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Protective Effect of Vitamin C on Cyclosporine-Induced Testicular Dysfunction and miR-21 Expression in Diabetic Male White Rats

LINA ABDULHUSSEIN A. ALLAITHI[1]

[1]*Ministry of Education, General Directorate of Al-Qadisiyah Education, Iraq*

Linamohhh@gmail.com

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Abstract

Diabetes is one of the most common chronic metabolic diseases affecting testicular function, hormone production, and spermatogenesis. Cyclosporine, on the other hand, is a drug widely used as an effective immunosuppressant in organ transplantation; however, it has been directly linked to adverse effects on body tissues, including the male reproductive system. Vitamin C is a potent antioxidant that protects lipid membranes and proteins from oxidative cell damage. Diabetes is also one of the most common chronic conditions affecting testicular function, hormone production, and spermatogenesis. Nevertheless, the molecular mechanisms linking cyclosporine exposure to diabetes and reproductive dysfunction require further experimental studies. miR-21-5p is one of the microRNAs important in regulating cellular processes; therefore, changes in testicular tissue are likely associated with its expression.

The aim of this study was therefore to evaluate the expression of miR-21-5p in testicular tissue in male rats with diabetes and those treated with cyclosporine, to investigate the protective effect of vitamin C, and to examine its relationship with indicators of reproductive function. miR-21-5p expression was assessed using qRT-PCR, alongside measurements of glucose, insulin, testosterone, LH, TNF-α, IL-6, MDA, and CAT in serum. These parameters were compared across the study groups, which comprised 32 male white rats weighing 210 ± 20 g. They were randomly allocated to four groups in which diabetes was induced by a high-fat diet (for 4 weeks) and subsequently treated with a reduced dose of streptozotocin at a concentration of 35 mg/kg for two weeks, except the first group: (C), which comprised animals given 1 ml of distilled water throughout the experiment; the second group (T1), which comprised animals in which diabetes was induced only during the experiment; the third group (T2), which comprised animals in which diabetes was induced and which were subsequently

administered cyclosporine at a concentration of (100 mg/kg)of body weight for 28 days; and the third group: T3, comprising animals in which diabetes was induced and which were administered cyclosporine at a concentration of (100 mg/kg) of body weight, followed by vitamin C at a concentration of (100 mg/kg) of body weight for 28 days.
The results showed a statistically significant decrease in the expression of miR-21-5p and the levels of testosterone, LH, and SOD, alongside an increase in MDA, TNF-α, and IL-6 in groups T1 and T2 compared with group T3, which showed an improvement in the aforementioned parameters.

keyword
Diabetic, vitamin C, miR-21-5p expression, Cyclosporine, streptozotocin.

Introduction:

Diabetes is one of the most common diseases today and is associated with high blood glucose levels. The imbalance in the body's utilisation of insulin results either from insufficient insulin secretion by the beta cells of the pancreas or from a complete absence of insulin. Diabetes is therefore divided into two types: the first is a chronic condition resulting from damage to the insulin-producing beta cells of the pancreas, leading to insulin deficiency (9).
Type 2 diabetes (T2DM), on the other hand, differs immunologically and genetically from type 1 diabetes; it is caused by insufficient insulin production, which leads to a disruption in the metabolism of carbohydrates, fats, and proteins in the body, and as the disease progresses, it causes damage to certain tissues and organs. Diabetes is also accompanied by disorders that result in an imbalance in the molecular composition of cells and tissues, which increases as the disease progresses (7).
The male reproductive system is one of the organs most susceptible to complications arising from uncontrolled diabetes. Uncontrolled diabetes is associated with adverse effects on the structure of the testes and epididymis, and consequently on spermatogenesis. A cross-sectional study of testicular tissue in mice with diabetes induced by STZ showed a gradual loss of spermatids, which coincided with damage to the seminiferous tubules (16). On the other hand, the drug cyclosporine is used for immunosuppression in organ transplantation and the treatment of autoimmune diseases; it is characterised by its high potency in inhibiting lymphokine synthesis, differentiation, and T-cell receptor signalling pathways (12). Studies have confirmed that treatment of experimental animals with cyclosporine harms the male reproductive system. The use of cyclosporine in rats has been associated with a reduction in certain indicators of testicular and sperm function, impaired spermatogenesis, and histological changes in the testis (13). Furthermore, a study conducted on rats in 2021 showed that cyclosporine contributed to the induction of oxidative stress and inflammation, as well as changes in the Nrf2/HO-1 and AKT/eNOS/NO pathways (1).
Vitamin C (ascorbic acid) is one of the most important non-enzymatic antioxidants, characterised by its high ability to reduce the harmful effects of

oxidative stress resulting from inflammation. Numerous studies have examined the positive effect of vitamin C on rats with induced diabetes associated with disorders of reproductive organ function. A study by Kamel and colleagues in 2025 confirmed that ascorbic acid contributed significantly to alleviating reproductive toxicity in male rats, with improvements in markers of oxidative stress and sperm production. (6).

These effects take on greater significance in the context of diabetes, as the hyperglycaemic environment itself is associated with increased oxidative stress and inflammation in the testis. It can therefore be hypothesised that the presence of diabetes may increase the susceptibility of testicular tissue to harmful pharmacological agents, which has led to increased interest in studying the protective effects of antioxidants (2).

Recent trends in scientific research have focused on molecular regulators important for gene expression, most notably microRNAs, which have been linked to the study of reproductive disorders resulting from diabetes; this makes the study of miR-21 particularly important for understanding the molecular disruption in testicular tissue associated with diabetes. A 2024 study confirmed that dysregulation of the expression of numerous miRNAs is associated with testicular damage, impaired spermatogenesis, and disrupted hormone production in animals with diabetes (10).

Investigating miR-21 expression in the testis may provide insight into the molecular changes associated with diabetes as well as exposure to cyclosporine; conversely, it assesses the protective role of vitamin C, which may coincide with these molecular-level alterations.

The present study therefore aims to evaluate the protective effect of vitamin C against cyclosporine-induced testicular dysfunction in male rats with streptozotocin-induced diabetes, whilst investigating changes in miR-21 expression, with the aim of exploring the relationship between the protective effect of vitamin C and the molecular changes associated with testicular damage.

Materials and methods

The study was conducted at the Animal House Laboratory of the Faculty of Science, Al-Qadisiyah University. The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines were followed, and we complied with the regulations.

The Foundation for the Care of Laboratory Animals. The experiment was conducted on 32 healthy adult male white rats weighing between 200±20 g and aged between 3 and 4 months. They were housed in a laboratory environment with appropriate temperature and humidity levels, along with light/dark cycles, and were allowed to acclimatise to the new environmental conditions for 14 days before the start of the experiment. They were provided with water and their standard diet throughout the acclimatisation period. At the start of the experiment, and for the purpose of inducing diabetes, the animals were fed a high-fat diet (HFD) for a period of four weeks, after which they were administered

streptozotocin. Following induction, they were randomly divided into four groups, each comprising eight animals in which diabetes was induced, except the control group, which was administered 1 ml of distilled water throughout the experiment. Following induction, group (T2) was administered cyclosporine at a concentration of 100 mg/kg body weight, and group (T3) was administered cyclosporine at a concentration of 100 mg/kg (8) of body weight, in conjunction with vitamin C at a concentration of 100 mg/kg (2) of body weight, throughout the 28-day duration of the experiment.

Induction of diabetes:

The animals were fed a high-fat diet (HFD) for four weeks, consisting of 34.9 g% fat (60 kcal%), 26.2 g% protein (20 kcal%), and 26.3 g% carbohydrates (20 kcal%), and was prepared in accordance with the AIN-93 standard.

Streptozotocin was purchased in powder form from Solgar, Inc. (Leonia, NJ, USA) and was then used to induce diabetes in the treated rats at a concentration of 35 mg/kg for two weeks, after being dissolved in citric acid and sodium citrate at a concentration of 0.1 molar at pH 4.5 (11).

The disease was induced in groups (T1, T2, T3), and blood glucose levels were then measured prior to the start of the experiment to confirm that the experimental rats had developed diabetes.

Experimental design:

The experimental animals were randomly allocated to four groups, each comprising 8 animals:

The first group served as the control group (C), comprising animals administered 1 ml of distilled water orally throughout the experiment; this was designated the negative control group. In groups (T1, T2, T3), diabetes was induced using streptozotocin at a concentration of 35 mg/kg; Group 2 (T1) was in which diabetes was induced for the duration of the experiment and was designated the positive control group; Group 3 (T2) was in which, following induction, cyclosporine was administered at a concentration of 100 mg/kg body weight for 28 days. Group 4 (T3): Following the induction of diabetes, cyclosporine was administered at a concentration of 100 mg/kg body weight in conjunction with vitamin C for a period of 28 days.

Sample collection:

At the end of the experiment, the rats were anaesthetised with chloroform to collect blood serum via a cardiac puncture for the necessary haematological tests; the animals were then sacrificed to obtain testicular tissue and measure the level of miR-21 therein. It should be noted that no unexpected deaths occurred during the experiment.

Measurement of miR-21-5p levels:

Samples were collected from testicular tissue and stored under appropriate conditions to protect the RNA from degradation. To analyse the samples, the miRcute miRNA Isolation Kit, designed for the extraction of miRNA from animal tissues (Tiangen, DP501), was used.

Once the extraction process was complete, a NanoDrop instrument was used to determine the purity and concentration of the RNA. The extracted RNA was then used as a template to prepare cDNA using the miRcute Plus miRNA First-Strand cDNA Synthesis Kit (Tiangen, KR211), which involves the addition of poly(A) to the miRNA followed by reverse transcription. rno-miR-21-5 was measured was measured following extraction; the concentration and purity of the RNA were then determined using a NanoDrop instrument. The extracted RNA was subsequently used as a template to prepare cDNA using the miRcute Plus miRNA First-Strand cDNA Synthesis Kit from Tiangen (KR211). This system relies on the addition of poly(A) to the miRNA, followed by reverse transcription.
Subsequently, the level of rno-miR-21-5p was measured using the miRcute Plus miRNA qPCR Detection Kit (SYBR Green; Tiangen, FP411).
After confirming that the reference gene (U6) was suitable for the study model, relative expression of miR-21-5p was calculated. Following verification of the suitability of U6 snRNA as an internal reference, miRNA expression was used to normalise the data, and relative rno-miR-21-5p levels were then calculated.
After verifying the suitability of U6 snRNA as an internal reference, it was used to normalise the miRNA expression data, following which relative expression was calculated. The sequence of the mature rno-miR-21-5p was adopted after verification in databases, and was as follows:
5′-UAGCUUAUCAGACUGAUGUUGA-3
The corresponding DNA sequence was used as the basis for the forward primer for rno-miR-21-5p, which is:
5′-TAGCTTATCAGACTGATGTTGA-3
U6 small nuclear RNA (U6 snRNA) was used as a reference for normalising the expression data (15).

Target	Primer	Sequence(5`-3`)	Role
rno-miR-21-5p	Forward	**TAGCTTATCAGACTGATGTTGA**	**Target miRNA**
rno-miR-21-5p	Reverse	**Universal Reverse Primer**	**Target miRNA**
U6 snRNA	Forward	**Specific U6 primer**	Endogenous reference
U6 snRNA	Reverse	**Specific U6 primer أو Universal Reverse Primer**	It is written as approved in Kit

U6 snRNA was used as an internal reference to normalise the expression of rno-miR-21-5p, and relative expression was calculated using the $2^{-}\Delta\Delta C$ method (Shi et al., 2022).

Glucose measurement:

A capillary blood sample was collected from rats with diet-induced diabetes (induced by a high-fat diet and streptozotocin) to measure blood glucose levels after a fast of 13–14 hours using a glucometer in accordance with the manufacturer's instructions.

Hormonal, Inflammatory and Antioxidant Parameters

Testosterone levels were measured using catalogue number (SL0668Ra), luteinising hormone (LH) using catalogue number (SL1093Ra), and insulin using catalogue number (SL0373Ra), TNF-α was measured using reference number (SL0722Ra) and IL-6 using reference number (SL0411Ra) in serum using a kit from Sunlong Biotech Co. Furthermore, MDA (reference number SL0475Ra) and CAT (reference number SL1084Ra) were measured using the same technique and a kit from Sunlong (China).

Statistical Analysis

The data were analysed using SPSS (version 25.0) to calculate the arithmetic mean and the LSD test. ANOVA and Tukey's test were also used to examine differences between the groups under study, and a p-value of < 0.05 was considered statistically significant.

Results and Discussion

Table (1). Relative gene expression of testes miR-21-5p in Cyclosporine-Induced Testicular Dysfunction and diabetic male rats.

Groups / Parameters	C	T1	T2	T3	LSD
miR-21-5p (2^-ΔΔCt)	1.00±0.12 ^d	4.43±0.48 ^b	6.32. ±0.42 ^a	3.89±0.40 ^c	0.38

Values are expressed as mean ± standard deviation (n=8). Means with different superscript letters within the same row are significantly different (p < 0.05). C: negative Control group. group; T1: positive control group induced diabetes. group; T2: induced diabetes then given Cyclosporine concertation 100mg/kg. group; T3 induced diabetes then given Cyclosporine concertation 100ml/kg and Vitamin C concertation 100mg/kg.

The results showed an increase in the relative expression of miR-21-5p in testicular tissue from diabetic rats, which rose to 4.43 ± 0.48 in the T2 group compared with 1.00 ± 0.12 in the C group. This finding indicates a clear alteration in the regulation of miR-21-5p in testicular tissue in the diabetic group, which is consistent with recent studies supporting a functional role for miR-21-5p in male reproductive tissues. A study published in 2022 showed that miR-21-5p, alongside miR-615, regulates the proliferation and apoptosis of Leydig cells, with this being associated with the targeting of SOX5, suggesting that this miRNA may be involved in regulating cellular function in testicular tissue (17).

More recent studies suggest that miR-21-5p plays a functional role in male reproductive tissues. A recent study has shown that miR-21-5p is present in

spermatogonial stem cells, and that its modification can affect the proliferation and survival of these cells, as well as spermatogenesis, in a testicular injury model. These findings suggest that miR-21-5p is not merely a random molecular marker, but may be involved in regulating testicular-related cellular processes. However, the model used in that study differs from that of the present study; therefore, it should not be regarded as direct evidence of the mechanism of change observed in diabetic rats.

A study by Zhang and colleagues also confirmed the direct link between the function of spermatogonial stem cells (SSCs) in the testis and miR-21-5p, and that experimentally increasing miR-21-5p improved the proliferation of these cells.

Its ability to counteract apoptosis, as well as its role in alleviating testicular dysfunction caused by Busulfan; furthermore, the modulation of miR-21-5p is not merely a random molecular marker, but may be involved in regulating testicular-related cellular processes and influence the proliferation, survival, and spermatogenesis of these cells in a testicular injury model. (17)

The results also showed a statistically significant increase in the level of miR-21-5p in the T2 group to 6.32 ± 0.42 compared with T1; these findings suggest a link between cellular and oxidative disturbances that may alter molecular regulation within tissues due to cyclosporine. This relationship has been confirmed by recent studies demonstrating a link between miR-21-5p dysregulation and processes associated with oxidative stress and inflammation. A recent study in 2025 examined miR-21-5p dysregulation in diabetic nephropathy and linked it to the inflammatory response, whilst another recent study investigated miR-21 in diabetes-associated pancreatic damage and the PTEN/PI3K- and AKT/caspase-3 pathways (3).

In contrast, miR-21-5p expression in the vitamin C-treated group fell to 3.89±0.40, representing a decrease compared with the diabetes + cyclosporine group, Our current study confirms the effect of vitamin C as an active antioxidant, which helped to mitigate the increase in miR-21-5p associated with diabetes and cyclosporine, A study using vitamin C at graduated doses of 15, 50 and 100 mg/kg demonstrated that it helped to improve the changes induced by STZ in the molecular structure of testicular tissue in diabetic rats. (2) However, expression levels remained higher than those in the control group; this may be attributed to the duration of the experiment and the dose used, suggesting that the treatment mitigated the disruption but did not fully restore it.

Table (2) shows the effect of of Vitamin C on Cyclosporine-Induced Testicular Dysfunction and miR-21 Expression in (glucose (in the serum of male rats in diabetic male rats.

Group / Parameters	C		T1	T2	T3	LSD
Glucose(mg/dL)	Pre	101.6±7.5 ^a	207.9±12.4 ^b	212±17.2 ^b	210.4±12.5 ^a	13.17

	Post	100.9±10.5 ^d	209.6±11.5 ^b	309.3±13.8 ^a	180.2±12.1 ^c	12.32

Values are expressed as mean ± standard deviation (n=8). Means with different superscript letters within the same row are significantly different (p < 0.05). C: negative Control group. Group; T1: positive control group induced diabetes. group; T2: induced diabetes then given Cyclosporine concertation 100mg/kg. group; T3 induced diabetes then given Cyclosporine concertation 100ml/kg and Vitamin C concertation 100mg/kg.

The results in Table (2) showed elevated glucose levels in the (HFD) and (STZ) treatment groups used to induce diabetes in groups (T1, T2, T3) before treatment with cyclosporine and vitamin C; following treatment, the results showed a statistically significant increase in glucose levels in group (T2); this is attributed to the induction of diabetes by streptozotocin and treatment with cyclosporine on the one hand, whereas the decrease observed in group (T3) is explained by treatment with vitamin C, which is known for its antioxidant properties that counteract the harmful effects of oxidative stress resulting from inflammation and oxidative stress (2).

Table (3) shows the effect of Vitamin C on Cyclosporine-Induced Testicular Dysfunction and miR-21 Expression (glucose &insulin) in the serum of male rats in diabetic male rats.

Parameter	C	T1	T2	T3	$LSD_{0.05}$
Insulin (mU/L)	5.93 ± 0.12^a	3.45± 0.8^c	2.98.± 0.04^c	4.87± 0.08^b	0.70
Testosterone(ng/mL)	2.85±0.30^a	1.45±0.06^b	0.97±0.09^c	1.45±0.06^b	0.29
LH(ng/mL)	0.82 ± 1.03^a	0.24 ± 0.02^b	0.17 ±0.06^b	0.54 ±0.03^ab	0.48

Values are expressed as mean ± standard deviation (n=8). Means with different superscript letters within the same row are significantly different (p < 0.05). C: negative Control group. Group; T1: positive control group induced diabetes. group; T2: induced diabetes then given Cyclosporine concertation 100mg/kg. group; T3 induced diabetes then given Cyclosporine concertation 100ml/kg and Vitamin C concertation 100mg/kg.

The results of the present study showed a marked reduction in serum insulin concentrations in diabetic rats, which were lower than those in the control group and also lower in the group treated with cyclosporine. This may be attributed to damage to pancreatic beta cells resulting from the rats being treated with STZ on the one hand and cyclosporine on the other, which is known to cause testicular dysfunction. A study published in 2021 on diabetic rats showed that levels of insulin, testosterone, and LH decreased with the occurrence of testicular damage, whilst markers of oxidative stress and inflammation increased (14).

In contrast, the results showed an improvement in insulin levels in group (T3), which is attributed to the effect of vitamin C as an active antioxidant that contributes to improving cellular tissue function by preventing the formation of free radicals and limiting their harmful effects on organs, including the pancreas, particularly changes associated with lipid peroxidation (4).

The results also showed a decrease in the levels of both testosterone and LH in group T1 compared with group C; this may be due to the induction of diabetes in the rats, which resulted in impaired testicular function. A study published in 2023 confirmed that diabetes contributed to reduced levels of testosterone, LH, and

FSH, as well as reduced antioxidant activity and elevated levels of MDA, IL-6, TNF-α, and markers of insulin resistance (5).

The reduction in testosterone and LH in the T2 group is explained by the harmful synergistic effect of diabetes and cyclosporine on testicular function. In contrast, the T3 group showed an improvement in outcomes, although this did not reach statistical significance; this improvement is associated with the role of vitamin C (4).

Table (4) shows the effect of Vitamin C on Cyclosporine-Induced Testicular Dysfunction and miR-21 Expression (glucose &insulin) in the serum of male rats in diabetic male rats.

Parameter	C	T1	T2	T3	$LSD_{0.05}$
TNF-α (pg/mL)	13.87± 0.25^d	24.65 ± 0.45^b	29.98± 0.88^a	17.64± 0.34^c	2.50
IL-6 (ng/L)	15.87 ± 0.26^d	21.83 ± 0.63^b	24.1 ±0.78^a	18.2 ±0.54^c	2.20

Values are expressed as mean ± standard deviation (n=8). Means with different superscript letters within the same row are significantly different (p < 0.05). C: negative Control group. Group; T1: positive control group induced diabetes. group; T2: induced diabetes then given Cyclosporine concertation 100mg/kg. group; T3 induced diabetes then given Cyclosporine concertation 100ml/kg and Vitamin C concertation 100mg/kg.

The results in Table (4) showed a statistically significant difference in the levels of (TNF-α) and (IL-6) in groups (T1) and (T2) compared with the control group (C), whilst group (T3) showed an improvement in TNF-α levels compared with groups (T1) and (T2). This is attributed to the role of vitamin C in protecting cells from the effects of pro-inflammatory factors resulting from the induction of diabetes and treatment with cyclosporine (9).

Table (5) shows the effect of Vitamin C on Cyclosporine-Induced Testicular Dysfunction and miR-21 Expression (glucose &insulin) in the serum of male rats in diabetic male rats.

Parameter	C	T1	T2	T3	$LSD_{0.05}$
MDA (ng/mL)	1.3 ± 0.2^d	6.2 ± 1.7^b	9.2± 0.8^a	3.2 ± 1.3^c	1.50
CAT(pg/mL)	27.4 ± 1.3^a	20.4 ± 1.5^b	19.3 ± 1.2^b	23.8±0.9^ab	5.00

Values are expressed as mean ± standard deviation (n=8). Means with different superscript letters within the same row are significantly different (p < 0.05). C: negative Control group. group; T1: positive control group induced diabetes. group; T2: induced diabetes then given Cyclosporine concertation 100mg/kg. group; T3 induced diabetes then given Cyclosporine concertation 100ml/kg and Vitamin C concertation 100mg/kg.

The results in Table 5 showed a statistically significant increase in MDA levels accompanied by a decrease in CAT levels in the diabetes groups (T1 and T2) compared with the control group; conversely, there was a statistically significant decrease in MDA levels accompanied by an increase in CAT levels in group (T3) compared with the (T1, T2) groups; this is attributed to the effect of vitamin C in scavenging free radicals and reducing lipid peroxidation (4).

Conclusion

The results demonstrated the protective role of vitamin C in rats in which diabetes was induced by streptozotocin and treated with cyclosporine, which was reflected in the dysregulation of miR-21-5p expression in testicular tissue, accompanied

by elevated levels of glucose, insulin, testosterone, and LH in the blood, alongside increased levels of inflammatory mediators (TNF-α) and (IL-6) along with (MDA) and reduced (CAT). The active role of vitamin C as an antioxidant and anti-inflammatory agent contributed to improved testicular function and reduced levels of inflammatory mediators and oxidants in the serum; furthermore, its positive role in regulating the miR-21-5p pathway in the pancreas also contributed to testicular tissue during the period of treatment with (HFD) and streptozotocin to induce diabetes, followed by treatment with cyclosporine.

References:

1-Arab, H. H., Eid, A. H., Gad, A. M., Yahia, R., Mahmoud, A. M., andKabel, A. M. (2021). Inhibition of oxidative stress and apoptosis by camel milk mitigates cyclosporine-induced nephrotoxicity: Targeting nrf2/ho-1 and akt/enos/no pathways. Food Science & Nutrition, 9(6), 3177-3190. https://doi.org/10.1002/fsn3.2277

2-Baloglu, F. K., Tas, D. G., Yilmaz, O., and Severcan, F. (2023). The recovery effect of vitamin C on structural alterations due to streptozotocin-induced diabetes in rat testicular tissues. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 288, 122149. https://doi.org/10.1016/j.saa.2022.122149

3-Chen, J., Zmijewska, A., Zhi, D., andMannon, R. B. (2015). Cyclosporine-mediated allograft fibrosis is associated with micro-RNA-21 through AKT signaling. Transplant International, 28(2), 232-245. https://doi.org/10.1111/tri.12471

4-Ebadi, H., Zakeri, M., Mousavi, S. M., Yavari, V., and Souri, M. (2021). The interaction effects of dietary lipid, vitamin E, and vitamin C on growth performance, feed utilization, muscle proximate composition, and antioxidant enzyme activity of white leg shrimp (Litopenaeus vannamei). Aquaculture Research, 52(5), 2048-2060. https://doi.org/10.1111/are.15056

5-Elsaeed, M. Y., Mehanna, O. M., Abd-Allah, E.-E. E., Hassan, M. G., Ahmed, W. M. S., Moustafa, A. E. G. A., Eldesoky, G. E., Hammad, A. M., Elgazzar, U. B., and Elnady, M. R. (2023). Combination therapy with enalapril and paricalcitol ameliorates streptozotocin diabetes-induced testicular dysfunction in rats via mitigation of inflammation, apoptosis, and oxidative stress. Pathophysiology, 30(4), 567-585. https://doi.org/10.3390/pathophysiology30040041

6-Kamel-Chouider, A., Hariti, M., Akdader-Oudahamne, S., andHamouli-Said, Z. (2025). The potential role of ascorbic acid in attenuating infertility induced by emamectin benzoate via suppressing oxidative stress and ameliorating sperm count in male rats. Reproductive Toxicology, 133, 108852. https://doi.org/10.1016/j.reprotox.2025.108852

7-Karimova, M. V., Vasiliev, A. V., and Vorotelyak, E. A. (2022). Type 2 diabetes mellitus: Pathogenic features and experimental models in rodents. Acta Naturae (англоязычная версия), 14(3), 57-68. https://doi.org/10.1016/j.reprotox.2025.108852

8-Khadhim, H. A., and Khadhim, W. M. (2022). Effect of naringin nanocomposite in improving reproductive system efficiency in white male rats exposed to cyclosporine-induced oxidative stress. Iranian Journal of Ichthyology, 9, 360-368. http://www.isi-org.ir

9- Küçük Baloğlu, F., Guldag Tas, D., Yılmaz, O., and Severcan, F. (2023). The recovery effect of vitamin C on structural alterations due to streptozotocin-induced diabetes in rat testicular tissues. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 288, 122149. https://doi.org/https://doi.org/10.1016/j.saa.2022.122149

10-Latifi, Z., Nikanfar, S., Khodavirdilou, R., Beirami, S. M., Khodavirdilou, L., Fattahi, A., and Oghbaei, F. (2025). MicroRNAs as diagnostic biomarkers in diabetes-related male infertility: A systematic review. Molecular Biology Reports, 52(1), 90.https://doi.org/10.1007/s11033-024-10197-1

11-Lee, M., Tan, Y., Li, H., Liu, D., andZhao, H. (2025). Huoxue-jiangtang decoction ameliorates type 2 diabetes in high-fat diet and streptozotocin-induced rats. Frontiers in Pharmacology, 16, 1675295. https://doi.org/10.3389/fphar.2025.1675295

12-Oyovwi, M. O., Ben-Azu, B., Edesiri, T. P., Arientare, R. R., Emojevwe, V., Nwangwa, K. E., Edje, K. E., and Adebayo, O. G. (2010). Lutein attenuates cyclosporin-induced testicular impairment in male rats through modulation of androgenic hormones and enzymes. Pharmacol Toxicol, 2(1), 12-24. DOI: 10.52406/ptnm.v2i1.17

13-Oyovwi, O. M., Ben-Azu, B., Tesi, E. P., Emojevwe, V., Rotu, R. A., Moke, G. E., Umukoro, E., Asiwe, J. N., and Nwangwa, K. E. (2024). Possible mechanisms involved in the protective effect of lutein against cyclosporine-induced testicular damage in rats. Heliyon, 10(3). DOI: 10.1016/j.heliyon.2024.e24989

14-Salah, M., Ismail, K. A., and Khadrawy, S. M. (2022). Nobiletin protects against diabetes-induced testicular injury via hypophysis–gonadal axis upregulation and amelioration of oxidative stress. Molecular Biology Reports, 49(1), 189-203. https://doi.org/10.21203/rs.3.rs-814954/v1

15-Shi, B., Zhang, J., andChen, Q. (2022). Liver and kidney surgical anatomy to verify the effect of miR-221 on organ damage in septic rats. Journal of Healthcare Engineering, 2022(1), 2814431. https://doi.org/10.1155/2022/2814431

16-Shrilatha, B., and Muralidhara. (2007). Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: Its progression and genotoxic consequences. Reproductive Toxicology, 23(4), 578-587. https://doi.org/https://doi.org/10.1016/j.reprotox.2007.02.001

17-Tang, Q., Zhang, Y., Yue, L., Ren, H., and Pan, C. (2022). Ssc-mir-21-5p and ssc-mir-615 regulate the proliferation and apoptosis of Leydig cells by targeting sox5. Cells, 11(14), 2253. https://doi.org/10.3390/cells11142253

18-Zhang, M., Wan, S., Chen, W., Yang, D., Wang, C., Li, B., Aili, A., Du, X., Li, Y., and Wu, W. (2025). miR-21-5p ameliorates busulfan-induced testicular dysfunction and maintains spermatogenesis. Journal of Integrative Agriculture, 24(12), 4744-4759. doi: 10.1016/j.jia.2024.02.004