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Comparative Anti-Diabetic Effects of Ocimium Gratissimum, Vernonia Amygdalina and Insulin on Testicular Architecture in Stz-Induced Diabetic Rats

Olorunfemi O. Joyce*[1], Nworah D. Chinwe[1], Egwurugwu J. Nnabufe[2], Pughikumo D. Tabot[3]., Joffa P.P. Kwaku[3].

1.Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Rivers State, Nigeria.

2.Department of Human Physiology, College of Medicine & Health Sciences, Imo State University, P.M.B. 2000, Owerri, Imo State, Nigeria.

3.Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

ABSTRACT

To study the effects of ethanolic extracts of Vernonia amygdalina(VA) and Ocimum gratissimum *(OG)*on the testes of diabetic rats, thirty-two male rats were used. Control consisted of eight (8) rats which served as non- diabetic control, receiving sodium citrate daily. The remaining rats were injected intraperitoneally with streptozotocin(65mg/kg) to induce diabetes. The rats confirmed diabetic were randomly divided into three experimental groups (1, 2 and 3) made up of eight rats each. Group 1 received 6IU/kg of insulin. Group 2 was given 100mg/kg body weight of Vernonia amygdalina and 200mg/kg body weight of Ocimum gratissimum combined daily. Group 3 served as the diabetic control and was given distilled water. The entire investigation lasted for 6 weeks. Results revealed typical testicular architecture in the normal control. Diabetic control exhibited alteration of germinal epithelium, distortion of seminiferous tubules as well as vacuolation of seminiferous tubules. The effects of the extracts on diabetic rats' testes showed improvements compared to the diabetic control group. It is therefore safe to speculate that the extracts of these plants used especially when combined exert some significant improvement in combating the adverse effects of diabetes on the testes of male rats.

Keywords: Vernonia amygdalina, Ocinum gratissimum, insulin, ethanolic extract, streptozotocin,diabetic, rats.

*Corresponding Author Email: talk2joyce2006@yahoo.com

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INTRODUCTION

Diabetes mellitus has been defined by the World Health Organization (WHO), on the basis of laboratory findings, as a fasting venous plasma glucose concentration greater than 7.8 mmol/l (140mg/dl) or greater than 11.1 mmol/l (200mg/dl) two hours after a carbohydrate meal or two hours after an oral ingestion of the equivalent of 75g glucose, even if the fasting concentration is norma[1]. It is a metabolic disease characterized by hyperglycaemia and glycosuria due to absolute or relative lack of insulin[2].

The non-pharmacological means (diet and exercise) and/or the pharmacological means (insulin and oral hypoglycaemics) may be used in the management of diabetes mellitus. The obvious limitations of these management methods necessitate a search for alternatives among the arsenal of herbs available to man. It was in this light that the World Health Assembly, in 1989, adopted among its resolutions, the support of national traditional medicine program, drawing attention to herbal medicines as being of great importance to the health of individuals and communities[3].

Streptozotocin (STZ; N-nitro derivative of glucosamine) is a naturally occurring, broad spectrum antibiotic and cyto-toxic chemical that is particularly toxic to the pancreatic, insulin producing beta cells in mammals[4, 5, and 6] Induction of experimental diabetes in the rat using streptozotocin is very convenient and simple to use[7, 8, 6] (Streptozotocin injection leads to the degeneration of the Langerhans islets beta cells[9, 10] Clinically, symptoms of diabetes are clearly seen in rats within 2-4 days following single intravenous or intraperitoneal injection of 60mg/kg STZ. Many minor components of foods, such as secondary metabolites, have been shown to alter biological processes which may reduce the risk of chronic diseases in humans.

Vernonia amygdalina (compositae) an edible rainforest plant native to the south eastern part of Nigeria has been widely used in folk medicine as anti-malaria, purgative, antiparasitic, treatment of eczema and for maintaining healthy blood glucose levels[11, 12.] In folk medicine, Ocimum gratissimum is extensively used throughout West Africa as a febrifuge, anti- malarial and anti-convulsant. The crushed leaf juice is used in the treatment of convulsion, stomach pain and catarrh. Oil from the leaves has been found to possess antiseptic, antibacterial and antifungal activities.[13]

Any factor that influences testicular structure indirectly affects male fertility.

In this study, the possible antidiabetic property of V. amygdalina and Ocimum gratissimum were investigated for their joint effect on testicular structure in the diabetic male in comparison with the conventional insulin, and to discover a possible use for prophylaxis and treatment of reduced

fertility commonly found in diabetic diseases.

MATERIALS AND METHODS

Plant Material

The fresh leaves of O. gratissimum and V. amygdalina were collected in August, 2010 from a local garden within the premises of the University of Port Harcourt, Nigeria. The plant was identified and authenticated by Dr. Goodie Uzo Obute- a senior botanist of the Department of Botany, University of Port Harcourt, Nigeria and a voucher specimen was deposited accordingly at the herbarium of the Department of Plant Science, University of Port Harcourt, Port Harcourt, Nigeria.

Extraction

The fresh leaves of the plants were separately air-dried, pulverized and extracted exhaustively in distilled water. The filtrate was concentrated and evaporated to dryness in vacuo at 40°C, using rotary evaporator. The yield was calculated and the dry extract was stored in a refrigerator at -4°C until use for the experiments. During the experiment, the crude extract was dissolved in distilled water and administered to the animals at 100mg/kg and 200mg/kg for Vernonia amygdalina and Ocimum gratissimum respectively (orally).

Animals

The animals used in this study were male Sprague-Drawley weighing between 150 - 270 g. The animals were obtained from the animal house of the Department of Pharmacology, University of Port Harcourt, Nigeria. The animals were randomly distributed into cages and allowed to acclimatize for 10 days in a well-ventilated room at a room temperature of 28.0 ± 2.0°C under natural lighting condition. The animals were fed with standard mouse chow and allowed free access to water daily. All animals used in this study were handled in accordance with the international, national and institutional guidelines for Care and Use of Laboratory Animals in Biomedical Research as promulgated by the Canadian Council of Animal Care (2009) and with the permission of Ethical Committee on The Use of Laboratory Animal for Research, University of Port Harcourt, Nigeria.

Experimental Protocol

Animals were divided into four main groups- Groups1, 2, 3 and control. Group 1 had insulin (6IU/kg) administered to them. Group 2 was given the two extracts of OG and VA combined. The crude extracts were dissolved in distilled water and administered to the animals (Vernonia amygdalina at 100mg/kg and Ocimum gratissimum at 200mg/kg orally) based on their respective

corresponding weights and acute toxicity tests response on the extracts.

Group 3 was the diabetic control.STZ was administered at 65mg/kg and complete signs and symptoms of diabetes appeared after 4 weeks. Administration of extract and insulin began after induction. The normal control group received only citrate daily for 2 weeks. No extract or insulin, but only distilled water was given to Group 3.At the end of the treatment course lasting 2 weeks, the animals were sacrificed by decapitation under pentobarbital anesthesia at 50 mg/kg, ip[14].

Histopathological Studies

Testis was carefully isolated, weighed, washed in buffered saline and fixed in 10% formalin. Testis sections (5 - 6 µm) were routinely processed by standard histological techniques, stained with hematoxylin and eosin (H and E), and examined by light microscope (Nikon Eclipse E400) to assess histopathological changes among control and experimental animals.

RESULTS AND DISCUSSIONS

The result of this study showed a significant ($P < 0.05$) decrease in fasting blood glucose level with an index of 235± 5.22mg/dl (diabetic control group) compared to 99 ± 1.84 mg/dl (group 1), 175 ± 6.12 mg/dl (group 2), 79 ± 1.84 (normal control) after one week of administration. The result also elucidated that there was a significant ($P < 0.05$) increase in fasting glucose level after induction of diabetes when comparing group 3 and the control group (as depicted by the bar chart in figure 1). The pattern of body weight across various groups showed a decline except group 1 treated only with insulin (table 1). This agreed with the fact that weight loss is closely associated with diabetic episodes[15]

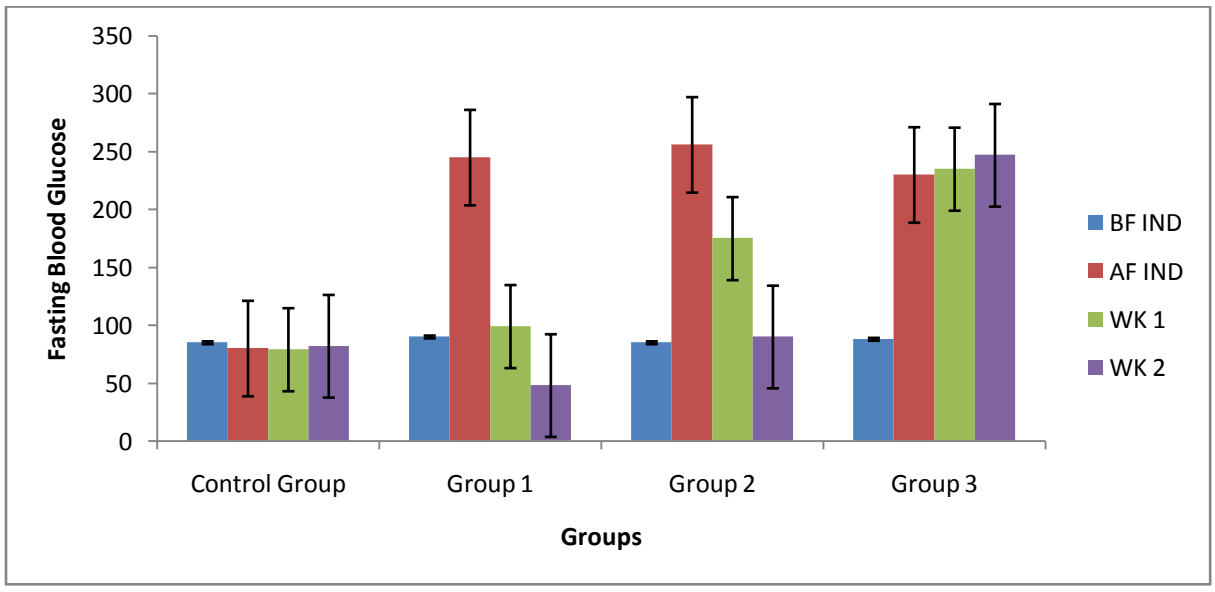

Figure 1: Bar chart showing the fasting blood glucose levels at different time intervals

Table 1: Showing the body weight at different time interval

Groups	Before induction (mg)	After induction (mg)	Week 1 (mg)	Week 2 (mg)
Control(group)(insulin-treated only)	261 ± 5.22	284 ± 5.22	287 ± 5.16	290 ± 5.07
Group 1(treated with og and va)	196 ± 7.84	160 ± 7.84	170.2 ± 8.11	173 ± 8.42
Group 2(diabetic control)	210 ± 5.22	191 ± 7.84	189 ± 8.18	190 ± 8.06
Group 3(distilled water only)	235.4 ± 5.22	204 ± 7.84	203 ± 7.40	198 ± 8.23

The hypoglycemic effect of OG and VA combined was observed, showing the validity of their anti-diabetic potential (Figure1). Sections of testes of control rats showed normal testicular architecture with distinct seminiferous tubules composed of both Sertoli and spermatogenic cells. The spermatogenic cells were seen to be at the spermiogenic stage of differentiation. Interstitial cells of Leydig were also prominently interspersed between the seminiferous tubules (PLATE C). The diabetic control (Group 3) showed alteration and distortion of both germinal epithelium and seminiferous tubules. The peritubular tissue surrounding the seminiferous tubules and interstitial cells were altered. There was presence of vacuoles within the seminiferous tubules (PLATE D). The testes of group 2 treated with VA and OG had more stable seminiferous tubules compared to group 3. Vacuoles were observed, but were not numerous as those observed in the diabetic control. The testes of group 2 rats treated with combination of VA and OG showed a significant improvement of testicular arrangement. There was increase in Leydig cells; well defined seminiferous tubules were seen (PLATE D). Lumen of seminiferous tubules was filled with active spermatids. In the insulin-treated rats, the testicular arrangement was almost the same as in the non-diabetic control, except for a few defects in the integrity of the seminiferous tubules (PLATE C). *Ibrahim et al*[16] reported a significant reduction in body weight of Wistar rats on chronic feeding with leaves of VA. However, they also reported that the nephritic, hepatic and testicular cells architecture were normal. This is in support of findings that VA does not have a deleterious effect on the testes of diabetic rats, but rather leads to an improvement of its cytoarchitecture. Regeneration of testicular tissue observed in the study after a combined administration of VA and OG may suggest that these plants have antioxidant properties that can mop up free radicals produced by Streptozotocin. This is in conformity with work carried out by *Iwalokun et al*[17] where certain phytochemicals of VA were isolated and their antioxidant properties were demonstrated. Although little information is available on the effect of OG on diabetes, in the work of *Atanghwo et al*[18] it was found that OG had a more positive and potent effect on the testes of diabetic rats compared to VA. This may be due to the high level of alkaloids and flavonoids found in OG. *Obianime et al*[19] actually reported that OG has an initially cytodestructive effect on the testes, which with time changes to a cytoprotective effect.

Alkaloids are known as the starting material in the manufacture of steroidal drugs[20]. Flavonoids are functional as disease resistant; OG also contains tannins that inhibit oxidation[21]. These components of OG put together may be responsible for the result obtained. The alkaloids may have triggered the production of testosterone, flavonoids may have acted by resisting the effect of diabetes and tannins may have inhibited oxidation by acting as an antioxidant. These potent properties of OG may have likely influenced the almost near to normal regenerative effects of testicular tissue observed in Group 2 that was given a combination of VA and OG. Since the effect of the combination is more promising than in the single administration common used in other works[18] it is safe to conclude that the use of both VA and OG work in synergism to produce endowed potentials which can be exploited in the development of anti-diabetic drugs which can be used as supplements

Table 2: Table showing the results of histopathological studies after 2 weeks

	Control Group	Group 1	Group 2	Group 3
Cell Structure Abnormalities	_	+	++	+++
Nucleo-cytoplasmic Ratio	Normal	Normal	Normal	Normal
Malignancy	-	-	-	-
Nuclear Pattern	Normal	Normal	Normal	Normal
Pigment/inclusion Bodies	-	-	-	-
Hemorrhage/Congestion	-	-	+	++
Neoplasm	-	-	-	-

Key:- + Slight, ++ Moderate, +++ Severe

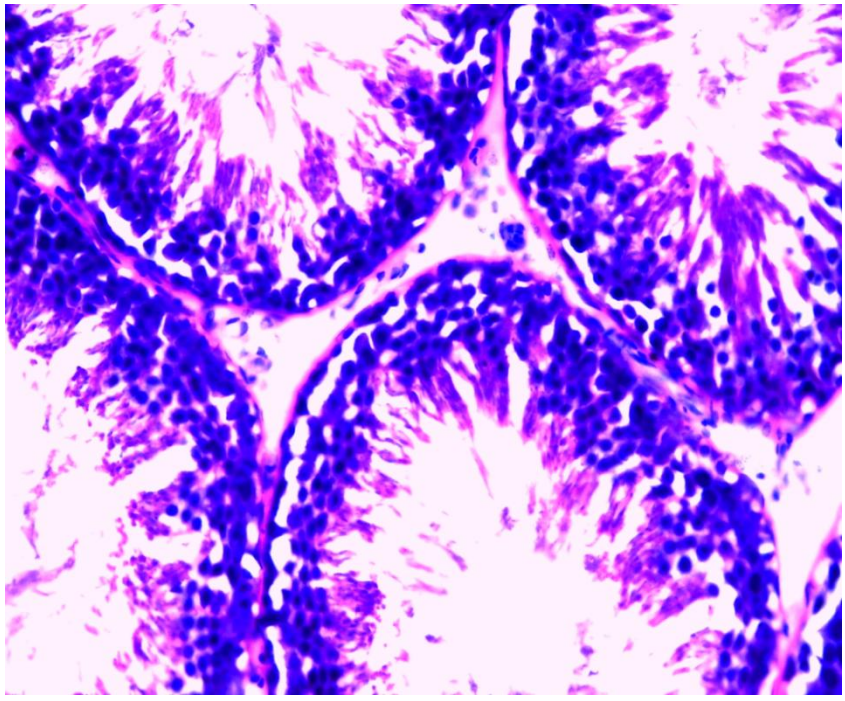

Plate A: Photomicrograph of testes of Group 1 after insulin administration for 2 weeks

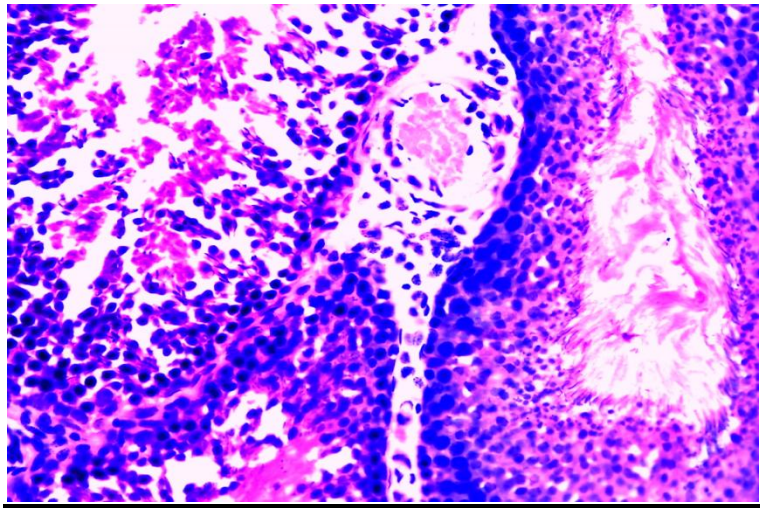

Plate B: Photomicrograph of Testes of Group 3 after Stz-Induced Diabetes

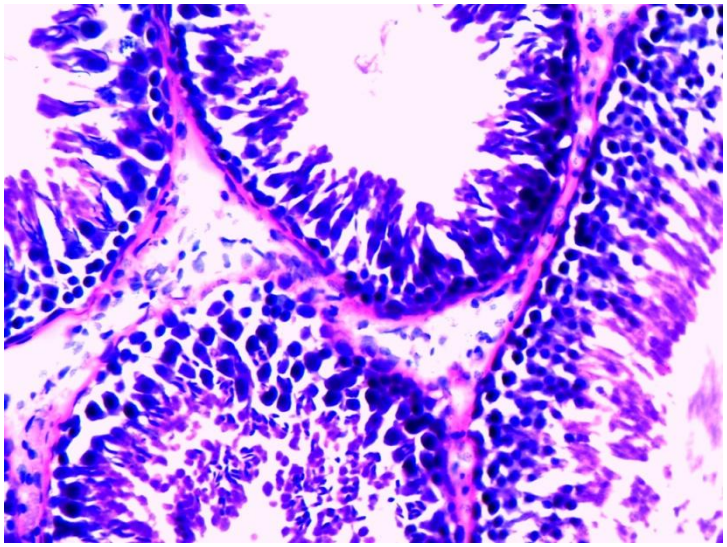

Plate C: Photomicrograph of testes of Group 1 after insulin administration for 2 weeks

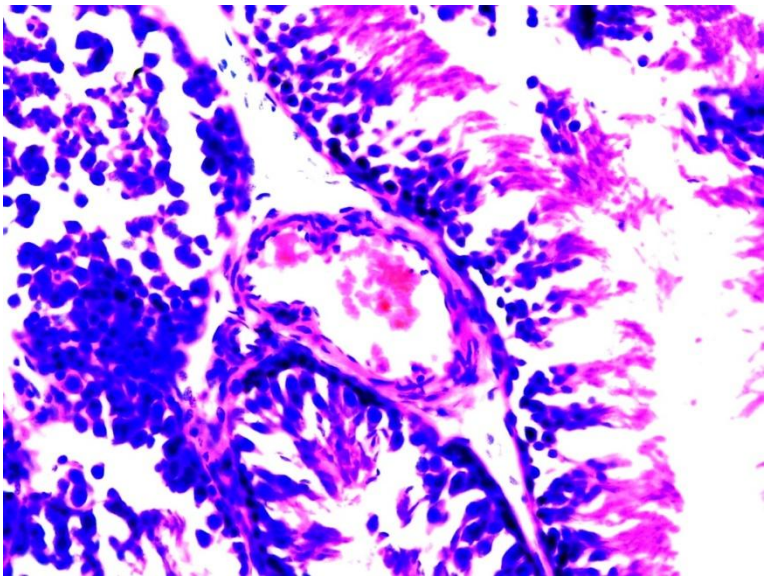

Plate D: Photomicrograph of testes of Group 2 after extract administration for 2 weeks.

CONCLUSION

The effects of the extracts on diabetic rats' testes showed improvements compared to the diabetic control group. It may be therefore safe to speculate that the extracts of these plants used especially when combined exert some significant improvement in combating the adverse effects of diabetes on the testes of male rats.

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