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ANNONA MURICATA LINN. ETHANOLIC LEAF EXTRACT AMELIORATES REPRODUCTIVE COMPLICATIONS IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

Diabetes mellitus is an endocrine and metabolic disorder of humans and animals characterized by hyperglycemia and low blood insulin levels or insensitivity of target organs to insulin and it's a major health problem affecting patient's quality of life due to its many complications. Infertility is one of the major secondary complications in diabetes. Although numerous drugs have been used for intervention studies on diabetes-induced infertility worldwide, there are currently no treatments for diabetes associated infertility in humans. This study was performed to investigate the effects of *Annona muricata* ethanolic leaf extract (AMELE) on fertility of male diabetic rats and levels of blood glucose. Twenty male Wistar rats (150-200g) were randomly distributed into 4 groups (n=5) treated thus: CTRL (control), DNT, DT1 and DT2 (diabetic, single intraperitoneal injection, streptozotocin, 60 mg/kg). Group DT1 and DT2 received AMELE orally at 100 mg/kg and 200 mg/kg respectively daily for fourteen days. Data were analysed using ANOVA at $\alpha_{0.05}$. The animals were sacrificed after 2 weeks via thiopental injection and testicular weights were recorded. Fasting blood glucose was determined using a digital glucometer. Sperm count, motility, viability and morphology were assessed microscopically. Testes were histologically evaluated. The results showed that oral administration of AMELE at 100 mg/kg and 200 mg/kg to diabetic male rats for fourteen days significantly decreased blood glucose level and also ameliorated diabetes-induced decreases in sperm functions in streptozotocin-induced diabetic male rats.

Keywords: *Annona muricata*, diabetes, streptozotocin, reproductive dysfunctions.

INTRODUCTION

Diabetes mellitus (DM) is an endocrine and metabolic disorder in which the body is unable to metabolize carbohydrates properly is increasing globally and now affects 7% of the world's adult population (Maiti *et al*., 2004; Philippe and Raccah, 2009). It is a major health problem affecting patient's quality of life due to its many complications. It is estimated that 382 million people suffer from diabetes and that the prevalence is 8.3% (Guariguata *et al*., 2014).

DM is grouped into two main types, type I diabetes mellitus (T1DM) and type II diabetes mellitus (T2DM). T1DM results from defective beta cells in the islets of Langerhans which could be caused by destruction from various infections, diseases and exposure to various toxic chemicals (Cooke and Plotnick, 2008; Eizirik *et al*., 2009; Akkati *et al*., 2011). Symptoms are polyphagia, polydipsia, polyuria and weight loss (Cooke and Plotnick, 2008; Akkati *et al*., 2011). T2DM is characterized by persistently high blood glucose due to the presence of insulin resistance and usually with relative insulin deficiency (Butler *et al*., 2003; Akkati *et al*., 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; Vlad *et al*., 2016) via actions at multiple levels including altered spermatogenesis, degenerative and apoptotic changes in testes (Ghanbari *et al*., 2015), 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), semen volume (Adedara *et al*., 2015), abnormal spermatozoa morphology (Afifi *et al*., 2015) and the disruption of seminiferous tubular 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 has been linked with increased oxidative stress (Freitas *et al*., 1997; Abou-Seif & Youssef, 2004) which may play an important role in the cause of diabetic complications.
Herbal remedies from medicinal plants have been used traditionally in many parts of the world where access to formal healthcare is limited. Several studies have described the medicinal purposes of *Annona muricata* and have outlined the social history of the plants' use (Ayensu, 1981). *A. muricata* (Linn.) (Family, Annonaceae) commonly called "Soursop" is a small, upright evergreen tree growing 5 to 6 meters in height. (Vasquez, 1990; de Feo, 1992).

It is indigenous to most of the warmest tropical areas in South and North America including Amazon and has become naturalized in many countries and throughout tropical and subtropical parts of the world, including western part of Nigeria. Ethnobotanically, all parts of *A. muricata* tree have been used medicinally in the tropics, including the bark, leaves, roots, fruits, and fruit seeds (Haddock, 1994; Mors, 2000).
Although numerous drugs have been used for intervention studies on diabetes-induced infertility worldwide, there are currently no

treatments for diabetes associated infertility in humans (Shi *et al*., 2017).

MATERIALS AND METHODS

Animals

Twenty male Wistar rats (150-200g) were used for this study. The rats were housed in the Experimental Animal Unit of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. 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 (Ladokun Feeds, Ibadan) and clean water was given *ad libitum*. There was a pre-experimental period of 2 weeks during which the rats were acclimatized. The rats received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" (Garber *et al*., 2011) and the study was approved by College of Veterinary Medicine Animal Ethics Committee.

Plant Material

The leaves of *Annona muricata L*. were collected from a farm in Abeokuta, Ogun State. Identification and authentication was done at Department of Pure and Applied Botany, College of Biosciences, Federal University of Agriculture, Abeokuta.

Plant Extraction

Annona muricata leaves were air-dried at room temperature for four weeks. The air-dried leaves were 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 60^0C using a vacuum rotary evaporator. The wet brown residue was allowed to evaporate *in vacuo* and stored in the refrigerator (4 ^{0}C) 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.

Experimental Procedure

The rats were randomly distributed into 4 groups (n=5) and treated as listed in Table 1.

Table 1: Animal groupings and daily protocols

GROUP	Protocols
CTRL	Control group received volume of distilled water (vehicle for the stock solution) commensurate with the body weight of rat average of 0.5 ml daily
DNT	Diabetic, intraperitoneal injection of streptozotocin 60 mg/kg once
DT1	Diabetic, intraperitoneal injection of streptozotocin 60 mg/kg once, AMELE (100 mg/kg rat/day) by oral gavage for 14 days
DT2	Diabetic, intraperitoneal injection of streptozotocin 60 mg/kg once, AMELE (200 mg/kg rat/day) by oral gavage for 14 days

Induction of diabetes in rats: Diabetes mellitus was experimentally induced in DNT, DT1 and DT2 by a single intraperitoneal injection of 60 mg/kg of STZ (Sigma St Louis, U.S.A) dissolved in cold 0.1M sodium citrate buffer pH 6.3 after an overnight fast (Akbarzadeh *et. al.,* 2007). CTRL were injected with equivalent volume of citrate buffer. The blood glucose concentration was monitored after 72 hours post STZ administration and rats with glucose concentration of 250 mg/dL and above were included

in the diabetic groups (DNT, DT1 and DT2).

***Blood glucose measurement*:** Fasting blood glucose level were estimated 3 times in a week during the treatment period for two weeks using blood sample obtained from the tail vein of the rats and determined in mg/dl by a digital glucometer (Accu-chekAdvantage, Roche Diagnotics, Germany).The animal 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 and a ventral midline abdominal incision was made to expose the reproductive organs. The testis were identified, carefully removed and processed further for sperm evaluation (Zemjanis, 1977) and testicular histology.

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using Statistical Package for the Social Science (SPSS) software (version 16.0; SPSS Inc., USA). Results were expressed as mean ± SEM for five rats in each group. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

a. Hypoglycaemic effects of AMELE on blood glucose

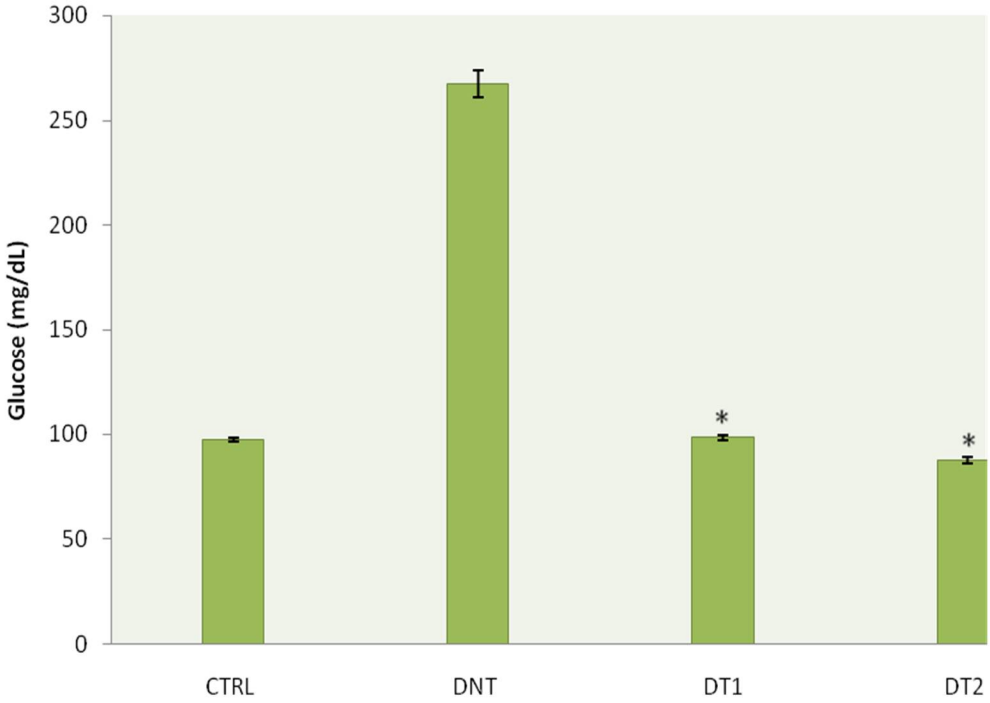


Figure 1: Effect of AMELE on blood glucose level of control
negative control (DNT) and test rats in (mg/dL), N = 5, *P<0.05 from DNT

b. Effects of AMELE on sperm variables

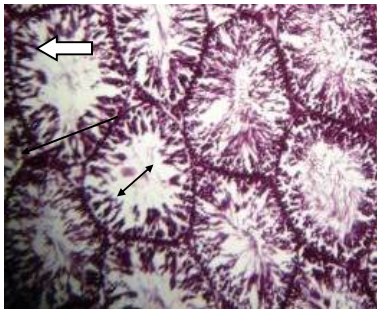
Table 2. Effect of AMELE administration on sperm parameters of streptozotocin-induced diabetic Wistar rats.
Mean ± S EM, n = 5, *P<0.05 from DNT.

Groups	CTRL	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*

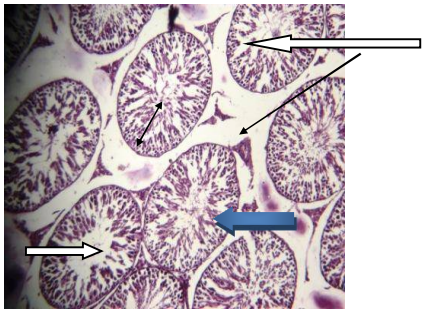
At the end of the experiment, the sperm viability, concentration, motility, morphology and testicular weights of rats in groups DT1 and DT2 differed significantly ($p< 0.05$) when compared with group DNT which are the diabetic non-treated rats.

c. Effects of AMELE on testicular histology

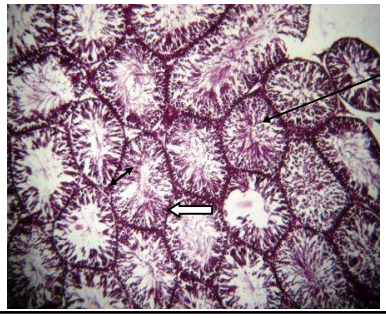
CTRL



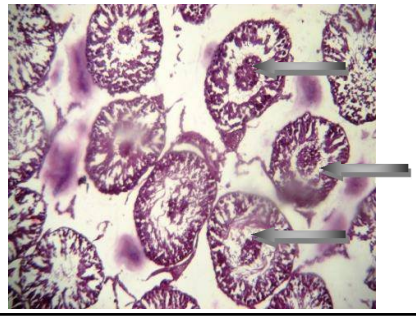
DT1



DT2



DNT[a]



DNT^{b}

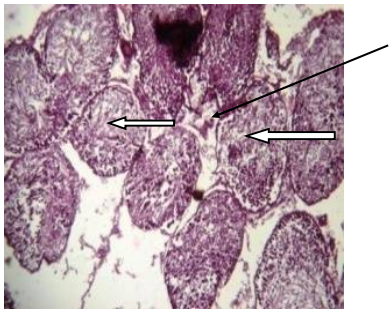


Figure 2: Transverse testicular section photomicrographs stained by Haematoxylin and Eosin, X100 magnification showing effects of AMELE administration on testis of streptozotocin-induced diabetic rats

CTRL, DT1 and DT2: Normal architecture with no visible lesions (Figure 2).
DNT^{a}: Seminiferous tubules with germ cells sloughed into the lumen, lack lumen and well defined germ cells layer (white arrow); spermatogonia cell (blue arrow) exhibit clear cytoplasmic halo and show maturation arrest at primary level (spanned), normal sertoli cells (red arrow) (Figure 2).
DNT^{b}: Several seminiferous tubules lack lumen and well defined maturing germ cell layer (white arrow) (Figure 2).

DISCUSSION

Management of diabetes without any side effect is still a challenge to the medical system. This has led to an increasing demand for natural products with anti-diabetic activity and fewer side effects (Kameswara *et al.*, 1999) and utilization of plants, plants extract, and the active compound from plants to cure diseases is very potential step in new drugs discovery (Rupeshkumar *et al.,* 2014). All parts of *Annona muricata* plant have been used empirically as herbal medicine, including the bark, leaves, roots, fruit, and seeds (Sawant and Dongre, 2014; Oladipo *et al*., 2018)). Prior study suggested that ethanolic extract of *Annona muricata* leaf has beneficial effect on diabetes by significant reduction in the blood glucose concentration (Adewole and Caxton-martins 2006; Adeyemi *et al.,* 2007, 2009[ab], 2010 and Suneel *et al*., 2015) and was in consonance with the results observed in this study (Figure 1).This could be as a result of improved glycemic control produced by *A. muricata* extract.

Although Oladipo *et al*. (2018) reported that the administration of *A. muricata* bark extract caused a reduction in sperm variables and also increased some secondary morphological sperm abnormalities in streptozotocin-induced diabetic rats, this was not so in this study because a lower dose of AMELE was used in contrast to their high dose (Oladipo *et al*., 2018).

Uno and colleagues (2017) suggested that *A. muricata* can have beneficial effect on sperm parameters and this was confirmed in the result of this present study indicative of AMELE's significant improvement in the sperm parameters of streptozotocin-induced

diabetic rats (Table 2). AMELE was observed to mitigate the effect of diabetes on sperm motility, sperm concentration and sperm viability. The mitigating effect of AMELE could be due to its rich phytonutrients, antioxidant content and vitamins which provide protective roles against oxidative stress (*Ekaluo et al.,* 2013). This showed that AMELE demonstrates a potent protective effect on sperm of streptozotocin-induced diabetic rats (Table 2) (Uno *et al.,* 2017).

Pathologies seen in DNT as shown in Figure 2 (seminiferous tubules with sloughed germ cells, lacking lumen and well defined germ cells layer; spermatogonia cell with clear cytoplasmic halo and showing maturation arrest at primary level) were absent in the treated groups DT1 and DT2 demonstrating a potent protective effect of AMELE on testicular components of streptozotocin-induced diabetic rats (Figure 2).

CONCLUSION

Reduction in blood glucose, improved testicular functions and morphology showed that AMELE can be used to manage diabetes and its associated reproductive dysfunctions in male Wistar rats which also supports the traditional usage of this plant leaves as an antidiabetic agent. These ameliorative actions on reproductive functions may be attributable to the antioxidant effect of AMELE.

Future work aims to confirm that the antioxidant actions of AMELE play a mechanistic role on its protection from reproductive dysfunctions in streptozotocin-induced diabetes

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