than the surgical control group ($P = 0.001$). The percentage ratio of liver weight to body weight in ovariectomized rats showed a

significant decrease compared to the surgical control group (P = 0.026). Also, the percentage ratio of liver weight to body weight in the ovariectomized diabetic and diabetic rats group was more than in the ovariectomized rats group (P = 0.05).

The amount of serum calcium in the group of diabetic and ovariectomized rats had a significant decrease compared to ovariectomized rats (P = 0.000). However, no significant change was observed compared to the surgical control group (P = 0.828). Also, the amount of serum calcium in ovariectomized diabetic rats showed a significant decrease compared to the sham group (P = 0.000). The amount of serum phosphorus in the ovariectomy group, diabetic ovariectomy group, and diabetic group did not show a statistically significant difference compared to the surgery control group (P>0.01). Body weight in ovariectomized rats was higher than in surgery control rats, diabetic rats, and diabetic ovariectomized rats (P = 0.002). The serum concentration of urea in female diabetic rats was higher than surgical control group and ovariectomized non-diabetic rats (P = 0.003) **(Table 1)**.

Table 1. Comparison of changes in serum urea, creatinine, and average kidney weight in the groups under study

Biochemical parameters \ Groups	Control	Ovariectomized female rat	Diabetic rat	Ovariectomized and diabetic female rats	P-value
Urea (mg/dl)	67.500±3.138^{c}	66.83±2.959^{c}	224±58.20^{a**}	145.600±16.154^{b}	0.001
Creatinine (mg/dl)	0.859±0.128^{a}	1.083±0.116^{a}	1.100±0.108^{a}	0.960±0.081^{a}	0.248
Percentage of weight of kidneys/body weight	0.749±0.018^{a}	0.614±0.033^{a}	1.065±0.077^{a}	0.922±0.109^{a}	0.300

Values are Mean ± SD. The number of samples in each group is 6 mice, non-similar letters (a, b, c) in each view of the table indicate a significant difference at the 95% confidence level.

** Statistically significant difference compared to the control group (P<0.01)

The concentration of serum creatinine in the rats of the experimental groups did not show a statistically significant difference compared to the surgical control group (P = 0.01) **(Table 1)**. The percentage ratio of kidney weight to body weight in diabetic female rats was more than the surgical control group (P = 0.022). Also, the average ratio of kidney weight to body weight in diabetic and ovariectomized rats showed a significant increase compared to the ovariectomized group (P = 0.001) **(Table 1)**. The amount of serum triglyceride in ovariectomized rats and diabetic rats showed a significant increase compared to the group of control rats (P < 0.01). Also, the number of triglycerides in diabetic female rats was more than that of the ovariectomized group (P = 0.002). However, the amount of triglyceride in ovariectomized diabetic rats was significantly lower than in diabetic rats (P = 0.000). The amount of serum cholesterol in ovariectomized rats was significantly higher than the surgical control group (P = 0.032) **(Table 2)**.

Table 2. Comparison of fat profiles in the studied groups.

Biochemical parameters \ Groups	Control	Ovariectomized female rat	Diabetic rat	Ovariectomized and diabetic female rats	P-value
Triglyceride (mg/dl)	42.833±3.33^{c}	104.500±9.704^{b**}	182.250±24.294^{a***}	84.00±7.463^{b}	0.000
Cholesterol (mg/dl)	53.66±5.684^{b}	84.33±7.596^{a}	63.750±4.697^{ab}	69.600±8.58^{ab}	0.053
HDL cholesterol (mg/dl)	42.43±7.681^{b}	87.300±12.107^{a}	46.775±6.144^{b}	51.42±6.48^{b}	0.008
LDL cholesterol (mg/dl)	19.833±2.243^{b}	37.316±4.260^{a*}	25.275±1.409^{b}	36.794±5.718^{a*}	0.027
VLDL (mg/dl)	8.56±0.666^{c}	20.90±1.940^{b}	36.45±4.857^{a}	16.8±1.492^{b}	0.000

Values are Mean ± SD. The number of samples in each group is 6 mice, non-similar letters (a, b, c) in each view of the table indicate a significant difference at the 95% confidence level.

** Statistically significant difference compared to the control group (P<0.01)

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The amount of high-density lipoprotein in the group of ovariectomized rats revealed a significant increase compared to the surgical control group (P = 0.016) **(Table 2)**. The amount of low-density lipoprotein in ovariectomized rats and diabetic ovariectomized rats revealed a significant increase compared to the surgical control group (P = 0.01) **(Table 2)**. Very low-density lipoproteins in diabetic rats revealed a significant increase compared to ovariectomized rats (P = 0.002) and rats of the surgical control group (P = 0.000). Also very low-density lipoproteins in ovariectomized rats, it was significantly higher than the surgical control group (P = 0.007) **(Table 2)**. The ratio of triglycerides to high-density lipoproteins in diabetic and ovariectomized diabetic rats was higher than in the surgical control group (P = 0.01) **(Table 3)**.

Table 3. Comparison of atherogenic indices in different groups under study.

Biochemical parameters \ Groups	Control	Ovariectomized female rat	Diabetic rat	Ovariectomized and diabetic female rats	P-value
TG/HDL	1.186±0.204[b]	1.259±0.136[b]	3.947±0.359[a**]	1.455±0.133[b]	0.000
HDL/LDL	2.338±0.192[a]	2.320±0.128[a]	1.887±0.312[b]	1.430±0.095[b]	0.004
HDL/Cholesterol	1.361±0.113[a]	0.969±0.0363[b*]	1.406±0.110[a]	1.362±0.048[a]	0.004
Atherogenic factor	0.325±0.106[a]	0.05±0.067[b]	0.407±0.112[a]	0.358±0.047[a]	0.011
Atherogenic index of plasma	0.033±0.0920[b]	0.088±0.044[b]	0.59±0.04[a]	0.22±0.023[b]	0.000

The ratio of high-density lipoproteins to low-density lipoproteins in the diabetic ovariectomized group was significantly lower than the surgical control group (P = 0.02). Also, the ratio of these two profiles in the diabetic ovariectomized group was significantly lower than the ovariectomized rats (P = 0.004). The ratio of cholesterol to high-density lipoproteins in ovariectomized rats was lower than in the surgical control group (P = 0.021). Also, this ratio was higher in diabetic rats than ovariectomized rats (P = 0.022). The ratio of cholesterol to high-density lipoproteins was higher in ovariectomized diabetic rats than in ovariectomized rats (P = 0.029) **(Table 3)**. The calculation of the plasma atherogenic index in female diabetic rats was significantly higher than in other groups (P = 0.00). Microscopic studies of the pancreatic tissue of the control group revealed the normal structure of the beta cells of the Langerhans islets. Inducing diabetes with alloxan in rats caused severe damage to beta cells and decreased the number of Langerhans islets and cell death. Pancreatic tissue in the ovariectomy group did not show any pathological changes.

The results of the current study showed that after intraperitoneal injection, alloxan monohydrate with a dose of 150 mg per kilogram of body weight induced diabetes and increased blood sugar in rats under study, which remained until the end of the study period. Also, the blood sugar of ovariectomized diabetic rats was significantly lower than the blood sugar of female diabetic rats. However, the absence of ovaries in female diabetic rats did not increase blood sugar compared to the diabetic group with ovaries. Experimental studies have shown that ovariectomy does not affect the glucose level in streptozotocin-induced diabetic rats, ovariectomy hardly affects glucose metabolism (Herrero *et al*., 1998), and ovariectomy of diabetic rats increases insulin resistance (Tawfik *et al*., 2015).

Insulin resistance is a condition in which the cells of the body do not respond properly to insulin, and as a result, the absorption of glucose is disrupted and, secondarily, it causes an increase in blood sugar (Pirola *et al*., 2004). However, the specific cellular mechanism behind the insulin resistance state and the role of the reduction of female sex hormones on blood glucose are not fully understood (Clegg *et al.*, 2006). The rapid absorption of alloxan by insulin-secreting cells (beta cells of pancreatic islets of Langerhans) and the production of oxygen free radicals cause islet necrosis in the pancreas. Compared to other tissues (such as the liver), beta cells are very sensitive to oxygen free radicals and cause a decrease in plasma insulin concentration. In ovariectomized rats, the ratio of liver weight to body weight was significantly reduced compared to other groups. The reason for this decrease can be irreversible damage such as necrosis and planned death (Unal *et al*., 2011). The second reason can be the increase in body weight of ovariectomized rats. Rearchers showed that in ovariectomized rats, food intake, body weight, and intra-abdominal fat accumulation increase, which is reversed by estradiol administration. Also, the lack of estrogen in women after menopause is one of the reasons for increasing visceral fat and insulin resistance and increasing the risk of cardiovascular diseases (Piche *et al*., 2005).

In the present study, changes in several lipid profiles including triglycerides, high-density lipoproteins, very low-density lipoproteins, the ratio of triglycerides to high-density lipoproteins, the cholesterol ratio to high-density lipoproteins, the atherogenic factor and index Atherogenicity was milder in ovariectomized rats than in diabetic rats. Changes in some lipid profiles including triglycerides of very low-density lipoproteins, the triglycerides ratio to very high-density lipoproteins, and atherogenic index were lower in long-term estrogen-reduced and diabetic rats than in non-depleted rats. Varicotomy was diabetic. The combination of ovariectomy and diabetes induction in rats did not have a significant impact on the weight of rats compared to the surgical control group. The histopathological results revealed that in diabetic rats, the increase in blood sugar is associated with the increase in degeneration and necrosis of liver hepatocytes, which can be due to the decrease in the activity of liver antioxidants. So the activity of serum alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) in the non-ovariectomized diabetic group was increased compared to the other groups under study. This finding is consistent with other reports; Chaudhry *et al*. (2007) reported that alloxan inhibits the antioxidant activity of superoxidase dismutase (SOD) and catalase, and liver dysfunction leads to the leakage of these enzymes from liver cells into the bloodstream.

Diabetic and ovariectomized rats had lower levels of serum albumin than rats of the ovariectomized and surgical control group, which can be justified due to changes in the cytoplasm and nucleus of liver tissue cells and damage to the granular endoplasmic reticulum structures. This is consistent with Nagae *et al*. (1991) studies. In the present study, the serum level of alkaline phosphatase enzyme in diabetic and ovariectomized diabetic rats was significantly higher than that in rats of the surgical and ovariectomized control group. Researchers have shown that increased serum levels of alkaline phosphatase are associated with liver, bone, and kidney disorders (Panteghini & Bais, 2007).

The findings of the current study showed that the induction of diabetes in rats is associated with an increase in the average kidney

weight to body weight, an increase in urea, and no change in serum creatinine. The increase in serum creatinine and urea in diabetic rats can be considered due to the increase in protein catabolism and as an indicator of kidney failure (Godsland *et al*., 1993; D'Eon *et al*., 2002). In this study, some lipid profiles in the groups under experimental study had significant changes, such that the amount of triglycerides, very low-density lipoproteins, and the triglycerides ratio to high-density lipoproteins in diabetic rats were It was significantly more diabetic than ovariectomized rats, which is a suitable predictive measure for coronary heart disease (Hokanson & Austin, 1996) and one of the main characteristics of insulin resistance and metabolic syndrome (Barzi *et al*., 2005).

In the present study, atherogenic indices were increased in diabetic rats. Atherogenic indices are predictors of heart diseases and cardiovascular complications that progress with the increase of this index (Frohlich & Dobiásová, 2003).

Conclusion

One of the metabolic diseases that occurs frequently in elderly women is diabetes. In this study, the effects of diabetes on some biochemical parameters and histopathological changes in rats were investigated. The findings showed that the long-term reduction of estrogen in ovariectomized diabetic rats can have beneficial changes in reducing the serum levels of glucose, aspartate aminotransferase, alanine aminotransferase, urea, gamma-glutamyl transferase, very low-density lipoprotein, and triglyceride compared to non-ovariectomized diabetic rats.

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