



REVERSAL OF DIABETIC COMPLICATIONS IN ANDROLOGY PARAMETERS OF ALLOXAN-INDUCED DIABETIC MALE WISTAR RATS TREATED WITH *CLEOME RUTIDOSPERMA* LEAVES

Oridupa, O. A.¹, Ovwighose, N. O.^{1,2}, Aina, O. O.³, Saba, A. B.¹

¹Department of Veterinary Pharmacology and Toxicology
University of Ibadan, Ibadan

²Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Jos, Jos

³Department of Veterinary Anatomy, University of Ibadan, Ibadan
Nigeria

olayinkaolumide2000@yahoo.com

ABSTRACT

Male infertility is one of the complications associated with *diabetes mellitus*. This study reported the effect of managing experimentally-induced diabetes in male Wistar rats with *Cleome rutidosperma* leaf extract at doses of 100 mg.kg⁻¹, 200 mg.kg⁻¹ or 400 mg.kg⁻¹ for 14 days. Further deterioration or amelioration of reproductive derangement was assessed by changes in sperm morphology, sperm characteristics, and testicular histopathology. Andrology profile of diabetic untreated rats showed significant ($P < 0.05$) derangements compared to normoglycaemic rats. The profile of diabetic rats treated with the extract of *C. rutidosperma*, especially at the 200 mg.kg⁻¹ dose showed a significant decrease in abnormal sperm cells, increases in sperm motility, live/dead ratio and count compared to the untreated diabetic rats. Histopathology showed spermatogenic arrest and

degeneration of Sertoli cells in the diabetic untreated rats, but was restored in the *C. rutidosperma* treated rats. This study showed evidence of infertility associated with diabetes and the ameliorative ability of *C. rutidosperma* on infertility demonstrated by improved andrological parameters. Traditional management of diabetes with *C. rutidosperma* leaves should therefore be encouraged in both humans and other animals, especially males with reproductive intentions.

Key words: andrology; *Cleome rutidosperma*; *diabetes mellitus*

INTRODUCTION

Up to now medicinal plants have provided mankind with remedies for disease prevention, treatment and/or

management. The role of plants in folklore medicine has been attributed to the presence of phytochemicals which are either nutritive or non-nutritive plant chemicals with disease preventive or curative properties [1, 20]. *Diabetes mellitus* has been successfully managed traditionally with medicinal plant preparations [5, 16]. Diabetes, usually characterized by persistent hyperglycemia, may lead to tissue/organ damages in the cardiovascular system, kidney, eye, nerve and reproductive organs [34]. Reproductive complications in the male associated with diabetes include disruption of male fertility, impotence, retrograde ejaculation and hypogonadism. The male hypothalamic-pituitary-gonadal axis was found by researchers to be regulated by pancreatic insulin, and this is essential for fertility [21, 22, 32].

Maintenance of normoglycemia in diabetics is imperative and has been achieved by orthodox and traditional therapeutic approaches. Many rural dwellers have opted for the traditional remedies with years of proven efficacy, good availability and affordability. *Cleome rutidosperma* DC is a medicinal plant traditionally used for the management of diabetes in Southern Nigeria. It is a terrestrial low-growing annular, erect or tufted herb, up to 70–100 cm tall with white or brown taproots [26]. *C. rutidosperma* is found in waste grassy grounds and has trifoliate leaves and small, violet-blue flowers. It is native to West Africa rainforest, from Guinea to Nigeria, Zaire and Angola, but naturalized in various parts of tropical America and South-east Asia [35]. Pharmacological properties of the plants reported include: analgesic, antipyretic, anti-inflammatory, diuretic and antioxidant activities [10, 11].

Despite proven therapeutic efficacy however, many medicinal plants have toxic potentials yet to be verified and these are important for drug safety and development. There is a sparsity of information on the reproductive toxicities that may accompany the management of diabetes with *C. rutidosperma*. Male infertility is one of the complications associated with diabetes. The management of diabetes should therefore consider the effect of treatment on the reproductive system, especially in males.

This study was designed to evaluate the reproductive toxicity potentials of the management of diabetes with *C. rutidosperma* leaves using changes in spermatozoa morphology, characteristics and Sertoli cell histopathology in male Wistar rats as indicators of toxicity.

MATERIALS AND METHOD

Plant preparation

Fresh leaves of *Cleome rutidosperma* were harvested from Ughelli, Delta State. It was identified and a voucher specimen deposited at the Department of Botany, University of Ibadan (Voucher-Number UIH-22548). The leaves were air dried, pulverized and macerated in methanol (96 %) for 72 hours. The decanted filtrate was concentrated using a rotary evaporator and the extract obtained was stored at 4 °C. The extraction was carried out according to the method described by Oridupa and Saba [28]. Fresh extract was reconstituted daily for dosing.

Experimental animals

Thirty male Wistar rats (140–180 g) were obtained from and housed at the Experimental Animal House, Department of Veterinary Pharmacology and Toxicology, University of Ibadan, Nigeria. They were fed with commercially available pelletized rat ration and allowed access to clean water ad libitum. Only normoglycaemic rats were included in the study. The fasting blood glucose concentration was determined in overnight fasted rats using a glucometer (Accu-Chek*Active, Roche Diagnostic, Germany). The study was conducted in accordance with the regulation of the Animal Care Use and Research Ethics Committee (ACUREC), University of Ibadan (UI-ACUREC/App/01/2017/005).

The rats were randomly distributed into six groups. Group 1 served as control normoglycaemic rats. Diabetes was induced in groups 2–6 of rats with alloxan monohydrate (100 mg.kg⁻¹; intraperitoneal; Sigma-Aldrich). Group 2 was administered Glibenclamide (0.07 mg.kg⁻¹; oral). Groups 3, 4 and 5 were orally administered *C. rutidosperma* extract (100 mg.kg⁻¹, 200 mg.kg⁻¹ or 400 mg.kg⁻¹), while Group 6 rats were untreated diabetics throughout the study. Minute quantities of blood was withdrawn from the tail vein to determine the blood glucose levels throughout the course of the study. The antidiabetic study was conducted according to the methods described by Folasire et al. [14].

Sample collection and analysis

All rats were sacrificed by cervical dislocation on day 15. Orchidectomy was performed by pre-scrotal or midline incision according to the method described by

Oyeyemi and Ubiogoro [29]. The *tunica vaginalis* was excised to expose the testicles which were milked out of the incision site. The spermatic cord was ligated, excised and sperm samples were collected from the caudal epididymis. The sperm morphology was determined from about 400 spermatozoa in smears stained (Wells and Awa stains). The sperm characteristics (sperm volume, motility, count and percentage livability) were also determined.

Routine histopathology was carried out according to the method described by Bancroft and Gamble [7]. The testes of the rats were also harvested and preserved in Bouin solution, followed by dehydration with grades of ethanol (70, 80, 90, 95 and 100 %). The samples were cleared in two changes of xylene, impregnated with 2 changes of molten paraffin wax and blocked out. The samples were cut, about 5–6 μm thickness, using a rotary microtome and mounted on glass slides. The slides were stained with haematoxylin and eosin stain, and examined under the light microscope (Olympus CH, Japan). Photomicrographs were taken with an Amscope camera fitted on an Accu-scope microscope and assessed with the aid of ToupView software.

Statistical Analysis

The data obtained were reported as means $\pm$ SD and analyzed using ANOVA with Tukey Kramer multiple comparison test using GraphPad Prism 5.0 (GraphPad Software, San Diego) and differences considered significant at $P < 0.05$.

RESULTS

Elevated blood glucose levels significantly decreased in the extract treated rats, especially in rats administered 200 mg.kg^{-1} extract compared to untreated diabetic rats and comparable to glibenclamide-treated rats (Table 1). The andrology profiles showed significant ($P < 0.05$) differences between normoglycaemic and diabetic rats.

Rudimentary tails (primary abnormality) observed were significantly increased in the diabetic untreated rats (3.21 ± 0.16 cells) compared to normoglycaemic rats (2.0 ± 0.41 cells) and the diabetic treated groups. Secondary sperm abnormalities seen included normal heads without tails, normal tails without heads, bent tails, curved tails, curved mid-pieces, bent mid-pieces and looped tails.

There were significant ($P < 0.05$) increases in the observed normal heads without tails, curved tails, curved mid pieces and bent mid pieces of diabetic untreated rats compared to normoglycaemic rats. There were significant ($P < 0.05$) increases in the total abnormal sperm count of all groups when compared with normoglycaemic rats. However, extract-treated rats at 200 mg.kg^{-1} (47.80 ± 1.36 cells) showed a significant ($P < 0.05$) decline in total sperm abnormalities compared with normoglycaemic (53.55 ± 2.71 cells) and diabetic controls (63.90 ± 1.46 cells) (Table 2).

The sperm motility and count of the diabetic control was significantly ($P < 0.05$) lower than in normoglycaemic control. However, there were increases in the extract-treated rats, especially those administered 200 mg.kg^{-1} , compared to normoglycaemic controls. The live-dead ratio and sperm volume of extract treated diabetic rats were similar to that of normoglycaemic control and significantly higher than untreated diabetic rats (Table 3).

The histopathology showed normoglycaemic rats had no observable lesions. Diabetic untreated rats showed spermatogenic arrest and degeneration of the Sertoli cells. Extract-treated rats at 100 mg.kg^{-1} had ectasia of tubular lumen and hypospermia, 200 mg.kg^{-1} showed tubular atrophy with sparing of pachytene spermatocytes, while ectasia of tubular lumen and hypospermia was observed in 400 mg.kg^{-1} treated rats. Diabetic rats administered glibenclamide showed no observable lesion (Fig. 1).

DISCUSSION

Diabetes mellitus is a metabolic derangement of glucose clinically characterized by hyperglycaemia. Prolonged cellular exposure to hyperglycemia is the primary factor implicated in most diabetic complications [34]. One of these complications in males include disruption of normal reproductive functions clinically presented as infertility and erectile disorders. Male infertility may be due to defects in sperm atozoa morphology and characteristics [22, 29]. The main focus of diabetes therapy is regulation of blood glucose levels aimed at reversal of the hyperglycaemic state and eventually the complications [14]. In this study, a significant decline in blood glucose levels was observed in *Cleome rutidosperma*-treated diabetic rats throughout the course of the experiment. Also, significant reversal of derangements in sperm morphology and characteristics

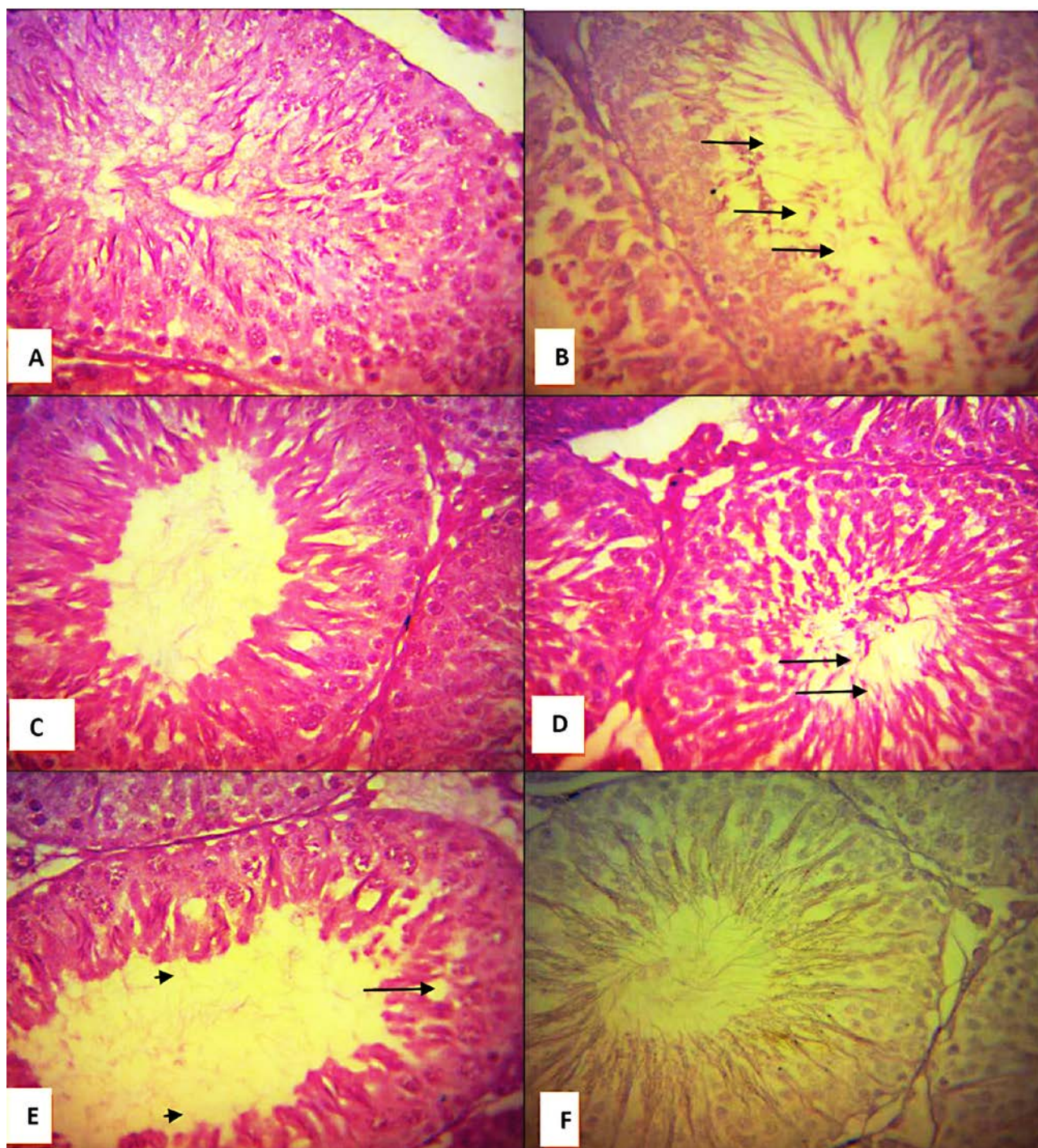


Fig. 1. Photomicrograph of diabetic Wistar rat testis administered with *Cleome rutidosperma* leaf extract

A (Control)—no observable lesion; **B** (Untreated Diabetic)—few immature stages found at the margin of the tubular lumen; **C** (Diabetic treated with 100 mg.kg⁻¹ *C. rutidosperma*)—no observable lesion; **D** (Diabetic treated with 200 mg kg⁻¹ *C. rutidosperma*)—immature stages found in the tubular lumen; **E** (Diabetic treated with 400 mg.kg⁻¹ *C. rutidosperma*)—relative widening of the seminiferous lumen (arrowheads), with interstitial oedema (arrows); **F** (Diabetic treated with Glibenclamide)—no observable lesion. H&E ×400

Table 1. Blood glucose levels (mg.dl⁻¹) of alloxan-induced diabetic rats administered with methanol extract of *Cleome rutidosperma* leaf observed within 14 days of administration

Group	Day 1	Day 3	Day 5	Day 7	Day 10	Day 14
Control	67.25±5.36	84.25±5.66	97.75±4.66	85.00±5.72	94.25±3.75	99.50±6.70
Diab Control	426.00±9.96	235.00±13.30	320.33±10.51	422.33±10.91	397.33±8.97	326.67±9.30
Diab + 100 mg.kg ⁻¹	466.33±8.70	281.00±9.90	192.67±14.73	191.67±8.60	195.33±15.60	179.67±10.73
Diab + 200 mg.kg ⁻¹	271.60±11.02	236.40±14.72	72.20±6.18*	69.20±7.81*	137.80±7.15	70.60±10.66*
Diab + 400 mg.kg ⁻¹	480.00±10.02	258.40±8.51	270.80±19.32	373.00±8.90	143.00±12.07	189.80±11.86
Diab + Gliben	252.25±14.56	234.50±13.97	194.50±7.75	253.75±7.20	284.25±10.56	156.00±10.80

*—Significantly lower (P < 0.05) blood glucose level compared to control in the same column; Diab—Diabetic rats; Gliben—Glibenclamide

Table 2. Spermatozoa abnormalities observed in alloxan-induced diabetic rats administered with methanol extract of *Cleome rutidosperma* leaf for 14 days

Group	Control	Diab Control	Diab + 100 mg.kg ⁻¹	Diab + 200 mg.kg ⁻¹	Diab + 400 mg.kg ⁻¹	Diab + Gliben
Rud tail	2.0±0.41	3.21±0.16 ^a	2.0±0.58 ^b	2.0±0.45 ^b	2.0±0.45 ^b	2.0±0.41 ^b
NHWT	4.75±0.63	6.0±0.34 ^a	4.67±0.88 ^b	4.60±0.51 ^b	4.60±0.68 ^b	4.75±0.63 ^b
NTWH	4.25±0.48	5.60±0.23 ^b	4.67±0.88 ^b	4.60±0.51 ^b	4.60±0.60 ^b	5.0±0.41 ^{a,b}
Bent tail	10.3±1.31	10.9±0.82	10.33±0.88	8.20±0.49 ^{a,b}	11.0±0.45	10.0±0.41
Curved tail	9.2.0±0.58	11.98±0.45 ^a	11.67±0.67 ^a	8.60±0.40 ^{a,b}	11.20±0.73 ^{a,b}	10.50±0.29 ^{a,b}
Curved MP	10.5±0.65	11.91±0.68 ^a	11.67±1.20 ^a	9.0±0.32 ^b	11.0±0.70 ^{a,b}	11.75±0.75 ^a
Bent MP	10.3±0.85	11.1±0.28 ^a	12.0±0.58 ^{a,b}	9.0±0.45 ^{a,b}	10.80±0.37 ^b	10.25±0.25 ^b
Looped tail	2.25±0.48	3.20±0.37	2.0±0.58	1.80±0.37	2.20±0.37	2.25±0.48
TASp count	53.55±2.71	63.90±1.46 ^a	59.01±2.08 ^{a,b}	47.80±1.36 ^{a,b}	57.40±1.75 ^{a,b}	56.50±1.66 ^{a,b}
TSp count	407.50±3.23	410.0±3.12	410.0±2.89	406±2.92 ^b	403.0±2.0 ^{a,b}	407.50±3.2
% Sp Ab	13.14 %	15.59 % ^a	14.39 %	11.77 % ^{a,b}	14.24 % ^a	13.87 %

Values differ significantly (P < 0.05) from normoglycaemic control^a or diabetic control^b;

Diab3—Diabetes; Gliben—Glibenclamide; Rud tail—Rudimentary tail; NHWT—normal head without tail; NTWH—normal tails without head; Curved MP—curved mid-piece; Bent MP—bent mid-piece; TA Sp count—total abnormal sperm count; TSp count—total sperm count; % Sp Ab—% sperm abnormality

Table 3. Sperm characteristics of alloxan-induced diabetic rats administered with methanol extract of *Cleome rutidosperma* leaf for 14 days

Group	Motility [%]	Live/Dead [%]	Volume [cm ³]	Count [%]
Control	72.50±5.00	96.50±1.73	5.18±0.05	100.0±12.75
Diab Control	57.50±3.54 ^a	79.00±1.41 ^a	5.07±0.01 ^a	97.00±1.40
Diab+100 mg.kg ⁻¹	73.33±5.77 ^b	96.00±1.73 ^b	5.13±0.06	116.00±7.20 ^{a,b}
Diab+200 mg.kg ⁻¹	89.00±5.48 ^{a,b}	96.80±1.64 ^b	5.18±0.04 ^b	133.20±11.30 ^{a,b}
Diab+400 mg.kg ⁻¹	68.00±4.47 ^b	96.80±1.64 ^b	5.18±0.04 ^b	106.00±17.01
Diab+Gliben	75.00±12.91 ^b	96.50±1.73 ^b	5.18±0.05 ^b	103.00±12.91

Values differ significantly (P < 0.05) from normoglycaemic control^a or diabetic control^b;

Diab—Diabetes; Gliben—Glibenclamide

associated with *diabetes mellitus* was observed in extract-treated diabetic rats especially at 200 mg.kg⁻¹, suggesting the extract has spermatogenic properties.

The increased incidence of sperm cell abnormality in untreated diabetic rats showed evidence of altered spermatogenesis and maturation which can be caused by decreased testosterone bioavailability and also deficient epididymal secretions [33]. The study by K a m a r u z a m a n et al. [17] concluded that *Gynura procumbens* significantly influenced sperm maturation in amelioration of diabetic male infertility by upregulation of related proteins. La V i g n e r a et al. [19] also mentioned that diabetes decreased the serum testosterone levels in association with a steroidogenetic defect in Leydig cells and increased oxidative stress by hyperglycemia which caused sperm nuclear and mitochondrial DNA damages. These researchers also related spermatogenesis derangement and germ cell apoptosis in type 1 diabetes to local autoimmune damage, while impaired sperm parameters and decrease testosterone serum levels in type 2 diabetics was associated with insulin resistance, obesity, and other related comorbidities [15, 19]. Studies by previous researches have reported medicinal plants such as *Teucrium polium*, *Eryngium caucasicum* and *Zingiber officinalis*, which reversed the decline in testosterone levels and attributed this effect to the ability of the plants to inhibit oxidative stress generated by the hyperglycemic state [2, 8, 31].

Various mechanisms may explain the sperm cell damage observed in patients with diabetes. These include: endocrine disorders, neuropathy, and increased oxidative stress [3, 25]. Insulin is the primary hormone responsible for cellular glucose metabolism including in spermatozoa where the glycolytic pathway for energy generation is a major mechanism employed for sperm motility [13]. Deficiency or insensitivity to insulin which is typical of diabetes also alters this mechanism in sperm cells [6]. The neurohormonal regulation of male reproduction is also altered in diabetes, as well as increased generation of free radical oxygen species [24]. The male reproductive organ is highly vascularized which predisposes the testes to substantial volume of hyperglycemic blood, thus increased exposure to reactive oxygen species, advanced glycation end products, amongst other toxicants [9].

Furthermore, diabetes-induced damage to Sertoli cells may be implicated in the production of defective spermatozoa as the Sertoli cells are responsible for spermatogen-

esis and part of sperm cell maturation [12, 30]. This study showed a reversal of Sertoli cell vacuolation and denser germinal centers in *C. rutidosperma*-treated diabetic rats. This was clinically demonstrated by higher sperm counts and a lower incidence of abnormal sperm cells. This correlates with the report of A l v e s et al. [4] that damaged Sertoli cells leads to the production of defective sperm cells which are unable to fertilize a mature ovum. *C. rutidosperma* also induced a significant increase in sperm motility which is important in the movement of the sperm cells through the female genital tract for fertilization. Similar reversal of spermatogenic damage in diabetes have been reported for *Alpinia galanga*, *Citrullus vulgaris* and *Xanthosoma sagittifolium*, amongst other medicinal plants [18, 23, 27].

CONCLUSIONS

In conclusion, the male reproductive complications associated with diabetes were reversed by treatment with *Cleome rutidosperma* leaves. The production of viable and motile spermatozoa increased. This study demonstrated that *C. rutidosperma*, especially at 200 mg.kg⁻¹ did not further progress infertility in the diabetic rats treated, but reversed diabetes-induced damages in the sperm cell morphology, characteristics and testicular tissue. These findings are relevant in males within the reproductive age and particularly animals intended for breeding.

REFERENCES

1. Adesuyi, A. O., Elumm, I. K., Adaramola, F. B., Nwoko-cha, A. F. M., 2012: Nutritional and phytochemical screening of *Garcinia kola*. *Adv. J. Food Sci. Tech.*, 4, 1, 9—14.
2. Afshari, M., Malayeri, A. R., Mohammadshahi, M., 2019: Effects of *Eryngium caucasicum* extract on testosterone, inflammation and oxidative status of nicotinamide-streptozotocin induced type-2 diabetes in male rats. *J. Contemp. Med. Sci.*, 5, 2, 77—81. Available at <<http://www.jocms.org/index.php/jcms/article/view/568>>. Date accessed: July 15, 2019.
3. Agarwal, A., Prabakaran, S. A., Said, T. M., 2005: Prevention of oxidative stress injury to sperm. *J. Androl.*, 26, 6, 654—660. DOI: 10.2164/jandrol.05016.
4. Alves, M. G., Martins, A. D., Cavaco, J. E., Socorro, S.,

- Oliveira, P. F., 2013: Diabetes, insulin-mediated glucose metabolism and Sertoli/blood-testis barrier function. *Tissue Barriers*, 1, 2: e23992. DOI: 10.4161/tisb.23992.
5. Bahmani, M., Zargaran, A., Rafieian-Kopaei, M., Saki, K., 2014: Ethnobotanical study of medicinal plants used in the management of *diabetes mellitus* in the Urmia, Northwest Iran. *Asian Pac. J. Trop. Med.*, 7, 1, S348—354. DOI:10.1016/S1995-7645(14)60257-1.
6. Ballester, J., Muñoz, M. C., Domínguez, J., Rigau, T., Guinovart, J. J., Rodríguez-Gil, J. E., 2004: Insulin-dependent diabetes affects testicular function by FSH- and LH-linked mechanisms. *J. Androl.*, 25, 5, 706—719. DOI: 10.1002/j.1939-4640.2004.tb02845.x.
7. Bancroft, J. D., Gamble, M., 2008: *Theory and Practice of Histology Techniques*. 6th edn., Churchill Livingstone Elsevier, Philadelphia, 83—134.
8. Banihani, S. A., 2018: Ginger and testosterone. *Biomolecules*, 8, 4, 119. DOI: 10.3390/biom8040119.
9. Bansal, M., Kaushal, N., 2014: *Oxidative Stress Mechanisms and their Modulation*. Springer New Delhi, India. 1—167. DOI: 10.1007/978-81-322-2032-9.
10. Bose, A., Mondal, S., Gupta, J. K., Ghosh, T., Debbhuti, D., Si, S., 2008: Antioxidant and free radical scavenging activities of *Cleome rutidosperma*. *Oriental Pharm. Exp. Med.*, 8, 2, 135—145. DOI 10.3742/OPEM.2008.8.2.135.
11. Bose, A., Saravanan, V. S., Karunanidhi, N., Gupta, J. K., 2004: Analgesic and locomotor activity of extracts of *Cleome rutidosperma* DC. *Indian J. Pharm. Sci.*, 66, 795—797.
12. Cooper, T. G., Noonan, E., Von Eckardstein, S., Auger, J., Baker, H. W., Behre, H. M., et al., 2010: World Health Organization reference values for human semen characteristics. *Hum. Reprod. Update*, 16, 3, 231—245. DOI:10.1093/humupd/dmp048.
13. Ding, G. L., Liu, Y., Liu, M. E., Pan, J. X., Guo, M. X., Sheng, J. Z., Huang, H. F., 2015: The effects of diabetes on male fertility and epigenetic regulation during spermatogenesis. *Asian J. Androl.*, 17, 6, 948—953. DOI: 10.4103/1008-682X.150844.
14. Folasire, O. F., Oridupa, O. A., Owolabi, A. J., Adepoju, O. T., 2016: Anti-hyperglycemic effect of cocoyam (*Xanthosoma sagittifolium*) corm in alloxan-induced diabetic albino rats. *Int. J. Nutr. Metabol.*, 8, 4, 24—29. DOI: 10.5897/IJNAM2016.0200.
15. Han, X. X., Jiang, Y. P., Liu, N., Jing, Wu, J., Yang, J. M., Li, Y. X., et al., 2019: Protective effects of Astragalin on spermatogenesis in streptozotocin-induced diabetes in male mice by improving antioxidant activity and inhibiting inflammation. *Biomed. Pharmacother.*, 110, 561—570. DOI: 10.1016/j.biopha.2018.12.012.
16. Kadir, M. F., Bin Sayeed, M. S., Shams, T., Mia, M. M. K., 2012: Ethnobotanical survey of medicinal plants used by Bangladeshi traditional health practitioners in the management of *diabetes mellitus*. *J. Ethnopharmacol.*, 144, 3, 605—611. DOI: 10.1016/j.jep.2012.09.050.
17. Kamaruzaman, K. A., Aizat, W. M., Noor, M. M., 2018: *Gynura procumbens* improved fertility of diabetic rats: Preliminary study of sperm proteomic. *Evid-Based Compl. Alt. Med.*, Vol. 2018, Article ID 9201539, 13 pp. DOI: 10.1155/2018/9201539.
18. Khaki, A., Fathiazad, F., Nouri, M., 2013: Effects of watermelon seed extract (*Citrullus vulgaris*) on spermatogenesis in rat. *Int. J. Women's Health Reprod. Sci.*, 1, 99—104.
19. La Vignera, S., Condorelli, R., Vicari, E., D'Agata, R., Calogero, A. E., Amaral, S., et al., 2012: *Diabetes mellitus* and sperm parameters. *J. Androl.*, 33, 2, 145—153. DOI: 10.2164/jandrol.111.013193.
20. Lata, N., Dubey, V., 2010: Preliminary phytochemical screening of *Eichhornia crassipes*: the world's worst aquatic weed. *J. Pharm. Res.*, 3, 6, 1240—1242.
21. Loeken, M. R., 2012: A new role for pancreatic insulin in the male reproductive axis. *Diabetes*, 61, 1667—1668. DOI: 10.2337/db12-0539.
22. Mallidis, C., Agbaje, I., McClure, N., Kliesch, S., 2011: The influence of *diabetes mellitus* on male reproductive function: a poorly investigated aspect of male infertility. *Urologe A*, 50, 33—37. DOI: 10.1007/s00120-010-2440-3.
23. Mazaheri, M., Shahdadi, V., Nazari, B. A., 2014: Molecular and biochemical effect of alcoholic extract of *Alpinia galanga* on rat spermatogenesis process. *Iranian J. Reprod. Med.*, 11, 765—770.
24. Muralidhara, S. B., 2007: Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: Its progression and genotoxic consequences. *Reprod. Toxicol.*, 23, 4, 578—587. DOI: 10.1016/j.reprotox.2007.02.001.
25. Olayemi, F. O., 2010: A review on some causes of male infertility. *Afr. J. Biotech.*, 9, 20, 2834—2842.
26. Onwueme, I. C., Sinha, T. D., 1991: *Feed Crop Production in Tropical Africa. Principles and Practice*. ITA Publisher Netherlands, 25—28.
27. Oridupa, O. A., Folasire, O. F., Owolabi, A. J., Aina, O., 2017: Effect of traditional treatment of *diabetes mellitus* with *Xanthosoma sagittifolium* on the male reproductive system of Alloxan-induced diabetic Wistar rats. *Drug Res.*, 67, 6, 337—342. DOI: 10.1055/s-0043-103575.

28. Oridupa, O. A., Saba, A. B., 2012: Relative anti-inflammatory and analgesic activities of the whole fruit, fruit bark, pulp and seed of *Lagenaria breviflora* Roberty. *J. Pharmacol. Toxicol.*, 7, 6, 288—297. DOI: 10.3923/jpt.2012.288.297.
29. Oyeyemi, M. O., Ubiogoro, O., 2005: Spermiogram and morphological characteristics in testicular and epididymal spermatozoa of Large White Boar in Nigeria. *Int. J. Morphol.*, 23, 3, 235—239.
30. Rato, L., Alves, M. G., Socorro, S., Duarte, A. I., Cavaco, J. E., Oliveira, P. F., 2012: Metabolic regulation is important for spermatogenesis. *Nat. Rev. Urol.*, 9, 330—338. DOI: 10.1038/nrurol.2012.77.
31. Salimnejad, R., Sazegar, G., Borujeni, M. J. S., Mousavi, S. M., Salehi, F., Ghorbani, F., 2017: Protective effect of hydroalcoholic extract of *Teucrium polium* on diabetes-induced testicular damage and serum testosterone concentration. *Int. J. Reprod. Biomed. (Yazd)*, 15, 4, 195—202.
32. Schoeller, E. L., Albanna, G., Frolova, A. I., Moley, K. H., 2012: Insulin rescues impaired spermatogenesis via the hypothalamic-pituitary-gonadal axis in Akita diabetic mice and restores male fertility. *Diabetes*, 61, 1869—1878. DOI: 10.2337/db11-1527.
33. Singh, S., Malini, T., Rengarajan, S., Balasubramanian, K., 2009: Impact of experimental diabetes and insulin replacement on epididymal secretory products and sperm maturation in albino rats. *J. Cell Biochem.*, 108, 1094—101. DOI: 10.1002/jcb.22337.
34. Soumya, D., Srilatha, B., 2011: Late stage complications of diabetes and insulin resistance. *Diabetes Metab.*, 2, 9, 167. DOI: 10.4172/2155-6156.1000167.
35. Waterhouse, B., Mitchell, A., 1998: *Northern Australia Quarantine Strategy Weeds Target List*. AQIS Miscellaneous Publication, Canberra, 29.

Received March 22, 2019

Accepted December 10, 2019