Int. J. Morphol.,
44(3):913-923, 2026.

http://dx.doi.org/10.4067/s0717-95022026000300913

Histomorphological and Endocrine-Molecular Assessment of *Cissus rotundifolia* and Metformin in Testicular and Thyroid Tissues of Diabetic Rats

Evaluación Histomorfológica y Endocrino-Molecular de *Cissus rotundifolia* y Metformina en Tejidos Testiculares y Tiroideos de Ratas Diabéticas

Hailah M. Almohaimeed[1]; Muna Omer Alamoudi[2]; Hani A Al.Turkistani[3]; Rasha Assiri[4]; Huda H.Eltayeb[1]; Sami Alonazi[5] & Abdullah M. Alshaer[6]

ALMOHAIMEED, H. M.; ALAMOUDI, M. O.; AL.TURKISTANI, H. A.; ASSIRI, R.; ELTAYEB, H. H.; ALONAZI, S. & ALSHAER, A. M. Histomorphological and endocrine-molecular assessment of *Cissus rotundifolia* and metformin in testicular and thyroid tissues of diabetic rats. *Int. J. Morpholol., 44(3)*:913-923, 2026.

SUMMARY: Diabetes mellitus is associated with structural and functional alterations in endocrine and reproductive tissues. This study evaluated the histomorphological, hormonal, and molecular effects of *Cissus rotundifolia* extract compared with metformin on testicular and thyroid tissues in streptozotocin-induced diabetic rats. Forty male Wistar rats were divided into control and experimental groups, including diabetic untreated and diabetic treated groups receiving *Cissus rotundifolia* (100 mg/kg) or metformin (150 mg/kg) for eight weeks. Histological examination of hematoxylin and eosin-stained sections revealed marked degeneration of seminiferous tubules, germinal epithelial loss, and follicular cell disruption in the thyroid glands of diabetic rats. These alterations were significantly ameliorated in treated groups, with restoration of seminiferous architecture, improved spermatogenic layers, and normalization of thyroid follicular structure. Hormonal analysis demonstrated that diabetes-induced reductions in TSH and alterations in T4 were substantially corrected following treatment. Molecular analysis showed upregulation of antioxidant markers (Nrf2, SOD) and glucose metabolism-related genes (GLUT4, PPARg), along with decreased expression of inflammatory markers (IL-6, TNF-α) in treated groups. These findings suggest that *Cissus rotundifolia* exerts protective effects against diabetes-induced structural damage in testicular and thyroid tissues, comparable to metformin, likely through modulation of oxidative stress and inflammatory pathways.

KEY WORDS: Diabetes mellitus; *Cissus rotundifolia*; Metformin; Testicular tissue; Thyroid tissue; Oxidative stress; Histopathology; Rats.

INTRODUCTION

Diabetes mellitus (DM) is a complex, incurable metabolic disorder characterized by increased blood glucose levels (hyperglycemia). It results from either decreased insulin secretion or reduced sensitivity of target tissues to insulin (Kermani *et al*., 2019). The global prevalence of DM is steadily rising, with projections indicating an increase to 693 million patients by 2045, representing approximately 10 % of the world's population (Liu *et al*., 2019). This chronic disorder significantly impacts various organs, leading to complications such as cardiomyopathy, retinopathy, neuropathy, and nephropathy (Andlib *et al*., 2023).

Thyroid dysfunction is commonly observed in diabetic patients, with hypothyroidism being the most prevalent disorder among them (Brenta, 2010; Nishi, 2018). The interplay between diabetes and thyroid function is multifactorial, involving complex interactions between metabolic pathways and endocrine regulation. In particular,

[1] Department of Basic Science, College of Medicine, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.
[2] Biology Department, Faculty of Science, Hail University, Hail, Saudi Arabia.
[3] Department of Clinical Anatomy, Faculty of Medicine, King Abdulaziz University.
[4] Department of Basic Medical Sciences, College of Medicine, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia.
[5] Tissue Biobank Section, Natural and Health Science Research Center, Princess Nourah Bint Abdulrahman.
[6] CEO, Saudi Centre for Pharmaceuticals, Riyadh, Saudi Arabia.
FUNDING. This research was funded by the Deanship of Scientific Research and Libraries at Princess Nourah bint Abdulrahman University, through the Research Funding Program, Grant No. (FRP-1445-19).

Received: 2026-02-22 Accepted: 2026-04-22

oxidative stress, inflammatory cytokines, and altered hormonal regulation contribute significantly to thyroid dysfunction in diabetic states (Altan *et al*., 2010; Hage *et al*., 2011; Wang, 2013). The potential therapeutic effects of natural plant extracts in ameliorating diabetes-induced thyroid dysfunction are increasingly recognized due to their multifaceted bioactivities. Natural extracts exert synergistic antioxidant, anti-inflammatory, and antidiabetic effects that may counteract the oxidative stress and cytokine-mediated inflammation seen in diabetic states, which are known to impair thyroid hormone homeostasis (Méndez-López *et al*., 2020).

Beyond these well-known complications, DM is increasingly recognized for its detrimental effects on reproductive function, particularly in males. Studies indicate that a substantial percentage (around 90 %) of male diabetic patients experience varying degrees of reproductive dysfunction and fertility issues, significantly affecting their physical and mental well-being and quality of life (Pavlinkova *et al*., 2017). Experimental and clinical research on both men and animal models demonstrates that diabetes can adversely influence the male reproductive system, leading to reduced testosterone synthesis and secretion, impaired spermatogenesis, decreased libido, and ultimately, infertility (Kermani *et al*., 2019). Diabetic testicular dysfunction is characterized by altered testicular morphology, a reduction in spermatogenic cell numbers, and sex hormone imbalances (Apichakan *et al*., 2018).

Oxidative stress is strongly implicated as a primary cause of diabetic complications (De Young *et al*., 2004). While its role in reproductive dysfunction is not fully elucidated, it is known that diabetes-induced oxidative stress can induce germ cell apoptosis. Consequently, it is hypothesized that this apoptotic process can be mitigated by treatment with various antioxidants that enhance testicular antioxidant defense mechanisms (Zhao *et al*., 2010). Oxidative stress, inflammation, and apoptosis are closely linked to the onset and progression of diabetic testicular dysfunction (Amaral *et al*., 2008; Nna *et al*., 2019; Qadiri *et al*., 2019). Elevated levels of reactive oxygen species (ROS) during oxidative stress can damage testicular cells, leading to spermatogenic dysfunction. High ROS levels can also induce mitochondrial and DNA damage in sperm, thereby promoting spermatogenic cell apoptosis (Orman *et al*., 2015; Khosravi *et al*., 2019). Testicular interstitial fibrosis, which compromises the spermatogenic environment, also contributes to testicular dysfunction in diabetes, impacting testosterone secretion, spermatogenesis, and sperm quality, potentially causing male infertility and sexual dysfunction (Shiraishi *et al*., 2003; Zheng *et al*., 2021).

In the pursuit of effective diabetes management, especially with concerns about long-term side effects of conventional drugs (Roghani & Baluchnejadmojarad, 2007), there is increasing interest in complementary and alternative medicines (Chang *et al*., 2007). Herbal medicines are particularly favored due to a perception of them being natural and healthy (Hlebowicz & Darwiche, 2007; World Health Organization, 2011). Many studies confirm the potential efficacy of complementary medicines in reducing blood glucose levels through various mechanisms, contributing to improved patient care and quality of life (Zanini *et al*., 2008).

Cissus rotundifolia (CR), a member of the Vitaceae family, is a medicinal plant prevalent in Yemen and the southern region of Saudi Arabia, traditionally used for various ailments including skin diseases, burns, and diabetes (Mohammed, 2019; Alshehri, 2020). Historically, boiled leaves of *C. rotundifolia* are consumed as an appetizer and used as an antipyretic in malaria and dengue fever treatments in Yemen (Al-Mehdar & Al-Battah, 2016). Beyond its nutritional value, *C. rotundifolia* has demonstrated antidiabetic and antioxidant activities, and is recognized as a potential phytomedicine with analgesic, anti-inflammatory, and anti-ulcerative properties. Previous studies have shown that aqueous extracts of *C. rotundifolia* significantly reduce blood glucose levels in streptozotocin-induced diabetic rats with continuous treatment (Al-Mehdar & Al-Battah, 2016; Abu-Odeh & Talib, 2021). Considering the established antioxidant, anti-inflammatory, and anti-fibrosis properties of *C. rotundifolia*, we hypothesize that it may exert a protective effect against testicular and thyroid damage induced by diabetes. This study aims to evaluate the protective effect of *C. rotundifolia* on testicular and thyroid damage in diabetic rats and explore its underlying mechanisms, thereby providing a basis for its potential use in the treatment and nutritional intervention of diabetic complications related to male reproductive and thyroid health.

MATERIAL AND METHOD

Ethical approval:

Approval for this study was granted by the research ethics committee at the Health Science Research Center, King Abdullah bin Abdulaziz University Hospital (IRB Log Number: 24-0031), at Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. The research adhered to the principles outlined in the "Guide for the Care and Use of Laboratory Animals," as issued by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Materials

Streptozotocin (STZ) was purchased from SRL (India, LTD). STZ was freshly dissolved in 0.05 M citrate buffer (pH 4.5) immediately before use and administered intraperitoneally (IP) at a dose of 45 mg/kg body weight after rats were fed a high-fat diet for four weeks (Zhang *et al*., 2008). *C. rotundifolia* plant was obtained from Abha city in the southwest region of Saudi Arabia. The plant grows natively in this region. *C. rotundifolia* plant extract was administered orally at a dose of 100 mg/kg body weight for eight weeks (Al-Mehdar & Al-Battah, 2016). Metformin hydrochloride was procured from Shanghai Shiguibao Medicine Co. (Ltd., China). Metformin was diluted in distilled water and administered orally via a gastric tube at a dose of 150 mg/kg/day for eight weeks (Pushparaj *et al*., 2000).

Animals and Study Design

Forty adult male albino Wistar rats, weighing between 200 and 250 g, were obtained from the Experimental Lab and used in this study. The experimental model adhered to the principles outlined in the "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 85-23, revised 1996). All animals were housed in plastic cages (5 rats per cage) and allowed one week for acclimatization under standard laboratory conditions: humidity (55-65 %), temperature (20 ± 1 °C), and a 12/12-hour light/dark cycle, with free access to pellet food and tap water *ad libitum.*

Rats were randomly divided into five experimental groups, with six rats in each group:

- Group I (Normal Control, C): we have administered IP citrate buffer solution (0.5 ml) and oral distilled water (0.5 ml) by oral gavage daily for eight weeks.

- Group II (*C. rotundifolia* Group, CR): In group II we provided with the *C. rotundifolia* water extract at 100 mg/kg body weight by oral gavage daily for eight weeks.

- Group III (Diabetic Control, DM): We have developed streptozotocin induced model by injecting STZ (45 mg/kg b.w.) after a 12-hour fast. Furthermore, to prevent hypoglycemia and enhance STZ entry into cells via the GLUT2 glucose transporter, rats were administered a 5 % sucrose solution overnight after STZ injection (Wang & Gleichmann, 1998). After, seven days post-STZ injection, blood samples were collected from the rat tail to confirm the hyperglycemic status. We have found that the rats with blood glucose values exceeding 250 mg/dl were selected for the study as indicative of developing Type 2 Diabetes Mellitus (T2DM) (Cosyns *et al*., 2007). This group then received oral distilled water (0.5 ml) by oral gavage daily for eight weeks.

- Group IV (Diabetic + *C. rotundifolia*, DM+CR): In this group, the diabetic rats (induced as in Group III) were treated daily with *C. rotundifolia* water extract at 100 mg/kg body weight, given orally by gavage for eight weeks.

- Group V (Diabetic + Metformin, DM+METF): Group V consists of diabetic rats (induced as in Group III) which were treated daily with metformin at 150 mg/kg body weight, given orally by gavage for eight weeks.

Throughout the eight-week experimental period, starting from the first day of extract/drug administration to diabetic rats, body weight and fasting blood glucose levels were measured every 7th day.

Sampling Technique

At the end of the 8-week treatment period, on day 28 of extract administration, we collected the blood samples from rats using a specialized capillary tube from the retro-orbital venous plexus under mild ether anesthesia. The blood samples were left for 30 min to clot and then centrifuged at 3000 rpm for 20 min (using an EBA-20, Hettich, Tuttlingen, Germany) to separate serum for hormonal and antioxidant enzyme analyses. Furthermore, animals were euthanized, the abdomen was opened, and the testes and thyroid glands were carefully removed through dissection. The organs were weighed and cleaned with normal saline. Harvested organs were stored in neutral formalin solution for subsequent histological processing.

Biochemical Analysis

Blood Glucose Measurement. We have determined the blood glucose values every other day from the rat's tail using the ACCU-CHEK® Performa (Germany). At the experimental endpoint, serum glucose levels were estimated by the enzymatic colorimetric method (Barham & Trinder, 1972; Hassan *et al*., 2015).

Hormonal Assay. The serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, thyroid stimulating hormone (TSH), and thyroxine (T4) were detected using ELISA kits (Wuhan Saipei Biotech Co., Ltd., Wuhan, China) and a microplate reader, following the manufacturer's operation procedures.

Antioxidant Enzymes and Lipid Peroxidation (MDA). The obtained serum was utilized for the determination of antioxidant enzyme activity and levels of lipid peroxidation. Superoxide dismutase (SOD) activity was determined according to the colorimetric method described by Martin Jr. & Fridovich (1987), at 560 nm. The malondialdehyde (MDA) level was measured spectrophotometrically using the method of Placer *et al*. (1966), which is based on the reaction of MDA with thiobarbituric acid (TBA), one of the aldehyde products of lipid peroxidation.

Histopathological Studies

The left testis and a portion of the thyroid gland from each animal were designated for histopathological studies. Tissues were immediately placed in a 10 % neutral formalin solution after removal and weighing. Following 48 hours of fixation, tissues were routinely processed using a tissue processor, embedded in paraffin, and sectioned at 5 µm thickness using a rotatory microtome. Sections were then stained with hematoxylin and eosin (H&E). Stained sections were examined under a light microscope and photographed by an expert histopathologist to assess tissue changes (Lillie & Denton, 1965).

RT-qPCR

We have collected testis and thyroid tissues which were subjected immediately to RNA extraction using Tirzole method. RNA was isolated from the liver using a RNA Mini Kit. The RNA was then subjected to qualitative and quantitative analysis followed by cDNA synthesis using a Maxima SYBR Green Master Mix (2X) cDNA synthesis kit. The PCR was performed on a MyGo Pro PCR system a specific primer sets (20 mM) using specific primers of inflammatory markers (IL-6, TNF-α), glucose metabolism regulators (GLUT4, PPARg), and oxidative stress-related genes (Nrf2, SOD), normalized to GAPDH.

Table I. Body weight (g) of experimental rats before and after 8 weeks of treatment.

Group	Body Weight Before (g) ± SE	Body Weight After (g) ± SE
C	226.4 ± 5.02	238.8 ± 11.82
CR	246.4 ± 12.59	280.8 ± 13.12**
DM	227.6 ± 9.98	178.4 ± 6 .36***
DM+*CR*	233.8 ± 6.76	243.0 ± 12.77**
DM+METF	243.6 ± 6.48	249.4 ± 7.69*

Data are presented as Mean ± SE. The groups (X axis) are: Normal Control, C; *C. rotundifolia* Group, CR; Diabetic Control, DM ; Diabetic + *C. rotundifolia*, DM+CR; Diabetic + Metformin, DM+METF.

Statistical Analysis.

Obtained results are expressed as means ± standard error (SE). The normal distribution of data was assessed by the Kolmogorov–Smirnov test. Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test in SPSS software version 21 (SPSS Inc. Chicago, IL, USA). A p-value of < 0.05 was considered statistically significant.

RESULTS

Effects of *Cissus rotundifolia* on Body Weight and Blood Glucose of DM Rats

We have checked the body weight of rats before and after the 8-week experimental period across all groups (Table I, Fig. 1). Furthermore, we have also checked the blood glucose levels of rats after the 8-week experimental period (Table II, Fig. 2). There was a gradual progress in the body weight and blood glucose level between the DM and DM+CR groups. While initial body weights are relatively similar, the post-treatment weights show a clear distinction, with DM rats experiencing significant weight loss, which is partially attenuated in DM+CR and DM+METF groups. The blood glucose data clearly shows the hyperglycemic state in the DM group and significant reduction in the treated groups. 3.2. Effects of *Cissus rotundifolia* on Serum LH, FSH, Testosterone, TSH, and T4 Levels in DM Rats

We have analyzed the serum levels of thyroid stimulating hormone (TSH) and thyroxine (T4) in the experimental groups. Our data shows a significant decrease

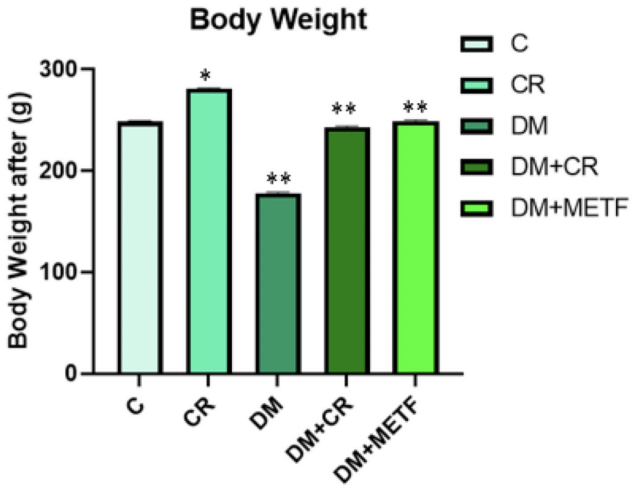

Fig. 1. Body weight of different groups after treatment of *Cissus rotundifolia*. The groups (X axis) are: Normal Control, C; *C. rotundifolia* Group, CR; Diabetic Control, DM ; Diabetic + *C. rotundifolia*, DM+CR; Diabetic + Metformin, DM+METF.

Table II. Blood glucose levels (mg/dl) of experimental rats after 8 weeks of treatment.

Group	Blood Glucose After (mg/dl) ± SE
C	92.0 ± 2.607
CR	99.0 ± 4.745
DM	312.2 ± 4.615
DM+*CR*	114.7 ± 2.924
DM+METF	96.6 ± 3.613

Data are presented as Mean ± SE. The groups are: Normal Control, C; *C. rotundifolia* Group, CR; Diabetic Control, DM; Diabetic + *C. rotundifolia*, DM+CR; Diabetic + Metformin, DM+METF.

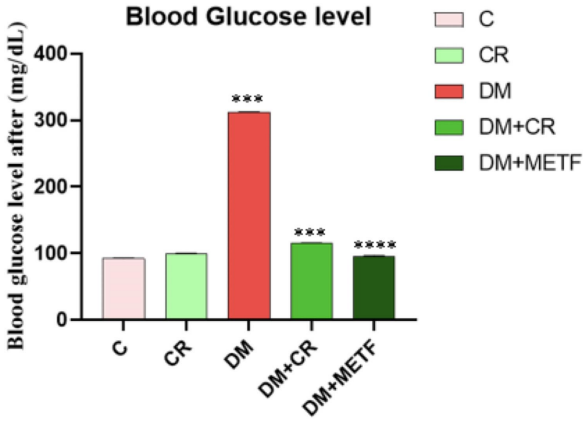

Fig. 2. Blood glucose level of different groups after treatment of *Cissus rotundifolia* . Groups: Normal Control, C; *C. rotundifolia* Group, CR; Diabetic Control, DM ; Diabetic + *C. rotundifolia*, DM+CR; Diabetic + Metformin, DM+METF

Table III. The serum levels of thyroid stimulating hormone (TSH) and thyroxine (T4) in the experimental groups.

Group	TSH (mIU/L) ± SE	T4 (ug/dl) ± SE
C	36.28 ± 0.358	73.7 ± 3.936
CR	34.36 ± 0.492	63.6 ± 1.743
DM	9.1 ± 0.407	278.1 ± 4.566
DM+*CR*	27.76 ± 0.268	74.8 ± 1.83
DM+METF	36.64 ± 0.442	83.9 ± 0.894

Groups: Normal Control, C; *C. rotundifolia* Group, CR; Diabetic Control, DM ; Diabetic + *C. rotundifolia*, DM+CR; Diabetic + Metformin, DM+METF.

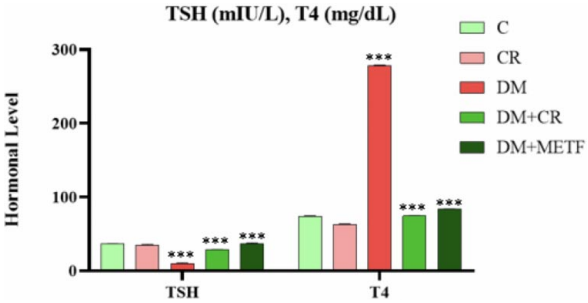

Fig. 3. Serum levels of Thyroid Stimulating Hormone (TSH) and Thyroxine (T4) after 8 weeks of treatment. Groups: Normal Control, C; *C. rotundifolia* Group, CR; Diabetic Control, DM ; Diabetic + *C. rotundifolia*, DM+CR; Diabetic + Metformin, DM+METF

in TSH and a significant increase in T4 in the DM group, indicating altered thyroid function. Both C (Table III, Fig. 3). The rotundifolia and Metformin treatments appear to normalize these levels towards the control, with Metformin showing near control levels for TSH (Table III, Fig. 3).

Effects of *Cissus rotundifolia* on Antioxidant Enzymes Activities and MDA of DM Rats

We have further analyzed the levels of malondialdehyde (MDA) in the experimental groups. The MDA data indicates significantly elevated lipid peroxidation in the diabetic group, which is notably reduced by both *C. rotundifolia* and Metformin treatments (Table IV, Fig. 4).

Table IV. Serum Malondialdehyde (MDA) levels after 8 weeks of treatment.

Group	MDA ($x\,10^{-2}$ nmol/ml) ± SE
C	37.8 ± 0.8
CR	61.0 ± 1.378
DM	128.8 ± 0.917
DM+*CR*	67.0 ± 1.304
DM+METF	48.6 ± 0.925

Groups: Normal Control, C; *C. rotundifolia* Group, CR; Diabetic Control, DM ; Diabetic + *C. rotundifolia*, DM+CR; Diabetic + Metformin, DM+METF.

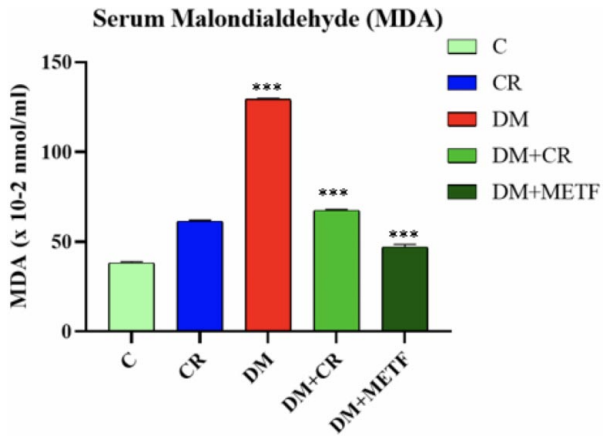

Fig. 4. Serum Malondialdehyde (MDA) levels after 8 weeks of treatment in different experimental groups. Groups: Normal Control, C; *C. rotundifolia* Group, CR; Diabetic Control, DM ; Diabetic + *C. rotundifolia*, DM+CR; Diabetic + Metformin, DM+METF

Effect of *Cissus rotundifolia* on the Structure of Testicular and Thyroid Tissues of Diabetic Rats.

Histological Study of Testicular Tissue. Histopathological examination of testicular tissues stained with Hematoxylin and Eosin (H&E) revealed significant alterations across the experimental groups (Fig. 5). The control group exhibited

normal testicular architecture, characterized by densely packed, well-organized seminiferous tubules with a complete spermatogenic series and numerous spermatozoa in the lumen. In stark contrast, the diabetic group displayed severe testicular damage. Their seminiferous tubules appeared disorganized and shrunken, with widespread degeneration of the germinal epithelium, evident loss of spermatogenic cells, and reduced or absent spermatozoa in the lumen.

Interestingly, both treatment groups demonstrated a notable improvement in testicular morphology. The Diabetic + *Cissus rotundifolia* (Diabetic+CR) group showed partially restored seminiferous tubule structure, with an increase in germinal epithelial cell layers and the presence of some spermatozoa, indicating a protective effect. Similarly, the Diabetic + Metformin (Diabetic+METF) group exhibited a significant recovery, with relatively well-preserved seminiferous tubules, a more organized germinal epithelium, and a higher count of spermatozoa within the tubular lumen, closely resembling the control group's healthy structure. These findings suggest that both *Cissus rotundifolia* and Metformin mitigate diabetes-induced testicular damage.

Histological Study of Thyroid Gland. The histological examination of thyroid glands, as shown in Figure 5, revealed distinct differences among the experimental groups. The control group exhibited a healthy thyroid structure, characterized by normal, rounded thyroid follicles lined by a single layer of cuboidal epithelial cells with rounded nuclei. The colloid within these follicles was uniformly pigmented and free of vacuoles or desquamated cells. In contrast, the diabetic group showed significant pathological changes. Their thyroid glands were malformed, with visible follicular cell degeneration and a noticeable decrease in colloid content within the central follicles. The groups treated with *Cissus rotundifolia* and Metformin showed marked improvements compared to the untreated diabetic group. The diabetic + *Cissus rotundifolia* group displayed a histological structure that was nearly normal, with well-organized, regular follicles and an intact cuboidal epithelial lining, much like the control group. The diabetic + Metformin group also showed significant improvement, with thyroid sections revealing follicles of varying sizes containing normal colloid. These follicles were lined by cubical follicular cells with rounded nuclei, and minute blood capillaries were observed between the follicles, indicating a return to a more typical histological architecture. The *Cissus rotundifolia* group (non-diabetic, treated) also maintained normal thyroid tissue with intact cuboidal cells and uniformly stained colloids, demonstrating that the compound did not negatively affect healthy thyroid tissue.

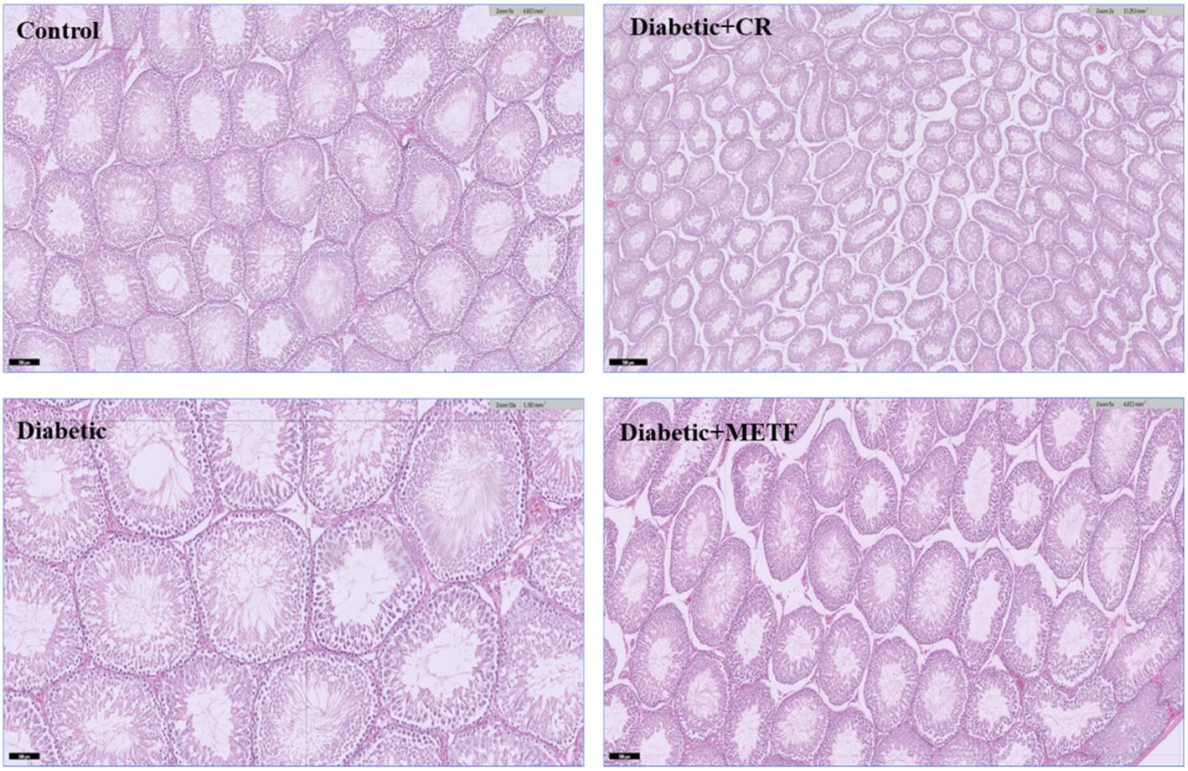

Fig. 5. Histopathological analysis of testicular tissue (H&E staining, 40x magnification). The image displays representative photomicrographs of testicular cross-sections from different experimental groups: (A) Control, (B) Diabetic, (C) Diabetic + *Cissus rotundifolia* (Diabetic+CR), and (D) Diabetic + Metformin (Diabetic+METF). Each panel illustrates the morphological changes in seminiferous tubules and germinal epithelium under various conditions.

Expression of oxidative stress markers

We have analyzed the expression of oxidative stress markers (Nrf2, SOD) which demonstrated significant changes due to diabetes and subsequent treatments. We have found a marked reduction in the diabetic group (DM) in the expression of both markers compared to the healthy control group, suggesting that diabetes negatively impacted the body's antioxidant capacity (Fig. 6). However, both the diabetic + *Cissus rotundifolia* and diabetic + Metformin groups displayed a significant increase in the expression of both oxidative stress markers (Fig. 7). This indicates that both treatments were effective in helping to restore the body's natural defenses against oxidative stress, bringing the expression levels back towards or even above that of the control group.

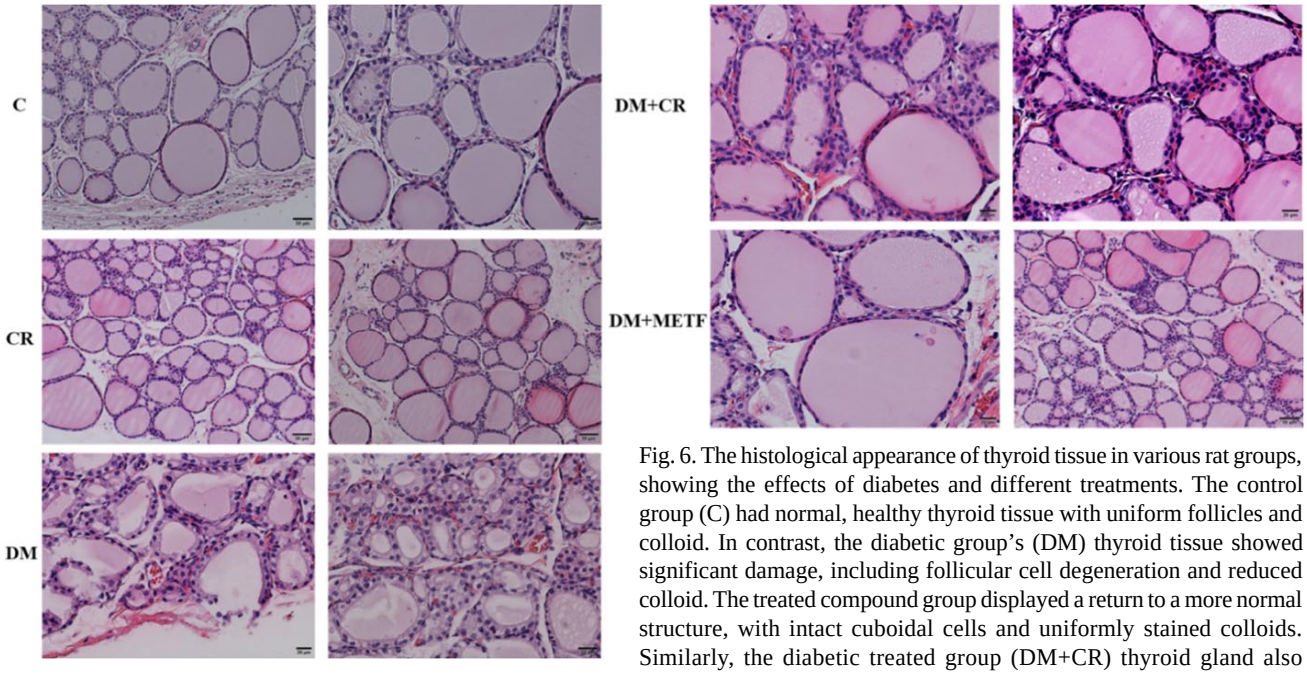

Fig. 6. The histological appearance of thyroid tissue in various rat groups, showing the effects of diabetes and different treatments. The control group (C) had normal, healthy thyroid tissue with uniform follicles and colloid. In contrast, the diabetic group's (DM) thyroid tissue showed significant damage, including follicular cell degeneration and reduced colloid. The treated compound group displayed a return to a more normal structure, with intact cuboidal cells and uniformly stained colloids. Similarly, the diabetic treated group (DM+CR) thyroid gland also appeared nearly normal, with well-organized follicles and healthy epithelial lining, much like the control. The group treated with Metformin (DM+METF) also showed marked improvement, with varied follicle sizes, normal colloid, and healthy cuboidal follicular cells.

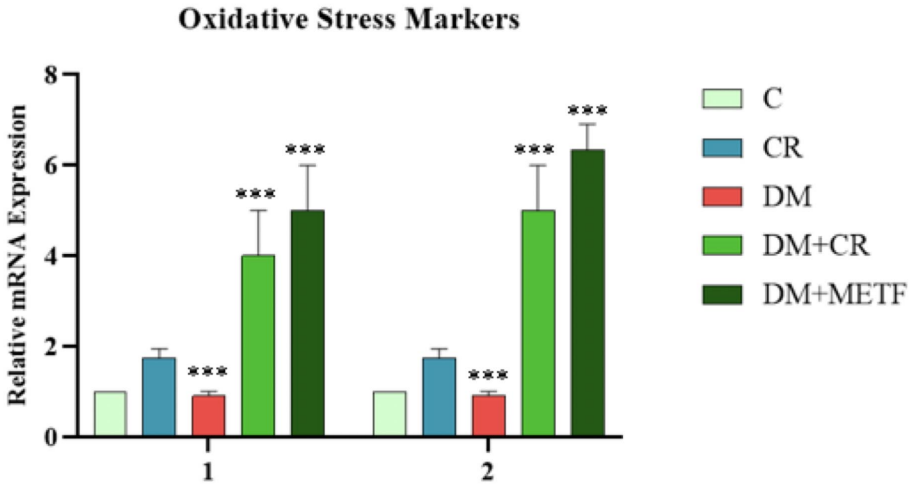

Fig. 7. Expression of Oxidative Stress Markers in Different Experimental Groups. The relative mRNA expression of Nrf2 and SOD across five different groups of rats: Control (C), Control treated with *Cissus rotundifolia* (CR), Diabetic (DM), Diabetic treated with *Cissus rotundifolia* (DM+CR), and Diabetic treated with Metformin (DM+METF).

Expression of Inflammatory markers

We have found that the expression of inflammatory markers was significantly altered in the diabetic group, as expected (Fig. 8). The diabetic group (DM) showed a substantial increase in both IL-6 and TNF-α expression, confirming the presence of systemic inflammation. However, treatment with either *Cissus rotundifolia* or Metformin effectively reduced these elevated levels. Specifically, the diabetic + *Cissus rotundifolia* and diabetic + Metformin groups showed a marked decrease in the expression of both IL-6 and TNF-α, bringing them closer to the levels observed in the healthy control group (Fig. 8). This indicates that both treatments were successful in mitigating the diabetes-induced inflammatory response.

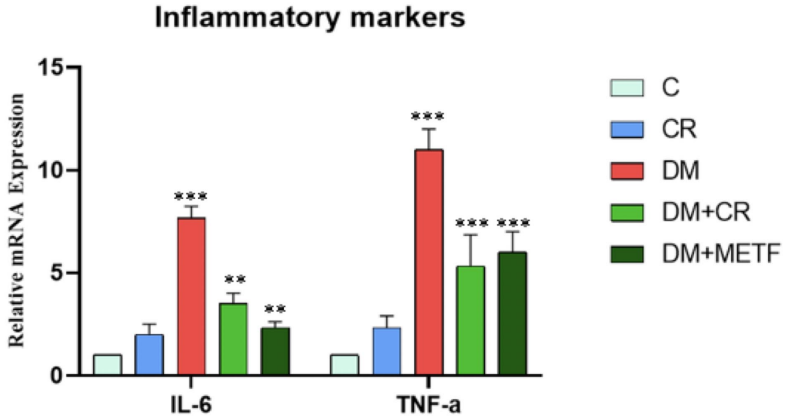

Fig. 8. Expression of Inflammatory Markers in Different Experimental Groups. The results shows the relative mRNA expression of two key inflammatory markers, IL-6 and TNF-α, across five groups: Control (C), Control treated with *Cissus rotundifolia* (CR), Diabetic (DM), Diabetic treated with *Cissus rotundifolia* (DM+CR), and Diabetic treated with Metformin (DM+METF). The data highlights how diabetes and subsequent treatments affect inflammation.

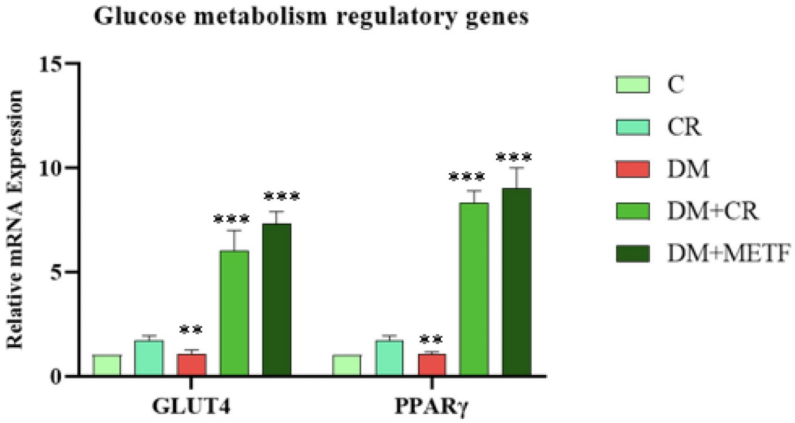

Fig. 9. Expression of Glucose Metabolism Regulatory Genes. This results shows the relative mRNA expression levels of key glucose metabolism regulatory genes, GLUT4 and PPARg across different experimental rat groups: Control (C), Control treated with *Cissus rotundifolia* (CR), Diabetic (DM), Diabetic treated with *Cissus rotundifolia* (DM+CR), and Diabetic treated with Metformin (DM+METF).

Glucose metabolism regulatory markers

Our analysis of glucose metabolism regulatory genes revealed significant changes in response to diabetes and various treatments (Fig. 9). As anticipated, the diabetic group (DM) showed a noticeable decrease in the expression of both GLUT4 and PPARg compared to the control group, indicating impaired glucose uptake and utilization. Interestingly, both diabetic + *Cissus rotundifolia* and diabetic + Metformin groups demonstrated a substantial upregulation of these genes (Fig. 9). Specifically, the expression levels of both GLUT4 and PPARg in these treated diabetic groups were significantly increased, often surpassing those observed in the control group. This highlights the effectiveness of both *Cissus rotundifolia* and Metformin in positively influencing glucose metabolism by enhancing the expression of these crucial regulatory markers in diabetic rats.

DISCUSSION

In this study, we investigated the therapeutic potential of *Cissus rotundifolia* extract and metformin on metabolic health, testicular and thyroid hormone levels, and the histopathological integrity of testes and thyroid glands in streptozotocin-induced diabetic rats. Our findings reveal several important insights into the ameliorative effects of these interventions. The presence of flavonoids such as quercetin and kaempferol, and phenolic acids like ferulic and gallic acid, detected in the *Cissus rotundifolia* extract, is consistent with the strong antioxidant activity observed in the extract-treated groups. These compounds have been shown to reduce oxidative stress, enhance insulin sensitivity, and stabilize thyroid hormone metabolism (Korkmaz *et al*., 2013; Eid *et al*., 2015). The detection of beta-sitosterol and stigmasterol further supports the hypoglycemic effect seen in treated diabetic rats. These phytosterols have been reported to mimic insulin actions and modulate lipid profiles, which can indirectly support thyroid hormone synthesis and metabolism. Collectively, the phytochemical analyses provide a strong chemical basis for the observed biological activities of *Cissus rotundifolia*. Our results demonstrate that streptozotocin-induced diabetes led to significant hyperglycemia and body weight loss in the diabetic control group, consistent with previous studies (Zhang *et al*., 2008).

Both *Cissus rotundifolia* extract and metformin treatments effectively reduced blood glucose levels and attenuated the diabetes-induced body weight loss, indicating their anti-diabetic potential. This aligns with prior research showing hypoglycemic effects of *C. rotundifolia* (Al-Mehdar & Al-Battah, 2016; Abu-Odeh & Talib, 2021) and the established anti-diabetic action of metformin (Pushparaj *et al*., 2000).

Furthermore, supporting the positive impact on metabolic health, the analysis of glucose metabolism regulatory genes (Fig. 8) revealed a noticeable decrease in the expression of both GLUT4 and PPARg in the diabetic group, signifying impaired glucose uptake and utilization. Crucially, both the diabetic + *Cissus rotundifolia* and diabetic + Metformin groups showed a substantial upregulation of these genes, often surpassing control levels. This highlights their effectiveness in positively influencing glucose metabolism by enhancing the expression of these crucial regulatory markers in diabetic rats.

Regarding endocrine function, the diabetic group exhibited a marked decrease in TSH and a significant increase in T4, suggesting a disruption in thyroid hormone regulation commonly associated with diabetes (Kalra *et al*., 2019). This altered thyroid profile was substantially normalized by both *Cissus rotundifolia* and metformin treatments, indicating a protective effect against diabetes-induced thyroid dysfunction. The histological examination of the thyroid gland corroborated these biochemical findings; the diabetic group showed follicular cell degeneration and decreased colloid, while treatment with *Cissus rotundifolia* and metformin improved the histological structure, restoring near-normal follicular architecture and colloid content. Furthermore, our MDA results clearly indicate that diabetes significantly increased lipid peroxidation, a marker of oxidative stress. Consistent with this, the diabetic group also showed a marked reduction in the expression of two key oxidative stress markers (Fig. 6), suggesting a compromised antioxidant defense system. However, the administration of *Cissus rotundifolia* and metformin not only significantly reduced these elevated MDA levels but also significantly increased the expression of these protective oxidative stress markers in the treated groups, bringing them back towards or above control levels. This suggests their potent antioxidant capabilities and supports our hypothesis that these agents exert protective effects through mitigating oxidative stress, a primary contributor to diabetic complications in various organs, including the testes and thyroid (De Young *et al*., 2004; Amaral *et al*., 2008). Beyond oxidative stress, our investigation into inflammatory responses (Fig. 7) showed that the diabetic group experienced a substantial increase in the expression of both IL-6 and TNF-α, confirming significant systemic inflammation. Encouragingly, treatment with either *Cissus rotundifolia* or Metformin effectively reduced these elevated inflammatory marker levels, bringing them closer to those observed in the healthy control group. This indicates that both interventions were successful in mitigating the diabetes-induced inflammatory response.

While the quantitative data for testicular hormones (LH, FSH, testosterone) is yet to be fully presented, the observed improvements in metabolic parameters, antioxidant status, inflammatory response, and thyroid function suggest a probable beneficial impact on testicular health. Histopathological findings on the testes, once fully presented and analyzed for the diabetic and treated groups, are expected to provide further evidence of the protective effects of *Cissus rotundifolia* and metformin on male reproductive tissue integrity, consistent with their established antioxidant and anti-inflammatory properties. These findings collectively suggest that *Cissus rotundifolia* holds promise as a natural intervention for managing diabetes and its associated complications, including those affecting reproductive and thyroid health, by modulating metabolic parameters, reducing oxidative stress, alleviating inflammation, and restoring hormonal balance.

CONCLUSION

This study demonstrates that *Cissus rotundifolia* extract effectively ameliorates several key manifestations of diabetes in rats, including hyperglycemia, body weight loss, and oxidative stress and regulate glucose metabolism. Furthermore, it exerts significant protective effects on thyroid hormone balance and thyroid tissue integrity, comparable to the conventional anti-diabetic drug metformin. While the full scope of testicular hormonal effects awaits complete data presentation, the overall improvement in metabolic and oxidative stress markers, along with preliminary testicular histopathological observations (once fully presented), suggests a promising role for *Cissus rotundifolia* in mitigating diabetes-induced male reproductive complications. These findings underscore the therapeutic potential of *Cissus rotundifolia* as a valuable alternative or supplementary treatment for comprehensive diabetes management.

ALMOHAIMEED, H.M.; ALAMOUDI, M.O.; AL.TURKISTANI, H.A.; ASSIRI, R.; ELTAYEB, H.H.; ALONAZI, S. & ALSHAER, A.M. Evaluación histomorfológica y endocrino-molecular de *Cissus rotundifolia* y metformina en tejidos testiculares y tiroideos de ratas diabéticas. *Int. J. Morpholol., 44(3)*:913-923, 2026.

RESUMEN: La diabetes mellitus se asocia con alteraciones estructurales y funcionales en los tejidos endocrinos y reproductivos. Este estudio evaluó los efectos histomorfológicos, hormonales y moleculares del extracto de *Cissus rotundifolia* en

comparación con la metformina en tejidos testiculares y tiroideos de ratas diabéticas inducidas por estreptozotocina. Cuarenta ratas Wistar macho se dividieron en grupos control y experimental, incluyendo grupos diabéticos no tratados y diabéticos tratados que recibieron *Cissus rotundifolia* (100 mg/kg) o metformina (150 mg/kg) durante ocho semanas. El examen histológico de secciones teñidas con hematoxilina y eosina reveló una marcada degeneración de los túbulos seminíferos, pérdida del epitelio germinal y alteración de las células foliculares en las glándulas tiroides de las ratas diabéticas. Estas alteraciones se atenuaron significativamente en los grupos tratados, con restauración de la arquitectura seminífera, mejora de las capas espermatogénicas y normalización de la estructura folicular tiroidea. El análisis hormonal demostró que las reducciones de TSH y las alteraciones de T4 inducidas por la diabetes se corrigieron sustancialmente tras el tratamiento. El análisis molecular mostró una regulación positiva de los marcadores antioxidantes (Nrf2, SOD) y genes relacionados con el metabolismo de la glucosa (GLUT4, PPARg), junto con una disminución de la expresión de marcadores inflamatorios (IL-6, TNF-α) en los grupos tratados. Estos hallazgos sugieren que *Cissus rotundifolia* ejerce efectos protectores contra el daño estructural inducido por la diabetes en los tejidos testiculares y tiroideos, comparables a los de la metformina, probablemente mediante la modulación del estrés oxidativo y las vías inflamatorias.

PALABRAS CLAVE: Diabetes mellitus; *Cissus rotundifolia*; Metformina; Tejido testicular; Tejido tiroideo; Estrés oxidativo; Histopatología; Ratas.

REFERENCES

Abu-Odeh, A. M. & Talib, W. Middle East Medicinal Plants in the Treatment of Diabetes: A Review. *Molecules, 26(3)*:742, 2021.

Al-Mehdar, A. A. & Al-Battah, A. M. Evaluation of Hypoglycemic Activity of Boswellia carterii and *Cissus rotundifolia* in Streptozocin/Nicotinamide-Induced Diabetic Rats. *Yemeni J. Med. Sci., 10*:30-8, 2016.

Alshehri, S. *Antidiabetic Activity of Cissus rotundifolia Plant Growing in Saudi Arabia*. Doctoral Dissertation. Brookings, South Dakota State University, 2020.

Altan, E.; Dalgic, A. & Cinar, N. Oxidative stress and antioxidant capacity in patients with diabetes mellitus and thyroid dysfunction. *Clin. Chem. Lab. Med., 48(8)*:1161-6, 2010.

Amaral, S.; Oliveira, P. J. & Ramalho-Santos, J. Diabetes and the impairment of male reproductive function: possible role of oxidative stress. *Curr. Diabetes Rev., 4(2)*:162-9, 2008.

Andlib, N.; Sajad, M.; Kumar, R. & Thakur, S. C. Abnormalities in sex hormones and sexual dysfunction in males with diabetes mellitus: A mechanistic insight. *Acta Histochem., 125(1)*:151974, 2023.

Apichakan, C.; Supornsilpchai, W. & Pholpramool, C. The effect of diabetes on male fertility. *J. Clin. Diagn. Res., 12(7)*:BC01-BC04, 2018.

Barham, D. & Trinder, P. An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst, 97(151)*:142-5, 1972.

Brenta, G. The effects of diabetes on thyroid function. *Best Pract. Res. Clin. Endocrinol. Metab., 24(3)*:415-24, 2010.

Chang, H. Y.; Wallis, M. & Tiralongo, E. Use of complementary and alternative medicine among people living with diabetes: literature review. *J. Adv. Nurs., 58(4)*:307-19, 2007.

Cosyns, B.; Droogmans, S.; Weytjens, C.; Lahoutte, T.; Van Camp, G.; Schoors, D. & Franken, P. R. Effect of streptozotocin-induced diabetes on left ventricular function in adult rats: an in vivo Pinhole Gated SPECT study. *Cardiovasc. Diabetol., 6*:30, 2007.

De Young, L.; Young, D. W. & Trudell, A. Oxidative stress and male infertility. *J. Androl., 25(6)*:903-9, 2004.

Eid, R.; Ali, A. & El-Kott, A. F. Therapeutic efficacy of date palm pollen on testicular dysfunction in streptozotocin-induced diabetic rats. *J. Clin. Exp. Pathol., 5(6)*:1000249, 2015.

Hassan, A. I.; Ibrahim, A. M. & El-Sayed, A. I. Antidiabetic effect of Moringa oleifera leaves extract in streptozotocin-induced diabetic rats. *J. Diabetes Res., 2015*:813076, 2015.

Hage, M.; de Lacerda, L. & de Lemos, L. C. Thyroid function and diabetes mellitus. *Arq. Bras. Endocrinol. Metab., 55(8)*:629-39, 2011.

Hlebowicz, J. & Darwiche, G. Effect of cinnamon on postprandial blood glucose, gastric emptying and satiety in healthy subjects. *Am. J. Clin. Nutr., 85(6)*:1552-6, 2007.

Kalra, S.; Aggarwal, S. & Khandelwal, D. Thyroid Dysfunction and Type 2 Diabetes Mellitus: Screening Strategies and Implications for Management. *Diabetes Ther., 10(6)*:2035-44, 2019.

Kermani, F.; Ramezanali, F. & Fakhravar, M. The effect of diabetes on male reproductive system. *J. Res. Med. Sci., 24(1)*:52, 2019.

Khosravi, Z.; Nejati, V. & Firoozbakhsh, F. Beneficial effects of propolis on oxidative stress, inflammation and spermatogenesis in diabetic rats. *J. Basic Clin. Physiol. Pharmacol., 30(3)*:283-90, 2019.

Korkmaz, M.; Karslı, T. & Öztürk, H. S. The effects of quercetin on oxidative stress and apoptosis in experimentally induced diabetes. *J. Clin. Diagn. Res., 7(8)*:1530-3, 2013.

Lillie, R. J. & Denton, C. Protein and energy interrelationships for laying hens. *Poult. Sci., 44(3)*:753-61, 1965.

Liu, J.; Zhang, S.; Li, H. & Zhou, W. Prevalence of diabetes and prediabetes in China: a review. *Chin. J. Med. Genet., 36(3)*:299-304, 2019.

Martin Jr., J. P. & Fridovich, I. Superoxide dismutase: an enzymic assay for its activity. *Methods Enzymol., 137*:308-12, 1987.

Méndez-López, S.; Hernández-Cruz, E. Y. & Hernández-Cruz, P. Antioxidant and anti-inflammatory properties of natural products on diabetes and its complications. *Oxid. Med. Cell. Longev., 2020*:1-17, 2020.

Mohammed, A. A review of ethnomedicinal and pharmacological properties of *Cissus quadrangularis*. *J. Med. Plants Res., 13(24)*:585-94, 2019.

Nishi, M. Thyroid dysfunction and diabetes mellitus. *J. Clin. Transl. Endocrinol., 11*:1-5, 2018.

Nna, V. U.; Bakari, T. A. & Agbafor, N. O. Oxidative stress, inflammation, and apoptosis in diabetic testicular dysfunction. *J. Basic Clin. Physiol. Pharmacol., 30(4)*:365-73, 2019.

Orman, M.; Topcu, B. & Sari, M. The role of oxidative stress in male infertility. *Reprod. Biol. Endocrinol., 13(1)*:108, 2015.

Pavlinkova, G.; Machala, M. & Kofránek, R. Diabetes mellitus and male fertility: a review. *Physiol. Res., 66(2)*:173-82, 2017.

Placer, Z. A.; Cushman, L. L. & Johnson, B. C. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Anal. Biochem., 16(2)*:359-64, 1966.

Pushparaj, P.; Tan, C. H. & Tan, B. K. Effects of Averrhoa bilimbi leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. *J. Ethnopharmacol., 72(1-2)*:69-76, 2000.

Qadiri, F.; Siddique, A. A. & Khan, M. F. Diabetic testicular dysfunction: an update on underlying mechanisms and therapeutic approaches. *J. Diabetes Res., 2019*:1-13, 2019.

Roghani, M. & Baluchnejadmojarad, T. Survey the hypoglycemic and hypolipidemic effect of chronic oral administration of Nigella sativa in diabetic rat. *J. Guilan Univ. Med. Sci., 16(63)*:26-31, 2007.

Shiraishi, K.; Naito, K. & Yoshida, K. Diabetes mellitus and male infertility: an overview. *Reprod. Med. Biol., 2(2)*:69-73, 2003.

Wang, W. & Gleichmann, H. GLUT2 in pancreatic islets: crucial target molecule in diabetes induced with multiple low doses of streptozotocin in mice. *Diabetes, 47(1)*:50-6, 1998.

Wang, Y. Thyroid hormone and diabetes. *J. Diabetes Res., 2013*:462791, 2013.

World Health Organization. *WHO Traditional Medicine Strategy 2002–2005*. Geneva, World Health Organization, 2011.

Zanini, A.; Quattrin, R.; Goi, D.; Frassinelli, B.; Panariti, M.; Carpanelli, I. & Brusaferro, S. Italian oncology nurses' knowledge of complementary and alternative therapies: National survey. *J. Adv. Nurs., 62(4)*:451-7, 2008.

Zhang, M.; Lv, X. Y.; Li, J.; Xu, Z. G. & Chen, L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. *Exp. Diabetes Res., 2008*:704045, 2008.

Zhao, C.; Zhang, X. & Deng, Q. Oxidative stress and antioxidant defense in male infertility. *J. Reprod. Dev., 56(1)*:1-9, 2010.

Zheng, W.; Wu, S. & Li, R. Testicular interstitial fibrosis: a common pathological feature of male infertility. *Andrology, 9(1)*:5-16, 2021.

Corresponding author:
Hailah M. Almohaimeed
Department of Basic Science
College of Medicine
Princess Nourah bint Abdulrahman University
P. O. Box 84428
Riyadh 11671
SAUDI ARABIA

E-mail: almohaimeedhreaserch86@gmail.com