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The effects of coadministration of curcumin and vitamin E on the reproductive system of diabetic male rats; An experimental study

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ABSTRACT

Introduction: Diabetes causes testicular damage due to oxidative stress. Nowadays, the use of vitamins and antioxidants is one of the common methods to treat this disease. Therefore, the aim of this study is to investigate the effect of single and combined administration of these two substances on the reproductive system of male diabetic rats.

Method and materials: In this study, 48 adult male Wistar rats weighing 250–270 grammes were divided into 6 groups: Control group, diabetic group, vehicle group, vitamin E, curcumin group, and vitamin E and curcumin group. The control group was the healthy group, and in the other groups, the rats were made diabetic by streptozotocin (60 mg/kg/ip). The vehicle group received 1 ml of olive oil, the vitamin E group (100 mg/kg/ip) received Vit.E, and the curcumin group (50 mg/kg/ip) received Cu. The group of rats received vitamin E and curcumin. At the end of the sixth week after treatment, blood was taken from the rats and biochemical analysis was performed to check the amount of malondialdehyde (MDA), LH hormones and serum testosterone, then the rats were killed and their testes and epididymides were removed. The weight of the testes and sperm parameters, the maturity of sperm nuclei and the integrity of their DNA were checked. The number of spermatogenic cells was determined by histological examination.

Results: This study showed that diabetes caused a decrease in testicular weight, sperm count, motility, and viability, an increased percentage of sperm with immature nuclei, and an increased percentage of sperm with denatured DNA. In addition, diabetes decreased the average number of matogenic sperm, and biochemical results showed that diabetes increased the level of MDA and decreased the level of the hormones LH and testosterone. Treatment with vitamin E, curcumin and their combination improved all these parameters, and this improvement was significant in the Toam group.

Conclusion: Combined administration of vitamin E and curcumin in diabetic rats significantly improves sperm parameters, matogenic sperm count, and improves MDA levels, LH, and serum testosterone compared with separate treatment.

1. Introduction

Diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia, poses a significant health burden worldwide [1,2]. It affects multiple organ systems, including the reproductive system, leading to impaired reproductive function and fertility issues in both males and females [3]. Among the complications associated with diabetes, reproductive dysfunction has gained considerable attention due to its profound impact on the quality of life and overall well-being of individuals affected [3–5].

In recent years, extensive research has focused on investigating the potential therapeutic interventions to mitigate the detrimental effects of

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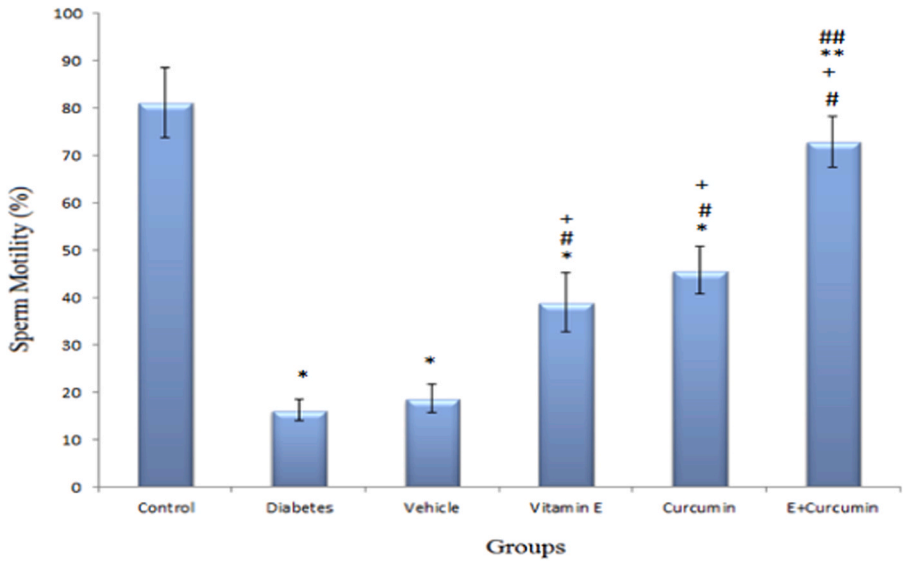

Chart 2. The average percentage of sperm motility. Significant difference with the control group at the 0.05 level *. Significant difference with vehicle group at 0.05 level +. Significant difference with diabetes group at 0.05 level #. Significant difference with vitamin E group at 0.05 level **. Significant difference with Curcumin group at 0.05 level ##.

diabetes on reproductive health [5]. In this regard, natural compounds and dietary supplements have emerged as promising candidates for adjunctive therapy in diabetes-related complications [4]. Two such compounds, curcumin and vitamin E, have gained attention for their potential protective effects against diabetic reproductive impairments [4,5].

Curcumin, a polyphenolic compound derived from the rhizomes of Curcuma longa, possesses potent antioxidant, anti-inflammatory, and anti-diabetic properties [6]. It has been widely studied for its diverse pharmacological effects and has shown promising results in ameliorating diabetic complications in various organ systems. Similarly, vitamin E, a lipid-soluble antioxidant, has been recognized for its protective role against oxidative stress-induced damage [7].

Considering the growing body of evidence on the individual benefits of curcumin and vitamin E in diabetes management, the exploration of their combined effects on the reproductive system of diabetic male rats is of significant interest [6]. Understanding the potential synergistic effects of these compounds may provide valuable insights into the development of novel therapeutic strategies to combat diabetes-associated reproductive impairments [7].

The reproductive system of males is particularly susceptible to the detrimental effects of diabetes, including impaired spermatogenesis, decreased sperm quality, and altered hormonal balance [6]. These alterations can ultimately lead to reduced fertility and reproductive dysfunction [7]. However, the exact mechanisms underlying the impact of diabetes on the male reproductive system and the potential protective effects of curcumin and vitamin E remain areas of active investigation [8].

Novel coronavirus has caused global concern due to its emergence and being unknown as well as its high mortality rate [9–11]. In this situation, the main responsibility of the medical staff is to implement strategies to adapt to these stressful conditions [12–14]. This study aims to evaluate the effects of co-administration of curcumin and vitamin E on the reproductive system of diabetic male rats. By examining various parameters such as sperm count, motility, morphology, hormonal levels, and testicular histology, we seek to elucidate the potential protective mechanisms of these compounds in mitigating diabetes-induced reproductive dysfunction.

Overall, this research endeavors to shed light on the potential benefits of co-administering curcumin and vitamin E in ameliorating the detrimental effects of diabetes on the male reproductive system. The outcomes of this study may provide valuable insights into the development of novel therapeutic approaches to address reproductive impairments in diabetic individuals, thereby improving their quality of life and reproductive well-being.

2. Material and method

2.1. Sampling and intervention

A total of 60 rats were randomly selected and divided into six groups, each consisting of 10 animals. The groups were as follows: Control group (C) (n = 10), which received an intraperitoneal injection of 1 ml of olive oil. Diabetic group (D) (n = 10), in which rats were induced with diabetes by intraperitoneal injection of streptozotocin (STZ) at a dosage of 60 mg/kg. Vehicle group (V) (n = 10), in which rats were induced with diabetes using STZ and received an intraperitoneal injection of 1 ml of olive oil. Curcumin (Cu) group (n = 10), in which rats were induced with diabetes using STZ and received an intraperitoneal injection of curcumin at a dosage of 50 mg/kg. Vitamin E group (n = 10), in which rats were induced with diabetes using STZ and received an intraperitoneal injection of vitamin E at a dosage of 100 mg/kg. Vitamin E and curcumin group (E + Cu) (n = 10), in which rats were induced with diabetes using STZ and received intraperitoneal injections of both vitamin E (100 mg/kg) and curcumin (50 mg/kg). The treatment groups received their respective doses three times weekly for a duration of four weeks (28 days) [15–17].

For the induction of diabetes and treatment groups, STZ (60 mg/kg) dissolved in 0.1 M citrate buffer was injected intraperitoneally [18]. After eighteen hours, blood glucose levels were measured using a glucometer and tail puncture method. Rats with blood glucose levels exceeding 300 mg/kg were considered diabetic and included in the study.

On day 42 after treatment, the rats were euthanized with a double injection of ketamine (160 mg/kg), and their testes and epididymides were surgically removed. The caudal part of the epididymis was used for evaluating sperm parameters, the right testis for histological studies, and the left testis for biochemical analysis.

2.2. Spermatozoa preparation and counting

On day 42 after treatment, rats were euthanized by intraperitoneal

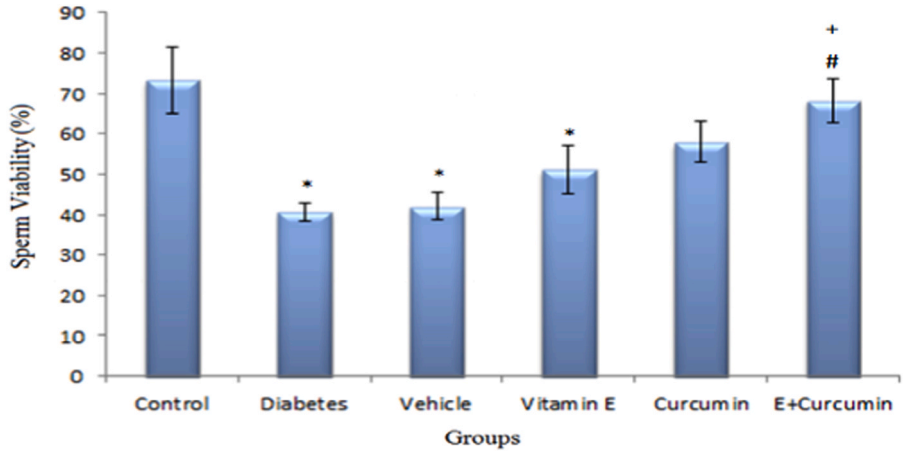

Chart 3. The average percentage of live sperm (viability) in the studied groups. Significant difference with the control group at the 0.05 level *. Significant difference with diabetes group at 0.05 level #. Significant difference with vehicle group at 0.05 level +.

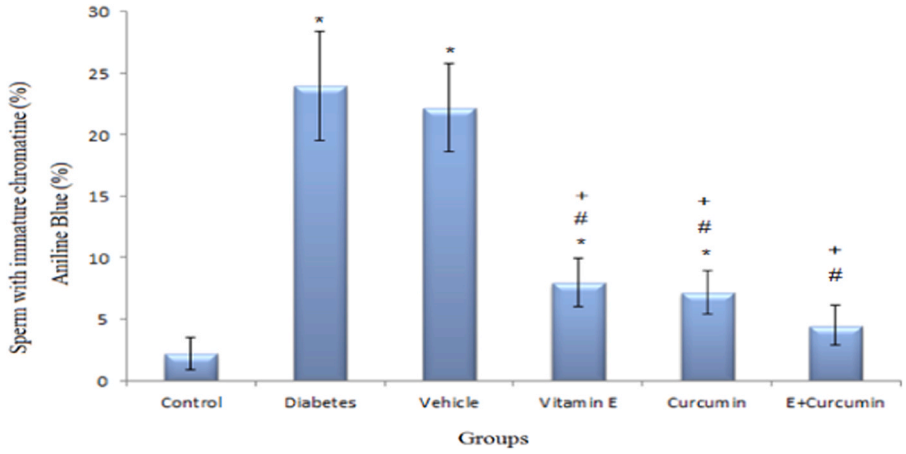

Chart 4. The average percentage of sperm with immature chromatin in the studied groups. Significant difference with the control group at the 0.05 level *. Significant difference with diabetes group at 0.05 level #. Significant difference with vehicle group at 0.05 level +.

injection of a double dose of ketamine and xylazine. The testes and epididymides were isolated by removing the surrounding connective tissue. The epididymal tail was placed in a Petri dish containing 1 ml of Human Tubal Fluid (HTF) culture medium supplemented with 4 mg/ml of bovine serum albumin (BSA) and incubated at 37 °C. Several incisions were made in the tail of the epididymis to release the sperm, which was then divided into smaller pieces and placed in the incubator. The testis was separated from the surrounding tissues and fixed in 10 % formalin for histological processing. After thirty minutes, the sperm was collected and distributed in the surrounding area. Sperm counting was performed by preparing a 1:20 dilution of sperm and adding 10 microliters of the diluted solution onto a Neobar slide. The sperm were then counted under a light microscope.

2.3. Sperm movement examination (motility)

To assess sperm motility, a drop of the sperm suspension was placed on a Neobar slide and examined under a light microscope at 400 × magnification. The number of sperm exhibiting rapid forward motion (RPFM), slow forward motion (SPFM), rotational motion (CM), and immobility (ml) were counted in different fields of the microscope, and the percentages of motile and immobile sperm were calculated. Ten different fields were analyzed for each sample [19].

2.4. Measurement of body weight and testes weight

The body weight of the animals was measured to monitor their general health, and the weight of the testes was also recorded.

2.5. Examination of sperm nucleus maturation

Aniline blue staining (AB) was performed to assess the maturation state of the sperm nuclei. Aniline blue specifically stains histones, which are present in immature sperm with uncondensed chromatin. A drop of the sperm suspension was placed on a slide and allowed to air dry. The samples were then fixed with 3 % glutaraldehyde and stained with 5 % aniline blue solution followed by incubation with 4 % acetic acid [20, 21]. The percentage of sperm stained with AB was determined by counting 400 sperm in each sample under a light microscope at 100× magnification (Charts 2–4).

2.6. Investigation of DNA damage

Acridine orange (AO) staining was employed to evaluate the extent of DNA damage in sperm. AO can distinguish healthy double-stranded DNA from denatured and unhealthy single-stranded DNA. A drop of the sperm suspension was placed on a slide and air-dried, followed by fixation in Carnoy's solution (methanol-acetic acid) for 2 h. The slides were then stained with AO for3 minutes and examined under a

Table 1
Mean ± standard deviation related to changes in body weight, testis weight and testis weight index in rats in different groups.

Variables	Control	Diabetes	Vehicle	Vitamin E	CU	E + CU
Weight of mice (gram)	264.7 ± 12.79	189.4 ± 0.31 a	190.3 ± 87.18 a	201.6 ± 12.31 abc	209.8 ± 62.35 abc	246.10 ± 25.32 ab
Testicle weight (gram)	47.06 ± 1.0	63.049 ± 0.0 a	64.07 ± 0.0 a	101.094 ± 1.0 abc	11.095 ± 1.0 abc	35.095 ± 1.0 b
Testicle weight index	56.034 ± 0.0	33.022 ± 0.0 a	33.035 ± 0.0 a	55.043 ± 0.0 b	53.046 ± 0.0 b	55.046 ± 0.0 b

* a indicates a significant difference with the control group * b indicates a significant difference with the diabetes group * c indicates a significant difference with the E + Cu group * ($p < 0.05$).

fluorescence microscope using a blue excitation filter. The percentage of sperm with DNA damage was determined by counting 400 sperm in each sample.

2.7. Evaluation of testicular histology

The fixed testes were processed and embedded in paraffin wax, and 5-μm-thick sections were prepared. The sections were stained with hematoxylin and eosin (H&E) for general histological examination. The slides were examined under a light microscope, and images were captured for analysis. The seminiferous tubules were evaluated for the presence of spermatogenic cells, germ cell maturation, and any histopathological changes.

2.8. Biochemical analysis

The left testis was homogenized in ice-cold phosphate buffer saline (PBS), and the homogenate was centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was collected for biochemical analysis. The levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) were measured using standard biochemical assays [22].

2.9. Statistical analysis

Data were analyzed using appropriate statistical methods such as analysis of variance (ANOVA) followed by post hoc tests. Results were expressed as mean ± standard deviation (SD), and p-values less than 0.05 were considered statistically significant.

3. Findings

3.1. The results of body weight, testicle weight and testicle weight index

The average weight of mice showed statistically significant differences between the control group and the diabetes, vehicle, vitamin E, Cu, and E + Cu groups ($P = 0.001$). Additionally, significant differences were observed in the average weight of rats between the diabetes group and the vitamin E ($P = 0.017$), Cu ($P = 0.001$), and Cu + E ($P = 0.001$) groups. The vitamin E group also exhibited a statistically significant difference in average weight compared to the Cu + E group ($P = 0.001$). However, no statistically significant difference in average weight was found between the vitamin E group and the Cu group ($P = 0.183$). Notably, a statistically significant difference in average weight was observed between the Cu group and the Cu + E group ($P = 0.001$).

Regarding testicular weight, a statistically significant difference was observed between the control group and the diabetes, vehicle, vitamin E, and Cu groups ($P = 0.001$). However, the average testicular weight between the control group and the Cu + E group did not reach statistical significance ($P = 0.052$). Furthermore, significant differences in average testicular weight were found between the diabetes group and the vitamin E ($P = 0.017$), Cu ($P = 0.001$), and Cu + E ($P = 0.001$) groups. The vitamin E group also exhibited a statistically significant difference in average testicular weight compared to the CU + E group ($P = 0.001$). Conversely, no statistically significant difference in average testicular weight was observed between the vitamin E group and the Cu group ($P = 0.999$). Lastly, a statistically significant difference in average testicular weight was found between the Cu group and the Cu + E group ($P = 0.001$).

Regarding the testicular index, a statistically significant difference was observed between the control group and the diabetes and vehicle groups ($P = 0.001$). However, no statistically significant difference was found in the average testicular index between the control group and the Cu + E ($P = 0.999$), vitamin E ($P = 0.995$), and Cu ($P = 0.738$) groups. The diabetes group showed statistically significant differences in average testicular index compared to the vitamin E, Cu, and Cu + E groups ($P = 0.001$), while no significant difference was observed between the diabetes group and the vehicle group ($P = 0.999$). Moreover, no statistically significant difference in average testicular index was found between the vitamin E group and the Cu ($P = 0.956$) and Cu + E ($P = 0.999$) groups. Lastly, the difference in average testicular index between the Cu group and the Cu + E group did not reach statistical significance ($P = 0.991$).

3.2. Sperm motility review

The difference in the mean percentage of sperm motility between the control group and the diabetes, vehicle, vitamin E and Cu groups is statistically significant ($p = 0.001$), while the difference in the mean percentage of sperm motility between the control group and the CU + E group is not statistically significant ($p = 0.694$). The difference in the average percentage of sperm motility between the diabetes group and the vitamin E ($p = 0.011$), CU ($p = 0.003$) and CU + E ($p = 0.001$) groups is statistically significant. The difference in the average percentage of sperm motility between the vitamin E and CU + E groups is statistically significant ($p = 0.001$), while the difference in the average percentage of sperm motility between the vitamin E group and CU group is not statistically significant ($p = 0.999$) (Table 1).

Table 2
Comparison of average sperm parameters in different groups.

Variables	Control	Diabetes	Vehicle	Vitamin E	CU	E + CU
Sperm count (106 × ml)	384 ± 0.14	202 ± 0.35 a	339.25 ± 1.21 a	5.03 ± 2.30 abc	59.94 ± 2.33 b	5.30 ± 4.37 b
Mobility percentage (%)	157.81 ± 7.39	159.16 ± 2.25 a	657.18 ± 3.08 a	941.38 ± 6.34 abc	714.45 ± 4.98 abc	941.72 ± 5.46 b
Viability percentage (%)	73.276 ± 8.33	40.645 ± 2.27 a	42.069 ± 3.25 a	51.183 ± 6.07 a	58.011 ± 4.907	68.311 ± 5.46 b
Immature sperm with chromatin (%)	2.2 ± 1.32	24 ± 4.42 a	22.2 ± 3.61 a	8 ± 1.94 ab	7.2 ± 1.75 ab	4.5 ± 1.58 b
Sperm with denatured DNA (%)	9.38 ± 9.17	29.5 ± 1.2 a	28.88 ± 1.96 a	25.63 ± 1.51 ab	22 ± 1.31 abd	19.758 ± 1.58 abde

* a indicates a significant difference with the control group * b indicates a significant difference with the diabetes group * c indicates a significant difference with the E + Cu group * d represents a significant difference with vitamin E group * e indicates a significant difference with the Cu group ($p < 0.05$).

Table 3
Comparison of changes in testicular tissue cells in the studied groups.

Groups	Spermatogonia	Spermatocyte	Spermatid	Leydig
Control	78.33 ± 7/89	95 ± 8.97	128.33 ± 1313	8.25 ± 1.91
Diabetes	32.83 ± 4.22 a	46.17 ± 6.62 a	77 ± 8.54 a	3 ± 1.81 a
Vehicle	31 ± 3.76 a	46.08 ± 5.14 a	78.92 ± 11.02 a	2.83 ± 1.47 a
Vitamin E	61.25 ± 5.93 abc	70 ± 5.27 abc	94.17 ± 8.24 abc	5 ± 2.17 ac
Cu	66.42 ± 5.9 abc	78.42 ± 10.05 abc	100 ± 14.18 ab	6 ± 1.71 ab
E + Cu	81.25 ± 10.79 b	92 ± 7.52 b	114 ± 15.94 b	8.08 ± 1.88 b

* a indicates a significant difference with the control group * b indicates a significant difference with the diabetes group * c indicates a significant difference with the E + Cu group * ($p < 0.05$).

3.3. Verification of sperm viability

The difference in the average percentage of live sperm between the control group and the diabetes, vehicle ($p = 0.001$), and vitamin E ($p = 0.004$) groups is statistically significant. In contrast, the difference in the average percentage of viable sperm between the control group and the CU group ($p = 0.81$) and the CU + E group ($p = 0.563$) is not statistically significant. The difference in the average percentage of viable sperm between the diabetes group and the CU + E group ($p = 0.009$) is statistically significant, while the difference in the average percentage of viable sperm between the diabetes group and the vitamin E ($p = 0.708$) and CU ($p = 0.142$) groups is not statistically significant. The difference in the average percentage of live sperm between the vitamin E group, the CU + E group ($p = 0.272$) and the Cu group ($p = 0.891$) is not statistically significant. Finally, the difference in the average percentage of live sperm between the CU group and the CU + E group is not statistically significant ($p = 0.882$). Table 2 and show the average percentage of live sperm (viability) in the studied groups. The results related to the percentage of live sperm in Table 2–4 show that diabetes causes a significant decrease in the percentage of live sperm compared to the control group. The combined administration of vitamin E and CU caused a significant increase in the percentage of live sperm compared with the diabetic group. Although separate administration of E and CU increased the percentage of live sperm compared with the diabetic group, this increase was not significant.

3.4. Examination of the quality of chromatin and maturity of the sperm nucleus (aniline blue staining)

The difference in the average percentage of sperm with immature chromatin between the control group and the diabetes, vehicle, vitamin E ($p = 0.001$) and CU ($p = 0.002$) groups was statistically significant. In contrast, the difference in the average percentage of sperm with immature chromatin between the control group and the CU + E group is not statistically significant ($p = 0.41$). The difference in the average percentage of sperm with immature chromatin between the diabetes group and the vitamin E, CU and CU + E groups is statistically

Table 4
Descriptive statistics of the parameters under study according to the groups under study.

Variable	Groups						Result
	Control	Diabetes	Vehicle	Vitamin E	Cucumin	Vitamin E & Curcumin	
Testosterone (ng/ml)	0.78 ± 0.01	0.18 ± 0.13 a	0.20 ± 0.18 a	0.31 ± 0.21 a	0.44 ± 0.36 a	0.53 ± 0.23 b	P < 0.001 *
LH (mIU/ml)	0.67 ± 0.90	0.29 ± 0.11 a	0.29 ± 0.08 a	0.36 ± 0.11 a	0.36 ± 0.13 a	0.45 ± 0.08 ab	P < 0.001 *
MDA (nmol/ml)	11.95 ± 1.64	81.13 ± 19.9 a	81.75 ± 17.09 a	68.63 ± 24.58 ac	63.63 ± 13.59 ac	49.13 ± 6.85 Ab	P < 0.001 *

*Statisticaly significance (p-value < 0/05).
* a indicates a significant difference with the control group * b indicates a significant difference with the diabetes group * c indicates a significant difference with the E + Cu group * ($p < 0.05$).

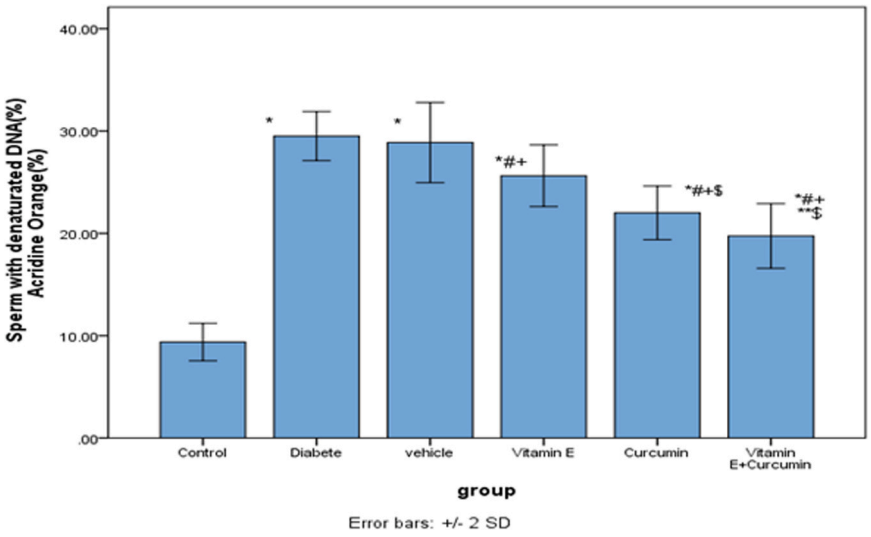

Chart 5. The average percentage of sperm with denatured DNA according to the groups under investigation. *Significant difference with the control group at $p < 0.05$ level. #Significant difference with diabetes group at $p < 0.05$ level. +significant difference with the vehicle group at $p < 0.05$ level. $significant difference with vitamin E group at $p < 0.05$ level. **Significant difference with curcumin group at $p < 0.05$ level.

significant ($p = 0.001$). The difference in the average percentage of sperm with immature chromatin between the vitamin E group and the CU ($p = 0.985$) and CU + E ($p = 0.057$) groups is not statistically significant. Finally, the difference in the average percentage of sperm with immature chromatin between the CU group and the CU + E group ($p = 0.238$) is not statistically significant. Table 2 and show the average percentage of spermatozoa with immature chromatin in the studied groups. The results related to the percentage of sperm with immature chromatin in Table 2 show that diabetes causes a significant decrease in the percentage of sperm with immature chromatin compared to the control group. Administration of vitamin E and CU and the combination of Endo caused a significant increase in the percentage of sperm with immature chromatin compared with the diabetic group. Although combined administration of vitamin E and CU decreased the number of sperm with immature chromatin compared with separate administration of these antioxidants, this increase was not significant.

3.5. Examination of the percentage of spermatozoa with denatured DNA (acridine orange stain)

The difference in the mean percentage of sperm with denatured DNA between the control group and the diabetes, vehicle, vitamin E, CU, and CU + E groups is statistically significant ($p < 0.001$). The difference in the mean percentage of sperm with denatured DNA between the diabetes group and the vitamin E, CU, and CU + E groups is statistically significant ($p < 0.001$). The difference in the average percentage of sperm with denatured DNA between the vitamin E group and the CU and CU + E groups is statistically significant ($p < 0.001$). Finally, the difference in the average percentage of sperm with denatured DNA between the CU group and the CU + E group is statistically significant ($p = 0.037$). Table 2 and 5 and Chart 5 show the average percentage of sperm with denatured DNA and its comparison in the studied groups. According to the results, diabetes led to a significant increase in the percentage of sperm with denatured DNA compared to the control group. Administration of vitamin E and CU alone and in combination resulted in a significant decrease in the percentage of sperm with denatured DNA compared to the diabetic group.

3.6. Histopathological examination

After fixation in 10 % formalin and tissue processing, 5-micron slices were prepared from the testes and stained with H&E, and the number of spermatogonial, primary spermatocytes, spermatids and lyid cells were counted under a light microscope. Table 3–4 shows the average number of spermatogonial cells, primary spermatocytes, spermatids in the seminiferous tubules and the average number of Lyid cells in the interstitial space. According to the ANOVA (Tukey) test, the following results have been obtained. The difference in the average number of spermatogonial cells between the control group and the diabetes, vehicle, vitamin E and CU groups is statistically significant ($p = 0.001$). While the difference in the average number of spermatogonial cells between the control group and the Cu + E group ($p = 0.9$) is not statistically significant. The difference in the average number of spermatogonial cells between the diabetes group and vitamin E, CU and Cu + E groups is statistically significant ($p = 0.001$). The difference in the average number of spermatogonial cells between vitamin E group and Cu + E group is statistically significant ($p = 0.001$). While the difference in the average number of spermatogonial cells between E group and CU group is not statistically significant ($p = 0.44$). Finally, the difference in the average number of spermatogonial cells between the CU group and the Cu + E group is statistically significant. According to the findings related to the number of spermatogonial cells in Table 3, diabetes caused a significant decrease in the number of spermatogonial cells compared to the control group. The administration of vitamin E and CU and a combination of endo caused a significant increase in the number of spermatogonial cells compared to the diabetic group. The combined administration of vitamin E and CU caused a significant increase in the number of spermatogonial cells compared to the separate administration of these two antioxidants.

4. Discussion

The objective of this study was to investigate the protective effect of vitamin E and curcumin alone and in combination on the genital tract in male diabetic rats.

A significant increase in body weight was observed in the alphatocopherol-treated group compared to the diabetic group. The researchers concluded that this weight gain was due to the maintenance of normal growth rates in alpha-copherol treated diabetic rats. This is because in diabetic rats, the growth rate decreases with age due to metabolic disturbances [23], and alpha-copherol prevented these metabolic disturbances and caused the rats to grow normally and increase in weight. Also, in Jarger's study, testicular weight loss was observed in the diabetic group, and it was concluded that this weight loss could be due to testicular atrophy in diabetic rats. Testicular atrophy is influenced by factors that interfere with spermatogenesis and reduce the number of gametes. One of these factors is the presence of oxygen free radicals, whose production has been shown to increase in diabetes due to hyperglycemia. As a potent antioxidant, vitamin E has been shown to prevent the harmful effects of free radicals in spermatogenesis and decrease the number of gametes, thereby preventing testicular atrophy and eventually their weight loss [24–26]. In a study, Momeni et al. [27] investigated the protective effect of vitamin E on sperm parameters of adult rats treated with an environmental pollutant called paranonylphenol. In their study, testicular weight increased significantly in the vitamin E treated group compared to the control group. The results of this study are consistent with the findings of our study on the increase in testicular weight after vitamin E administration. It was found that vitamin E can increase the thickness of the germinal epithelium and increase the number of spermatogonia and spermatocytes [28].

4.1. Study of sperm parameters

The results of the present study showed that diabetes caused a significant decrease in sperm count, sperm motility, and sperm viability compared with the control group. Administration of vitamin E caused a significant increase in these parameters compared to the diabetic group. Studies have shown that oxidative stress increases in diabetes due to increased production of reactive oxygen species (ROS) and decreased efficiency of the antioxidant defense system [29].

Excessive increase in ROS and free radicals negatively affects sperm motility and fertility, i.e., oxidative damage to sperm lipids and DNA leads to a decrease in sperm motility and fertility [30]. Oxygen free radicals cause DNA damage in the last mitochondria and lead to damage of the middle part of spermatozoa [31]. Damage to mitochondria in the middle part of spermatozoa decreases sperm motility [32]. According to the studies conducted on the increase of oxidative stress in diabetes and the subsequent increase of MDA, the end product of lipid peroxidation by reactive oxygen species (ROS). It can be concluded that the production of ROS disrupts the cell cycle and promotes the process of apoptosis, resulting in a decrease in daily sperm production and total sperm count [33,34].

In the present study, vitamin E, as a potent antioxidant in the vitamin E group, was shown to significantly prevent the deleterious effects of diabetes, such as decreased sperm motility, decreased sperm viability, and decreased sperm count. Consistent with the results of the present study, a study showed that a water-soluble analog of vitamin E called Trolox promoted sperm motility and preserved the integrity of their mitochondrial membrane [35].

Table 5
Pairwise comparisons of measured variables according to the groups under investigation with Tukey Test.

Groups	Testestrone	LH	MDA	AO
Control & Diabetes	< 0.001	< 0.001	< 0.001	< 0.001
Control & vehicle	< 0.001	< 0.001	< 0.001	< 0.001
Control & Vit E	0.001	< 0.001	< 0.001	< 0.001
Control& curcumin	0.033	< 0.001	< 0.001	< 0.001
Control& curcumin + vit E	0.231	0.001	< 0.001	< 0.001
Diabete & vehicle	1	1	1	0.953
Diabete& Vit E	0.85	0.692	0.624	< 0.001
Diabete& curcumin	0.208	0.737	0.262	< 0.001
Diabete & curcumin + vit E	0.028	0.03	0.003	< 0.001
Vehicle& Vit E	0.901	0.778	0.574	0.001
Vehicle & curcumin	0.261	0.817	0.228	< 0.001
Vehicle & curcumin + vit E	0.039	0.044	0.002	< 0.001
Vit E& curcumin	0.855	0.521	0.988	< 0.001
Vit E& curcumin + vit E	0.334	0.171	0.164	< 0.001
curcumin& curcumin + vit E	0.940	0.103	0.466	0.037

4.2. Study of sperm nucleus maturation and sperm DNA damage

Sperm nuclei maturation was checked by aniline blue staining. In the maturation process of sperm nucleus, histone protein is converted into protamine with the increase of disulfide bonds. As a result of the staining of the nucleus at the histone stage, it becomes dark blue and with the completion of the protamine process, it becomes light blue. This staining indicates protamine deficiency [36,37]. In the present study, the highest percentage of sperm with damaged DNA was observed in the diabetes group, whereas administration of vitamin E resulted in a significant decrease in the percentage of sperm with DNA damage. In general, there are several reasons for sperm DNA fragmentation, including Poor recombination during spermatogenesis [38], Exposure of mature sperm to excessive amounts of ROS produced by immature sperm during migration from the seminiferous tubules to the epididymis which can lead to DNA fragmentation [39], and abnormal spermatid maturation that can also lead to sperm DNA fragmentation.

Oxidative stress is the most important cause of DNA damage in sperm, which can take the form of changes in organic bases, shifting and formation of new sites, and deletions [40]. If the damage is minor, the sperm can repair it, but if it is extensive, it leads to apoptosis by DNA fragmentation [41].

Pourentezari et al. [42] showed in a study that treatment with antioxidants such as vitamin E improved sperm nucleus DNA integrity in laboratory mice that suffered sperm DNA damage, which is consistent with the results of our study. One of the most important causes of DNA fragmentation and sperm damage is oxidative stress and excessive production of ROS, which occurs in diabetes after hyperglycemia [43,44] (Table 5).

5. Conclusion

In summary, the results of this study show that administration of curcumin and vitamin E to diabetic rats with STZ improved sperm parameters (number, motility, viability, percentage of sperm with immature nucleus, percentage of sperm with denatured DNA). In addition, their administration improved histopathological symptoms of testicular tissue (increase in the number of spermatogonia, primary spermatocytes, spermatids and lydian cells) and also biochemical parameters (MDA levels, LH hormones and serum testosterone). These beneficial effects were far more evident and significant in the combined treatment with curcumin and vitamin E, so the present study recommends the combined administration of these antioxidants as an effective treatment method for diabetes. Further studies in clinical trials on the use of these substances in the treatment of diabetics seem to be necessary.

CRediT authorship contribution statement

Kamal Rahimi, Navid Faraji, and Behnam Babamiri: Study concept, data collection, writing the paper and making the revision of the manuscript following the reviewer's instructions. **Maryam Pourheidar and Somaye Nabavi:** Study concept, reviewing and validating the manuscript's credibility.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data that has been used is confidential.

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