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Ameliorative effects of *Allium cepa* Linn. scaly leaves extract on reproductive dysfunctions in streptozotocin-induced diabetic Wistar rats

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ABSTRACT

Diabetes mellitus, an endocrine and metabolic disorder characterized by hyperglycemia and low blood insulin or target organs insensitivity to insulin affects life quality due to its complications. Infertility is a complication in diabetes. Various agents have been used for research on diabetes-induced infertility globally, but there are little documented treatments for diabetes associated infertility. *Allium cepa* scaly leaves extract (ACSLE) possess anti-oxidant and anti-diabetic activities. This study investigated the effects of ACSLE on reproductive dysfunctions in male diabetic rats. Twenty eight male rats were assigned to 4 groups (n=7): CT (control); DNT (streptozotocin, 60 mg/kg once, intraperitoneal), DT1 and DT2 (streptozotocin, 60 mg/kg once, intraperitoneal, ACSLE 125 and 250 mg/kg rat/day respectively). Organ samples were obtained after 2 weeks and testicular weights recorded. Fasting blood glucose was determined using a digital glucometer. Sperm count, motility, viability and morphology were assessed microscopically. Testes were histologically evaluated. Glucose levels were reduced in DT1 and DT2 compared with DNT. Testes weights increased in DT1and DT2 compared with DNT. Sperm concentration increased in DT1 and DT2 compared with DNT motility increased in DT1 and DT2 compared with DNT; viability increased significantly in DT1 and DT2 compared with DNT. Abnormal sperm morphology decreased in DT1 and DT2 compared with DNT. Testes showed degenerated cells in DNT and no lesions in DT1 and DT2. Reduced blood glucose, improved testicular functions and morphology showed that ACSLE ameliorated reproductive dysfunctions associated with streptozotocin-induced diabetes in male Wistar rats.

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Introduction

Diabetes mellitus (DM), a disorder in which the body is unable to properly metabolize carbohydrates is increasing globally and now affects 9.3 % of the world's adult population (Saeedi et al., 2019). It is estimated that 463 million people had diabetes in 2019 (Saeedi et al., 2019) and without urgent and sufficient actions, it is predicted that 578 million people will have diabetes in 2030 and the number will increase by 51% (700 million) in 2045 (Saeedi et al., 2019).

The disease is characterized by excessive amounts of sugar in the blood and urine; inadequate production and / or utilization of insulin; thirst, hunger and loss of weight (Maiti et al., 2004). DM is a complex metabolic

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disorder that results from defects in insulin secretion, action, or a combination of both (Akkati et al., 2011). DM is grouped into two main types, type I diabetes mellitus (T1DM) and type II diabetes mellitus (T2DM) (American Diabetes Association, 2019).

T1DM results from defective beta cells in the islets of Langerhans which could be caused by destruction by various infections, diseases and exposure to various toxic chemicals (Cooke and Plotnick, 2008; Eizirik et al., 2009; Akkati et al., 2011). Major symptoms are polyphagia, polydipsia, polyuria and weight loss (Cooke and Plotnick, 2008; Akkati et al., 2011; Ramachandran, 2014).

T2DM is characterized by persistently high blood glucose caused by insulin resistance and usually with relative insulin deficiency (Butler I., 2003; Akkati I., 2011). Risk factors for T2DM are genetic predisposition, hyperlipidemia, lifestyle factors (including obesity, lack of physical activity, poor diet, stress, urbanization) and history of gestational diabetes (Kahn and Hull, 2006; Riserus et al., 2009; Akkati et al., 2011).

Glucose metabolism is an important event in spermatogenesis in DM and deleterious effects of DM leads to male infertility (Amidu et al., 2013; Koroglu et al., 2015) via actions at multiple levels including altered spermatogenesis, degenerative and apoptotic changes in testes, altered glucose metabolism in blood-testes barrier, reduced testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) synthesis and secretion (Tsounapi et al., 2016); decreased spermatozoa motility (Saumya et al., 2016) and semen volume (Adedara et al., 2015); abnormal spermatozoa morphology (Afifi et al., 2015) and the disruption of seminiferous tubule morphology (Rashid et al., 2015); ejaculatory dysfunction and reduced libido (Sexton and Jarow, 1997; Baccetti et al., 2002; Cavallini, 2006; Scarano et al., 2006; Agbaje et al., 2007; Kilarkaje et al., 2014; Ghanbari et al., 2015).

Diabetes is linked with increased oxidative stress (Freitas et al., 1997; Abou-Seif and Youssef, 2004) which may play an important role in the cause of diabetic complications.

Physiologically, the balance between reactive oxygen species (ROS) production and antioxidant activity is maintained in the body, but when ROS accumulates in excessive amounts either due to increased generation or impaired clearance, they can cause deleterious effects on sperm cells which can lead to sperm dysfunction (Marti et al., 2007; Agarwal et al., 2008; Tremellen, 2008).

The management of DM without negative effects is still challenging to the medical system, which has increased demands for natural and other products with anti-diabetic activity and little or no side effects (Kameswara et al., 1999; Philippe and Raccah, 2009; Akkati et al., 2011). Plants, plants extract, and the active compound from plants usage to manage diseases are crucial in new drugs discovery (Rupeshkumar et al., 2014).

Allium cepa (onion) is a plant that belongs to the Alliaceae family (Shaath and Flores, 1998). It is the most widely cultivated species of the genus Allium (Bindu and Podikunju, 2015) and contains pharmacologically active con-stituents, including flavonoids (quercetin), organosul-fur compounds (propyl thiosulfinate), and phenol com-ponents, fructooligosaccharides (FOS) compounds (Slimestad et al., 2007). *Allium cepa* possess anti-allergic (Roldan et al., 2008; Marefati et al., 2018), anti-inflammatory, and antioxidant activities (Griffiths et al., 2002; Lee and Jung, 2016; Marefati et al., 2018). Peak concen-trations of quercetin is attained 7.0 ± 2.9 hours after ingestion, and its elimination half-life is about 11 hours. Also, the plant matrix influences both the rate and ex-tent of absorption (Marefati et al., 2018).

Flavonoids are the major phenolics in onions, which can be classified to different subclasses (flavones, flavanones, flavonols, isoflavones, flavanonols, flavanols, chalcones, and anthocyanins) on the basis of the degree of unsaturation and the degree of oxidation of the central ring (P´erez-Gregorio et al., 2010). Flavonoids subclasses can be further differentiated on the basis of the number and nature of substituent groups attached to the rings (P´erez-Gregorio et al., 2010). Flavonols are the most abundant in onions, present as their glycosides, that is, quercetin and kaempferol (Prakash et al., 2007; Santas et al., 2010; Nile et al., 2020) in higher concentration (280-400 mg/kg) than other vegetables (i.e., 100 mg/kg in broccoli, 50 mg/kg in apple) (de Ancos et al., 2015). Anthocyanins, belonging to anthocyanidins, are mainly present in red onions (250 mg/kg), besides having a composition rich in flavonols as yellow onions (de Ancos et al., 2015).

Quercetin which is one of the most common flavonoids in *A. cepa* has been reported to improve diabetic status by decreasing oxidative stress (Mahesh and Menon, 2004; Coskun et al., 2005; Dias et al., 2005) and by reducing the disturbance of hepatic gene expressions (Kobori et al., 2009; Jiet al., 2011).

The objectives of this study were to study the anti -diabetic, antioxidant activity and free radical scavenging effects of *Allium cepa* scaly leaf extract on high blood glucose level and impaired testicular functions and morphology associated with streptozotocin-induced diabetic male Wistar rats.

Materials and methods

Ethical statement: This experiment was conducted in the experimental animal house at the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria according to the guidance and approval of the committee on animal care ethics and use, Federal University of Agriculture, Abeokuta (CACEU/2019/3224).

Animals: Twenty eight male Wistar rats (150-200g) were used for this study. The rats were housed in the experimental animal house of the college. They were kept in well ventilated standard rat cages at ambient temperature and 12 hour light/darkness period was maintained. The rats were fed standard pelleted rat chow and clean water was given *ad libitum*. They were kept for 10 days for adaptation prior to start of the experiment.

Plant material: Allium cepa L. (onion) were obtained from a market in Abeokuta, Ogun State. Identification and authentication was done at Department of Pure and Applied Botany, College of Biosciences, Federal University of Agriculture, Abeokuta (FUNAABH0029).

Plant extraction: Allium cepa L. scaly leaves were removed from the onion and reduced into coarse particles. The coarse particles were soaked in 97% ethanol for 72 hours at room temperature. The mixture was filtered using Whatman's filter paper and the filtrate was evaporated at 600C using a vacuum rotary evaporator. The wet brown residue i.eAllium cepa scaly leaves extract (ACSLE) was allowed to evaporate in vacuo and stored in the refrigerator (40C) until ready to use. Thereafter, 1 g of the residue was dissolved in 20 mL of distilled water to give a concentration of 50 mg/mL.

Plant phytochemical analysis: Standard screening tests for the extract was performed for various plant constituents. Presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and anthraquinones were screened for using standard procedures(Edeogaet al., 2005).

Diabetes induction: Streptozotocin (STZ) (Sigma Chemical Co., St. Louis, MO, USA) was used to induce diabetes in rats. It was injected intra-peritoneally at a single dose of 60 mg/kg of body weight after being freshly prepared in ice-cold citrate buffer of pH 4.5 (Akbarzadeh et. al., 2007). Animals with glucose levels above 300 mg/dL were considered diabetic and were included in the study.

Experimental Procedure: The rats were randomly distributed into 4 groups (n=7) after diabetes induction and treated as listed in Table 1. Blood glucose measurement

Fasting blood glucose levels were estimated 3 times within a week during the treatment period of two weeks using blood sample obtained from the tail vein of the rats and determined in mg/dL by a digital glucometer (Accu-chek Advantage, Roche Diagnotics, Germany). The animals were fasted for a period of 16 hours before their blood glucose levels were measured.

At the end of the 14 days treatment, the rats were euthanized with thiopental injection (50 mg/kg) and a ventral midline abdominal incision was made to expose the reproductive organs. The testes were identified, carefully removed and processed further for sperm evaluation; sperm motility, concentration, viability and morphology as described by Jequier, (2010) and testicular histology.

Statistical Analysis: The differences between the means were analyzed statistically with one-way analysis of variance, followed by Tukey's comparison test (ANOVA; 95% confidence interval). Values of $P<0.05$ were taken to imply statistical significance. Results were expressed as mean and standard error of mean and were presented in bar charts. Statistical Package for the Social Science (SPSS) software (version 16.0; SPSS Inc., USA) was used in all data analysis.

Results

Phytochemical studies: Tannins, saponins, anthraquinones, flavonoids, sterols, alkaloids and terpenoids were present in the extract (Table 2).

Hypoglycemic effects of ACSLE on blood glucose: The mean values of blood glucose levels revealed a reduction in the DT1 (175.3 ± 2.3 mg/dL) and DT2 (165.0 ± 1.8 mg/dL) than that in the DNT (440.0 ± 13.0 mg/dL) after 14 days (Figure 1).

Effects of ACSLE on sperm variables: On administration of ACSLE for 14 days, the mean values of sperm motility (77.7 ± 1.5, 80.0 ± 0.0 %), viability (70.0 ± 0.0, 80.0 ± 0.00 %), concentration (130.0 ± 5.9,

Table 1. Animal groupings and daily protocols

Group	**Protocols**
CT	Control group received distilled water of 0.5 ml daily.
DNT	Diabetic group received 60 mg/kg STZ once
DT1	Received 60 mg/kg STZ once and treated with 125 mg/kg ACSLE (O.E.C.D., 2008), 2 weeks
DT2	Received 60 mg/kg STZ once and treated with 250 mg/kg ACSLE (O.E.C.D., 2008), 2 weeks

130.7 ± 7.3 x 106 cell/mL) and testis weight levels (1.0 ± 0.04, 1.0 ± 0.03 g) revealed a significant increase in the DT1 and DT2 than that in the DNT (57.0 ± 1.5 %; 52.7 ± 1.3 %; 98.3±2.3 x 106 cell/mL; 0.7 ± 0.03 g) (Table 2). Sperm abnormal morphology revealed a significant decrease in the DT1 (13.0 ± 0.0) and DT2 (8.8 ± 0.4 %) than that in the DNT (19.8 ± 0.9 %) (Table 3).

Table 2. Basic phytochemical constituents of aqueous extract of *Allium cepa* Linn. scaly leaf.

Phytochemicals	Reaction
Tannins	+
Glycosides	-
Saponins	+
Anthraquinones	+
Flavonoids	++
Sterols	+
Alkaloids	+
Terpenoids	+

+ represents presence, - represents absence

Effects of ACSLE on testicular histology: In CT, DT1 and DT2, there was normal architecture with no visible lesions (Figure 2) while DNT revealed a testicular section showing severe degeneration and necrosis of germinal epithelial cells (arrow) of diabetic non-treated group (Figure 2).

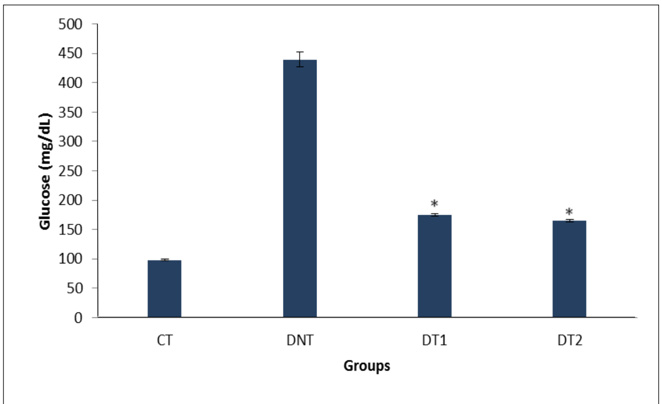

Figure 1. Effect of ACSLE on blood glucose level of control, negative control (DNT) and test rats in (mg/dL), N = 5, *P<0.05 from DNT. CT = Control, DNT = 60 mg/kg STZ once,DT1 = 125 mg/kg ACSLE, DT2 = 250 mg/kg ACSLE.

Discussion

Diabetes mellitus is a global health problem due to its serious health complications which includes hyperglycaemia and male infertility (Melendez-Ramirez et al., 2010). Streptozotocin-induced diabetes in laboratory animals has been widely used for research on diabetes mellitus (Tamtaji et al., 2017), which was employed in this present study and results from this study aligned with this documented finding. The blood glucose level of the diabetic non treated group was significantly increased compared to the control group (Figure 1).

Administration of Streptozotocin within one week to ten days induces stable diabetes which indicates irreversible destruction of Langerhans islets cells (Akbarzadeh et al., 2007) and diabetic patients have been documented to have increased reactive oxygen species in circulation which ultimately makes them suffer from an increased risk of free radical-mediated damage (Maxwell et al., 1997). The levels of plasma lipid peroxide products, including malondialdehyde in diabetic patients, increased compared to control subjects as documented by Freitas et al. (1997) and this may be one of the reasons for increased glucose concentrations in DNT.

Previous studies have suggested that quercetin derivatives in *Allium cepa* possess a strong antioxidant activity (Dias et al., 2005; Machavarapu et al., 2013), which is responsible for its beneficial effect on hyperglycemia and defects in male fertility caused by diabetes mellitus (Smith et al., 2003; Khaki et al., 2010; Chae et al., 2017). Daily oral administration of 125 mg/kg and 250 mg/kg of ACSLE to diabetic rats for 14 consecutive days caused a statistically significant decrease in the blood glucose level of diabetic animals (Figure 1). This decrease in glucose level may be due to the potent antioxidant actions of ACSLE.

High glucose level impairs male fertility, affecting quality of sperm (Scarano et al. 2006; Kim and Moley 2008; Navarro-Casado et al. 2010; Amaral et al., 2014), multiplication rate or inhibits Sertoli cells maturation, and eventually the functionality having repercussions on spermatogenesis and spermatozoa (Tavareset al, 2017). Sertoli cells contain insulin receptors (Oonk and Grootegoed, 1987) which have effects on their metabolic functions (Oonk et al. 1985; Oliveira et al. 2012). Therefore, distortions in concentrations of insulin because of high blood glucose affect functions of Sertoli cell and spermatogenesis negatively (Schoeller et al. 2012).

The results of this present study indicated that there was a significant improvement in the sperm parameters (motility, viability, morphology and sperm concentration) of streptozotocin-induced diabetic rats by ACSLE administration (Table 3), this is in accordance with Khaki et al. (2010) who documented that Allium cepa increases the epididymal sperm number and the percentages of motile and viable spermatozoa in streptozotocin-induced diabetic rats. Also, Chae et al., (2017) documented that onion peel extract possesses beneficial properties on sperm motility that could be used in development of drugs to

Table 3. Effect of ACSLE administration on sperm parameters of streptozotocin-induced diabetic Wistar rats. Mean ±SEM, n = 5,*P<0.05 from DNT.

Groups	CT	DNT	DT1	DT2
Sperm concentration (x10^6 cell/mL)	132.4 ± 0.68	98.3 ± 2.3	130.0 ± 5.9*	130.7 ± 7.3*
Abnormal sperm morphology (%)	10.4 ± 0.51	19.8 ± 0.9	13.0 ± 0.9*	8.8 ± 0.4*
Sperm motility (%)	95.0 ± 0.05	57.0 ± 1.5	77.7 ± 1.5*	80.0 ± 0.0*
Sperm viability (%)	97.2 ± 0.58	52.7 ± 1.3	70.0 ± 0.0*	80.0 ± 0.0*
Testes weight (g)	1.06 ± 0.01	0.7 ± 0.03	1.0 ± 0.04*	1.0 ± 0.03*

CT=Control, DNT=60 mg/kg STZ once,DT1=125 mg/kg ACSLE, DT2=250 mg/kg ACSLE.

treat male infertility in tandem with the findings of this present study. Daily oral administration of 125 mg/kg and 250 mg/kg of ACSLE to diabetic rats for 14 consecutive days caused a statistically significant increase in the values of the sperm variables of diabetic animals when compared to that of the diabetic non-treated group (Table 3).

Therefore, ACSLE administration was observed to ameliorate the deleterious effects of diabetes mellitus on sperm variables. These deleterious effects could be due to direct actions of high glucose concentration in circulation because abnormal glucose homeostasis has adverse outcomes for the reproductive function in the male gametes (Agbaje et al., 2007) or effect of reactive oxygen species (ROS) generation on sperm cells because there are documentations showing

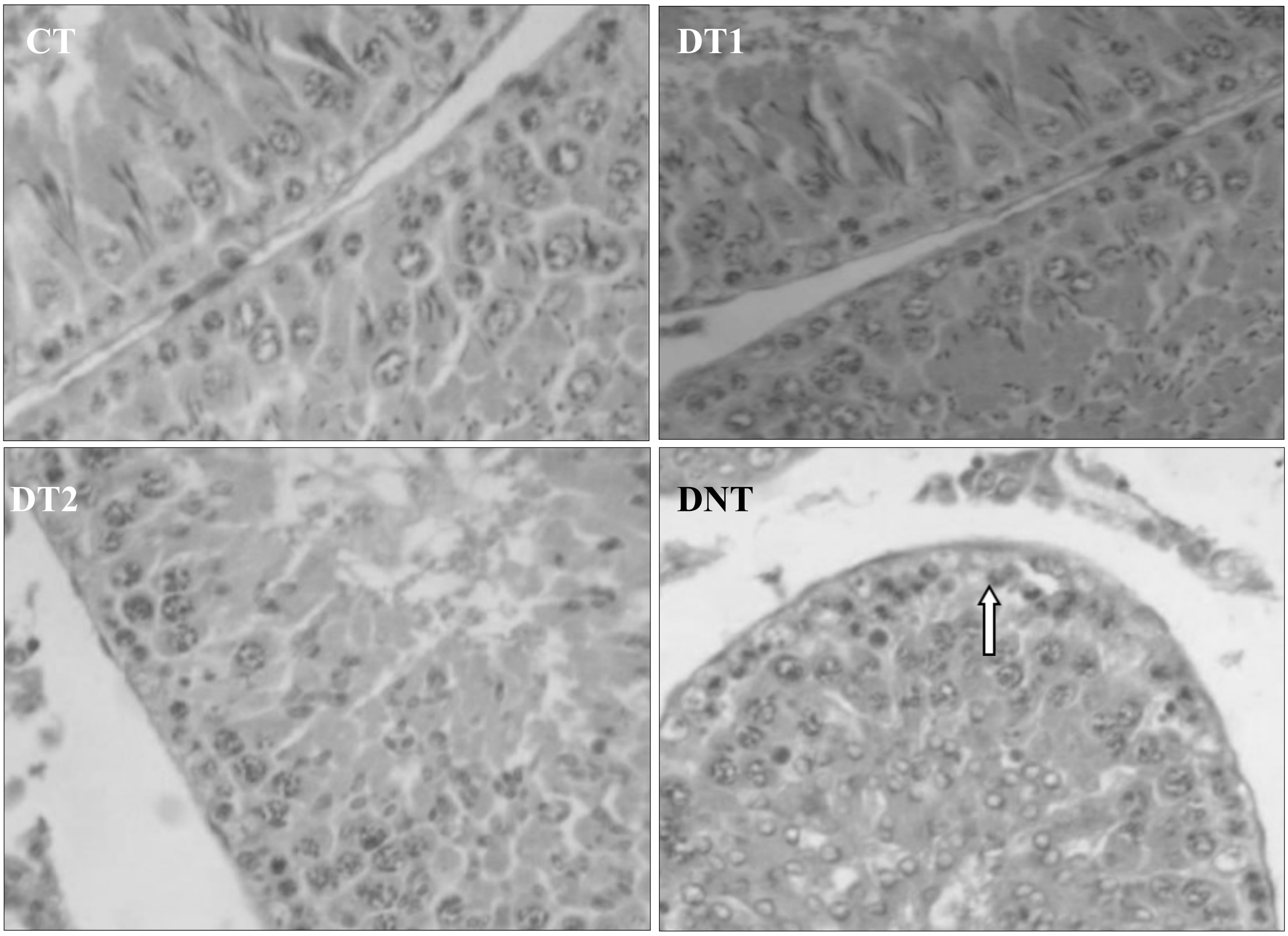

Figure 2: Transverse testicular section photomicrographs stained by Haematoxylin and Eosin, X100 magnification showing effects of ACSLE administration on testis of streptozotocin-induced diabetic rats. CT=Control, DNT=60 mg/kg STZ once,DT1=125 mg/kg ACSLE, DT2=250 mg/kg ACSLE

diabetes strongly associated with increased oxidative stress (Freitas et al., 1997; Abou-Seif and Youssef, 2004; Palsamy and Subramanian, 2010; Folli et al., 2011; Muthukumaran et al., 2018). Energy in sperm cells is mainly used to maintain the motility to complete capacitation and subsequent acrosome reaction (Yanagimachi, 1994; Flesch and Gadella, 2000; Harrison and Gadella, 2005). Quercetin, the major derivative in *Allium cepa* is a strong antioxidant (Dias et al., 2005; Machavarapu et al., 2013; Nile et al., 2020), which help to ameliorate defects in male fertility arising from diabetes mellitus (Smith et al., 2003; Khaki et al., 2010; Jiet al., 2011). This potent antioxidant action of ACSLE may be the mechanism of ameliorating diabetic complications seen in the reproductive system.

There was a significant increase in the testicular weight of streptozotocin-induced diabetic rats administered with ACSLE in this study. Daily oral administration of 125 mg/kg and 250 mg/kg of ACSLE to diabetic rats for 14 consecutive days caused a statistically significant increase in testicular weight when compared to that of the diabetic non-treated group. The reduction in weight of testes is often attributed to decreased population of germ cells including spermatogonia, spermatids and spermatocytes at various stages (Kanter et al., 2013). Studies have shown that *Allium cepa* improves testicular weight by increasing the number of germ cells through reduction of oxidative stress caused by diabetes mellitus (Mahesh and Menon, 2004) which suggested why there was a significant increase in testicular weight of diabetic rats treated by ACSLE.

Finally this present study revealed normal histological structure of most of the seminiferous tubules with normal spermatogenic series of the testes under microscopic examination after 14 days of treatment with ACSLE at 125 mg/kg and 250 mg/kg to streptozotocin-induced diabetic rats (Figure 2). The diabetic non treated group revealed severe degeneration and necrosis of germinal epithelial cells of the seminiferous tubules. This variation shows that ACSLE significantly improved histological testicular degeneration observed in streptozotocin-induced diabetic rats and this complies with previous reports by Park et al. (2007) and Khaki et al. (2010).

The ameliorative effect of ACSLE may be due to its high concentration of quercetin derivatives especially in the outer layers of *Allium cepa* (Park et al., 2007; Melendez-Ramirez et al., 2010), which was why ACSLE was used in this present study. *Allium cepa* improves diabetic status of animals by decreasing oxidative stress (Coskun et al., 2005) and by also reducing the disturbance of hepatic gene expressions (Kobori et al., 2009).

In conclusion, the results of the present study showed that ACSLE possesses ameliorative effects on high blood glucose level and impaired testicular functions and morphology associated with diabetes mellitus.

Recommendations

The whole data made available in this work supports the use of ACSLE has an antidiabetic agent with low reproduction complications. However, further investigations are required to study the exact mechanism of the ameliorative actions of ACSLE on reproductive dysfunctions in streptozotocin-induced diabetic Wistar rats with great emphasis on quercetin.

References

Abou-Seif, M. A. & Youssef, A. (2004). Evaluation of some biochemical changes in diabetic patients. *Clinica Chimica Acta,* 346, 161-170.

Adedara, I. A., Awogbindin, I. O., Anamelechi, J. P. & Farombi, E. O. (2015).Garcinia kola seed ameliorates renal hepatic, and testicular oxidative damage in streptozotocin-induced diabetic rats. *Pharmaceutical Biology,* 1, 695-704.

Afifi, M., Almaghrabi, O. A. & Kadasa, N. M. (2015). Ameliorative effect of zinc oxide nanoparticles on antioxidants and sperm characteristics in streptozotocin- induced diabetic rat testes. *Biomedical Research International,* 2, 1–6.

Agarwal, A., Makker, K. & Sharma, R. (2008). Clinical relevance of oxidative stress in male factor infertility: An update. *American Journal of Reproduction and Immunology,* 59, 2–11.

Agbaje, I. M., Rogers, D. A., McVicar, C. M., McClure, N., Atkinson, A. B., Mallidis, C. & Lewis, S. E. M. (2007). Insulin dependent diabetes mellitus: implications for male reproductive function. *Human Reproduction,* 22, 1871-1877.

Akbarzadeh, A., Norouzian, D., Mehrabi, M. R., Jamshidi, Sh., Farhangi, A., Allah Verdi, A., Mofidian, S. M. A. & Lame Rad, B. (2007). Induction of diabetes by streptozotocin in rats. *Indian Journal of Clinical Biochemestry,* 22 (2), 60-64.

Akkati, S., Sam, K. G. & Tungha, G. (2011). Emergence of promising therapies in diabetes mellitus. *Journal of Clinical Pharmacology,* 51, 796–804.

Amaral, S., & Ramalho-Santos, J. (2014). Free radical biology andreproductive health in diabetes. In *'Systems Biology of Free Radicalsand Anti-Oxidants'.*

and diagnosis of diabetes: Standards of Medical Care in Diabetes. (2019). *Diabetes Care, 42*(Suppl. 1): S13–S28.

Amidu, N., Owiredu, W. K., Alidu, H., Sarpong, C., Gyasisarpong, C. K., & Quaye, L. (2013). Association between metabolic syndrome and sexual dysfunction among men with clinically diagnosed diabetes. *Diabetology and Metabolic Syndrome,* 5, 242-250.

Baccetti, B., Marca, L. A., & Piomboni, P. (2002). Insulin -dependent diabetes in men is associated with hypothalamo-pituitary derangement and with impairment in semen quality. *Human Reproduction,* 17, 2673-2677.

Bindu, B., & Podikunju, B. (2015). Performance evaluation of onion (*Allium Cepa* L. Var. Cepa) varieties for their suitability in kollam district. *International Journal of Research in Agricultural Science, l*(1), 18-20.

Butler, A. E., Janson, J., Bonner Weir, S., Ritzel, R., Rizza, R. A., & Butler, P. C. (2003). Beta�cell deficit and increased beta�cell apoptosis in humans with type 2 diabetes. *Diabetes,*52, 102-110.

Cavallini, G. (2006). Male idiopathic oligo asthenoteratozoospermia. *Asian Journal of Andrology.* 8, 143–157.

Chae, M. R., Kang, S. J., Lee, K. P., Choi, B. R., Kim, H. K., Park, J. K., Kim, C. Y., & Lee, S. W. (2017). Onion (*Allium cepa* L.) peel extract (OPE) regulates human sperm motility via protein kinase C-mediated activation of the human voltage-gated proton channel. *Andrology,* 5(5), 979–989.

Cooke, D. W. & Plotnick,L. (2008). Type 1 diabetes mellitus in pediatrics. *Pediatric Review,* 29, 374–384.

Coskun, O., Kanter, M., Korkmaz, A., & Oter, S. (2005). Quercetin; a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. *Pharmacology Research,* 51, 117-123.

deAncos, B., Colina-Coca, C., Gonzalex-Pena, D. & Sanchez-Moreno, C. (2015). Bioactive compounds from vegetable and fruit by-products. In *Biotechnology of Bioactive Compounds. Sources and Applications. Section I*, V. K. Gupta and M. G. Tuohy, Eds., pp. 3–36,Wiley Blackwell, 2015.

Dias, A. S., Porawski, M., Alonso, M., Marroni, N., Collado, P. S. & Gonzalez-Gallego, J. (2005). Quercetin decreases oxidative stress, NF-kappaB activation, and iNOS overexpression in liver of streptozotocin-induced diabetic rats. *Journal of Nutrition,* 135, 2299-2304.

Edeoga, H. O., Okwu, D. E. & Mbaebie, B. O. (2005).Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology, 4* (7), 685-688.

Eizirik, D. L., Colli, M. L., & Ortis, F. (2009).The role of inflammation in insulitis and beta�cell loss in type 1 diabetes. *Nature Reviews Endocrinology,* 5, 219-26.

Flesch, F. M. & Gadella, B. M. (2000). Dynamics of the mammalian sperm plasma membrane in the process of fertilization. *Biochimica et Biophysica Acta,* 1469, 197-235.

Folli, F., Corradi, D., Fanti, P., Davalli, A., Paez, A. . Giaccari, A Perego C., & Muscogiuri, G. (2011). The Role of Oxidative Stress in the Pathogenesis of Type 2 Diabetes Mellitus Micro- and Macrovascular Complications: Avenues for a Mechanistic-Based Therapeutic Approach. *Current Diabetes Review,* 7 (5), 313-324.

Freitas, J. P., Filipe, P. M., & Rodrigo, F. G. (1997). Lipid peroxidation intype 2 normolipidemic diabetic patients. *Diabetes Research and Clinical Practice*, 36, 71-75.

Ghanbari, E., Nejati, V., Najafi, G., Khazaei, M., & Babaei, M. (2015). Study on the effect of royal jelly on reproductive parameters in streptozotocin-induced diabetic rats. *Internal Journal of Fertility and Sterility,* 1, 113-120.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B., & Smith, B. (2002). Onions-a global benefit to health. *Phytotherapy Research*, *16*(7), 603-615.

Harrison, R. A., & Gadella, B. M. (2005). Bicarbonate �induced membrane processing in sperm capacitation. *Theriogenology,* 63, 342-51.

Jequier, A. M. (2010). Semen analysis: a new manual and its application to the understanding of semen and its pathology. *Asian Journal of Andrology,* 12(1), 11–13.

Ji, Y. J., Yeni, L., Min, S. M., Ji, Y. K., & Oran, K. (2011). Effect of quercetin derivatives on diabetic animals. *Nutrition & Metabolism,* 8, 18-25.

Kahn, S. E., Hull, R. L., & Utzschneider, K. M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature.* 444, 840–846.

Kameswara, R. B., Kesavulu, M. M., Guiri, R. & Apparao, C. H. (1999). Hepatic key enzyme in experimental diabetes. *Journal of Ethnopharmacology, 91*(1), 109-113.

Kanter, M., Aktas, C. & Erboga, M. (2013). Curcumin attenuates testicular damage, apoptotic germ cell death, and oxidative stress in streptozotocin-induced diabetic rats. *Molecular Nutrition and Food Research,* 57, 1578-1585.

Khaki, A., Fathiazad, F., Nouri, M., Maleki, N.A., Khamnei, H. J., & Ahmadi, P. (2010). Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats *Phytotherapy Research,* 24, 1285-1291.

Kilarkaje, N., Al-Hussaini, H., & Al-Bader, M. M. (2014). Diabetes‐induced DNA damage and apoptosis are associated with poly (ADP ribose) polymerase 1 inhibition in the rat testis. *European Journal of Pharmacology,* 737, 29–40.

Kim, S. T., & Moley, K. H. (2008). Paternal effect on embryo quality in diabetic mice is related to poor sperm quality and associated with decreased glucose transporter expression. *Reproduction.* 136, 313-322.

Kobori, M., Masumoto, S., Akimoto, Y. & Takahashi, Y. (2009). Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. *Molecular Nutrition and Food Research,* 53, 859-868.

Koroglu, P., Senturk, G. E., Yucel, D., Ozakpinar, O. B., Uras, F., & Arbak, S. (2015). The effect of exogenous oxytocin on streptozotocin (STZ) induced diabetic adult rat testes. *Fertility and Sterility,* 2, 47–54.

Lee, B.K. & Jung,Y.S.(2016).Allium cepaextract and quercetin protect neuronal cells from oxida-tive stress via PKC-ε inactivation/ERK1/2 ac-tivation. *Oxidative Medicine and Cellular Longevity,* 2016, 2495624.

Machavarapu, M., Sindiri, M. K., & Vangalapati, M. (2017). Optimization of Physico-chemical Parameters for the Extraction of Flavonoids and Phenolic Components from the Skin of *Allium cepa*. Optimization. *Journal of Physiology,* 2(7), 110-121.

Mahesh, T., & Menon, V. P. (2004). Quercetinallievates oxidative stress in streptozotocin-induced diabetic rats. *Phytotherapy Research,* 18, 123-127.

Maiti, R., Jana, D., Das, U. K., & Ghosh, D. (2004). Antidiabetic effect of aqueous extract of seed of tamarindusindica in streptozotocin induced diabetic rats. *Journal of Ethnopharmacology,* 92, 85-91.

Marefati, N., Eftekhar, N., Kaveh, M., Boskabadi, J., Beheshti, F., Boskabady, M. H. (2018).The Effect of *Allium cepa* extract on lung oxidant, boskabadi, antioxidant, and immunological biomarkers in ovalbumin-sensitized rats. *Medical Principles and Practice,* 27,122–128.

Marti, E., Mara, L., Marti, J. I., Muiño-Blanco, T., & Cebrián-Pérez, J. A. (2007). Seasonal variations in antioxidant enzyme activity in ram seminal plasma. *Theriogenology.* 67, 1446–1454.

Maxwell, S. R. J., & Lip, G. Y. H. (1997). Free radicals and antioxidants in cardiovascular disease. *British Journal of Clinical Pharmacology,* 44,307-317.

Melendez-Ramirez, L. Y., Richards, R. J. & Cefalu, W. T. (2010). Complications of type 1 diabetes. *Journal of Endocrinology.* 39, 351-400.

Muthukumaran, J., Ramachandran, V., Ranga, R. A., Baojun, X., Stephen, S. M. C. (2018). Guava leaf extract diminishes hyperglycemia and oxidative stress, prevents‐cell death, inhibits inflammation, and regulates NF-kB signaling pathway in STZ Induced diabetic rats. *Biomedical Research International,* 5, 1-14.

Navarro-Casado, L., Juncos-Tobarra, M. A., Cha´fer-Rudilla, M., de Onzono, L. I., Bla´zquez-Cabrera, J. A., & Miralles-Garcı´a, J. M. (2010). Effect of experimental diabetes and STZ on male fertility capacity study in rats. *Andrology,* 31, 584–592.

Nile, A., Gansukh, E., Park, G. S., Kim, D. H., & Hariram Nile, S. (2020). Novel insights on the multi-functional properties of flavonolglucosides from red onion (Allium cepaL) solid waste-In vitro and in silicoapproach. *Food Chemistry.* 127650.

O.E.C.D. (Organization for Economic Co-operation and Development). (2008): OECD guidelines 407 for the testing of chemicals: Repeated Dose 28- day oral toxicity studies in rodents. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecdtg407-2008.pdf

Oliveira, P. F., Alves, M. G., Rato, L.,Laurentino, S., Silva, J., Sa´,R., Barros, A., Sousa, M., Carvalho, R. A., Cavaco, J. E., & Socorro, S. (2012). Effect of insulin deprivation on metabolism and metabolism associated gene transcript levels of in vitro cultured human Sertoli cells. *Biochimica et Biophysica Acta,* 1820, 84-89.

Oonk, R. B., Grootegoed, J. A., & van der Molen, H. J. (1985). Comparison of theeffects of insulin and follitropin on glucose metabolism by Sertoli cells fromimmature rats. *Molecular and Cellular Endocrinology,* 42, 39-48.

Oonk, R. B., & Grootegoed, J. A. (1987). Identification of insulin receptorson rat Sertoli cells. *Molecular and Cellular Endocrinology,* 49, 51-62.

Palsamy, P., Sivakumar, S., & Subramanian, S. (2010). Resveratrol attenuates hyperglycemia-mediated oxidative stress, proinflammatory cytokines and protects hepatocytes ultrastructure in streptozotocin-nicotinamide-induced experimental protects hepatocytes ultrastructure in streptozotocin-nicotinamide-induced experimental diabetic rats. *Chemico-Biologic Interaction,* 186 (2), 200-210.

Park, J., Kim J., & Kim, M. K. (2007). Onion flesh and onion peel enhance antioxidant status in aged rats. *Tokyo Journal of Nutrition Science and Vitaminology,* 53, 21-29.

P´erez-Gregorio, R. M., Garc´ıa-Falc´on, M. S., Simal-G´andara, J., Rodrigues, A. S., & Almeida, D. P. F. (2010). Identification and quantification of flavonoids in traditional cultivars of red and white onions at harvest. *Journal of Food Composition and Analysis,* 23(6), 592–598.

Philippe, J., Raccah, D. (2009). Treating type 2 diabetes: how safe are current therapeutic agents? *International Journal of Clinical Practice,* 63, 321–332.

Prakash, D., Singh, B. N. & Upadhyay, G.(2007). Antioxidant and free radical scavenging activities of phenols fromonion (Allium cepa). *Food Chemistry, 102*(4), 1389–1393.

Ramachandran, A. (2014). Know the signs and symptoms of diabetes. *Indian Journal of Medical Research, 140*(5), 579-5816.

Rashid, K. & SilP. C. (2015). Curcumin ameliorates testicular damage in diabetic rats by suppressing cellular stress-mediated mitochondria and endoplasmic reticulum-dependent apoptotic death. *Biochimica Biophysica Acta,* 3, 70–82.

Riserus, U., Willett, W. C. & Hu, F. B. (2009). Dietary fats and prevention of type 2 diabetes. *Progress in Lipid Research,* 48, 44-51.

Roldan, E., Sanchez-Moreno, C.,de Anco,B., & Cano, M. P. (2008). Characterisation of onion (AlliumcepaL.) by-products as food ingredients with antioxidant and antibrowning properties. *Food Chemistry, 108* (3), 907-916.

Rupeshkumar, M., Kavitha, K., & Haldar, P. K. (2014). The role of herbal plants in the diabetes mellitus therapy: an overview. International *Journal of Applied Pharmacology,* 6, 1-3.

Saeedi, P., Petersohn, I., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., ShawJ. E., Bright, D., & Williams, R. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice, 157* . 107843.

Santas, J., Almajano, M. P., & Carb´o, R. (2010). Antimicrobial and antioxidant activity of crude onion (Allium cepa, L.) extracts. *International Journal of Food Science and Technology, 45*(2), 403–409.

Saumya, S. & Basha, P. M. (2016). Fluoride exposure aggravates the testicular damage and sperm quality in diabetic mice: protective role of Ginseng and Banaba. *Biological Trace Element Research,* 2, 1–14.

Scarano, W. R., Messias, A. G., Oliva, S. U., Klinefelter, G. R., & Kempinas, W. G. (2006). Sexual behaviour, sperm quantity and quality after short-term streptozotocin-induced hyperglycaemia in rats. International *Journal of Andrology,* 29, 482-488.

Schoeller, E. L., Albanna, G., Frolova, A. I., &Moley, K. H. (2012). Insulin rescues impaired spermatogenesis via the hypothalamicpituitary-gonadal axis in Akita diabetic mice and restores malefertility. *Diabetes*, 61, 1869-1878.

Sexton, W. J., & JarowJ. P. (1997). Effect of diabetes mellitus upon male reproductive function. *Urology Research,* 49, 508-513.

Shaath, N. A., & Flores, F. B. (1998). Egyptian onion oil, developments in Food Science. *Journal of Nutrition*, 40, 443-453.

Slimestad, R., Fossen,T., & Vagen, I. M. (2007). Reviews. Onions: a source of unique dietary flavonoids. *Journal of Agriculture and Food Chemistry*, 55 (25), 10067–10080.

Smith, C., Lombard, K. A. Peffley, E. B., & Liu. W. (2003). Genetic analysis of quercetin in onion (Allium cepa L.) 'Lady Raider'. *Texas Journal of Agriculture and Natural Resources,* 16, 24-28.

Tamtaji, O. R., Hossan, H., Sagyed, A. T., Mohamed, B., Seyed, M. T., Moshen, T. & Reza, A. (2017). Protective effects of red onion ethanolic extract on learning and memory impairments in animal of diabetes. *Journal of Medicine,* 6(3), 249-257.

Tavares, R. S., Portela Joana, M. D., Sousa, M. I., Mota Paula, C., Ramalho-Santos, J., & Amaral, S.(2017). High glucose levels affect spermatogenesis: an in vitro approach. *Reproduction Fertility and Development,* 29(7), 1369-1378.

Tremellen, K. (2008). Oxidative stress and male infertility a clinical perspective. *Human Reproduction Update, 14*(3),243-258.

Tsounapi, P., Honda, M., Dimitriadis, F., Kawamoto, B., Hikita, K., Muraoka, K., Saito, M., Sofikitis, N., & Takenaka, A. (2016). Impact of antioxidants on seminal vesicles function and fertilizing potential in diabetic rats. *Asian Journal of Andrology,* 2, 1-10.

Yanagimachi, R. (1994). Mammalian fertilization. *Physiology of Reproduction,* 1, 189-317.