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# Effect of Humulin (Insulin) on the Pancreas of Streptozotocin Induced Diabetic Wistar Rats.

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**ABSTRACT**

Several substances including orthodox pharmacological agents and traditional herbs have been employed in the management of diabetes. In this study the effect of humulin (insulin) on the pancreas of streptozocin induced diabetic wistar rats was investigated. Twenty-four (24) albino rats were divided equally into 3 groups A (Normal control), B (Diabetic control), and C (Diabetic rats treated with Humulin). Diabetes was induced with sreptozotocin. Humulin was administered subcutaneously daily at a dosage of 5iu/kgbw to rats in group C while Groups A and B rats received placebo for 28 days. At the end of the experiment the animals were sacrificed under chloroform vapour. The pancreas was collected for histological studies and serum for blood glucose assay. Results showed a significant weight loss ($p<0.05$) among group B animals and a significant weight gain ($p<0.05$) in the group C animals compared to the normal control group. Also the glucose levels of the group B animals was significantly higher ($p<0.05$) than group A while that of group C was significantly reduced ($p<0.05$) compared to group B. Histologically, the pancreatic islets of group B animals was distorted with shrunken cell mass which appeared degenerated as against that of group A animal where there were prominent islet cells with a normal exocrine pancreas. In rats treated with Humulin, there was no observable difference in the cytoarchitecture of the pancreatic islet compared to the diabetic control group.

**Key word:** Diabetes, insulin (Humulin), pancreas, wistar rats.

Disbetes Mellitus (DM) is a metabolic disorder characterized by a high level of glucose in the blood, usually above 6.0mmol/L, resulting from either a defect in insulin secretion, action or both (Fox, 2004).

Elevated levels of blood sugar (hyperglycaemia) results in spillage of glucose into the urine, hence the term "sweet-urine" disorder. Normally, blood glucose levels are strictly controlled by insulin, a hormone produced by the islet cells of the pancreas. Insulin make it possible for the cells in the body to utilize glucose, thus lowering the blood glucose levels. However, in patients with DM, the absence or insufficient production of insulin makes it difficult for the cells to take up glucose and this among other things is responsible for the symptoms experienced by the diabetic. Such symptoms include weight loss, excessive thirst, excessive urination and general fatigue (Fox 2004, Larsen *et al* 2003)

There are 2 major forms of DM. Type 1 is the insulin dependent diabetes and accounts for about 10% of all cases of diabetes and occurs in people below the age of 40 years. It was once known as juvenile onset diabetes because it was usually diagnosed in young people (Fox 2004, Armstrong 2006). There is usually a defect in insulin production and there may be a family history of diabetes among sufferers who have to depend on daily injections of insulin. The type 2 diabetes accounts for about 90% of all cases of DM and is usually familiar. In this case, there may be normal or high levels of insulin but a defect in insulin action. Sufferers are usually above 40 years of age and commonly obessed. (Fox 2004, Larsen *et al* 2003). In experimental diabetes models where certain chemical agents are used to induce type 1diabetes, the pancreatic beta cells of the subjects used selectively destroyed leading to a total lack on deprived insulin production, hence chronic hyperglycaemia.

The relationship between DM and hyperglycaemia was discovered around the 18th century despite the fact that the condition was known to be characterized by passage of "sweet urine" since the 2nd century. In 1776, Matthew Dobson discovered that the sweetness of the diabetic urine was due to the presence of sugar which previously existed in the serum but not formed by the kidney (Milner *et al* 1972). The link between DM and the pancreas was discovered by Cawley in 1988. He found out that there was massive destruction and calcification of the pancreatic islet tissues at post-mortem examination of diabetic patients.

Following the discovery of the relationship

between the pancreas and diabetes, several other studies were then carried out which culminated in the discovery of insulin by Banting & Best in 1922.

**Insulin Therapy**

So far, insulin in said to be the ultimate in the management of DM, (Davis and Granner 2003) and it is available in various presentations such as Humulin, Humalog etc. all of which are administered subcutaneously.

Insulin is a hormone that is produced by the â-cells of the pancreatic islet majorly in response to meals. It acts by binding to the â-subunit of the receptor, thereby activating the tyrosine kinase activity of the receptor subunit. This initiates a cascade of biochemical reactions which it turn results in several physiological events, including the inhibition of hepatic glucose output. Stimulation of hepatic glucose uptake and the stimulation of glucose uptake by peripheral tissues (Mayerson and Inzucchi 2002)

Insulin therapy however is not without undesirable effects, the most bothersome being hypoglycemia and undue weight gain. In a 6-month trial with insulin it was reported that 62% of patient had experienced at least one episode of hypoglycemia and an average weight gain of 6kg (Mayerson and Inzucchi 2002) For proper and more successful control of blood sugar, as close to euglycaemia as possible in diabetics, adequate nutrition, rest and exercise are also very important. Exercise is said to increase tissue sensitivity to insulin thus reducing insulin requirement of those with type 1 diabetes (Chamlers *et al* 2007)

## MATERIALS AND METHOD

**Experimental Animals**

24 male albino rats of Wistar strain weighing about 140-180g were obtained from the animal houses of the Department of Zoology and Environmental Biology, University of Calabar, Calabar. The animals were allowed to acclimatize for three weeks in the animal house of the Department of Anatomy during which they were housed in well ventilated cages (wooden bottom and wire mesh top) and kept under controlled environmental conditions of temperature ($25 \pm 5^0$C), relative humidity ($50 \pm 5\%$) and 12 hour light/dark cycle. The 24 rats were divided into 3 equal groups Normal control (A), Diabetic control (B) and group treated with Humulin (C) (Table 1)

**Table 1: Experimental Design and Protocol**

| Group | No of rats | Drug Administered | Qty (mg/kgw) |
|---|---|---|---|
| A (Normal control) | 8 | Normal saline | 0.2ml |
| B (Diabetic Group) | 8 | Normal saline | 0.2ml |
| C | 8 | Humulin | 5iμ/kg |

**Glucose Estimation**

This was done using the Randox-assay kit (GOD-PAP) method based on Barham and Trinder (1972). The principle involves the enzymatic oxidation of glucose in sample by the enzyme Glucose oxidase to generate hydrogen peroxide and gluconic acid. The concentration of $H_2o_2$ released is proportional to initial amount of glucose in the sample and it reacts under catalysis of peroxidase, with phenol and 4-amino phenazone to form a red violet quinoneimine dye whose colour intensity reflects the concentration of glucose in the sample.

Glucose $+V_2 + H_2O$ Glucose oxidase gluconic acid + $H_2O_2$ $2H_2O_2$ + 4 aminophweazone phenol peroxidase quinoneimine $+ 4H_2O_2$

**Collection of Samples for Analysis**

At the end of the 28 days, food was withdrawn from the rats and they were fasted overnight but the animals had free access to water. They were then euthanized under chloroform vapour and sacrificed. The pancreas was surgically removed. It as immediately blotted using filter paper to remove traces of blood and then weighed with an analytical balance. Thereafter, the tissues were suspended in 10% formal saline for fixation preparatory to histological processing.

**Statistical Analysis**

One way anova and the LSB post hoc test were used for the statistical analysis.

**Histopathological studies**

The fixed pancreatic tissues were sectioned (5-micon thickness) and sections firstly stained with basic dyes, Heamatoxylin and Eosin (H&E) according to Conn procedure and later pancreatic section were specifically stained for beta cells by the aldehyde fuschin procedure as described by Gomori and photomicrographs at (x 400) developed (as shown below).

**Induction of Experimental Diabetes**

Prior to diabetes induction, the rats were subjected to 12hr fast, and then diabetes was induced by intraperitoneal injection of 65mg/kg b.w. with streptozotocin (STZ) (Sigma St. Louis, MO, U.S.A) reconstituted in saline solution. Control animals received saline only. Seven days after STZ treatment, diabetes was confirmed in STZ treated rats with a fasting blood sugar concentration = 200mg/dl. This was estimated using One Touch ® Glucometer (Lifescan, Inc. 1995 Milpas, California, U.S.A) with blood obtained from the tail vein of the rats.

**Experimental Protocol:** Diabetic and non-diabetic animals were grouped as shown in Table 1 and accordingly, treated with extracts and insulin. Insulin dose, (5iu/kg b.w.) was as previously used by Sonia and Srinivasan (1999) and was administered once per day post prandial (6.00pm). The animals were maintained on pelletised growers mash obtained from Vital Feeds, Jos, Plateau State, Nigeria, and tap water. Both the fed and water provided *ad libitum* and environmental conditions maintained as stated earlier throughout the 28 days.

## RESULTS

**Morphological Observation**

**Table 2:** Variation in weight of the animals in the various groups

| GROUP | INITIAL (G) | FINAL (G) | % INCREASE |
|---|---|---|---|
| A | 148.93±2.99a | 170.58±3.51b | 6.8% |
| B | 147.58±3.05a | 119.73±2.71b | -10.5% |
| C | 146.23±2.23a | 176.20±6.02b | 8.9% |

Values are expressed as mean ± SEM

N=8

a=p<0.05 Vs b

From the result above, there was a significant variation in the weight of the animals in the groups. Animals in the diabetic control group showed a significant decrease in the final body weight ($p<0.05$) when compared to their initial body weight, while those in the normal control and in the group treated with humulin (HU) showed a significant increase ($p<0.05$) in the final body weight. Also, treatment HU caused a significant increase in weight ($p<0.05$) compared to the diabetic and Normal control groups.

**Biochemical Observations**

**Table 3:** Blood glucose of animals in the various groups at end of the experiment

| Group | No of Animals | Mean |
|---|---|---|
| NC | 8 | 65.62±2.47 |
| DC | 8 | 244.62±8.64a |
| HC | 8 | 54.25±1.33b |

Values are expressed as Mean±SEM

N=8

a=p<0.05 Vs NC

b=p<0.05 Vs DC/NC

The table above shows the final blood glucose levels of the animals in the various groups. Compared to the normal control group, there is a significant increase ($p<0.05$) in the blood glucose level of the animals in the diabetic control group. Blood glucose level of animals in the treated group was significantly reduced ($p<0.05$) compared to the Normal control and the diabetic group.

**Histological Observations**

The effect of the extracts on the cyto-architecture of the pancreas in this work was studied using H/E staining technique and the Gomori Aldehyde Fuschin (GAF) staining method for pancreatic islet cells. Stained sections were examined using the light microscope.

**Haematoxylin Eosin (H&E) Stain:**

GROUP A **Normal Control (PLARE 1):** The pancreatic ducts are properly outlined, the islet cells are prominent and well circumscribed. Also, there are clearly defined secretory acini, centoracinar cells and excretory ducts.

GROUP B **Diabetic Control (PLATE 2):** The secretory acini and centroachinar cells are present, the lobules are distorted and the islet cells are necrotic and appear degenerate.

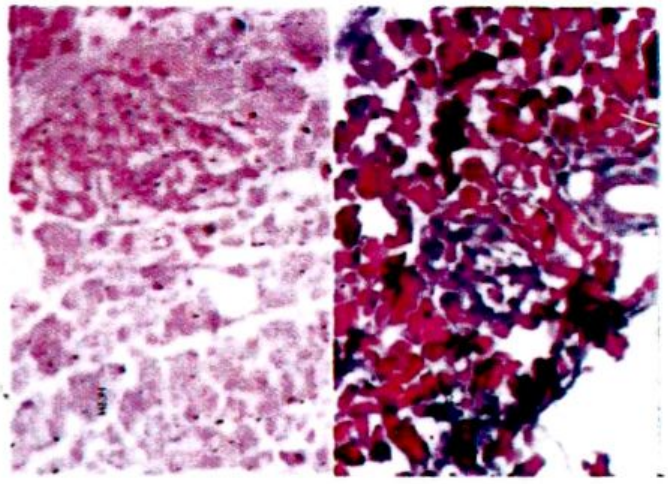
GROUP C **Humulin (PLATE 3):** Presence of vacuolations, with necrotic acinar cells and islet cells which appear degenerated.

**Gomori Aldehyde Fuschin Stain:**

PLATE 1 **Normal Control:** Islet cells are clearly defined, prominent and well circumscribed.

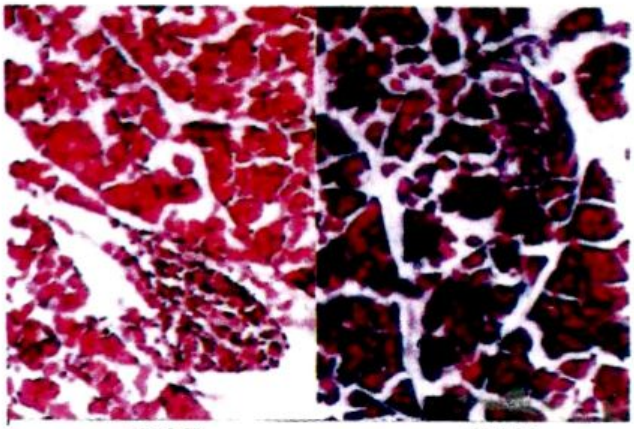
PLATES 2 **Diabetic Control:** Islet cells appear completely necrotic with dictation of the pancreatic cyto-architecture.

PLATE 3 **Humulin:** The Islet cells are distorted, appearing degenerated with presence of vacuolatins.



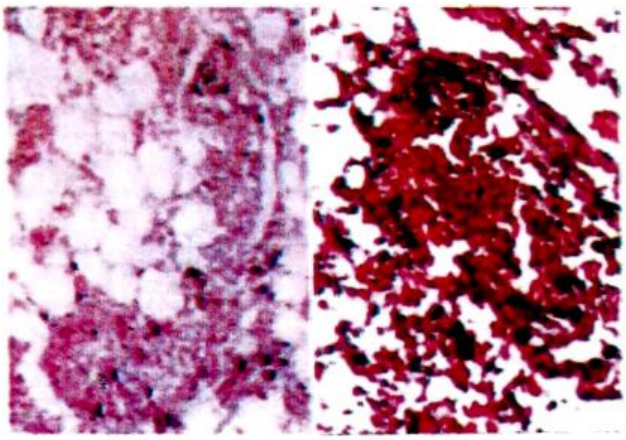
**H&E** **G&E**

**PLATE 1:** Photomicrographs of pancreas of Normal control rats given placebo treatment (X 400). H&E = Haematoxilin and Eosin, GAF=Gomori Aldehyde Fuschin Stains



**H&E** **G&E**

**PLATE 1:** Photomicrographs of pancreas of Diabetic rats given placebo treatment x 400. H&E=Haematoxylin and eosin, GAF=Gomori Aldehyde Fuschin Stains



**H&E** **G&E**

**PLATE 3:** Photomicrograph of pancreas of diabetic rats treated with Humulin (Insulin) 5iu/hg (X 400) H&E=Haemtoxylin and eosin, GAF=Gomori Aldehyde Fuschin Stain

## DISCUSSION:

The results from this study revealed significant loss of weight of group B (Diabetic Control) animals compared to group A (Normal Control). This may be due to the loss in muscle and adipose tissue resulting from excessive breakdown of protein and starch (Granner, 1996). There has been report by Granner (1996) that glycosuria causes a significant loss of calories for every gram of glucose excreted and that this loss results in severe weight loss in spite of increased appetite, especially when it is coupled with loss of muscle and adipose tissue due to excessive breakdown of protein. However, the animals in group C (those treated with Humulin) tended to gain more weight than in other groups. This agrees with reports by Makimattila *et al* (1999) that improved glycaemic control by insulin promotes weigh gain by decreasing both metabaolic rate and glucosuria.

The histology of the pancreas in the normal control group showed normal pancreatic tissues with endocrine and exocrine parts. The endocrine portion showed normal islet cells. In the diabetic control group, there was a marked distortion of the pancreatic cyto-architecture with necrotic and degenerated islet cells. This is in line with the report by Bolkent *et a,* (2002) and Noor *et al* (2008) that streptozocin damages the pancreatic tissues especially the islet cells. Also in the treatment group (with insulin), the islet cells, appeared distorted and degenerated with vocuolations in the exocrine pancreas. There was no observable difference in the pancreatic islet cytoarchitecture compared to the diabetic control group. This is an accordance with earlier reports by Pirola *et al* (2004) that insulin

does not act directly on the pancreas to reduce blood sugar levels but on peripheral tissues, facilitating the uptake of glucose into the cells, thereby reducing blood glucose levels.

Hyperglycaemia was observed in the Diabetic Control Group (B) which suggests that the insulin producing cells of the pancreatic islets were destroyed. This aggress with reports by Rodrigues *et al.*, (1999) that STZ selectivity destroys insulin producing cells of the pancreas and it induces a prototype of Type 1 diabetes. Treatment with insulin (Humulin) significantly reduced the blood glucose to below normal levels. This is in accordance with reports by Rother, (2007) that treatment with insulin is usually a common cause of hypoglycaemic attacks in diabetic subjects.

In conclusion, although treatment with insulin reduced the blood glucose of experimental animals remarkably, and caused significant weight gain, it was observed that the drug did not have any positive impact on the histology of the pancreatic islet cells.

## REFERENCES


Armstrong C (2006). Standards of medical care for patients with diabetes. *American Famiy Physicican, 74(5): 871-874.*

Barham D, Trinder P (1972). An improved colour regent for the determination of blood glucose by the oxidase system. *Analyst, 97, 142-145*

Bolkent R, Yanarda A, Tabakolu-Ouz O, Ozsoy S (2000). Effect of chard (beta-vulgaris L. Varcida) Extract in pancreatic beta cells in streptozocin diabetic rats: Morphyological and biochemical study. *Journal of Ethnophamacology,* **73** (1-2): 251-259.

Chalmers J, Patel A, MacMahon S, Netal B, (2007). Effects of a fixed combination of perindopril and indapoamide on macrovascular and macrovasular outcomes in patients with type 2 diabetes mellitus (The advanced trial). **370**: 829-840.

Conn HJ, 1946. Biological Stains: A handbook on the nature and uses of the dyes employed in the biological laboratory N.Y. Biotech publication.

Davis SN, Granner DK (2003). Insulin, oral hypoglycemic agents and the pharmacology of the endocrine pancreas. In G. Goodman and N. Gulmon (eds), *The pharmacological basis of therapeutics* (10th ed., 1679-1714). New York: McGraw Hill.

Fox IS (2004). *Human physiology* (8th ed). New York: McGraw Hill.

Granner DK (1996). Hormones of the pancreas and gastrointestinal tract. In DK Granner (Ed), *Harper's biochemistry* (25th ed., 610-626). New York: McGraw Hill.

Larsen PR, Kronberg HM, Schlomo M (2003). *Williams textbook of endocrinology* (10th ed). St. Louis: WB Saunders.

Makimattila S, Nikkila K, Yki-Jarvinen H (1999). Causes of weight gain during insulin thereapy with and without metformin in patients with type II diabetes mellitus. *Diabetologia,* **42**(4): 406-412.

Mayerson AB, Inzucchi SE,(2002). Type II Diabetes mellitus: A pathophysiology based approach. *Postgraduate Journal of Medicine, III* (3): 83-92

Milner RDG, Ashworth MA, Barson AJ, (1972). Artherosclerosis in Diabetes. *Journal of Endocrinologu,* **52**: 497-499.

Noor A, Gunasekeran S, Manicckan AS, Vijayalakshrin, MA (2008), MA, (2008). Anti-diabetic activity of aloe Vera and histology of organs in streptozocin induced diabetic rats. *Current Issues in Science,* 94 (8): 1070-1076.

Pirola L, Johnston AM, Oberghen EV, (2004). Modification of insulin action. *Diabetologia.* **47**, 170-184

Rodrigues B, Poucheret P, Betell, ML, Mc Neill JN, (1999). Streptozcin induced diabetes: Induction, mechanisms and dose dependency. In J. H. Mc Neill (Ed.), *Experimental models of diabetes* (3-7). Florida: CRC Press

Rother KI, (2007). Diabetes treatment-bridging the divide. *North England Journal of Medicine,* **365** (15): 1499-1501.

SONIA B, Scrinivasan BD, (1999). Investigations into the anti-diabetic activity of *Azadirachta indica. Indian Journal of Pharmacoology,* **31**: 138-141.

World Health Organization (2004). *Facts about diabetes:* WHO information sheet on diabetes Geneva: World Health organization.