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DIABETES- A NEURODEGERATIVE DISEASE: AN INVESTIGATION INTO THE AMELIORATIVE EFFECT OF ETHANOLIC EXTRACT OF *COPAIFERA SALIKOUNDA*

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ABSTRACT: *Objective: This study aims at investigating the possible ameliorative effects of the fruit pod of Copaifera salikounda on the histoarchitectural changes of the limbic system in diabetes induced rats which may affect cognition and learning. Materials and methods: Group 1 served as control and received normal saline. Group 2, the diabetic untreated group received a single intraperitoneal injection of 200 mg/kg body weight of alloxan monohydrate. Group 3 was treated with low dose (200mg/kg) body weight of the extract. Group 4 was the treated high dose (400mg/kg) body weight of the extract. Results: On examination, the histological section revealed various things about the limbic system of diabetic rat. The non-diabetic group showed normal histological appearance. The diabetic untreated group showed disorganization and dispersion of pyramidal cells with eosinophilic cytoplasm, haemorrhage and some areas of cell loss. They were cell regeneration in the groups that received Copaifera salikounda especially as it affects pyramidal and glial cells. The dentate gyrus became more prominent as the dosage increased and compact arrangement of the granule cells. Conclusion: The extract may be good in cell regeneration and diabetic high risk individual may need some daily dose of Copaifera salikounda fruit pods as part of their meal.*

KEYWORDS: Alloxan; Ameliorative; *Copaifera salikounda*; Diabetes; Limbic system; Regeneration.

INTRODUCTION

A lot of ailments has befallen men in this our present age but out of all diabetes stands out and is fast becoming a family name without any particular treatement but can only be managed. This is due to the fact that much attention is being directed to avoid its devastating effects on humans. The prevalence of diabetes mellitus is increasingly on the rising and all over the world it is being highlighted (Mohammed and Askary, 2017). It is one of the diseases without a definite treatment but can only be managed. According to Wild *et al*., 2000, in the year 2000, 2.8% (percent) that is about 171 million people of the world population had diabetes but they suggested that the number

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could increase to almost double rising to about 4.4% as many as 366 million people who will be down with the disease by 2030 and supported by ADA, 2012.

Hyperglycemia is very deadly to the cells of the body including that of the brain cells but uncontrolled hyperglycemia has been identified as the cause of severe damage to almost all the major organs of the body such as retinopathy, neuropathy, nephropathy and vasculopathy (ADA, 2012). The nervous system is one of the most vital systems of the body and could be said to control other systems as well. Earlier on, the only known nervous system complication that was associated with diabetes was peripheral neuropathy but was debunked when researchers discovered that any alteration on the nervous system can alter cognitive, learning and behavioral functions and also causes neuronal degenerations, abnormal expression of hypothalamic neuropeptides, etc (Biessels *et al*., 1998; Saravia *et al*., 2004; Selvarajah *et al*., 2011; Matough *et al*., 2012; Nagayach *et al*., 2014).

The limbic system serves as a learning and memory centre of the brain and the hippocampus is one of the complex formations of this system (Kiernam, 2009; Ugwuja *et al*., 2010; Li *et al*., 2015). Any distortion of this part of the nervous system leads to various forms of impairments. According to Saravia *et al*., 2004; greenwood and Winocur, 2005; Kienam, 2009, rats induced with diabetes was discovered to have impaired learning ability. It has been discovered that intensive insulin therapy can help normalize blood glucose levels in the events of diabetes complications (Raimund *et al*., 2013). The brain with no doubt needs glucose as the most predominant source of energy substrate although the brain is capable of synthesizing its own energy from other alternative sources such as lactate and β-hydroxybutyrate which help maintain the energy requirements of the brain cells in an event of hypoglycemia (Dienel, 2012; Raimund *et al*., 2013). Based on the reports of Venaman *et al*., 1994; Maram *et al*., 2000; Page *et al*., 2009; these alternative sources of energy are very essential in improving cognitive functions.

Copaifera salikounda is a tall tree with very many medicinal values especially in West African region. The pulp of the leaves are applied to sores directly (Costa, 2009) while the dried powdered leaves and bark are mixed with baked and powdered clay and applied to ulcers. A macerated fruit valves is taken as a drink in order to purify the blood (N'guessan *et al*., 2011). It has not been used to check for any diabetic therapeutic effects. Hence, this present study aim at investigating the possible ameliorative effects of the fruit pod of *Copaifera salikounda* on the histoarchitectural changes of the limbic system in diabetes induced rats which may affect cognition and learning.

MATERIALS AND METHODS

Collection, Identification and Preparation of Extract

The seed pods of *Copaifera salikounda* used in this study were bought from Abakaliki Local Government Area of Ebonyi State, Southeastern part of Nigeria. This plant material was identified as *Copaifera salikounda* at the Department of Biology, Alex Ekwueme Federal University Ndufu-Alike, Ikwo, Ebonyi State, Nigeria. They were washed with clean water to remove dirt. Thereafter, they were opened with a sharp, knife and the seeds were carefully removed from the pods. They were air dried for three weeks and grinded into powder. About 103.3g was weighed and soaked in

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2.5 liters of absolute ethanol at room temperature for 48 hours in a container. The content was filtered using Whitman's filter paper No. 10. The filtrate was concentrated using vacuum evaporator at 40^0 to get a powdered substance that was stored until required for use.

Experimental Animals

The male albino rats used for this study weighing between 110 and 150g were obtained from a private animal holding facility in Nsukka, Enugu State, Nigeria. The rats were housed in netted cages of the animal house of Alex Ekwueme Federal University Ndufu-Alike, Ikwo (AE-FUNAI), Ebonyi State, Nigeria. They were kept in well-ventilated cages at room temperature of 25 ± 1°C in the laboratory environment under controlled light cycles of 12 hours light and 12 hours dark. The rats were allowed to feed on standard rat pellet (Vital Feed Nig., Ltd) and water *ad litium*. The rats were acclimatized to the animal house for a period of seven days prior to the induction of experimental diabetes.

Diabetes Induction

After the acclimatization period of 14 day, the rats were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared alloxan monohydrate in 0.9% NaCl solution (normal saline). Group 1 served as the normal control and was not injected with alloxan but was placed on normal saline alone. However, groups 2 to 4 were induced with diabetes by a single intraperitoneal injection of 200mg/kg body weight of Alloxan monohydrate (Richard *et al.*, 1983).

Experimental protocol

A total of number of 24 male albino Wistar rats were used and they were randomly assigned into 4 groups of 6 male albino rats in each group for the evaluation of anti-diabetic activity of the seed pods of ethanol extract of *Copaifera salikounda*. Group 1 served as control and received water and normal saline. Groups 2,3 and 4 received a single intraperitoneal injection of 200mg/kg body weight of alloxan monohydrate while group 2 served as the diabetic untreated a group 3 served as diabetic treated with low dose (200mg/kg) of extract and group 4 as the diabetic treated high dose (400mg/kg) of extract. The extract and normal saline were administered by the use of oral Gavages and lasted for 14 days.

Results

Histological examination of the tissues revealed various intriguing things to behold about alloxan induced diabetes and the limbic system. The non-diabetic group showed normal histological appearance (Figure 1). The diabetic untreated group showed disorganization and dispersion of pyramidal cells, most of which were eosinophilic cytoplasm and some areas of cell loss. This group also showed of haemorrhagic areas (Figure 3). The group 3 and 4 that received *Copaifera salikounda* showed regeneration of pyramidal and glial cells and vesicular nuclei. The dentate gyrus became more prominent as the dosage was increased. The compacted arrangement of the granule cells were obvious with rounded pale vesicular nuclei of the dentate gyrus.

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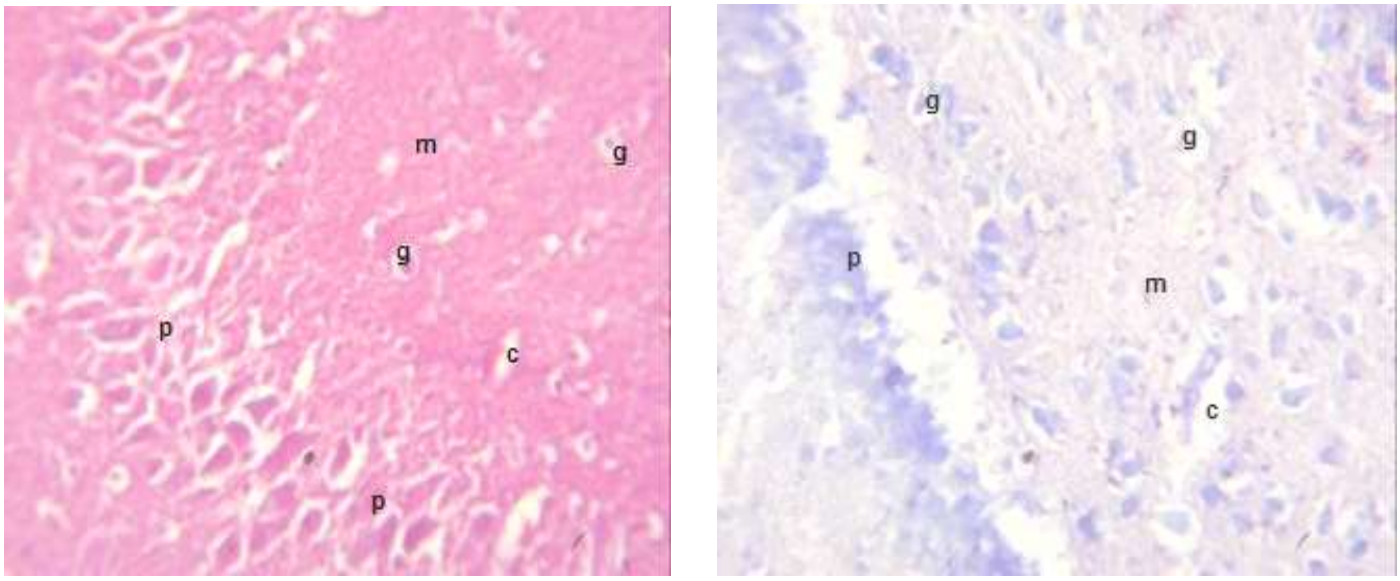

Figure1: Photomicrograph of a coronal section of the limbic system of the control showing pyramidal cells region and molecular layer with the well apparent processes of pyramidal cells (p), glial cells (g) and capillaries (c). 1a H & E and 1b niessle stain; X 400

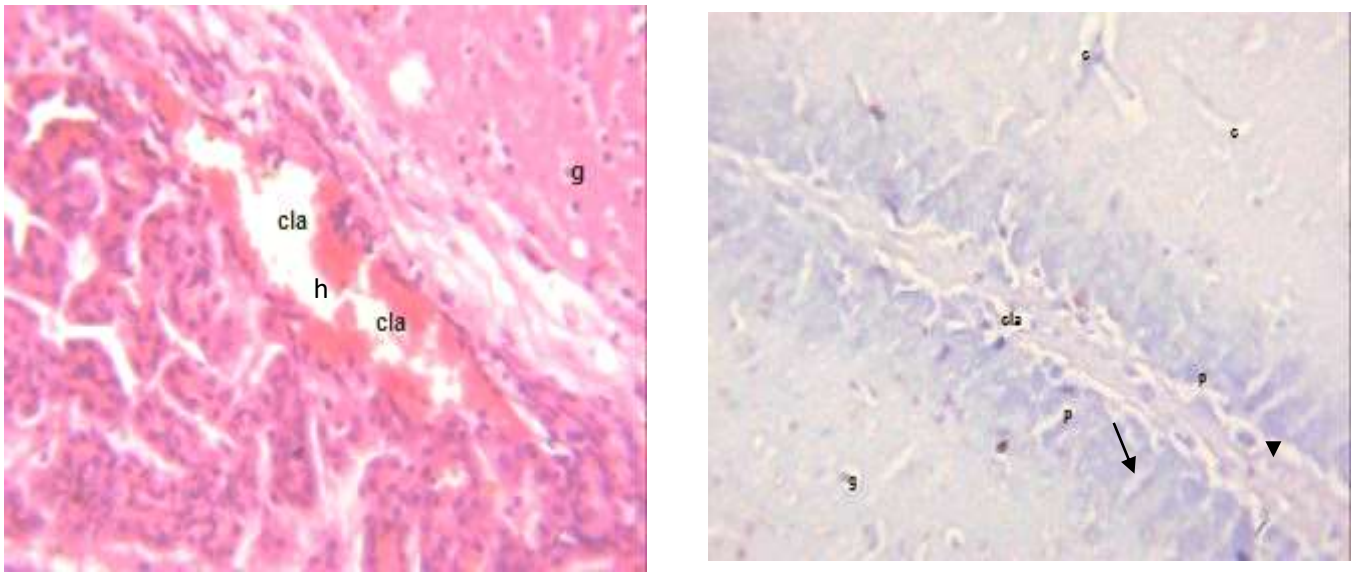

Fig**.** 2 Photomicrograph of a coronal section of the limbic system of diabetic rat showing disorganization and areas of cell loss (cla), contracted pyramidal cells (P), pyknotic nuclei (arrows) and hyperchromatic nuclei (arrow head), dilated capillaries (C), scattered haemorrhage (h) and increased glial cells (g). 2a H & E and 2b niessle stain; X 400

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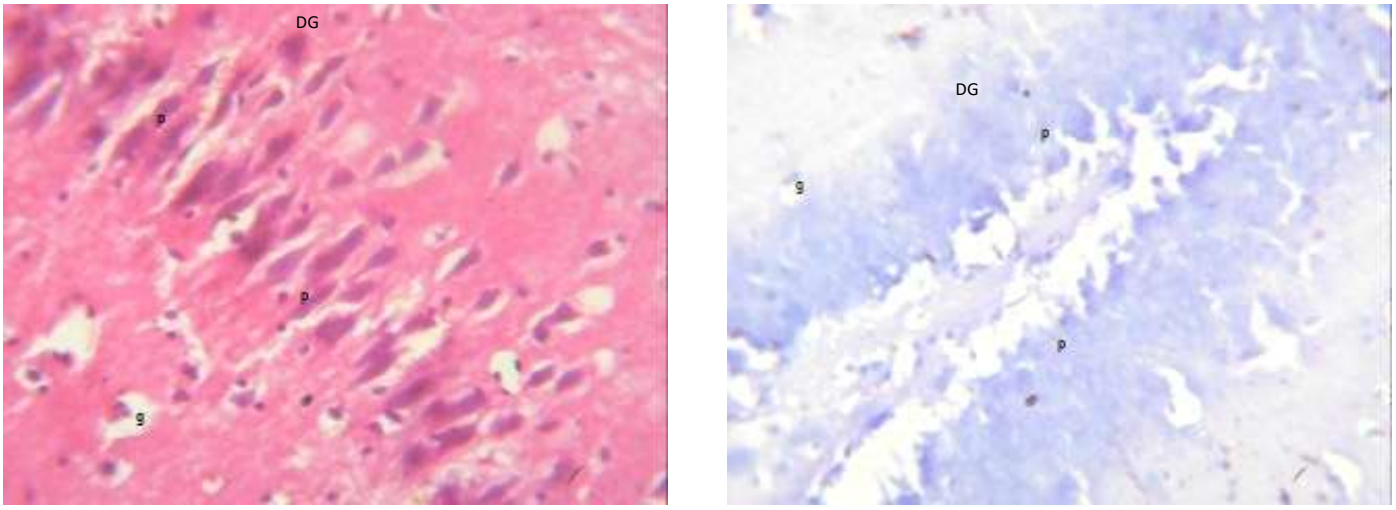

Fig. 3: Photomicrograph of a coronal section of limbic system of a treated diabetic rat showing regeneration of most of pyramidal cells. 3a H & E and 3b niessle stain; X 400

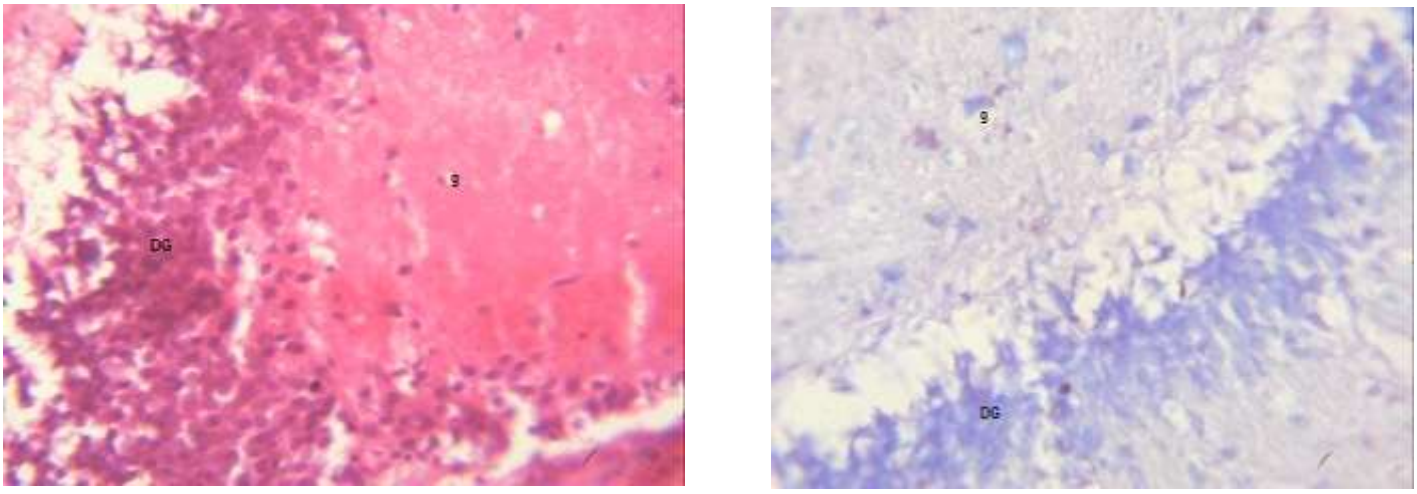

Fig. 4: Photomicrograph of a coronal section of the limbic system of a diabetic rat received *Copaifera salikounda* group showing restoration of the dentate gyrus (DG)**.** 4a H & E and 4b niessle stain; X 400.

DISCUSSION

Low cognitive function and increased risk of dementia has been mostly identified to be associated with diabetes mellitus (Mohammed and Askary, 2017). Dementia is a very risky situation that its incidence in the diabetic patients are on the rise ranging between 50 – 100% (Ho *et al*., 2013) this could be due to the alterations that diabetes causes to the hippocampus which is one of the crucial

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component of the limbic system and due to its sensitivity it is highly susceptible to the metabolic disturbances present in diabetes (Sibiya and Mabandla, 2017; Candy and Szatkowski, 2000; Klausberger and Somogyi, 2008; Zlokovic, 2011). According to Pintana, 2012; these alterations seen on the limbic system is as a result of sustained hyperglycemia.

In this present experiment, the limbic system under the microscopic examination showed various histoarchitectural degenerations caused by induced diabetes. The mostly affected part is the pyramidal cells which agree with the report of Mohammed and Askary, 2017. The control showed normal brain (limbic system) without alteration. All the pyramidal and glial cells looked healthy and there was no neurodegeneration seen under the microscopic examination. The diabetic untreated rats revealed marked alterations ranging from hemorrhage, disorganization, diminished number of granule cells to vacuolation (Mohammed and Askary, 2017). Our finding is in agreement with the report of Amin *et al*., 2013; Mohammed and Askary, 2017. Untreated diabetes damages the hippocampus within two weeks or even earlier than that while in prolonged cases, untreated diabetes causes severe hippocampal neurodegeneration according to Pamidi and Satheesha, 2013. The glial cells are given the task of surrounding neurons and keeping them firm, insulating neurons, to nurture the neurons, and to destroy and remove dead neurons; a process that is very vital for neuronal plasticity and stability (Mohammed and Askary, 2017). The glial cells were seen to be tampered with in the diabetic untreated rats as shown is figure 1.

In our study, the treated diabetic group that received *Copaifera salikounda* extract revealed that the histological structures of the limbic system and dentate gyrus were all preserved as seen in figure 3 and 4. The low dose showed restoration of pyramidal and glial cells. There were still areas of disorganizations noticed in the structures (figure 3) which may be due to the low dosage administered. The cell regeneration of the dentate gyrus, glial and pyramidal cells became more pronounced when the dosage was increased in the diabetic treated group 4 (figure 4). The cells became aggregated better than in the high dose group. The large pyramidal cells increased in number and are more prominent and visible. This present study could be a pointer that *Copaifera salikounda* might induce proliferation, regeneration and migration of neuronal progenitor cells which may help in the repair of hippocampus and limbic system at large. This may help in to improve cognition and learning in diabetic patients.

CONCLUSION

Our result might be suggesting that *Copaifera salikounda* have ameliorative effects. This could be beneficial as it will help in the management of diabetes and regenerate the worn out cells. Hence, diabetic subjects and high risk individual may need some daily dose of *Copaifera salikounda* fruit pods as part of their daily meal.

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