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A preliminary study on the evaluation of the ameliorative effect of *Mucuna pruriens* on ovulation, serum gonadotropins and oxidative stress markers in STZ-induced diabetic rats.

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ABSTRACT

The ovary is the female gonad and it produces sex hormones that are responsible for the release of ovum during the menstrual cycle. Diabetes has been reported to have deleterious effect on the structure and function of the ovary. *Mucuna pruriens* is a plant that has been reported to have hyperglycemic property and rich antioxidant activities. This study was carried out to determine the effect of ethanolic extract of *Mucuna pruriens* on the function of the ovary in STZ-induced diabetes. Fifteen female Sprague-Dawley rats averagely weighing 150 g were used for this study. The animals were divided into 3 groups. Extract of *Mucuna pruriens* was given orally for two weeks at doses of 50 and 100 mg/kg body weight. Control animals received distilled water. At the end of the experiment animals were sacrificed by cervical dislocation. Blood was collected for hormonal assay studies and the oviducts were excised; ova count and biochemical assay for oxidative stress markers were performed respectively. An increase in the serum levels of FSH and LH were observed in the treated groups. However, this increase was not significant. The result also showed increased antioxidant activities of catalase and superoxide dismutase in the experimental groups when compared with control. Furthermore, the number of ova shed at ovulation in the treated groups was comparable with the control group. In conclusion, *Mucuna pruriens* seed extract ameliorates the deleterious effect of diabetes mellitus on ovarian function.

Keywords: STZ; Mucuna pruriens; diabetes; antioxidants; ovulation; follicle stimulating hormone; luteinizing hormone.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia and insulin resistance. Diabetes impacts both directly and indirectly on the fertility of couples[1]. Consequently, DM impairs both male and female reproductive functions at multiple levels as a result of its effects on the endocrine system as well as on vasculature[2]. Diabetes has been reported to cause alterations in ovarian function by reducing serum levels of FSH and LH which consequently induces a decrease or even absence of ovulated oocytes in female rats and in turn impairs fertility[3-5]. DM has been associated with several mechanisms, one of which is oxidative stress. Oxidative stress plays an important role in the development and progression of diabetes and its complications[6-8]. Hyperglycemia is the link between diabetes with diabetic complications. Hyperglycemia results in overproduction of oxygen free radicals, which contributes to the progression of diabetes[9,10]. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms then ensues[11]. Damage to the cells ultimately results in the secondary complications associated with diabetes[12,13].

Mucuna pruriens belongs to the family *Fabaceae* and has been described as a multipurpose plant which is used extensively both for its nutritional and medicinal properties. *M. pruriens* seeds are a rich source of L-Dopa and its metabolites. In vitro antioxidant assays have supported the antioxidant property of L-Dopa[14]. Dopamine, a product of L-Dopa metabolism, has also been found to possess strong anti-oxidant capacity and free radical scavenging activity[15,16]. Phytochemical screening of the plant revealed that it contains alkaloids, flavonoids, tannins saponins, cardiac glycosides, anthraquinones and carbohydrates[17-20]. L-Dopa and its metabolite dopamine, have been reported to stimulate the hypothalamus and forebrain to secrete gonadotropin-releasing hormone (GnRH)[21-23]. This ultimately activates the anterior lobe of the pituitary gland to secrete follicle stimulating hormone and luteinizing hormone.

Literature is replete with the antihyperglycemic action

of *Mucuna pruriens*[24-28], its excellent scavenging ability that mops up excessive production of reactive oxygen species and free radicals[14-16, 19, 22, 29-31, 32] and its ability to activate the secretion of gonadotropins[17,30,32-34].

An earlier study had reported that ***M. pruriens*** seed extract has the potential to enhance fertility by increasing serum levels of FSH and LH which in turn increases the number of oocytes released at ovulation possibly through its antioxidant properties[32].

This study was carried out to showcase *Mucuna pruriens* as a cheap, easily accessible, non invasive remedy of plant origin for the treatment of infertility in diabetic women.

MATERIALS AND METHODS

Animals

Fifteen female Sprague-Dawley rats averagely weighing 150 g were obtained from the Animal Holding Centre, Badagry Lagos State, Nigeria and were transferred to the Animal House of the Department of Anatomy, College of Medicine of the University of Lagos. The animals were housed in special clear sided cages and left to acclimatize for a period of 2 weeks. All the animals were given pelleted rat feed purchased from Agric Farms, Lagos Nigeria and water was provided Ad-libitum. They were exposed to twelve hours light and dark cycles at standard temperature (26°C-28°C). The animals were identified by different ear tags. All experimental procedures and techniques were approved by the Departmental Committee on the use and care of animals and tissue collection.

Plant Source

Mucuna pruriens plant with mature seeds was obtained from Mushin market Lagos, Nigeria.
They were identified and authenticated in Department of Botany of the University of Lagos. Voucher specimen with accession number LUH 4922 was deposited in the herbarium of the Department of Botany of the University of Lagos.

Seed Extraction

The extraction was carried out in the Pharmacognosy Department of the Faculty of Pharmacy, University of Lagos Nigeria. Briefly, seeds were obtained from the pods, air-dried and grounded into fine powder using the mortar and the pestle. 1.5 Kg of fine powder was mixed with alcohol and placed in the Soxhlet apparatus. The powder obtained (147.8 g yield) was stored at room temperature of 25°C before use. All dilutions of the extract were made in distilled water.

The animals received a single oral dose of *Mucuna prurients* at 50 and 100 mg/kg body weight at 2 p.m. daily for two weeks using an oro-gastric tube. Distilled water was given to the control animals.

Induction of Diabetes.

Rats were fasted overnight for 12 hrs before diabetes was induced using streptozotocin (STZ). Briefly, 50 mg/kg body weight of STZ was dissolved in citrate buffer (pH 4.5) and injected intraperitoneally within 10 minutes after preparation. Symptoms of diabetes were clearly seen within 2-4 days after induction.

Experimental Design

The animals were divided into 3 groups with 5 animals in each group.
Group l; Received distilled water only, served as control.
Group II; Received oral dose of *Mucuna pruriens* at 50 mg/kg daily.
Group III; Received oral dose of *Mucuna pruriens* at 100 mg/kg daily.

At the end of the 2 weeks study, oestrous cycle was performed daily in all the groups to determine the animals in the proestrus phase. Blood was collected from the angular vein of the eye at 6 p.m. on proestrus and stored at -80°C for hormonal assay studies.

The next day, the rats were sacrificed by cervical dislocation. A ventral laparotomy was performed and the oviducts and the ovaries were excised. The right ovaries were immediately placed in 10% formal saline for histological studies while the left ovaries were stored in -80°C for biochemical analysis.

Determination of the Oestrous Cycle

The phases of the oestrous cycle were determined daily between 8 a.m. and 9:30 a.m. using the vaginal smear method. Vaginal secretion was collected with a plastic pipette filled with 10 µl of normal saline (NaCl 0.9%). The vagina was flushed two or three times with the pipette and the vaginal fluid was placed on a glass slide. A different slide was used for each animal. The unstained secretion was observed under a light microscope.

Ovulation study

The dissected oviduct was placed on glass slides with a drop of saline and covered with cover-slips. This was squeezed with both sides being gently rocked and each ovum found in the distended ampulla was counted under a light microscope[35].

Antioxidant study

The right ovaries were homogenized using a Potter–Elvehjem homogenizer. A 20% (1/5 w/v)homogenate of the tissue was prepared in 50m MTris-HCl buffer (pH 7.4) containing 1.15%potassium chloride and centrifuged at 10,000 rpm at 4°C for 10 min.

Superoxide dismutase was assayed utilizing the technique of[36]. A single unit of enzyme was expressed as 50% inhibition of Nitrobluetetrazo-lium (NBT) reduction/min/mg/protein.

Catalase was assayed colorimetrically at 620 nm and expressed as µmoles of H2O2 Consumed/min/mg/protein as described by[37].

Hormonal Studies (Follicle Stimulating Hormone and Luteinizing Hormone)
Blood was obtained from the angular vein of the eye of the Sprague-Dawley rats at 6 p.m. in the evening of proestrus and collected into heparinised bottles. Each blood sample was spun at 2,500 rpm for 10 minutes in an angle-head desktop centrifuge at temperatures of 25°C.

Serum samples were assayed in batches with control sera at both physiological and pathological levels by Standard Quantitative Enzyme- Linked Immunosorbent Assay (ELISA) technique with Micro well kits from Syntro Bioresearch Inc., California, USA.

Statistical Analysis
Results were analyzed and expressed as Mean ± SD and were subjected by Student T- test and one-way ANOVA. Statistical significance was considered at P >0.05.

RESULTS

During the course of this experiment, the animals showed signs and symptoms of diabetes such as high blood glucose levels, weight loss, frequent urination and loss of fur. However, two animals died in the experimental group after the induction of experimental diabetes using STZ as a diabetogenic agent.

Effect of ethanolic extract of *Mucuna pruriens* on the number of ova shed in STZ induced- diabetic rats: Table 1 shows the number of ova shed in the oviduct across the treated groups. The result revealed that the number of ova released in the animals treated with *Mucuna pruriens* after induction of diabetes at both dosages (50 and 100 mg/kg body weights) were comparable with control values.

Table 1: Effect of ethanolic extract of *Mucuna pruriens* administered after two weeks of STZ-induced diabetes on the number of ova shed in the oviduct in Sprague-Dawley rats.

TREATMENT GROUPS	NUMBER OF OVA SHED
Control	6.00 ±1.00
Diabetic +50 mg/kg	6.00 ± 0.00
Diabetic +100 mg/kg	6.00 ± 1.00

Values are represented as Mean ± SEM, N=5.

Effect of ethanolic extract of *Mucuna pruriens* on oxidative stress markers in STZ- induced diabetic rats:
An increase in the activities of the antioxidant status of SOD and catalase were observed in all the treated groups when compared with the control. However, this increase was not statistically significant (Table 2).

Table 2: Effect of ethanolic extract of *Mucuna pruriens* administered after two weeks of STZ-induced diabetes on SOD and catalase activities in S-D rats.

Treatment Group	SOD (min/mg protein)	Catalase (Mmol/min/mg protein)
Control	280.14 ± 39.33	1.75 ± 0.37
Diabetic +50 mg/kg	399.65 ±25.60	1.78± 0.77
Diabetic +100 mg/kg	364.48 ± 26.99	2.44 ± 0.20

Values represented as Mean ± SEM, N=5.

Effect Of Ethanolic Extract Of *Mucuna Pruriens* On Serum Levels Of Fsh And Lh In Stz Induced Diabetic Rats: The serum levels of both FSH and LH were increased in this study when compared to the control values (Table 3) but were not statistically significant.

Table 3: Effect of ethanolic extract of *Mucuna pruriens* administered for two weeks of STZ-induced diabetes on serum levels of FSH and LH in S-D rats.

Treatment groups	FSH (mIU/ml)	LH (mIU/ml)
Control	0.45 ± 0.09	0.84 ± 0.04
Diabetic +50 mg/kg	0.64 ± 0.22	1.28 ± 0.22
Diabetic +100 mg/kg	0.79 ± 0.51	0.89 ± 0.09

Values represented as Mean ± SEM P, N=5.

DISCUSSION

Diabetes Mellitus is a disease in which increased oxidative stress plays an essential pathogenic role. In this present study, the antioxidant activities of SOD and catalase increased in the STZ-induced diabetic + *Mucuna pruriens* groups compared with the control. This up-regulation in the production of SOD and catalase is in response to the increasingly build-up of free radicals production in the ovary. An earlier study had reported that the administration of *Mucuna pruriens* for 28 days increased the antioxidant activities of SOD and catalase in female Sprague-Dawley rats[32]. These investigators proposed that the antioxidant properties inherent in the *Mucuna* plant may be responsible for this action.

Diabetes has been reported to cause alterations in ovarian function by reducing serum levels of FSH and LH which consequently induces a decrease or even absence of ovulated oocytes in female rats and in turn impairs fertility[3-5]. Serum levels of FSH and LH in the STZ-induced diabetic + *Mucuna pruriens* groups increased compared to the control group in this study. L-Dopa and its metabolite dopamine have been reported to stimulate the hypothalamus and forebrain to secrete gonadotropin-releasing hormone (GnRH)[21-23]. This ultimately will activate the anterior lobe of the pituitary gland to secrete FSH and LH. In concert with our report, many studies on both animals and humans have reported significantly increased levels of FSH and LH following the administration of *Mucuna pruriens in males*[17,30,33,34].

FSH and LH are responsible for the growth and maturation of follicles and the release of oocyte at ovulation respectively. In this study, it was recorded that the number of oocytes released at ovulation in the STZ-induced diabetic + *Mucuna pruriens* groups were comparable with values in the control group. An earlier study had reported the release of a comparable number of oocytes when *Mucuna pruriens* was administered for 28 days at 50 and 100 mg/kg body weight compared with the control[32]. The increase in serum levels of LH in the STZ-induced diabetic + *Mucuna pruriens* groups may be responsible for the number of oocytes released at ovulation. This report is supported by Ojo *et al*[32] who reported that increasing levels of circulating luteinizing hormone from the anterior pituitary produced by the administration of *Mucuna pruriens* was responsible for the increase in the number of oocytes released at ovulation.

CONCLUSION

In conclusion, it can be deduced from this study, that the ethanolic extract of seeds of *Mucuna Pruriens* has the potential to ameliorate the deleterious effects of diabetes mellitus on the ovary. Thus, the long term usage of *Mucuna Pruriens* seems promising in the treatment of diabetes-related infertility.

REFERENCES

1. Glenn DR, McClure N, Lewis SE. The hidden impact of diabetes on male sexual dysfunction and fertility. Hum Fertil (Camb) 2003; 6(4): 174–179.
2. Sexton WJ, Jarow JP. Effect of diabetes mellitus upon male reproductive function. Urology. 1997l 49(4): 508–513.
3. Bestetti GE, Locatelli V, Tirone F, Rossi GL, Muller EE. One month of streptozotocin-diabetes induces different neuroendocrine and morphological alterations in the hypothalamo-pituitary axis of male and female rats. *Endocrinology. 1985;* 117: 208–216.
4. Ballester J, Munoz MC, Domınguez J, Rigau T, Guinovart JJ, Rodrıguez-Gil JE. Insulin-dependent diabetes affects testicular function by FSH- and LHlinked mechanisms. *J. Androl*. 2004; 25: 139–152.
5. Hussein Z, Al-Qaisi J. Effect of Diabetes mellitus Type 2 on Pituitary Gland Hormones (FSH, LH) in Men and Women in Iraq. Journal of Al-Nahrain University science. 2012; 15(3): 57–79.
6. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes. 1991; 40: 405–412.
7. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes. 1999; 48:1–9.
8. Ceriello A. Oxidative stress and glycemic regulation. Metabolism. 2000; 49(2, Suppl 1): 27–29.
9. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001; 414: 813–820.
10. Rolo AP, Palmeira CM. Diabetes and mitochondrial function: Role of hyperglycemia and oxidative stress. Toxicology and Applied Pharmacology. 2006; 212: 167–178.

11. Maritim AC, Sanders RA, Watkins III JB. Diabetes, oxidative stress, and antioxidants: a review. J. Biochem. Mol. Toxicol. 2003; 17: 24–38.
12. Hunt JV, Dean RT, Wolff SP. Hydroxyl radical production and autoxidative glycosylation. Glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and ageing. Biochem. J. 1988; 256(1): 205–212
13. Jaganjac M, Tirosh O, Cohen G, Sasson S, Zarkovic N. Reactive aldehydes—second messengers of free radicals in diabetes mellitus. Free Radical Research. 2013; 47(1): 39–48.
14. Gulcin I. Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa. Amino Acids. 2007; 32: 431–438.
15. Gow-Chin Y, Chiu-Luan H. Antioxidant effects of dopamine and related compounds. Biosci Biotech Biochem. 1997; 61:1646–1649.
16. Kazuki K, Hiroyuki S. High content of dopamine, a strong antioxidant, in Cavendish banana. J Agric Food Chem. 2000; 48: 844–848.
17. Vadivel V, Janardhanan K. Nutritional and Anti-nutritional Composition of Velvet Bean: an Under-Utilized Food Legume in South India. Inter J Food and Sci Nutr. 2000; 51:279–287.
18. Nebedum JO, Udeafor PC, Okeke CU. Comparative effects of ethanolic extracts of Ficus carica and Mucuna pruriens leaves on haematological parameters in albino rats. Biokemistri. 2010; 22:77–84.
19. Agbafor KN, Nwachukwu N. Phytochemical analysis and antioxidant property of leaf extracts of Vitex doniana and Mucuna pruriens. Biochem Res Inter. 2011; Article ID 459839, 4 pages.
20. Nwaoguikpe RN, Braide W, Ujowundu CO. The Effects of Processing on the Proximate and Phytochemical Compositions of Mucuna pruriens Seeds (Velvet Beans). Pakistan J Nutr. 2011; 10: 947–951.
21. Shukla KK, Mahdi AA, Ahmad MK, Shankhwar SN, Rajender S, Jaiswar Sp. Mucuna Pruriens improves male fertility by its action on the hypothalamus-pituitary-gonadal axis. Fertil Steril. 2009; 92: 1934–1940.
22. Singh AP, Sarkar S, Tripathi M, Rajender S. Mucuna pruriens and Its Major Constituent L-DOPA Recover Spermatogenic Loss by Combating ROS, Loss of Mitochondrial Membrane Potential and Apoptosis. PLOS ONE. 2013; 8(1):54655.
23. Vermes I, Toth EK, Telegdy G. Effects of drugs on brain neurotransmitter and pituitary testicular function in male rats. Horm Res. 1979; 10: 222–232.
24. Murugan M, Uma C, Reddy M. Hypoglycemic and hypolipidemic activity of leaves of *Mucuna pruriens* in alloxan induced diabetic rats. J. Pharmaceutical Sci. Technol. 2009; 1(2): 69–73.
25. Anusha B, Nithya V, Vidhya VG. Phytochemical evaluation by GC-MS and antihyperglycemic activity of *Mucuna pruriens* on Streptozotocin induced diabetes in rats. *J. Chem. Pharm. Res., 2011; 3(5): 689–696.*
26. Majekodunmi SO, Oyagbemi AA, Umukoro S, Odeku OA. Evaluation of the anti-diabetic properties of Mucuna pruriens seed extract. Asian Pacific Journal of Tropical Medicine. 2011; 4(8): 632–636.
27. Eze ED, Mohammed A, Musa KY Tanko Y. Evaluation of Effect of Ethanolic Leaf Extract of *Mucuna pruriens* on Blood Glucose Levels in Alloxan-Induced Diabetic Wistar Rats. Asian J.Med. Sci. 2012; 4(1): 23–28.
28. Bhadra B, Morshed T, Roney SI, Rahman FB, Rahmatullah M, Hossain S, Haque S. *Mucuna pruriens*: An evaluation of antihyperglycemic potential of seeds. Advances in Natural and Applied Sci. 2013; 7(3): 243–247.
29. Tripathi YB, Upadhyay AK. Effect of the alcohol extract of the seeds of Mucuna Pruriens on free radicals and oxidative stress in albino rats. Phytother Res. 2002; 16: 534–538.
30. Shukla KK, Mahdi AA, Ahmad MK, Jaiswar SP, Shankwar SN, Tiwari SC. Mucuna Pruriens reduces stress and improves the quality of semen in infertile men. Evid Based Complement Alternat Med. 2010; 7:137–144.
31. Suresh S, Prithiviraj E, Prakash S. Effect of Mucuna pruriens on oxidative stress mediated damage in aged rat sperm. Int J Androl. 2010; 33: 22–32.
32. Ojo TN, Gbotolorun SC, Oremosu AA. Fertility enhancing potential of Mucuna pruriens seeds in female Sprague-Dawley rats. British J. Med. Med. Res. 2012; 4 (16): 3148–3157.
33. Gupta A, Mahdi AA, Ahmad MK, Shukla KK, Bansal N, Jaiswar SP et al. A proton NMR study of the effect of Mucuna Pruriens on seminal plasma metabolites of infertile males. J Pharm Biomed Anal. 2011; 55: 1060–1066.
34. Jayanthi Abraham. Effect of Mucuna pruriens Seeds on Fertility of Male Abino Rats. Rattus norvegicus. J Pharma Res. 2011; 4: 233–236.
35. Kim CY, Wakabayashi K, Nobunaga T. Time-dependent ovulation-blocking effect of ether anesthesia differs from pentobarbital in rats. Tohoku J Exp Med. 1994; 172: 237 242.
36. Kakkar P, Dos B, Viswnathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem. 1984; 21: 130- 132.
37. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972; 47: 389- 394.