DIABETES-RELATED TESTICULAR DYSFUNCTION AND THE EFFECTS OF VINCAMINE

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Abstract –
Diabetes mellitus causes problems of various organ systems, including retinopathy, neuropathy and nephropathy. However, patients may experience changes in their sexual function, such as decreased libido and fertility. Vincamine, a monoterpenoid indole alkaloid, has hypoglycemic and antioxidant effects. This study evaluated the effect of vincamine on testicular dysfunction in alloxan-induced male rats by measuring fasting blood glucose, oxidative stress, seminal analysis, and histological examination of the testis. The rats were randomly divided into the following groups: untreated-healthy, untreated diabetics, vincamine-treated (20mg/kg) diabetics, vincamine-treated (40mg/kg) diabetics, and clomiphene-treated diabetics (5mg/kg). On day 14, rats were sacrificed and semen/blood samples were collected. Sperm count, motility and morphological abnormalities were recorded by microscopic examination. The testis was examined histopathologically and evaluated using the Johnsen score. Compared to the untreated diabetic group, the 40 mg/kg dose of vincamine produces a significant reduction in FBC. In addition, the vincamine groups showed a dose-dependent antioxidant effect. Compared with the untreated diabetic group, vincamine-treated rats produced higher plasma testosterone levels and Johnsen scores. The results suggest that vincamine may have antioxidant activity and a potential preventative effect against diabetes-related reproductive problems.

Keywords – Rat, Spermatogenesis, Diabetes Mellitus, Testis, Testosteron

I. INTRODUCTION

It is estimated that the prevalence of diabetes in the world is 8.3 percent and 382 million people worldwide suffer from this disease [1]. Diabetes mellitus (DM) is idenfied by hyperglycemia induced by a deficiency of insulin secretion (DM-type I) or insulin activity (DM-type II), and it causes a variety of systemic abnormalities, including kidney impairments, neuropathies, ophthalmopathies, neuropathies, reproductive/cardiovascular diseases [2]. The vast majority of diabetics are infertile or sub-infertile as a result of testicular problems, reduction in libido, ejaculation, erection, and spermatogenesis issues [3]. It has also been shown that serum testosterone levels are impaired, as well as testicular and epididymal structural abnormalities in experimental DM models [4].

Numerous oxidants form oxidized and nitrated intermediates that inhibit enzymes and cause tissue damage, inflammation, necrosis, and apoptosis [7].ROS can affect steroidogenic enzymes by decreasing the synthesis of steroid hormones [8]. Decreased insulin production alters spermatogenesis by altering blood levels of FSH and LH in diabetic rats. Testosterone is an essential male sex hormone produced and released largely by Leydig cells and is essential for male growth and reproduction. Decreased insulin in diabetes causes a significant decrease in testosterone levels [9].

Some phytochemicals have gotten a lot of interest because they have fewer side effects and can have numerous positive effects, such as stimulating insulin production and regenerating pancreatic islets [10].
Vincamine is defined as a monoterpenoid indole alkaloid, that extrated from Catharanthus roseus (Vinca rosea). According to the literature, vincamine has anticoagulant, memory enhancer, nootropic, hypoglycemic, hypolipidemic, vasodilator and antioxidant effects along with some biological effects [12]. Vincamine has been shown to improve brain metabolism by affecting ATP production and glucose and oxygen utilization, and increase short-term defense against hypoxia and ischemia.
Vincamine is a health care product with a nootropic effect commercially marketed in the United States [13,14].
In light of this, the study was carried out to investigate whether vincamine may have a beneficial effect on the relief of testicular injury in alloxan-diabetic rats, as well as whether inflammation, oxidative stress, and apoptosis could have played a role in the process.

II. MATERIALS AND METHODS

A. Chemicals

Sigma-Aldrich provided the vincamine and alloxan (St. Louis, MO). Commercial diagnostic kits from Sigma, Germany, were used to estimate biochemical parameters. All of the other compounds employed in

the research were analytical grade and originate at well-known commercial sources.

B. Experimental animals and Experimental diabetes induction

Wistar albino rats weighing 250-270 g were collected from Istanbul Medipol University MEDITAM. Animals were housed in cages with unlimited water and feed at 25±2°C. The animals were preserved as in the NIH Guidelines for the care and use of laboratory animals, and the experiment was also approved by the Istanbul Medipol University Ethics Committee (No: 38828770-604.01.01-E.53853). Rats were unsystematically seperated into five groups (n=8): untreated-healthy, untreated-diabetes, vincamine-treated (20mg/kg) diabetics, vincamine-treated (40mg/kg) diabetics, and clomiphene-treated diabetics (5mg/kg). After the rats were diagnosed with diabetes, vincamine was dissolved in 0.5 percent dimethyl sulfoxide (DMSO) and administered once daily through gavage (0.1 ml) for 14 days.

Fasting blood glucose (FBG) levels were assessed in all rats after a 12-hour fast with free access to water, with alloxan monohydrate (150 mg/kg) dissolved in sterile saline sodium chloride (0.9%) pH 7, injected intraperitoneally into rats for induction of hyperglycemia. was done. Glucose solution (5%) was given in water bottles for 24 hours to prevent hypoglycemia. rats were considered hyperglycemic when FPG levels were above 250 mg/dl [15,16].

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C. Biochemical analyses of serum parameters

Following the last dose, the animals were fasted overnight and sacrificed under anesthesia. Their blood was collected and centrifuged. The testosterone content in the blood was determined using an enzyme-linked immunosorbent assay (ELISA) kit [17]. The FBG level was analyzed by determining the blood sample from the tail vein with a glucometer (Accu-Chek, Germany) using the glucose oxidase technique.

D. Sperm concentration and motility analysis

To isolate and clean the epididymis, the adipose tissue around the epididymis was excised, the cauda portion was excised, and placed in a Tyrode buffer preheated to 37°C before use and examined under a microscope for sperm motility and concentration. Results were expressed in million/ml using the sum of the A, B, and C motility rates according to the defined parameters and criteria

E. Histopathological evaluation

Each testis was removed after sacrification and placed in Bouin's solution for stability. Tissue samples were then dehydrated using graded ethanol alcohols and xylene and embedded in paraffin. Sample blocks were sectioned into 5 µm sections using a microtome ((Thermo Scientific, HM340E). Slides were stained with hematoxylin and eosin (H&E) and examined with Nikon Eclipse (Nikon® Instruments Inc., USA). Testicular sections associated with seminiferous tubule injury It was quantitatively scored to determine the extent of histological changes [19].

F. Statistical analysis

The GraphPad Prism 7.0 tool was used to analyse the data. All results are imported as mean ± standard error of the mean (S.E.M.) and statistical examination was carried out utilizing a one-way analysis of variance, pursued by Tukey test to compare the factors in various groups. The outcomes were deemed significant at $p<0.05$.

III. RESULTS

A. Biochemical analyses of serum parameters

The efficacy of vincamine on FBG levels in diabetic rats is presented in Fig 1. Alloxan-induced diabetic rats displayed significant rise in blood sugar level compared to the untreated-healthy group as typical feature of hyperglycemia.

An oral medication of 40mg/kg vincamine for 14 days considerably ($p<0.05$) reduced high FBG concentrations as compared to the untreated-diabetics. The results also showed vincamine decreased FBG concentrations in a dose-dependent manner in diabetic rats. The clomiphene treated group did not produced any meaningful effect on FBG concentrations.

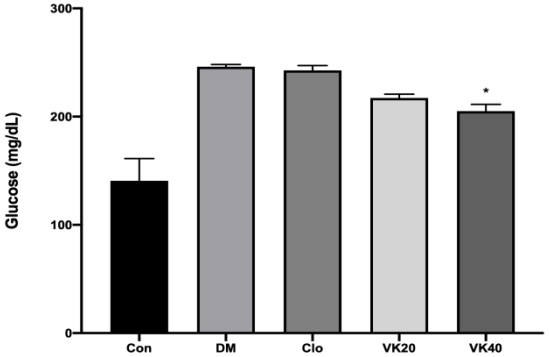


Fig. 1. Effect of vincamine on FBG level in diabetic and non-diabetic rats. All the values are represented in mean ± S.E.M. *p<0.05 vs. diabetic control. Con: untreated-healthy control, DM: untreated-diabetic control, VK20: vincamine-treated (20mg/kg) diabetics, VK40: vincamine-treated (40mg/kg) diabetics, Clo: clomiphene-treated diabetics (5mg/kg).

The data clarified in Fig 2 declared a significant decline ($p<0.05$) of testosterone level in diabetic rats compared with control group. However, treatment with clomiphene increased testosterone level significantly ($p<0.001$) in diabetic rats. Moreover, 20 mg/kg ($p<0.01$) and 40 mg/kg ($p<0.001$) vincamine administration to diabetic rats significantly avoided diabetes-induced serum testosterone reduction in a dose-dependent manner.

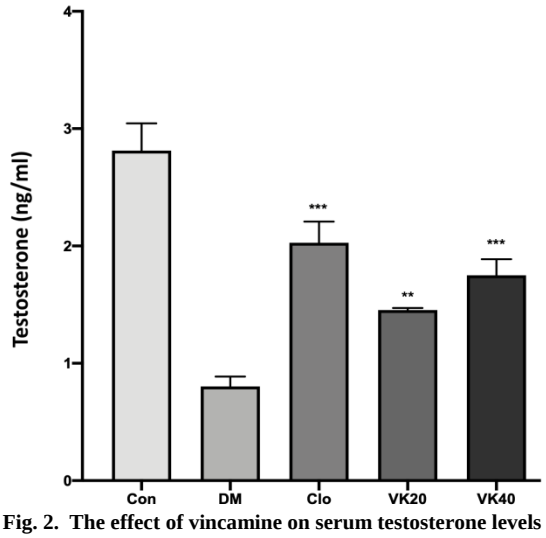


Fig. 2. The effect of vincamine on serum testosterone levels of diabetic rats and non-diabetic rats. All the values are represented in mean ± S.E.M. **p＜0.01, ***p＜0.001 vs. diabetic control. Con: untreated-healthy control, DM: untreated-diabetic control, VK20: vincamine-treated (20mg/kg) diabetics, VK40: vincamine-treated (40mg/kg) diabetics, Clo: clomiphene-treated diabetics (5mg/kg).

B. Sperm parameters

While there was no statistically significant difference between the tested groups in terms of sperm concentration and total motility, when we calculated the average rates for sperm concentration, the results showed that the sperm concentration in the DM group decreased by 2 times compared to the control group.

C. Histopathological evaluation

Johnsen scoring and histopathological evaluation are shown in Figure 3, respectively. The seminiferous epithelium in the diabetes mellitus (DM) group was thinner than the control and clomiphene groups, and vacuolization occurred between the seminiferous epithelium. Openings were seen in the interstitial space. In the vincamine 40mg/kg (VK40) group, morphology similar to the control group was observed in the seminiferous tubules.

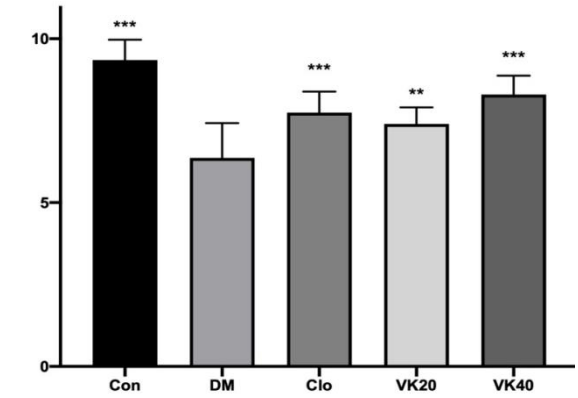


Fig. 3. Johnsen's score of testis tissues in each group. All the values are represented in mean ± S.E.M. **p＜0.01, ***p＜0.001 vs. diabetic control. Con: untreated-healthy control, DM: untreated-diabetic control, VK20: vincamine-treated (20mg/kg) diabetics, VK40: vincamine-treated (40mg/kg) diabetics, Clo: clomiphene-treated diabetics (5mg/kg).

IV. DISCUSSION

Reproduction is essential for humanity's long-term survival. On the other hand, total fertility rates have plummeted due to a variety of lifestyle and environmental variables [22]. The inability to have children affects more than 80 million people globally, with the male component accounting for around 51.2 percent of all instances [23]. DM lowers testosterone levels while increasing gonadotropin levels. Both in vivo and in vitro evidence suggests that these alterations are linked to a steroidogenetic abnormality in Leydig cells. In addition, diabetes-related neuropathy can cause tonia of seminal vesicles, bladder, and urethra [6].

Dietary phytochemicals are a diverse group of physiologically active substances found in plants, many of which have been claimed to have medicinal effects. [24]. Natural components have been demonstrated to play an essential impact in the prevention of human illnesses in epidemiological investigations. Among them, vincamine has been reported to have therapeutic potential for cerebral metabolic and circulatory disorders [25]. This paper was the first research about potential reaction of vincamine on alloxan-induced diabetic rat with spermatogenic dysfunction.

Clomiphene citrate is a non-steroidal fertility medicine that stimulates testosterone and sperm production when taken orally. Several studies reported that clopmiphene citrate treatment was improved testosterone levels and spermatogenesis in infertile and hypogonadal men [26]. Clomiphene could be applied to improve sperm parameters in clinical therapy by binding to the oestrogen receptor and suppressing normal oestrogenic negative feedback on the hypothalamus-pituitary and hence spermatogenesis. This is in line with the data of this research, which found that clomiphene enhanced sperm parameters in diabetic rats. Thus, clomiphene exerted a beneficial controlled effect on the spermatogenic dysfunction observed in diabetes testes, as a result of the findings [27,28].

The findings of the current examination revealed that FBG concentrations are substantially decreased after the application of vincamine 20 mg/kg and 40 mg/kg compared with diabetic control group. This results are consistent with the outcomes of previous researches. Nandini et al. demonstrated that oral treatment of vincamine (20 and 30 mg/kg) for a month resulted in a significant drop in FBG concentrations in diabetic rats, which was comparable to the reduction observed in the glibenclamide-treated animals [12]. Wang et al showed that vincamine exhibited good protective activity against STZ-induced apoptosis in pancreatic β-cells and vincamine showed significant effects on lowering the level of fasting blood glucose, improving oral glucose tolerance, and decreasing the level of HbA1c and LDL in serum, restoring the histology structure of damaged islet of pancreas and

increasing positive regions of insulin on the models of STZ/HFD-induced type 2 diabetic mice and db/db type 2 diabetic mice [23]. Vincamine's ability to lower blood sugar levels in diabetic rats might help to reduce the risk of aberrant spermatozoa and oxidative stress in sperm.

One of the primary causes for the onset of diabetic spermatogenic diseases is oxidative stress. Antioxidant enzymes have been found to be significantly reduced in both animal models of type 1 and 2 DM [29]. Sexual germ cells were affected by oxidative stress and it disorders their division and differentiation, impairing the number of spermatogonia on the basement membrane and reducing the number of primary and secondary spermatocytes, spermatids and mature spermatozoa. Hyperglycemia modifies sperm characteristic and impairs the stability of the DNA by rising the generation of oxidative stress and free radicals [30]. Antioxidants are believed to increase the quality of sperm by preventing oxidative damage [31].

The lower antioxidant activity seen in diabetic control rats in this study imply that their testicular tissue was subjected to oxidative stress. Meanwhile, diabetic rats testes were treated with vincamine, which restored their antioxidant activity. Accordingly, our findings suggested that vincamine may be effective in strengthening the antioxidant defence system in the testicles of diabetes-affected rat.

The antioxidant regulator nuclear factor erythroid 2-related factor 2 (Nrf2) is involved in the regulation of the redox state for the protection of intracellular oxidative damage. Heme oxygenase 1 (HO-1), a phase II detoxifying enzyme controlled by Nrf2, has been found to be a major beneficial regulator in disorders caused by oxidative stress [31]. Nrf2 displayed protective activity in the process of oxidative stress in animal testes. It has been revealed that HO-1 of Leydig cells is implicated in the testicular response to stress. [32,33].

Diabetes-induced spermatogenesis dysfunction is linked to lower levels of Nrf2 and HO-1 expression in the testis [29]. El-Sayed et al. found that vincamine could reactivate Nrf2/HO-1 signaling pathway. It displayed antioxidant features by restoring balance between pro and antioxidants of myeloperoxidase (MPO), malondialdehyde (MDA), Nrf2 and HO-1. Also, vincamine inhibited the inflammatory cascade via triggering Toll-like receptor 4 activation (TLR4) - the interferon gamma (IFN)-CD44 cell pathway, as well as the transforming growth factor beta (TGF1) route [34].

Spermatogenesis relies heavily on testosterone as the primary androgen, and its enhancement has a considerable impact on the number and quality of sperm [35]. Diabetes was shown to have a significant suppressive effect on FSH and LH levels in the blood. The release of TST from the Leydig cells is suppressed by changes in LH levels in the blood. Previous studies have connected diabetes to disturbance of the hypothalamic–pituitary–gonadal axis, which alters TST, FSH, and LH levels in males. [29]. In this investigation, diabetic rats showed reduction of serum testosterone when compared to control. Moreover, vincamine was significantly prevented reduction of serum testosterone levels in diabetic rats.

It has been well demonstrated that pro-inflammatory variables including interleukin-1 (IL-1), tumor necrosis factor- (TNF-α), and oxidative stress have been shown to be elevated in patients with diabetes mellitus. Additionally, in vitro and in vivo investigations shown that IL-1 and TNF- hindered Leydig cell activity, hence inhibiting testosterone synthesis produced by LH [36]. On the other hand, it was found that herbal medicines might enhance reproductive function by boosting testosterone production by inhibiting inflammation and oxidative stress [37].

In conclusion, it was reported that vincamine has antioxidant, antidiabetic and testosteron-improved, on diabetic rats. These actions might be explained, at least in part, by its antioxidant and anti-inflammatory properties, which could be a viable purpose for the treatment or prevention of testicular reproductive harm. More research is needed to determine the specific mechanism of vincamine's protective action against alloxan-induced testicular dysfunction.

REFERENCES

[1] G. L. Ding et al., “The effects of diabetes on male fertility and epigenetic regulation during spermatogenesis,” Asian Journal of Andrology, vol. 17, no. 6, pp. 948–953, 2015, doi: 10.4103/1008-682X.150844.

[2] M. E. Okur, I. D. Karantas, and P. I. Siafaka, “Diabetes Mellitus: A Review on Pathophysiology, Current Status of Oral Pathophysiology, Current Status of Oral Medications and Future Perspectives,” Acta Pharmaceutica Sciencia, vol. 55, no. 1, p. 61, 2017, doi: 10.23893/1307-2080.APS.0555.

[3] B. D. Anawalt, “Approach to the Patient: Approach to Male Infertility and Induction of Spermatogenesis,” The Journal of Clinical Endocrinology and Metabolism, vol. 98, no. 9, p. 3532, Sep. 2013, doi: 10.1210/JC.2012-2400.

[4] C. C. Maresch, D. C. Stute, M. G. Alves, P. F. Oliveira, D. M. De Kretser, and T. Linn, “Diabetes-induced hyperglycemia impairs male reproductive function: a systematic review,” Human Reproduction Update, vol. 24, no. 1, pp. 86–105, 2018, doi: 10.1093/humupd/dmx033.

[5] G. Yigitturk et al., “The antioxidant role of agomelatine and gallic acid on oxidative stress in STZ induced type I diabetic rat testes,” Biomedicine and Pharmacotherapy, vol. 87, pp. 240–246, 2017, doi: 10.1016/j.biopha.2016.12.102.

[6] J. Ballester, M. C. Muñoz, J. Domínguez, T. Rigau, J. J. Guinovart, and J. E. Rodríguez-Gil, “Insulin-Dependent Diabetes Affects Testicular Function by FSH- and LH-Linked Mechanisms,” Journal of Andrology, vol. 25, no. 5, pp. 706–719, Sep. 2004, doi: 10.1002/J.1939-4640.2004.TB02845.X.

[7] F. Gholizadeh, S. Dastghaib, F. Koohpeyma, E. Bayat, and P. Mokarram, “The protective effect of Stevia rebaudiana Bertoni on serum hormone levels, key steroidogenesis enzymes, and testicular damage in testes of diabetic rats,” Acta Histochemica, vol. 121, no. 7, pp. 833–840, 2019, doi: 10.1016/j.acthis.2019.08.001.

[8] N. Tran, B. Pham, and L. Le, “Bioactive Compounds in Anti-Diabetic Plants: From Herbal Medicine to Modern Drug

Discovery," Biology, vol. 9, no. 9, pp. 1–31, Sep. 2020, doi: 10.3390/BIOLOGY9090252.

[9] H. S. Nandini and P. R. Naik, "Antidiabetic, antihyperlipidemic and antioxidant effect of Vincamine, in streptozotocin-induced diabetic rats," European Journal of Pharmacology, vol. 843, pp. 233–239, 2019, doi: 10.1016/j.ejphar.2018.11.034.

[10] T. Du et al., "Vincamine as a GPR40 agonist improves glucose homeostasis in type 2 diabetic mice," Journal of Endocrinology, vol. 240, no. 2, pp. 195–214, 2019, doi: 10.1530/JOE-18-0432.

[11] S. Al-Rashed et al., "Vincamine, a safe natural alkaloid, represents a novel anticancer agent," Bioorganic Chemistry, vol. 107, p. 104626, Feb. 2021, doi: 10.1016/j.bioorg.2021.104626.

[12] N. A. Khouri and H. Daradka, "Antidiabetic effect of Orchis anatolica root extracts on alloxan-induced diabetic rats," Comparative Clinical Pathology, vol. 22, no. 3, pp. 347–354, 2013, doi: 10.1007/s00580-012-1415-8.

[13] Y. Kumar, K. Nandakumar, M. Handral, S. Talwar, and D. Dhayabaran, "Hypoglycaemic and anti-diabetic activity of stem bark extracts Erythrina indica in normal and alloxan-induced diabetic rats," Saudi Pharmaceutical Journal, vol. 19, pp. 35–42, 2011, doi: 10.1016/j.jsps.2010.10.001.

[14] H. Oghbaei, M. R. Alipour, G. Hamidian, M. Ahmadi, V. Ghorbanzadeh, and R. Keyhanmanesh, "Two months sodium nitrate supplementation alleviates testicular injury in streptozotocin-induced diabetic male rats," Experimental Physiology, vol. 103, no. 12, pp. 1603–1617, Dec. 2018, doi: 10.1113/EP087198.

[15] S. G. Johnsen, "Testicular biopsy score count – a method for registration of spermatogenesis in human testes: Normal values and results in 335 hypogonadal males," Hormone Research in Paediatrics, vol. 1, no. 1, pp. 2–25, 1970, doi: 10.1159/000178170.

[16] Y. Cheng et al., "Total flavonoids of Epimedium ameliorates testicular damage in streptozotocin-induced diabetic rats by suppressing inflammation and oxidative stress," Environmental Toxicology, vol. 35, no. 2, pp. 268–276, Feb. 2020, doi: 10.1002/tox.22864.

[17] J. Wang et al., "Design, synthesis and biological evaluation of vincamine derivatives as potential pancreatic β-cells protective agents for the treatment of type 2 diabetes mellitus," European Journal of Medicinal Chemistry, vol. 188, 2020, doi: 10.1016/j.ejmech.2019.111976.

[18] S. La Vignera, R. Condorelli, E. Vicari, R. D'Agata, and A. E. Calogero, "Diabetes mellitus and sperm parameters," Journal of Andrology, vol. 33, no. 2. John Wiley & Sons, Ltd, pp. 145–153, Mar. 04, 2012. doi: 10.2164/jandrol.111.013193.

[19] L. Wu, M. Ye, and J. Zhang, "Vincamine prevents lipopolysaccharide induced inflammation and oxidative stress via thioredoxin reductase activation in human corneal epithelial cells," Am J Transl Res, vol. 10, no. 7, pp. 2195–2204, 2018.

[20] J. Han, Q. Qu, J. Qiao, and J. Zhang, "Vincamine Alleviates Amyloid-β 25–35 Peptides-induced Cytotoxicity in PC12 Cells," Pharmacognosy Magazine, vol. 13, no. 49, p. 123, Jan. 2017, doi: 10.4103/0973-1296.196309.

[21] O. A. Wahab, A. C. Princely, A. A. Oluwadamilare, D. O. Oore-Oluwapo, A. O. Blessing, and E. F. Alfred, "Clomiphene citrate ameliorated lead acetate-induced reproductive toxicity in male wistar rats," Jornal Brasileiro de Reproducao Assistida, vol. 23, no. 4, pp. 336–343, 2019, doi: 10.5935/1518-0557.20190038.

[22] K. Chenniappan and K. Murugan, "Therapeutic and fertility restoration effects of Ionidium suffruticosum on sub-fertile male albino Wistar rats: Effects on testis and caudal spermatozoa," Pharmaceutical Biology, vol. 55, no. 1, pp. 946–957, 2017, doi: 10.1080/13880209.2016.1278453.

[23] H. Ghanem, O. Shaeer, and A. El-Segini, "Combination clomiphene citrate and antioxidant therapy for idiopathic male infertility: a randomized controlled trial," Fertility and Sterility, vol. 93, no. 7, pp. 2232–2235, May 2010, doi: 10.1016/J.FERTNSTERT.2009.01.117.

[24] S. Saeedan et al., "Artemisia judaica L. diminishes diabetes-induced reproductive dysfunction in male rats via activation of Nrf2/HO-1-mediated antioxidant responses," Saudi Journal of Biological Sciences, vol. 28, no. 3, p. 1713, Mar. 2021, doi: 10.1016/J.SJBS.2020.12.013.

[25] Jalili, M. Kamani, S. Roshankhah, H. Sadeghi, and M. R. Salahshoor, "Effect of Falcaria vulgaris extracts on sperm parameters in diabetic rats," Andrologia, vol. 50, no. 10, 2018, doi: 10.1111/and.13130.

[26] L. Wang and L. Zhuang, "Astaxanthin Ameliorates the Lipopolysaccharides-Induced Subfertility in Mouse via Nrf2/HO-1 Antioxidant Pathway," Dose-Response, vol. 17, no. 3, Jul. 2019, doi: 10.1177/1559325819878537.

[27] X. Shi and L. Fu, "Piceatannol inhibits oxidative stress through modification of Nrf2-signaling pathway in testes and attenuates spermatogenesis and steroidogenesis in rats exposed to cadmium during adulthood," Drug Design, Development and Therapy, vol. 13, p. 2811, 2019, doi: 10.2147/DDDT.S198444.

[28] W. J. Bae et al., "Protective Effect of Decursin Extracted from Angelica gigas in Male Infertility via Nrf2/HO-1 Signaling Pathway," Oxidative Medicine and Cellular Longevity, vol. 2016, 2016, doi: 10.1155/2016/5901098.

[29] R. M. El-Sayed, R. E. Abo El Gheit, and G. A. Badawi, "Vincamine protects against cisplatin induced nephrotoxicity via activation of Nrf2/HO-1 and hindering TLR4/ IFN-γ/CD44 cells inflammatory cascade," Life Sciences, vol. 272, May 2021, doi: 10.1016/J.LFS.2021.119224.

[30] D. Haghmorad et al., "Improvement of fertility parameters with Tribulus Terrestris and Anacyclus Pyrethrum treatment in male rats," International Brazilian Journal of Urology : official journal of the Brazilian Society of Urology, vol. 45, no. 5, p. 1043, 2019, doi: 10.1590/S1677-5538.IBJU.2018.0843.

[31] K. Nasiri, A. Akbari, M. Nimrouzi, M. Ruyvaran, and A. Mohamadian, "Safflower seed oil improves steroidogenesis and spermatogenesis in rats with type II diabetes mellitus by modulating the genes expression involved in steroidogenesis, inflammation and oxidative stress," Journal of Ethnopharmacology, vol. 275, 2021, doi: 10.1016/j.jep.2021.114139.

[32] N. Asadi, M. Bahmani, A. Kheradmand, and M. Rafieian-Kopaei, "The impact of oxidative stress on testicular function and the role of antioxidants in improving it: A review," Journal of Clinical and Diagnostic Research, vol. 11, no. 5. Journal of Clinical and Diagnostic Research, pp. IE01–IE05, May 01, 2017. doi: 10.7860/JCDR/2017/23927.9886.

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