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RESEARCH ARTICLE

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Histological Effects of Melatonin and *Azadirachta Indica* Administration on the Pancreatic tissue in Streptozotocin-induced Diabetic Wistar Rats

E. T. Godam[1], M.O.A. Samaila[3], A.O. Ibegbu[2], W.O. Hamman[2] and S.A. Musa[2]

[1] *Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Bingham University, Karu, Nasarawa State, Nigeria*
[2]*Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria-Nigeria.*
[3] *Department of Pathology and Morbid Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria*

ABSTRACT

This study was aimed at evaluating the histological effects of melatonin and Azadirachta indica leaves ethanolic extract administration on the pancreatic tissue in streptozotocin-induced diabetic Wistar rats. Forty five male Wistar rats were used in the study. The animals were divided into two main groups control group A and diabetic group B. The animals in Group A were sub divided into group 1, 2, 3 and 4 and group B animals subdivided into group 5, 6, 7, 8 and 9 comprises of five rats per group respectively. Group 1 received placebo orally once daily. Group 2 (extract control) received 200mg/kg body weight of extract orally daily, Group 3 (melatonin control) received 10mg/kg body weight of melatonin intraperitoneally (IP) daily. Group 4 (extract and melatonin control) received 200mg/kg body weight of extract orally and 10mg/kg body weight of melatonin IP daily. Group 5 (diabetic control) received 10ml/kg body weight normal saline daily, group 6 (extract treated) received 200mg/kg body weight of extract orally daily, Group 7 (melatonin treated) received 10mg/kg body weight of melatonin IP daily, group 8 (extract and melatonin treated group) received 200mk/kg body weight of extract orally and 10mk/kg body weight of melatonin IP daily while group 9 (metformin treated) received 500mg/kg body weight of metformin orally daily. At the end of 21 days of treatment, the animals were sacrificed and the pancreatic tissues were harvested and histologically processed and stained using H&E. The histological studies showed regeneration of the pancreatic islets in all treated diabetic groups.

Key words: Melatonin, *Azadirachta Indica*, Streptozotocin, Induced Diabetes, Adult Wistar Rats.

INTRODUCTION

Diabetes mellitus (DM) is a disease common in all parts of the world and recognized as one of the leading causes of death in the world [1]. The number of people suffering from diabetes worldwide is increasing at an alarming rate. It is predicated that about 366 million people are likely to be diabetic by the year 2030 [2]. This is because that none of the antidiabetic drugs could give a long term glycaemic control without causing any adverse side effects [3]. Various hypoglycemic drugs, such as sulfonylurea, metformin are being used for the treatment of diabetes but their use is restricted by their limited action and accompanying side effects such as hypoglycaemic shock and weight gain. Insulin treatment also fails to prevent the long term complication [4]. Plants and plant products continue to play a dominant role in traditional remedies against ailments from antiquity [5]. Conventional measures used in the management of diabetes usually aim at improving glucose homeostasis and delay the onset of complications but, these measures are not curative [5].While undue weight gain, drug resistance to Insulin and hypoglycaemia are some

side effects associated with conventional measures. Many complications of diabetes result due to an increased free radical load [6]. The search for natural antioxidative agents that will ameliorate the harmful effects associated with hyperglycaemia still continues in spite of considerable progress in the management of diabetes mellitus by synthetic drugs. There is therefore an increasing preference for whole plant extract among patients and professionals as these rarely produce side effects, rather they tend to protect the patient from the usual degenerative changes [7]. The naturally endowed antioxidant components of plants including flavonoids, vitamins A and C and other secondary metabolites may alleviate these complications when used as whole extract. Over 400 traditional plants have been used in the treatments of diabetes [8]. Opinion about the mechanism of action of plants include: presence of insulin-like substances, interference with carbohydrate absorption, inhibition of insulinase activity and increase in beta cells in the pancreas [9].

Azadirachta indica of the family- Meliaceae melioideae, is a medium-sized tree that is found throughout the South Asian region, Africa and in Northern Nigeria. It is one of the most versatile medicinal plants having a wide spectrum of biological activities. Previous studies have reported the beneficial effect of *Azadirachta indica* leaves in the management of diabetes mellitus and the amelioration of the oxidative stress associated with the disease [10]. This was explained by the presence of terpenoids and saponins which have been found to be potentially useful for the treatment of hyperglycaemia.

Melatonin has been shown to be a major scavenger of both oxygen and nitrogen based radicals [11]-[13], including peroxynitrite anion ($ONOO^-$) [14-[15]. Melatonin mayinfluence diabetes and associated metabolic disturbances not only by regulating insulin secretion, but also by providing protection against reactive oxygen species, since pancreatic β-cells are very susceptible to oxidative stress because they possess only low-antioxidative capacity [16]. In developing countries adequate treatment measures for diabetes mellitus are often unavailable or too expensive hence the need to test for the viability of *Azadirachta indica* ethanolic leaves extract and melatonin a known potent antioxidant as alternatives to conventional antidiabetic drugs. However, the effects of melatonin and ethanolic extract of *Azadiracta indica* leaves on pancreatic islets and Liver of diabetic subjects are yet to be reported. The aim of the present study was to evaluate the histological effects of melatonin and *Azadirachta indica* administration on the pancreatic tissue in streptozotocin-induced diabetes in Wistar rats.

MATERIALS AND METHODS

Animal care and management

Forty five young adult male Wistar rats, weighing approximately 140g each were obtained from the Faculty of Pharmaceutical Sciences of Ahmadu Bello University, Zaria. They were kept in plastic cages and allowed to acclimatize for 2 weeks in the Faculty of Pharmaceutical Sciences Animal house before the experiment, and maintained under laboratory conditions of temperature, humidity and light. They were allowed free access to water and standard pellet diet obtained from Grand Cereals Ltd, Jos Plateau State. The animals were divided into nine groups of five animals each.

Acquisition and extraction of plant material

Leaves of fresh *Azadirachta indica* were harvested from Ahmadu Bello University Faculty of Medicine Zaria in the month of April 2012 and authenticated at the Department of Biological Sciences, Ahmadu Bello University Zaria, with a voucher specimen number 900151. The Fresh leaves of *A. indica* were air dried, minced and powdered using laboratory mortar. 1000g of the powdered leaves was extracted in 1.5 liters of 80% ethanol using a soxhlet extractor. This was filtered using a Whatman filter paper (24mm). The filtrate was dried in a laboratory water bath set at 67°C and total yield of 46.8g was obtained per 1000g of the powdered leaves.

Chemicals

Melatonin M5250-1G (Sigma Aldrich USA), Streptozocin SP0130 (Sigma Aldrich, USA)

Diabetes induction

A baseline blood glucose levels was taken for all the control and test animals before grouping them. This was done to ensure that the animals were all normoglycaemic. Thirty three Wistar rats were randomly selected and were given a single dose of intra peritoneal injection of streptozotocin, (STZ) (Sigma, Aldrich, USA), at 55mg/kg body weight in citrate buffer (0.1M, pH 4.5). The solution (STZ in citrate buffer) was used within 5 minutes to induce chemical diabetes in the wistar rats after overnight fasting of twelve hours. Blood samples were collected at 72 hours after

STZ treatment from the dorsal vein of the tail and the blood glucose levels detected using a One Touch Ultra 2 Glucometer, (Lifescan, CA, USA). Streptozotocin treated adult Wistar rats with fasting blood glucose level at 11mmol/L and above was considered diabetic. Twenty eight Wistar rats in this group were found to be chemically diabetic giving 84 % diabetic induction. These animals were further grouped into five groups of five Wistar rats each (Group 5, 6, 7, 8, 9) called the diabetic group while group 1, 2, 3, and 4 were the normal control groups with five rats per group as shown below.

Experimental protocol

Twenty five (25) diabetic animals and twenty (20) normoglycaemic were randomly selected and divided into nine groups of five (5) animals each as follows:

Group 1: Normal control + normal saline orally
Groups 2: Normal + *A.Indica* (200mg/kg bw) orally
Group 3: Normal + Melatonin (10 mg/kg bw) IP
Group 4: Normal + Melatonin (10 mg/kg b w) IP + *A. Indica* (200 mg/kg b w) orally
Group 5: Diabetic control + normal saline orally
Group 6: Diabetic + *A. Indica* 200 mg/kg b w orally
Group 7: Diabetic + Melatonin (10 mg/kg b w) IP
Group 8: Diabetic + *A. Indica* 200 mg/kg b w orally + Melatonin (10 mg/kg b w) IP
Group 9: Diabetic + Metformin (500 mg/kg b w) orally

The extract and drug were administered once daily for a period of three (3) weeks respectively. Streptozotocin was selected to chemically induce diabetes since it potentially destroys the β-cells of the Pancreas to produce diabetes signified by sustained hyperglycaemia above 200mg/dl [17]. The extract (200mg/kg b w) was administered by orogastric intubation once daily for three weeks while melatonin (10mg/kgbw) was administered intraperitoneally once daily for three weeks in all treated groups while metformin was the standard drug (500mg/kg b w) [17].

Histological studies

The tissues excised after the animal had been sacrificed were fixed in buffered 10% Neutral formal saline in plastic containers and embedded in paraffin wax. They were sectioned with Leica rotary Microtome to produce serial sections of 5µ thickness. Haematoxylin and Eosin stains were used to stain the pancreas to demonstrate the pancreatic islets. Photomicrographs were obtained using a microscope eye piece attached to a computer monitor and observations made.

RESULTS

Results of histological observations of the Pancreatic section of normal saline control (NC) group (Plate I) showed a normal section of pancreas with normal pancreatic islet. There was no observable pancreatic lesion of pancreatic islets. Pancreatic section of STZ induced diabetic normal saline control group (Plate II) showed areas of islet necrosis and degenerated islet cell mass as compared with normal saline control group. Pancreas section of *A. indica* extract treated (Plate III), melatonin treated (Plate IV), extract and melatonin treated group (Plate V) all showed restoration of pancreatic islet cells. When these groups (Plates III and IV) were compared with the normal control group (Plate I) showed histological features similar to normal saline control group and extract and melatonin treated control groups (VII, VIII, and IX).

Microscopic findings of effects of ethanol leave extract of *Azadirachta indica* and melatonin on the histology of pancreatic tissue of normoglycaemic and streptozotocin-induced diabetic wistar rats.

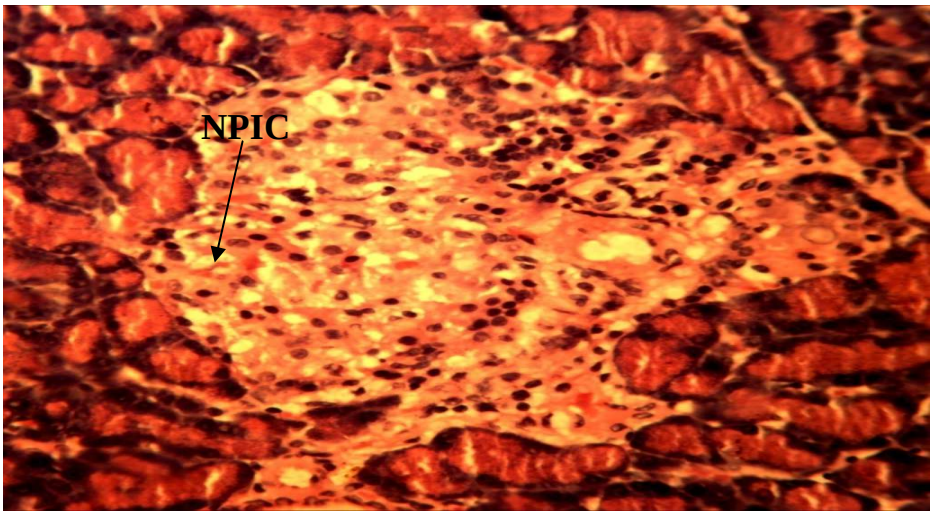

Plate I: Section of Pancreas from normal control group (NC) showing normal pancreatic islet cells (NPIC) of langerhans (H & E, x 400).

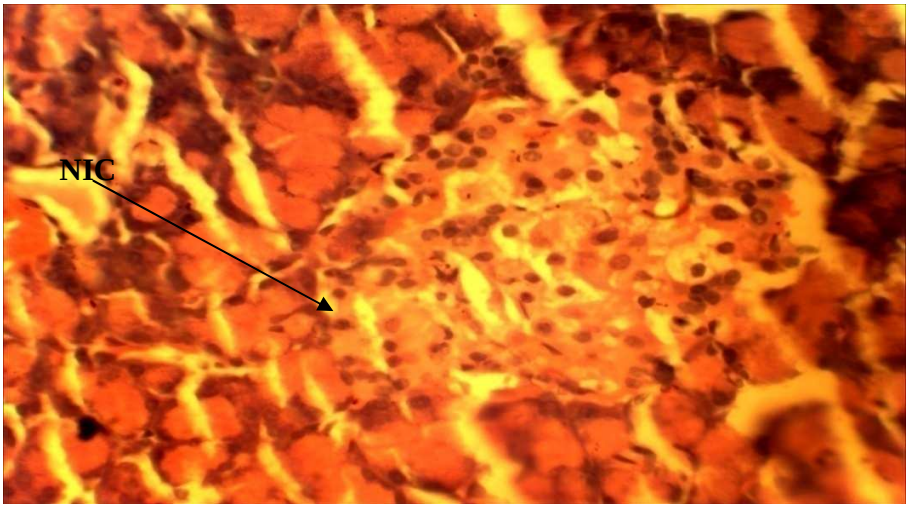

Plate II: Section of Pancreas from STZ induced diabetic control group showing necrosis of islets cells (NIC). (H & E, x400)

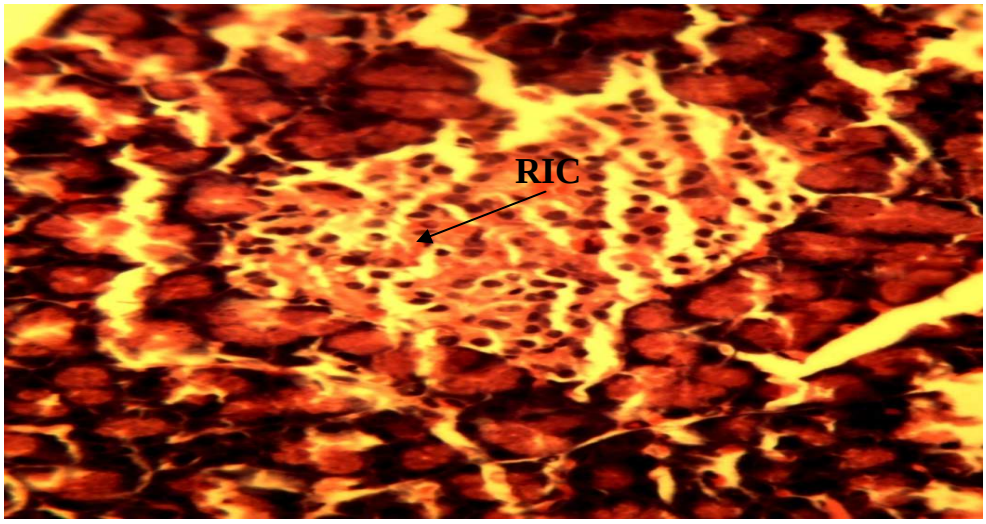

Plate III: Section of Pancreas from STZ induced diabetic rat treated with *Azadirachta indica* (200mg/kgbw) (DAI) showing regenerated islet cells (H&E, x400)

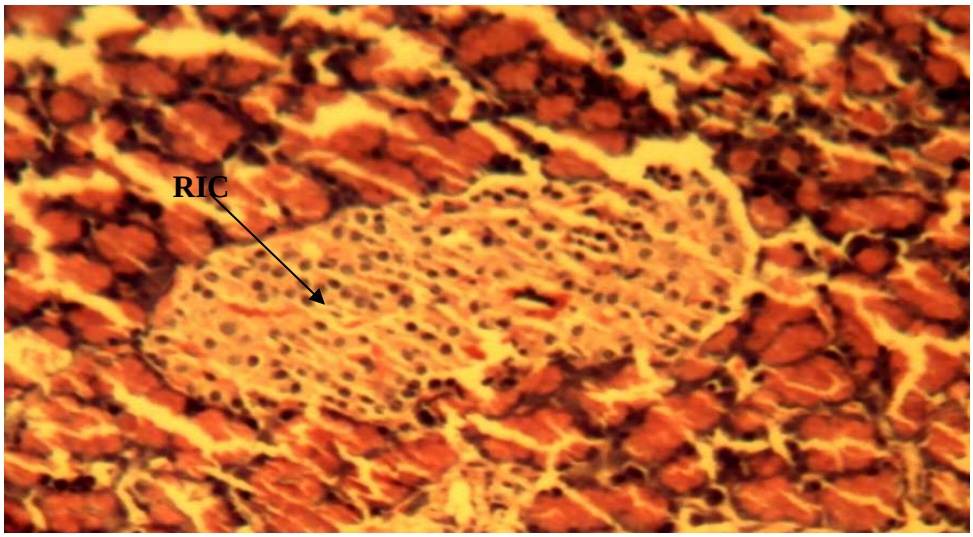

Plate IV: Section of Pancreas from STZ induced diabetic rat (DML) treated with melatonin (10 mg/kgbw) showing regenerated islet cells (H&E, x400).

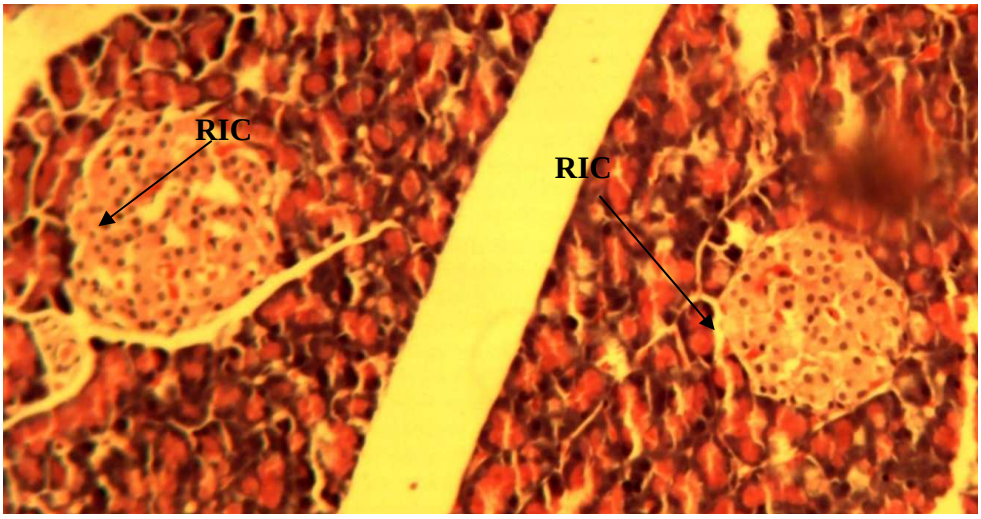

Plate V: Section of Pancreas from STZ induced diabetic rats treated with *Azadirachta indica* and melatonin combined group (DAI/DML) showing well regenerated islet cell (RIC) mass and number (H & E, x250)

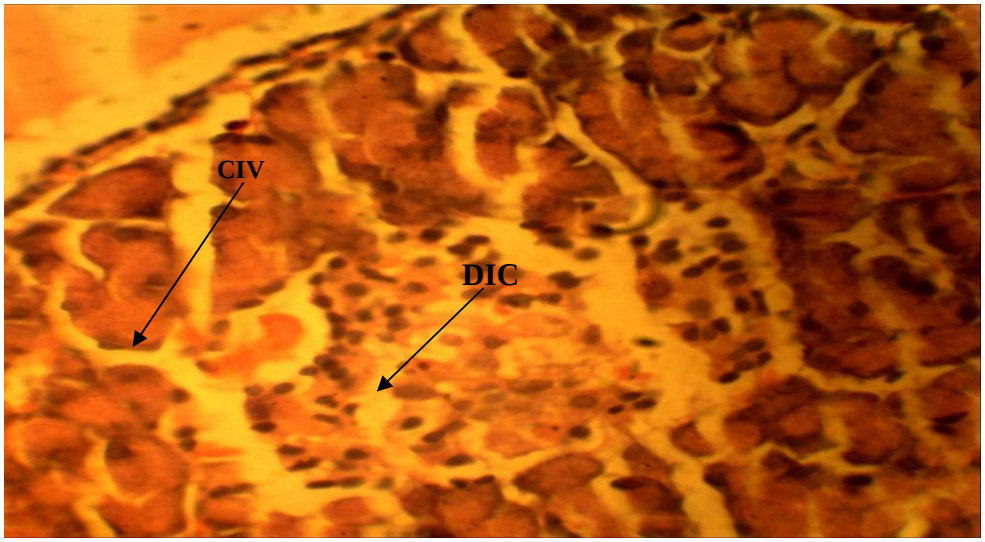

Plate VI: Section of Pancreas from STZ induced diabetic rats treated with metformin (DMF) group showing poorly regenerated islet cells and congestion of islet veins (CIV). Areas of islet necrosis are observed, DIC (Degenerated islet cells) (H&E x400).

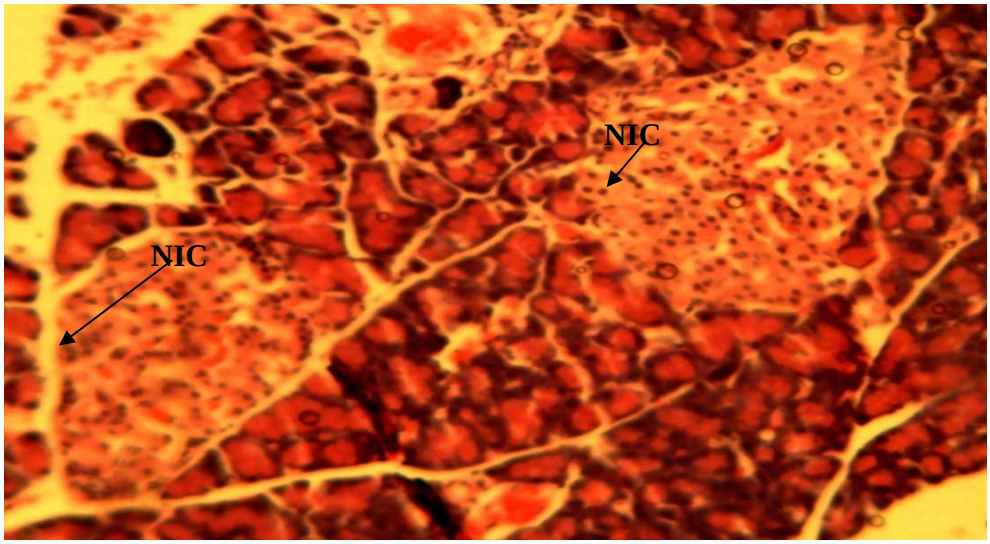

Plate VII: Section of Pancreas of extract treated Normal control group (NAI) showing well preserved pancreatic islets. The plate showed no necrotic changes (H&E, x250).

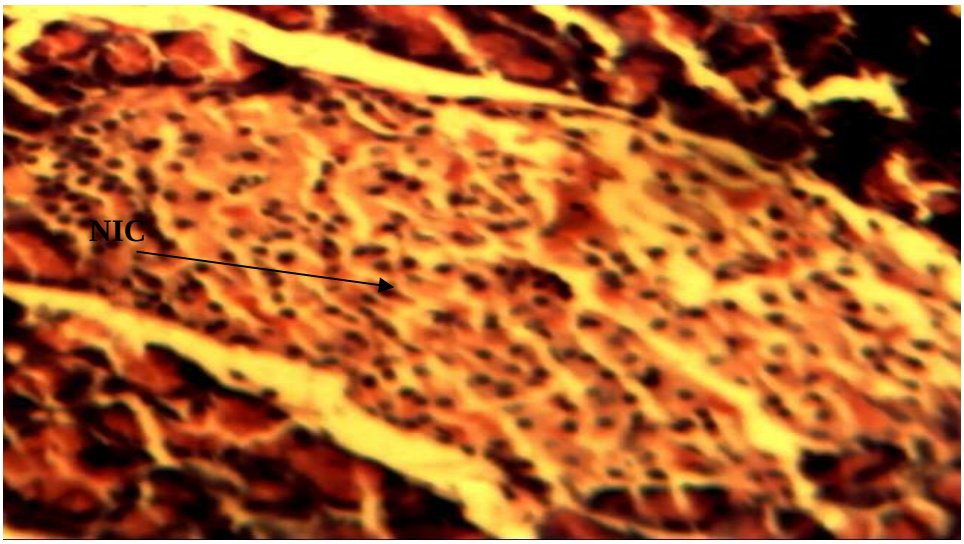

Plate VIII: Section of melatonin treated control group (NML). The section showed no necrotic change in pancreatic islet. There is preservation of the islet cells (NIC), (H&E, 400).

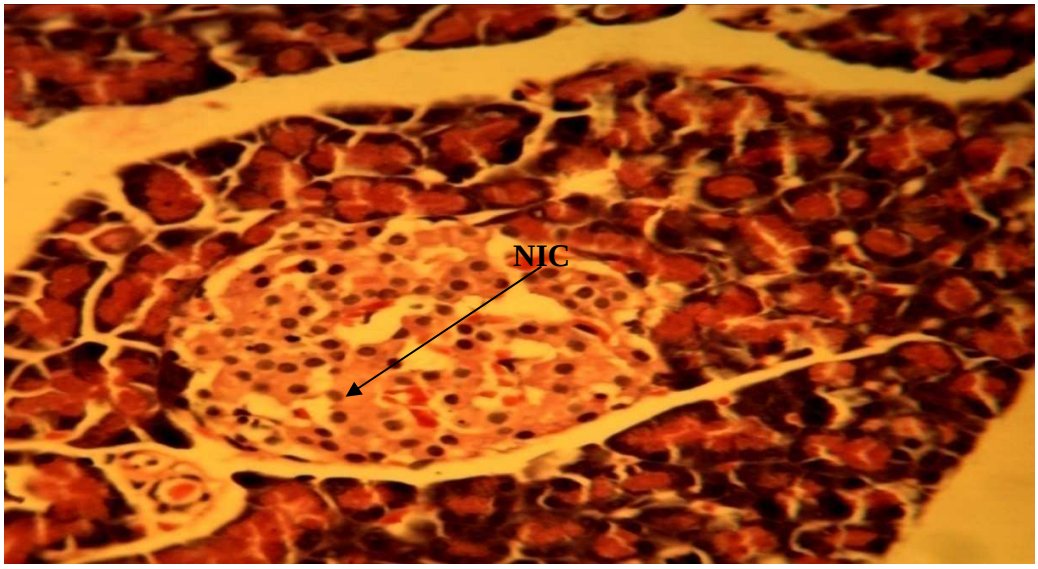

Plate IX: Section of Pancreas of *Azadirachta indica* and melatonin treated control group (NAI/NML) showed preservation of the pancreas and islet cells, (NIC). (H&E x 400)

DISCUSSION

The histology of pancreatic islet cells was normal in treated and untreated normal control groups while degenerative and necrotic changes and shrunken islets of langerhans cell mass was observed in STZ-induced untreated diabetic group. The Pancreas of the treated diabetic group showed regenerated pancreatic cell mass of islets of langerhans. The result is comparable with the findings by Vijayanand and Wesely [18] and Maisaa and Al-Rawi [19] where A. *indica* reversed and restored degenerative changes associated with pancreas in alloxan induced diabetic Wistar rats, and that melatonin participated in pancreatic regeneration in STZ-induced diabetes which was due to its ability to scavenge free radicals as an antioxidant by stimulating messenger ribonucleic acid (mRNA) levels and activities of SOD and GPx. The results of the treated group with melatonin and A. *indica* extract showed synergistic interaction between and among components of the extract and melatonin with respect to pancreatic islets of langerhans cell recovery. The results further demonstrated that regeneration and restoration of pancreatic langerhans cell mass is one of its anti-diabetic mechanisms.

CONCLUSION

It can be concluded that administration of *Azadirachta indica* ethanolic leave extracts and melatonin separately and when they are combined, possess pancreatic-protective effects as evidenced by pancreatic tissue regeneration and significant increased islet cell mass in streptozotocin-induced diabetes in wistar rats.

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