

Effect of Ethanolic Stem-Bark Extract of *Anacardium Occidentale* (Cashew) on the Histology of the Pancreas of Diabetic Wistar Rats.

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Abstract

This study was designed to investigate the effect of aqueous ethanolic bark extract of *Anacardium Occidentale* (Cashew) on the histology of the pancreas of streptozotocin induced diabetic wistar rats. Twenty-four wistar rats weighing 150-170g were grouped into four (A, B, C and D) of six rats each. Streptozotocin was used in the production of diabetic models in groups B, C and D by subcutaneous injection of 65mg/kgbw after an overnight fast.

Groups A and B served as the normal and diabetic controls respectively and received 0.5ml of normal saline, twice daily. Groups C and D are the treatment groups that were treated with 300mg/kgbw and 500mg/kgbw of plant extract pre os respectively, twice daily. All the rats were given growers' mash and water. The experiment lasted for 28 days. After the expiration of extract administration, rats were anaesthetized using chloroform and the peritoneum stripped open to excise the pancreas which were prepared for histological studies using haematoxylin and eosin staining technique. The result of this study showed regeneration of beta cells, but it was more significant in the group that received 500mg/kgbw of the extract. The extract is therefore dosage dependent.

Keywords: *Anacardium occidentale*, Pancreatic (beta) Cells, Diabetes mellitus, streptozotocin, wistar rats and Histology

1.Introduction

Herbal medicine is known to be the oldest form of healing. It originated from ancient Greek as far back as 1600BC (Baker, 1970). It involves the use of plant materials such as flowers, bark, leaves, seeds or root to improve, maintain or restore health and wholeness (Taylor, 2005).

The plant kingdom offers a wide field to look for oral hypoglycemic agent which could be used in the management of diabetes mellitus without unpleasant side effects such as Lipoatrophy seen with subcutaneous insulin therapy, hypoglycemic coma e.t.c.

Anacardium occidentale is a member of the Anacardiaceae family. Initially, it was planted in India to reduce erosion (Johnson, 1973) but today, it is extensively used locally in treating diabetes mellitus, dyspepsia, impotence, diarrhoea, urinary disorders, venereal diseases, Leishmaniasis (Franca, 1993) and Syphilis – related skin disorders (Akinpelu, 2001). Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, oxalate and phytate (Eliakim-Ikechukwu *et al.*, 2010).

Diabetes mellitus is a syndrome characterized by disordered metabolism and inappropriately high blood sugar (hyperglycaemia) resulting from either low level or resistance to insulin (Guyton and Hall, 2002). Insulin is produced by the beta cells of the endocrine part of the Pancreas. These cells are sensitive to cytotoxic action of streptozotocin which induces experimental diabetes mellitus in animals (Ganda, 1976). Alloxan could also be used. (Abdel, 1997).

The primary objective of this study is to validate the use of *Anacardium occidentale* in the management of diabetes mellitus in traditional medical practice.

2.Materials and Methods

Experimental animals: A total of twenty-four wistar rats of average weight 90g were procured. They were bred to desired experimental weight, of 150g-170g in a ventilated and hygienic environment. The rats were housed in plastic cages with wire gauze top. **Plant material and extract preparation:** bark of cashew tree (*Anacardium occidentale*) were collected, cut into thin slices, washed free of debris, air-dried and pulverized into powder form using manual blender. 1000g of the powder was soaked in 3500ml of 80% ethanol, agitated for 3-5 minutes in an electronic blender and stored in a refrigerator for 48 hours. The mixture was filtered with a cheese cloth twice using Whatmann's filter paper and the filtrate was concentrated using a rotator evaporator to yield 150g of extract. (0.15% yield) the extract was reconstituted up to 100ml with 50g of the extract was reconstituted up to 100ml with normal saline.

Experimental protocol and administration of extract: Twenty-four wistar rats (150g-170g) were divided into four groups (A, B, C and D) of six rats each. Groups A and B served as the normal and diabetic controls respectively while groups C and D were the experimental groups. Groups B, C and D were induced with treatment diabetes by subcutaneous injection of 65mg/kgbw of streptozotocin after an overnight fast (Ganda, 1976). Hyperglycaemic state (21mmol/L) was ascertained 72 hours later using a glucometer and glucose test strips.

Groups A and B were administered 0.4 ml normal saline twice a day while groups C and D were administered 300mg/kgbw and 500mg/kgbw of extract twice a day via orogastric tube. The rats were fed with growers' mash and given water liberally. The experiment lasted for 28 days.

Collection of experimental tissue: At the end of the experimental period, rats were anaesthetized using Chloroform. A midline incision was made, the peritoneum stripped open, and pancreas dissected from each group was collected for histological study.

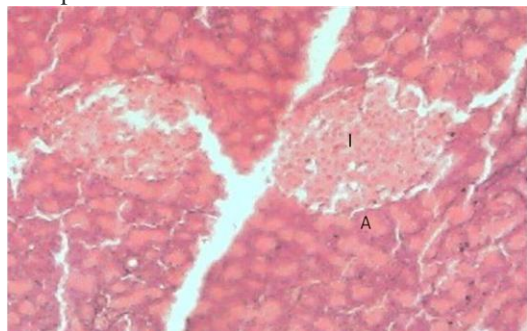
2.1. Histological Technique

The pancreas tissue were fixed in Bouin's fluid for 24hours and processed routinely for histological studies using haematoxylin and eosin-staining technique. Slides were viewed under the light microscope and photomicrographs taken.

3. Results

3.1. Normal Control

Group A: Received 0.4ml normal saline



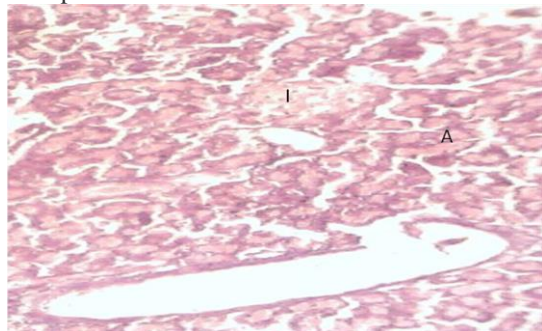
Stain: H/E mag. x 100

Photomicrograph reveals:

Numerous pancreatic acini (A) containing homogenous eosinophilic secretions and prominent islets of Langerhans (I) with a distinct capsule.

3.2. Diabetic Control

Group B: Received 0.4ml normal saline



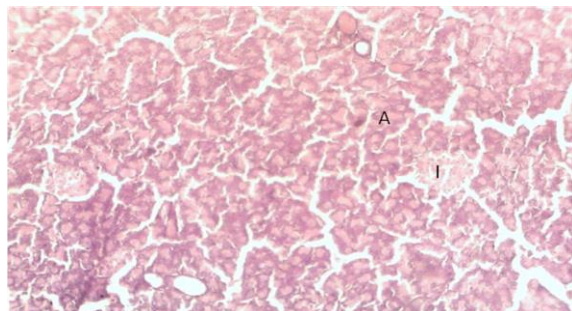
Stain: H/E mag x100

Photomicrograph reveals:

Prominent pancreatic acini (A) and poorly defined islets of Langerhans (I) with reduced cell population.

3.3. Diabetic Group C

Received 300mg/kg of extract



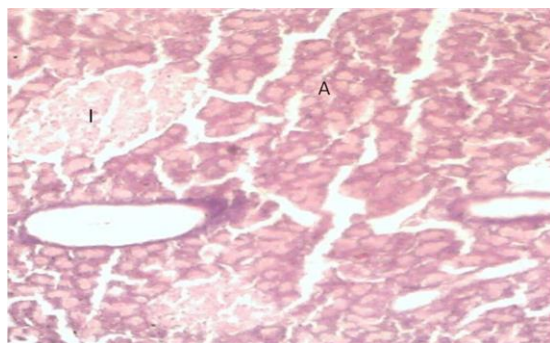
Stain: H/E mag. x100

Photomicrograph reveals:

Prominent pancreatic acini (A) and islet of Langerhans(I) with good cell population but of small volume.

3.4.Diabetic Group D

Received 500mg/kg of extract



Stain: H/E mag. x 100

Photomicrograph reveals:

Numerous pancreatic acini (A) and distinct and well populated Islet of Langerhans (I)

4. Discussion

Insulin produced by beta cells of the pancreas is the principal hormone that regulates uptake of glucose from the blood. Therefore deficiency of insulin or the insensitivity of its receptor plays a central role in all forms of diabetes mellitus.

In this research work streptozotocin was used to selectively destroy beta cells. The synergistic action of nitric oxide and reactive radicals generated are responsible for necrotic changes seen in beta cells.

The increase in beta cell population in the treated groups regenerative role of *anacardium occidentale* bark extract.

Regeneration may be attributed to the presence of tannins (Mota, 1985), anacardic acid, phenol (Alexander, 2008) and also flavonoids, alkanoids, saponins present in the extract have been documented to have antioxidant effects (Olaleye et al., 2007). The herb also contains amino acids which may have contributed to the formation of

polypeptide growth factors needed for differentiation of beta stem cells in the pancreas. This may have led to the regeneration process.

From results obtained, it can be concluded that the regenerative effect of *Anacardium occidentale* extract on beta cells may be due to the phytochemical components of the herb.

5. References

- Abdel, M. A. El fek, M. and Salah, E. (1997).
'Effect of Nigella Sativa, fish oil and Gliclazide on alloxan diabetic rats. Biochemical and histopathological studies', *J. Egy ger soci*, 23: 237.
- Akinpelu D. A. (2001)
'Antimicrobial activity of Anacardium Occidentale bark', *Filoterapia*, 72 (3): 286-87.
- Baker, H. G. (1970). *Plants and Civilization*. 2nd ed. Macmillan Press Limited, New York.
- Eliakim-Ikechukwu, C.; Obri, A.; Akpa, O.(2010).
'Phytochemical and micronutrient composition of *Anacardium Occidentale* Linn (cashew)stem-bark extract and its effect on the fasting blood glucose levels and body weight of diabetic wistar', *J. Nutrition and wellness*. Vol. 10, No. 1.
- Franca, F; Cuba, C. A.; Moreira, E. A.; Miguel, O. and Almeida, M. (1993).
'An evaluation of the effect of a bark extract from the cashew (*Anacardium Occidentale* L.) On infection by Leishmania (Viannia) Braziliensis', *Rev. Soc. Bras. Med. Trop*; 26 (3): 151-155.
- Ganda, O. P., Rossi, A. A. and Like, A. A. (1976).
'Studies on Streptozotocin diabetes', *Diabetes*, 25:595-603.
- Guyton, A. C. and Hall, J. K. (2002).
Textbook of Medical Physiology. 10th ed. SB Saunders Company, London.
- Johnson, D. (1973).
'Cashew Cultivation in Brazil', *Agron. Mocamb. Lourenco Marques*, 7 (3):119-129.
- Mota, M. L., Thomas, G. and Barbosa, J. M. (1985).
'Anti-inflammatory actions of tannins isolated from the bark of *Anacardium Occidentale*', *J. Ethnopharmacol*; 13 (3):289-300.
- Olaleye, M. T., Kolawole, A. O. and Ajeje, J. O. (2007).
'Antioxidant properties and glutathione S-Transferases inhibiting activity of A. Cordifolia leaf extract in acetaminophen Induced Liver Injury', *Iranian J. Pharm. Ther.* 6:63-66.
- Singh, I. (2002). *Textbook of Human Histology*. 4th ed. Jaypee Brothers Medical Publishers Ltd.
- Taylor, L. (2005). *The healing power of Rain forest Herbs*. Dioscorides Press, Canada.
- Tullo, A. H. (2008). 'A Nutty Chemical', *Chemical and Engineering News* 86 (36):25-27.