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ANTIHYPERGLYCEMIC EFFECTS OF PROCESSED SEEDS OF *PENTACLETHRA MACROPHYLLA* ON HIGH FAT DIET AND STREPTOZOTOCIN- INDUCED DIABETIC WISTAR RATS

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ABSTRACT

The antihyperglycemic effects of Pentaclethra macrophylla (PM)seeds on high fat diet (HFD) and streptozotocin (STZ) induced diabetic wistar rats were investigated. Feeds consisting of 50% , 30% and 20% PM, sucrose and lard respectively and were given to the rats for 4 weeks and subsequent intraperitoneal injection of STZ, 40mg/kg body weight for induction of diabetes. The PM seeds were processed in different stages, of raw, 1st cooking (16-18 hours and dehulled), 2nd cooking (dehulled sliced, cooked for 3 hrs and soaked for 12h). These processed samples were dried, ground and used to compound diet for feeding of animals for Eight (8) weeks. A known antidiabetic drug, metformin (50mg/kg body weight) was used as a referenced drug. After Eight (8) weeks of Pentaclethra macrophylla administration, blood samples were collected for glucose estimation and the pancreas excised for histhopathological examination. The result obtained showed that animals fed with various stages of processed seeds of had significant ($p<0.05$) decrease in plasma glucose concentration with 2^{nd} cooking being at the 8^{th} week showing glucose reduction comparable to the normal control and metformin(positive test) groups. Histological examinations of the pancreas revealed that the hyperglycemic group had distortion of the islets cells while the groups fed with the processed seeds of Pentaclethra Macrophylla revealed regenerated islets. Thus it may be deduced that the processed seeds of Pentaclethra macrophylla exhibited antihyperglycemic potentials which could be compared to metformin a standard antihyperglycemic agent.

KEYWORDS: *Diabetes, hyperglycemia, streptozotocin, glucose, of Pentaclethra macrophylla.*

INTRODUCTION

Diabetes mellitus is a serious disease that afect the metabolism of generally high energy rich compounds such as fats, carbohydrate and lipids. The disease emanates from an abnormality in insulin secretion and related impairment associated to hyperglycemia. The metabolic disturbances in the body results to rapid morbidity and mortality and affects about three hundred and eighty one million people and this figure has been predicted to be doubled by 2030 (Chen *et al.,* 2012). Many of the conditions resulting to diabetes are generally referred to as pathophysiological and are multifarious ranging from immune mediated, genetic, beta cell dysfunction, insulin resistance, etc (Beverley and Eschwège, 2003). Due to inadequate medical facilities, most of the people depend on herbal medicines for health care. In some cases, other treatment options available are more expensive and associated with many serious complications of frequent use. Most plant parts ingested indiscriminately by many local populations posses adequate healing potential in disease states without either knowing the means through which they act or function . Data given by the WHO indicates that about 80% of individuals in the world of developing nations depends on herbal remedies for their basic health care needs (Murray, 2004; Joy *et al.*, 1998). In the past, traditional medicines have given very useful synthetic clues to modern drugs (Gullo *et al.*, 1994). Plants have being used as therapeutic agents for the following goals: (a) to adequately isolate phyto compounds which are bioactive and medicinal to man for direct use as drug substances e.g. morphine, vinblastine, digoxin etc. semi synthesis, e.g. metformin, which is based on galegine, garlic. About 25% of all the drugs prescribed today are from plants (Raskin and Ripoll, 2004).

Oral hypoglycemic drugs employed in controlling diabetic complications abound. Although, these agents are very effective in control the glucose level, they are not efficient in relieving clinical symptoms and controlling diabetic complications. There is also the problem of drug resistance, various side effects contra indicatory especially during pregnancy (Mooy *et al.*, 1995). The growing number of diabetics and the harsh side effects of some synthetic drugs have led to the increasing search for alternatives, which are relatively.

Pentaclethra macrophylla, it is a genus- *Pentaclethra,* leguminous naturally found in West Africa and is cherished in southeast Nigeria for improvement on soil physiochemical factors (Latha and Pari, 1995). It is the only member of the leguminosae family and sub-family mimosoideae. The seeds are eaten as oil bean "salad" or used as soup condiment. They also serve as animal feed. To maximize the potential of animal and human nutrition, its nutritional and phytochemical properties have been evaluated. The study was thus undertaken to scientifically evaluate hypoglycaemic effects of this leguminous seeds, *Pentaclethra macrophylla* used locally for many ailments; its impact on some biochemical parameters and organs taking the various processing stages into consideration.

MATERIALS AND METHODS

Collection of Plant Samples

The seeds of *Pentaclethra macrophylla* were obtained from Nkwo-Orodo in Mbaitoli Local Government Area of Imo state, identified by Prof. B.E Okoli in the Plant Science and Biotechnology Department, University of Port Harcourt, Rivers State, Nigeria. The seeds were scrutinized and the bad ones were removed and the good ones were stored in air tight bags for subsequent use.

Preparation of Seed/ Processing the Seeds

The seeds were processed and the different products were used for the analysis. The raw samples of the seed were stored and used subsequently. For the second treatment referred to as first cooking, the raw seeds were boiled in water for 16-18h and the rough testae were removed. This is called dehulling. The dehulled seeds were sliced, dried and ground into powder and stored for subsequent use. For the third treatment referred to as second cooking, the cotyledons were boiled again for about 30 minutes and left overnight in water at room temperature. They were dried and ground into powder and stored for subsequent use.

Procurement of Experimental Animals and Compounding of Feeds

Wistar rats weighing between 175-240g were used for this research work at the animal house of the Department of Biochemistry, University of Port Harcourt, Choba, and Rivers State, Nigeria. 50% rat feed was substituted with 50% of the various *Pentaclethra macrophylla* treatments used to feed the animals. The rats were weighed and divided into six (6) groups of sixteen rats each, such that the average weights were approximately equal as presented in Table1. The animals were housed in plastic metal top cages in the Animal House of the Department of Biochemistry, University of Port Harcourt. The rats were fed standard diet and allowed access to clean water for a period of one week acclimatization. Rats in groups 2 to 6 were fed with high fat diet (20% sucrose +30% lard + 50% standard diet) for four weeks and then injected with 40mg/kg streptozotocin (STZ) in distilled water to induce diabetes. The blood glucose level was analyzed 6 days after STZ injection and the treatment which lasted for eight (8) weeks.

Metformin was administered daily by intra-gastric gavages. At the end of weeks 2, 4, 6 and 8 weeks, four rats from each group were fasted overnight, anaesthetized by exposure to chloroform and then sacrificed to excise the pancreas for histhopathological examination.

Table 1: Experimental Design for the Anti- hyperglycemic Screening

S/No.	Group Identity	Treatment
1.	Normal control	Normal feed + water
2 .	Negative control	High fat diet + streptozotocin (40mg/kg)
3	Metformin (Positive control)	High fat diet + streptozotocin (40mg/kg) + metformin(50mg/kg)
4	Raw	High fat diet + streptozotocin (40mg/kg) +feed compounded with the raw sample
5	1st cooking	High fat diet + streptozotocin (40mg/kg) + feed compounded with second treatment
6	2nd cooking	High fat diet + streptozotocin (40mg/kg) + feed compounded with the third treatment

Assay of Plasma Glucose Concentration

The plasma glucose was assayed using the multiCareinTMglucose strips and glucometer.

Principle

The glucose contained in the sample reacted with the glucose oxidase (enzyme) in the glucose electrode strips which resulted in the oxidation of glucose to gluconic acid and the temporary transfer of two electrons from glucose to the enzyme. The reduced enzyme next reacted with the mediator, transferring a single electron to each of two mediator ions, thus returning to its original state. At the electrode surface, the reduced mediator was re-oxidized providing amperometric signal, whose magnitude was directly proportional to the glucose concentration, and thus was measured and used to determine the concentration of glucose in the sample.

Procedure

The data chip from the strip pack was inserted into the space on the side of the glucometer. The strip was then inserted into the strip holder. The tails of the rats were cut and blood samples were dropped on appropriate portion of the strip. The displayed results were recorded.

Histopathological Observation of the Pancreas

The histopathology was carried out on small piececs of pancreas that were kept in 10% formalin for proper fixation and then embedded in paraffin wax. Sections of 5-6 µm in thickness were cut mounted on slide and after stained with hematoxylin and eosin, then examined via light microscopy at ×400 magnification.

Statistical Analysis

The data were analyzed for statistical differences between treatment groups, by means of one-way analysis of variance (ANOVA). In all, $P < 0.05$ was considered significant. Data are presented as Mean± S.E.M. (standard error in the mean).

Results

Different Processing Stages of *Pentaclethra Macrophylla* Seed on Plasma Glucose

Different processing stages of *Pentaclethra macrophylla* seeds on the plasma glucose concentration of high fat diet (HFD) and streptozotocin (STZ)- induced diabetic rats and *Pentaclethra macrophylla* (PM) treated rats is presented in Table 2. Glucose level in the hyperglycemic group (Negative control) increased at a significant level ($p < 0.05$) when it was compared to the normal control group in all the six (6) weeks of treatment. In week 2 and week 4, all the groups treated with different processed *Pentaclethra macrophylla* seeds showed significant ($p < 0.05$) decrease in the plasma glucose levels when compared to the hyperglycemic group. However, in comparison to metformin, results obtained for treated groups results were not significantly ($P>0.05$) different except in the 6th week. The result obtained for the 2nd cooking group in week 8 showed significant ($p < 0.05$) glucose reduction when compared to the raw and 1st cooking and is comparable to normal control and metformin groups.

Histopathological Results Obtained for the Pancreas

The histopathological results are presented in Figures 1-2. There was marked alterations and damage to the pancreas in the negative control (hyperglycemic) group showing distorted and shrunken pancreatic islet with atrophic vacuoles. Treatment with processed *Pentaclethra macrophylla* seeds brought about pancreatic islet showing normal acinoductal system and islets with good cellularity.

Table 2: Fasting Blood Glucose Concentration (mg/dl) of Diabetic Wistar Rats Fed with Processed *Pentaclethra macrophylla* Seeds

Duration	Normal Control	Negative Control	Metformin	Raw	1st Cooking	2nd Cooking
2nd Week	91.75 ± 3.09^{a}	161.00 ± 10.23^{b}	115.25 ± 7.71^{cdefgh}	123.00 ± 3.74^{cdefgh}	124.25 ± 3.09^{cdefgh}	117.25 ± 6.23^{cdefgh}
4th Week	91.75 ± 3.09^{a}	139.25 ± 3.30^{b}	113.25 ± 9.10^{cdefgh}	120.75 ± 3.68^{cdegh}	117.50 ± 2.38^{cdefgh}	112.25 ± 6.23^{cefgh}
6th Week	91.75 ± 3.09^{ac}	136.00 ± 5.35^{b}	96.75 ± 2.50^{ac}	121.00 ± 3.74^{de}	114.75 ± 4.99^{defh}	113.25 ± 4.03^{efh}
8th Week	91.75 ± 3.09^{acfg}	129.25 ± 0.50^{b}	88.75 ± 6.18^{acfg}	109.75 ± 10.87^{deh}	112.75 ± 10.50^{deh}	92.25 ± 2.63^{acfg}

Values presented are mean ± standard deviation of four determinations. Mean values in each row with different small letter superscripts are statistically significant

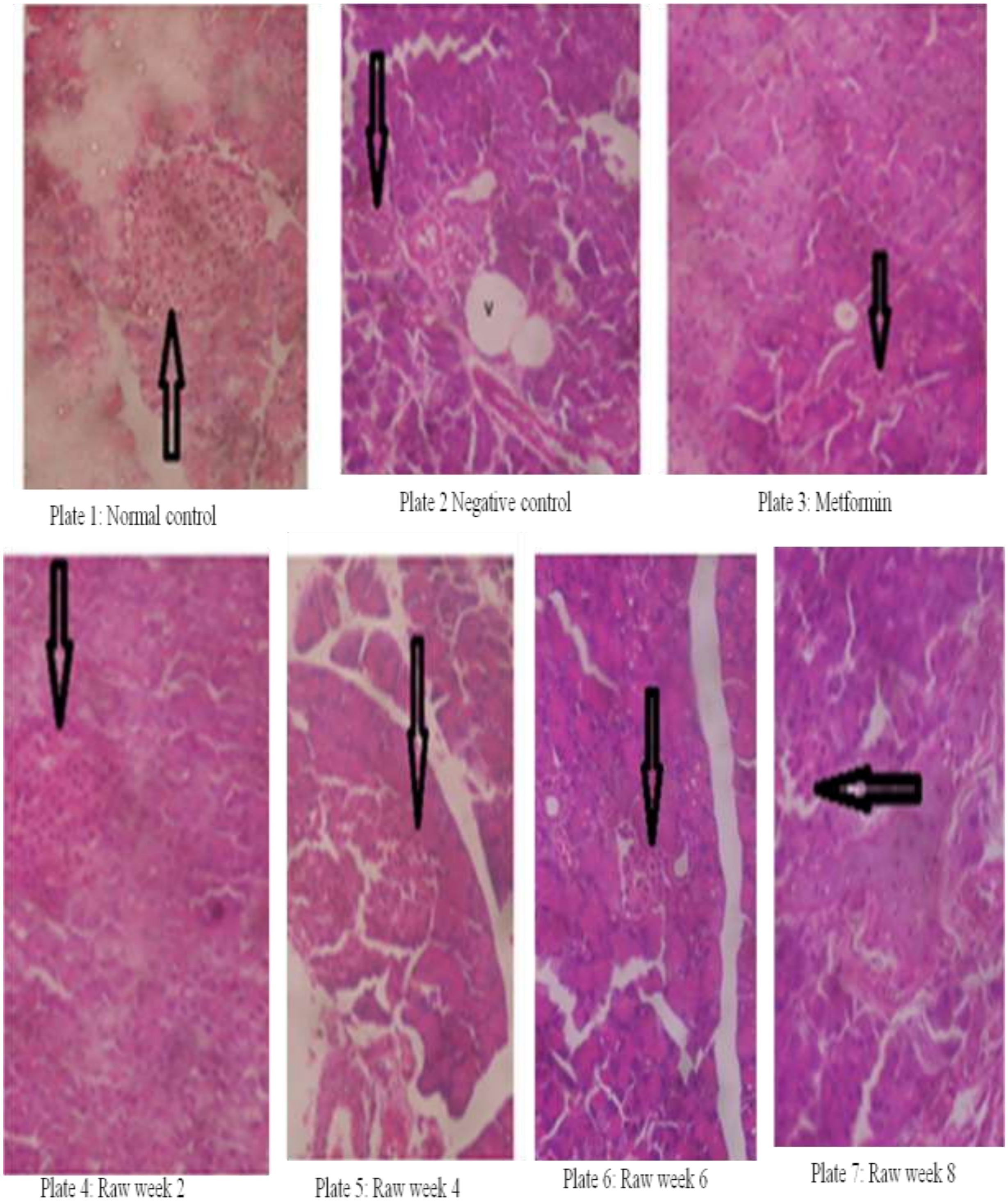

Figure 1: Histomicrographs of Different controls and raw cooking treated groups (× 400)

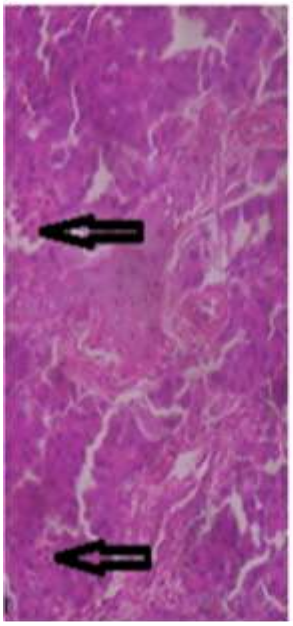

Plate 8: 1st cooking week 2

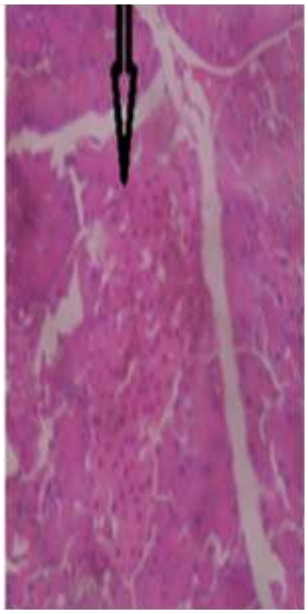

Plate 9: 1st cooking week 4

Plate 10: 1st cooking week 6

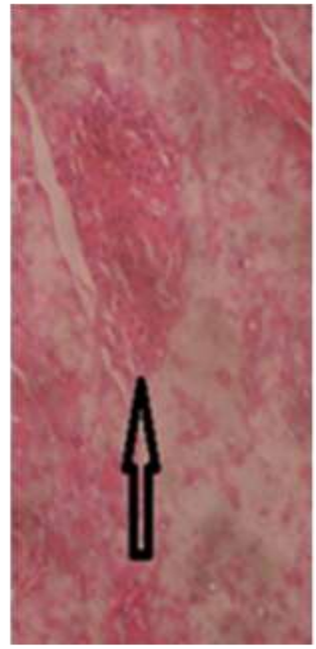

Plate 11: 1st cooking week 8

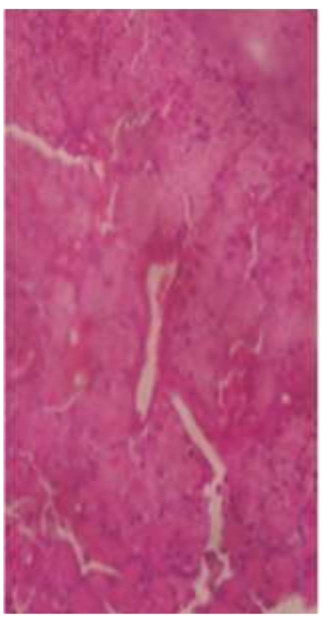

Plate 12: 2nd cooking week 2

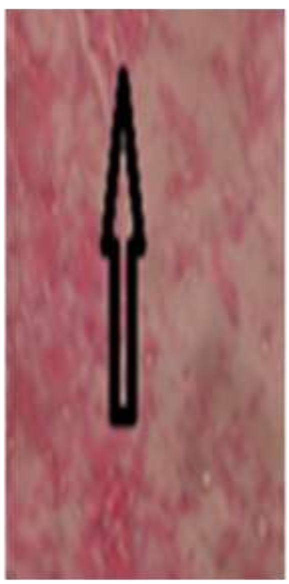

Plate 13: 2nd cooking week 4

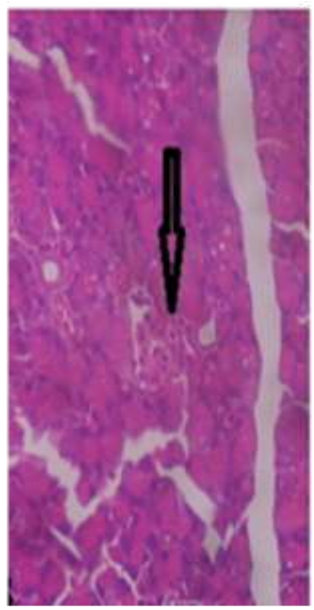

Plate 14: 2nd cooking week 6

Plate 15: 2nd cooking week 8

Figure 2: Histomicrographs of 1st and 2nd cooking treated groups (× 400)

DISCUSSION

Since the origin of humankind people have relied primarily on plants for nourishment and health care such that have threatened their existence and survival (Philipeon, 2001). Herbal medicine is the oldest form or type of medical practice which has stood the test of time, currently, receiving recognition as beneficial in coronary heart disease, obesity, diabetes, cancer, osteoporosis and many chronic and degenerative diseases (Murray, 2004). The goals of using plants for therapeutic agents are use as pharmacological tools/ to become a base chemical agents or drugs (Evans, 2005).

The measurement of blood glucose is the most important biological marker diagnosis and monitoring of diabetes mellitus both in clinical and experimental settings (Mayfield, 1998). According to literatures induced diabetes in animals and this often times result to high amount of glucose, triglyceride and cholesterolof groups administered with chemical diabetic agents (Nwanjo and Oze, 2007; Ayinla *et al.,* 2011). The result obtained indicated that the glucose profile of the negative control group increased in all the weeks of treatment. Processed *Pentaclethra macrophylla* seeds brought about drastic change of the fasting blood glucose levels. The 2nd cooking sample in 8th week of treatment showed better result and was more effective in the reduction of the blood glucose level (hypoglycemic) compared to 1st cooking. The processed seeds may have exerted anti-hyperglycaemic activity possibly by directly or indirectly facilitating glucose uptake at the peripheral tissues preventing hyperglycemia. In conclusion, these seeds could be used in therapeutic formulation of food supplements and hypoglycemic agents to impede diabetes related complications.

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