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The Protective Effect of Catechin on Fertility in Streptozotocin-Induced Diabetic Male Mice

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ABSTRACT

Background and Objective: Diabetes affects many physiological systems of the body and can lead to fertility disorders. Since catechin, as a strong antioxidant, can improve sperm parameters and increase fertility, this study was conducted to investigate the effect of catechin on oxidative stress, sperm parameters and in vitro fertilization (IVF) of diabetic mice.

Methods: In this experimental study, 48 adult male NMRI mice were divided into 6 groups of 8: oral control, injection control, diabetic, diabetic with a low dose of catechin (25 mg per kilogram of body weight), diabetic with a medium dose of catechin (50 mg per kilogram of body weight) and diabetic with a high dose of catechin (100 mg per kilogram of body weight). Diabetes was induced via intraperitoneal injection of streptozotocin (50 mg per kilogram of body weight) and after the 30-day treatment period, sperm parameters, testosterone hormone level, catalase level, total oxidative capacity, malondialdehyde and IVF were evaluated.

Findings: In diabetic mice, sperm count (28.63±1.24) and sperm motility percentage (53.47±1.46) and live sperms (55.96±1.34), as we as zygote (57.68±1.56), two-cell embryos (62.93±3.92), blastocysts (41.69±1.49) and hatched embryos (34.51±2.98) were significantly reduced compared to the control group ($p<0.05$). In the diabetic group, the percentage of immature sperm (13.41±0.30) and damaged DNA (8.35±0.42) increased. Administering catechin improved sperm parameters, fertilization rate and embryo growth.

Conclusion: Considering the harmful effects of diabetes on fertility, catechin can increase conception and fertility due to its antioxidant ability and inhibition of free radicals.

Keywords: *Diabetes Mellitus, Mice, Oxidative Stress.*

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Introduction

Diabetes is a chronic disease and one of the most common endocrine diseases in the body, which is often caused by the decrease in insulin secretion by the beta cells in the pancreatic islets of Langerhans (1). This disease causes dysfunction of the testicles and ovaries, including changes in estrous behavior, follicular growth, oocyte maturation, and reduced ovulation or anovulation (2). These changes are caused by disturbances in the axis of the hypothalamus, pituitary gland, and testes and ovaries, which leads to a decrease in the levels of sex hormones and a disturbance in the production of sex cells and fertility (3).

Research has shown that in diabetic rats treated with streptozotocin (STZ), due to disruption in spermatogenesis, the number and motility of sperms are greatly reduced (4). Several studies have shown that the increase in oxidative stress and the decrease in the levels of antioxidants are among the most important causes of the problems of diabetics (5) and the lack of definitive treatment of diabetes is associated with disorders in the reproductive system and sexual activities at diverse degrees in patients (6, 7).

Green tea (*Camellia sinensis*) has three major groups of polyphenols including catechins, theaflavins and thearubigins. Catechins are a type of antioxidant and are considered one of the most important flavonoids, and they comprise 25 to 35% of the dry weight of green tea (8). Based on previous studies, the use of green tea extract can improve the motility percentage, the normal morphology of the sperm, the diameter of the spermatogenic tubes, and the thickness of the germinal epithelium in rats consuming sodium arsenite (9). In addition, the use of green tea extract has increased the motility and number of sperms and the number of normal sperms following the induction of heat stress in rats due to the antioxidants present in it, especially catechins (10).

Considering that the effects of catechin on in-vitro fertilization (IVF) and embryo growth in mice have not been studied so far, in this research the effects of catechin on oxidative stress, sperm parameters and IVF in streptozotocin-induced diabetic mice.

Methods

In this experimental study, 48 male mice (NMRI) with an average weight of 26±2 grams and 8 weeks of age were obtained from the Laboratory Animal Center of the Faculty of Veterinary Medicine of Urmia University and kept under conditions of 12 hours of daylight and 12 hours of darkness at a temperature of 22±3 degrees Celsius, and fed with a special plate for mice. Water was also freely provided to them. This study was conducted based on the guidelines approved by the ethics committee of Urmia University with the code of ethics 3/Pad/67-IR-UU-AEC and all the ethical issues related to working with laboratory animals were observed. After a week of adaptation to the environment, the animals were randomly divided into 6 groups of 8:

Oral control group: In this group, the animals received citrate buffer (0.5 ml) orally (via gavage).

Injection control group: In this group, the animals received physiological serum (0.1 ml) via intraperitoneal injection.

Diabetic group: In this group, mice were made diabetic by intraperitoneal injection of STZ (50 mg per kg of body weight).

Diabetic group with low dose of catechin: STZ-induced diabetic mice received catechin at a dose of 25 mg per kilogram of body weight daily orally (gavage).

Diabetic group with medium dose of catechin: STZ-induced diabetic mice received catechin at a dose of 50 mg per kilogram of body weight daily orally (gavage).
Diabetic group with high dose of catechin: STZ-induced diabetic mice received catechin at a dose of 100 mg per kilogram of body weight daily orally (gavage). The treatment period was 30 days (11).

Diabetes was induced by intraperitoneal injection of 50 mg/kg body weight (Sigma, Germany) of STZ dissolved in citrate buffer. Blood glucose measurement was performed to detect the induction of diabetes using blood from the tail vein with the help of a glucometer kit 72 hours after STZ injection. Mice with blood sugar more than 250 mg per deciliter were considered as diabetic. All mice in all 6 groups were anesthetized by intraperitoneal injection of ketamine and xylazine 24 hours after the last treatment and then euthanized by cervical vertebrae displacement (12).
Preparation of sperm from the epididymal tail: after making an incision in the abdomen, the epididymal tail was separated from the testicle and after making several cuts in it, it was transferred to a sterile falcon tube containing one milliliter of human tubal fluid (HTF) culture medium and 4 mg bovine serum albumin (BSA). Then, it was transferred to an incubator with 5% CO2 and a temperature of 37 °C. After one hour, the extracted sperms were used to evaluate sperm parameters and IVF (13).
Evaluation of sperm motility: After extracting sperms from the epididymal tail, 10 µL of the diluted solution (1:20) containing sperms was placed on a slide and the sperms were examined for their motility percentage. In order to calculate the motility percentage, 10 light microscope fields at 400X magnification were examined on the slide and the average of all moving sperms in 10 light microscope fields was expressed as the motility percentage (13).
Evaluation of sperm viability: Eosin-Nigrosin staining was used to evaluate live sperms and distinguish live sperms from dead ones. 20 µL of the sperm sample that was prepared from the tail of the epididymis was placed on a clean slide, and then 20 µL of Eosin were added to it, and after 5 seconds, 50 µL of Nigrosin were added. They were then mixed together and expanded. After drying the slide, the percentage of live and dead (stained) sperm was calculated using an optical microscope at 400X magnification (13).
Assessment of sperm nucleus maturity: Aniline blue staining was used for this purpose. After preparing the spread (5 µL) from the sperm extracted from the tail of the epididymis, the slides were dried in air and the samples were fixed with 3% glutaraldehyde fixing solution in 0.2 M phosphate buffer (pH=7.2). The samples were stained by 5% aniline blue solution combined with 4% acetic acid (pH=3.5) for 5 minutes. Then, the slides were washed and examined with a light microscope with a 100X objective lens. 100 sperm were counted from each slide and the results were expressed as percentage. Immature sperms were visible in blue color and mature sperms were visible with less staining (14).
Evaluation of sperm DNA damage: acridine orange staining was used for this purpose. In this staining, when examining the slides with a fluorescent microscope, healthy DNA is seen in green, discontinuous double-stranded DNA is seen in yellow, and single-stranded DNA is seen in red. After preparing the spread from the sperms that were extracted from the epididymal region and drying in air, the samples were fixed by Carnoy's solution for at least 2 hours, and then acridine orange dye, with a concentration of 0.19 mg in citrate phosphate buffer was used for staining for 10 minutes. After washing, the slides were examined by a fluorescent microscope in a dark environment with a filter of 450-490 nm and the results were expressed in percentage (15).
Oocyte retrieval and IVF: In order to stimulate ovulation in adult mice, 10 international units of Pregnant Mare Serum Gonadotropin (PMSG) were injected intraperitoneally. After 48 hours, human chorionic gonadotropin (HCG) in 10 international units was injected intraperitoneally. 10 to 12 hours after HCG injection, mice were euthanized after anesthesia with ketamine-xylazine. After opening the abdominal area, the oviducts were separated from the mice and placed in the HTF culture medium. Then, using a 28-gauge

insulin syringe, oocysts were taken from the oviduct area under a stereomicroscope along with the cumulus mass. After washing, the eggs were placed in drops of HTF fertilization culture medium containing bovine serum albumin (BSA) under mineral oil. The isolated sperms of all groups were added separately to one million per milliliter of culture medium after undergoing the capacitation process. Fertilization took place about 4 to 6 hours after adding sperm. After this period, the number of zygotes formed in each group was checked and expressed as percentage of fertilization in each group. Examination of the percentage of two-cell embryos 24 hours after fertilization, as well as the percentage of morula, developed blastocysts and hatched embryos on day 5 after fertilization were evaluated (16, 17).

Testosterone serum concentration measurement: To evaluate the testosterone level, blood samples were taken from the hearts of mice, then centrifuged (3000 rpm in 5 minutes) and serum samples were prepared, and the level of testosterone hormone in the serum was measured by radioimmunoassay technique using a kit (Pishtaz Teb, Iran) and 2010 Elecsys device (18).

Measuring the amount of malondialdehyde (MDA) in the testicular tissue: after the mice were euthanized, the testicular tissue was separated and kept at -80 °C until the experiments. At the time of the experiment, the frozen testicular tissues were homogenized in 0.05 M phosphate buffer at 0 °C with a pH=4.7 and a concentration of 10% (W/v). Then, the obtained solutions were centrifuged at 1000g and the supernatant was used to measure the amount of lipid peroxidation products. The amount of lipid peroxidation was determined based on the formation of MDA. 150 µL of the homogenized tissue was added to 300 µL of 10% trichloroacetic acid and centrifuged for 10 minutes at 1000g at 4 °C. 300 µL of 0.67% thiobarbituric acid (TBA) was added to 300 µL of the supernatant and boiled for 20 minutes in a bain-marie. After the cooling of the solution, a pink color appeared due to the reaction of MDA with TBA and the absorbance of the pink solution was read at the wavelength of 535 nm. The amount of MDA was calculated using the absorption coefficient of malondialdehyde and was expressed as micromoles per gram of tissue (19).

Measurement of total antioxidant capacity (TAC) of testicular tissue: TAC evaluation based on the ability of plasma to reduce Fe^{3+} ion to Fe^{2+} was measured at a wavelength of 593 nm and the data were expressed in nanomoles per milligram of protein (20).

Measurement of catalase (CAT) enzyme activity of testicular tissue: CAT enzyme activity was determined based on its ability to break down H_2O_2 in homogenized testicular tissue. For this purpose, 30 mM hydrogen peroxide was used as a substrate and 50 mM phosphate buffer (pH=7) was used as a substrate substitute in the blank solution. The assay solution contained 2 ml of tissue homogenous solution and 1 ml of hydrogen peroxide solution. The reaction was started by adding H_2O_2, the decrease in absorbance was checked using a spectrophotometer at a wavelength of 240 nm for 30 seconds, and the values were expressed in units per gram of tissue (21).

Statistical analysis: In this research, SPSS version 21 was used for statistical analysis of data. The data were analyzed by one-way analysis of variance and Tukey's test, and $p<0.05$ was considered significant.

Results

The number of epididymal tail sperms: the number of epididymal tail sperms in the diabetic group (28.63±1.24) showed a significant decrease compared to the oral control (56.22±1.66) and injection (57.56±0.99) control groups ($p<0.05$). In the diabetic groups, with all three doses of catechin, an increase in the number of sperms was seen, but this increase showed significant difference only in the doses of 50 mg (34.25±0.44) and 100 mg (35.27±0.43) of catechin compared to the diabetic group ($p<0.05$) (Table 1).

Sperm viability: the percentage of viable sperms in the diabetic group decreased (55.96±1.34) and there was a significant difference compared to the oral (95.23±0.60) and injection (95.06±0.98) control groups ($p<0.05$). In the diabetic groups with all three doses of catechin, an increase in the number of live sperm was observed, but this increase showed a significant difference only in the dose of 100 mg of catechin (81.65±1.34) compared to the diabetic group ($p<0.05$). Also, a significant difference was observed between the diabetic group with catechin dose of 100 mg compared to the diabetic groups with catechin dose of 50 mg (63.93±0.53) and 25 mg (65.93±2.83) ($p<0.05$) (Table 1).

Sperm motility: in the diabetic group (53.47±1.46), the percentage of sperm motility significantly decreased compared to the oral (93.29±1.94) and injection (92.2±1.60) control groups ($p<0.05$). In addition, the sperm motility rate in diabetic groups receiving catechin at doses of 25 mg (59.88±0.83), 50 mg (64.91±1.30) and 100 mg (65.58±0.99) compared to the diabetic group showed a significant increase ($p<0.05$), but there was no significant difference between the diabetic groups with catechin in all three doses (Table 1).

Sperm nuclear maturation and the number of immature sperms: in diabetic rats (13.41±0.30), the percentage of immature sperms compared to oral (6.16±0.44) and injection (5.42±0.56) control groups showed a significant increase ($p<0.05$). In the diabetic groups with catechin in all three doses of 25, 50 and 100 mg, a decrease in the percentage of immature sperm was seen, but only in the diabetic groups with catechin at doses of 50 mg (10.54±0.35) and 100 mg (11.07±0.73), this decrease revealed a significant difference compared to the diabetic group ($p<0.05$). No significant difference was observed between the diabetic group and the diabetic group with catechin at a dose of 25 mg (Table 1).

Sperms with damaged DNA: the increase in the percentage of sperms with damaged DNA in the diabetic group (8.35±0.42) showed a significant difference compared to the oral (3.51±0.46) and injection (3.40±0.16) control groups ($p<0.05$). While there was a decrease in the number of sperms with damaged DNA, no significant difference was observed in the diabetic groups with catechin in all three doses of 25, 50 and 100 mg compared to the diabetic group. Although the reduction in the number of sperms with damaged DNA was greater in the doses of 50 and 100 mg compared to the dose of 25 mg, it did not show a significant difference (Table 1).

Table 1. Mean parameters of epididymal tail sperms in different experimental groups

Groups	Sperm count ($\times 10^6$)	Sperm motility percentage Mean±SD	Percentage of live sperm Mean±SD	Percentage of immature sperm Mean±SD	Percentage of sperm with damaged DNA Mean±SD
Oral control	56.22±1.66^{a}	93.29±1.94^{a}	95.23±0.60^{a}	6.16±0.44^{a}	3.51±0.46^{a}
Injection control	57.56±0.99^{a}	92.23±1.60^{a}	95.06±0.98^{a}	5.42±0.56^{a}	3.40±0.16^{a}
Diabetic	28.63±1.24^{b}	53.47±1.46^{b}	55.96±1.34^{b}	13.41±0.30^{b}	8.35±0.42^{b}
Diabetic+catechin 25 mg	29.62±0.83^{b}	59.88±0.83^{c}	65.93±2.83^{b}	12.55±0.32^{bc}	7.57±0.25^{b}
Diabetic+catechin 50 mg	34.25±0.44^{c}	64.91±1.30^{c}	63.93±0.53^{b}	10.54±0.35^{c}	6.72±0.27^{b}
Diabetic+catechin 100 mg	35.27±0.43^{c}	65.58±0.99^{c}	81.65±1.34^{c}	11.07±0.73^{c}	6.77±0.26^{b}

Different letters (a, b, c) indicate significant differences in each column between different groups.

Testosterone serum level: The amount of testosterone hormone decreased significantly in the serum of diabetic mice (0.30±0.11) compared to oral (0.51±0.01) and injection (0.49±0.26) control groups. However, in diabetic mice with catechin in doses of 50 mg (0.38±0.02) and 100 mg (0.39±0.02) of catechin, an increase in the amount of testosterone hormone was seen, which was a significant difference compared to the diabetic group ($p<0.05$) (Table 2).

CAT level of testicular tissue: the level of CAT activity in the testicular tissue in the diabetic group (319.28±15.05) decreased significantly compared to the oral (495.34±4.15) and injection (485.14±7.68) control groups ($p<0.05$). There was an increase in the amount of CAT in diabetic groups with all three doses of catechin, and this increase was significantly different only in the diabetic group with 50 mg of catechin (371.52±9.46) compared to the diabetic group ($p<0.05$) (Table 2).

The amount of TAC of testicular tissue: the mean level of TAC of testicles decreased significantly in the diabetic group (2.20±0.23) compared to the oral (3.40±0.10) and injection (3.55±0.34) control groups ($p<0.05$). In the diabetic groups receiving catechin in all three doses of 25, 50 and 100 mg, there was an increase in TAC level and this increase in the catechin 50 mg (2.98±0.06) and 100 mg (2.90±0.12) showed a significant difference compared to the diabetic group ($p<0.05$) (Table 2).

MDA level of testicular tissue: the mean level of MDA of testicular tissue revealed that there was a relative decrease in the diabetic group receiving catechin in all three doses; this decrease was significant in the groups of 50 mg (16.83±0.15) and 100 mg (16.77±0.22) catechin compared to the diabetic group (17.76±0.26) ($p<0.05$) (Table 2).

Table 2. Mean biochemical parameters measured in serum and testicular tissue in different experimental groups

Groups	CAT (µ/mg) Mean±SD	MDA (µmol/gr tissue) Mean±SD	TAC (nmol/mg.protein) Mean±SD	Testosterone (ng/ml) Mean±SD
Oral control	495.34±4.15^{a}	15.79±0.30^{a}	3.40±0.10^{a}	0.51±0.01^{a}
Injection control	485.14±7.68^{a}	15.48±0.39^{a}	3.55±0.34^{a}	0.49±0.26^{a}
Diabetic	319.28±15.05^{b}	17.76±0.26^{b}	2.20±0.23^{b}	0.30±0.11^{b}
Diabetic+catechin 25 mg	303.32±4.04^{bc}	17.01±0.22^{cb}	2.83±0.06^{cb}	0.32±0.02^{cb}
Diabetic+catechin 50 mg	371.52±9.46^{c}	16.83±0.15^{c}	2.98±0.06^{c}	0.38±0.02^{c}
Diabetic+catechin 100 mg	331.96±7.63^{bc}	16.77±0.22^{cb}	2.90±0.12^{c}	0.39±0.02^{c}

Different letters (a, b, c) indicate significant differences in each column between different groups.

Fertilization rate: The percentage of fertilization in the group that was affected by diabetes (57.68±1.56) had a significant decrease compared to the oral (92.19±1.27) and injection (93.43±0.90) control groups ($p<0.05$). The percentage of fertilization in the diabetic group with catechin increased in all three doses, and this increase was significant in the dose of 50 mg (72.70±2.62) and 100 mg (71.73±3.93) of catechin compared to the diabetic group. No significant difference was observed between the dosages of catechin in diabetic groups ($p<0.05$) (Figure 1 and Table 3).

Two-cell embryos: in the diabetic group (62.93±3.92), the percentage of two-cell embryos decreased significantly compared to the oral (87.23±3.36) and injection (89.06±2.30) control groups ($p<0.05$). The increase in the percentage of two-cell embryos in the diabetic group with catechin at doses of 50 mg (68.46±1.85) and 100 mg (42.1±1.53) also showed a significant difference compared to the diabetic group ($p<0.05$) (Figure 1 and Table 3).

Morula and blastocyst stage embryos: The percentage of morula (56.92±3.02) and blastocyst (41.69±1.49) in the diabetic group showed a significant decrease compared to the control groups ($p<0.05$). In the diabetic group with catechin in the dose of 50 mg, morula percentage (63.79±2.92) and blastocyst percentage (53.50±2.61) and in the dose of 100 mg, morula percentage (63.24±1.89) and blastocyst percentage (55.06±2.54) showed a significant increase compared to the diabetic group ($p<0.05$) (Figure 1 and Table 3).

Hatched embryos: the percentage of hatched blastocysts (34.51±2.98) in the diabetic group showed a significant decrease compared to the oral (64.16±0.18) and injection (65.18±1.09) control groups ($p<0.05$). In the diabetic group with catechin in all 3 doses, the percentage of hatched embryos increased, but it did not show a significant difference compared to the diabetic group. Also, no significant difference was observed between different doses of catechin (Figure 1 and Table 3).

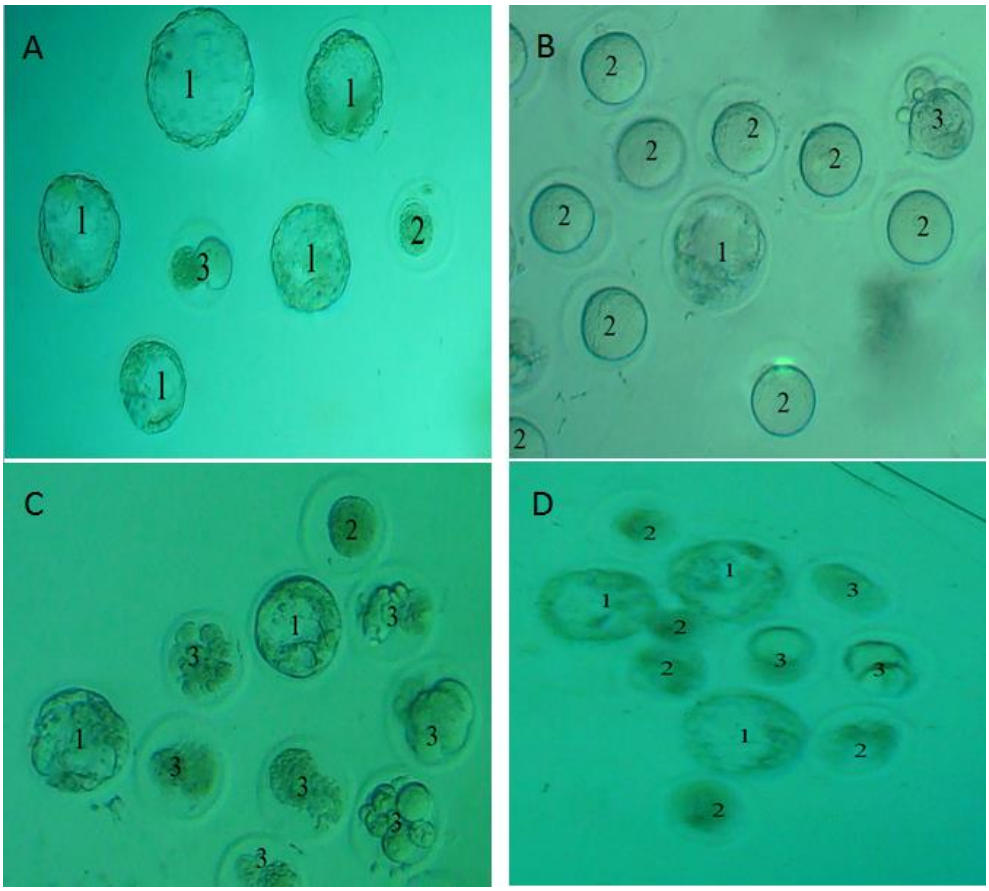

Figure 1. Fetal growth on day 5 after fertilization in different experimental groups. Blastocyst stage embryos (1), unfertilized oocytes (2) and embryonic arrest (3) in control group (A), diabetic group (B), diabetic+low dose catechin group (C) and diabetic+medium dose catechin group (D) (200x magnification).

Table 3. The mean results obtained from the evaluation of in vitro fertilization in different experimental groups

Groups	Fertilization percentage Mean±SD	Percentage of two-cell embryos Mean±SD	Percentage of morula stage embryos Mean±SD	Blastocyst percentage Mean±SD	Percentage of hatched embryos Mean±SD
Oral control	92.19±1.27[a]	87.23±3.36[a]	78.44±1.12[a]	74.73±1.62[a]	64.16±0.18[a]
Injection control	93.43±0.90[a]	89.06±2.30[a]	81.83±1.89[a]	76.33±1.64[a]	65.18±1.09[a]
Diabetic	57.68±1.56[b]	62.93±3.92[b]	56.92±3.02[b]	41.69±1.49[b]	34.51±2.98[b]
Diabetic+catechin 25 mg	59.25±1.83[bc]	63.55±3.15[bc]	59.30±3.20[bc]	45.17±0.20[bc]	37.52±1.53[b]
Diabetic+catechin 50 mg	72.70±2.62[c]	68.46±1.85[c]	63.79±2.92[c]	53.50±2.61[c]	43.22±2.91[b]
Diabetic+catechin 100 mg	71.73±3.93[c]	71.42±1.53[c]	63.24±1.89[c]	55.06±2.54[c]	43.39±4.59[b]

Different letters (a, b, c) indicate significant differences in each column between different groups.

Embryonic arrest: the percentage of embryonic arrest in the diabetic group (51.76±13.41) significantly increased compared to the oral (16.64±2.10) and injection (16.72±2.97) control groups ($p<0.05$). In the groups receiving catechin in all three doses 25 mg (28.59±3.68), 50 mg (28.30±4.13) and 100 mg (0.94±2.71), the reduction of embryonic arrest showed a significant difference compared to the diabetic group ($p<0.05$). However, no significant difference was observed between all three doses of catechin. The percentage of type 1 embryonic arrest increased in the diabetic group (42.06±4.82) and compared to the oral (23.33±1.66) and injection (19.76±3.09) control groups, the difference was significant ($p<0.05$). However, only in the group receiving catechin in a dose of 100 mg (18.05±3.67), there was a significant difference in the number of type 1 embryonic arrest compared to the diabetic group ($p<0.05$). There was no significant difference in the percentage of type 2 embryonic arrest between the studied groups. The percentage of type 3 embryonic arrest in the diabetic group decreased (24.20±5.51) and compared to the oral (53.33±3.33) and injection (55.71±2.97) control groups, the difference was significant ($p<0.05$). Despite the increase in the percentage of type 3 embryonic arrest in all three doses of catechin, only in the dose of 100 mg (55.55±5.55), a significant difference was observed compared with the diabetic group ($p<0.05$) (Figure 1 and Table 4).

Table 4. Mean quality of embryonic arrest in different experimental groups

Groups	Percentage of embryonic arrest Mean±SD	Percentage of type 1 embryonic arrest Mean±SD	Percentage of type 2 embryonic arrest Mean±SD	Percentage of type 3 embryonic arrest Mean±SD
Oral control	16.64±2.10[a]	23.33±1.66[a]	23.33±1.66[a]	53.33±3.33[a]
Injection control	16.72±2.97[a]	19.76±3.09[a]	24.52±2.48[a]	55.71±2.97[a]
Diabetic	51.76±13.41[b]	42.06±4.82[b]	33.72±5.15[a]	24.20±5.51[b]
Diabetic+catechin 25 mg	28.59±3.68[c]	28.96±2.41[ba]	52.38±2.38[b]	18.64±3.25[b]
Diabetic+catechin 50 mg	28.30±4.13[c]	31.11±5.87[ba]	31.11±5.87[a]	37.77±2.22[b]
Diabetic+catechin 100 mg	22.94±2.71[c]	18.05±3.67[ca]	26.38±6.05[a]	5.55±5.55[ca]

Different letters (a, b, c) indicate significant differences in each column between different groups.

Discussion

The present study showed that diabetes, with its adverse effect on sperm parameters, decreases fertility, while catechin increases the fertility and quality of embryos, by reducing oxidative stress and improving sperm parameters. Despite the decrease in the amount of testosterone hormone in diabetic mice, catechin as an antioxidant in diabetic mice increased the amount of testosterone, which plays a very important role in sperm production and fertility. Considering the importance of testicles in the production of testosterone and sperm, the testicles are one of the organs whose structure is affected by diabetes and ultimately the individual's fertility is disturbed (22).

Diabetes can reduce or stop the secretion of gonadotropins and cause disruption in the production of sperm or eggs and inhibition of reproduction. The two hormones LH and FSH, along with testosterone, play a very important physiological role in maintaining the continuity of ovulation and sperm production. The increase in oxidative stress and the decrease in the levels of antioxidants are among the important causes of the problems diabetic people experience (23), and through the production and increase in reactive oxygen species (ROS), lead to the development of insulin resistance and type 2 diabetes (24). Furthermore, antioxidants with different chemical properties can strengthen each other in the antioxidant network and improve intracellular antioxidant defense by inducing antioxidant enzymes (25).

Research has shown that the induction of experimental diabetes by STZ in rats causes a decrease in testosterone levels compared to the control group, while some studies have reported that following experimental diabetes, testosterone levels in rats remain unchanged (26). In this study, the amount of CAT and TAC decreased in diabetic mice, while the amount of MDA increased. Previous research has shown that free radicals produced in diabetic mice are important causes of fetal arrest and cell death (27). In addition, it has been reported that the developmental arrest of two-cell embryos occurs after the increase in lipid peroxidation (28).

Much evidence indicates the significant role of oxidative stress in infertility. In the present study, the number of sperm and the percentage of sperm motility and live sperm decreased, while the percentage of immature sperm and damaged DNA increased in the diabetic group. Furthermore, the percentage of fertilization and two-cell embryos, morula, blastocyst and hatched embryos decreased in diabetic mice. The percentage of embryonic arrest also increased in the diabetic group. Since oxidative stress is strongly related to diabetes and its complications, antioxidants can play an important role in protecting the body against diabetes. Various studies have shown that antioxidants can improve the quality and quantity of sperm and eggs. The destructive effects of free radicals are controlled or inhibited by the intracellular antioxidant system such as glutathione, ascorbic acid and enzymes including superoxide dismutase, catalase and glutathione peroxidase (29). Laboratory investigations have confirmed the role of antioxidants in reducing the production of ROS and improving fetal development (30). Research has also shown that food additives with antioxidant properties improve the quality of fertilization and increase the maturation of oocytes in vitro (31).

Recent research has shown that the use of green tea increases the activity of glutathione peroxidase and catalase enzymes in mice receiving thioacetamide. The increase in the activity of antioxidant enzymes in mice receiving green tea indicates its antioxidant property, which is attributed to the catechin present in green tea (32). In mice with hypertriglyceridemia and hypercholesterolemia, receiving green tea decreased the serum levels of liver enzymes, increased the activity of antioxidant enzymes, and decreased the amount of lipid peroxidation in the liver tissue (33).

By inhibiting the accumulation of free radicals, catechin prevents the spread of lipid peroxidation and reduces the serum level of MDA (34). In the present study, it was found that catechin consumption in diabetic rats decreased the amount of MDA in testicular tissue and also increased the amount of CAT and

TAC. In the groups receiving catechin, the concentration of testosterone increased and sperm count, sperm motility and live sperms increased. In addition, the percentage of immature sperms and sperms with damaged DNA decreased and the percentage of fertilization and two-cell embryos, morula, blastocyst and hatched embryos increased.

The results of this study showed that diabetes causes oxidative stress by disturbing the oxidation-reduction balance and increases the damage to the testicular tissue and reduces the rate of fertility and conception and embryo production. At the same time, catechin, due to its strong antioxidant properties, improves the disorders caused in the testicles under the influence of diabetes and increases fertility by inhibiting free radicals.

Conflict of interest: In this research, no conflict of interest is reported by the authors.

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