Original Article

Effects Of Annona Muricata (Linn) On The Morphology Of Pancreatic Islet Cells Of Experimentally-Induced Diabetic Wistar Rats

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Keywords

annona muricata, diabetes, islets of langerhans, morphology, pancreas, streptozotocin

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Abstract

The study was designed to evaluate the effects of methanolic extract ofAnnona muricata leaves on the pancreatic islet cells of streptozotocin induced- diabetic rats. Thirty adult Wistar rats were randomly assigned into three groups (A, B and C) of ten rats each. Group A was the control, Group B was untreated diabetic group and group C was A. muricata-treated group. Diabetes mellitus was experimentally induced in groups B and C by a single intra-peritoneal injection of 80mg/kg streptozotocin dissolved in 0.1M citrate buffer. Group A rats were intraperitoneally injected with equivalent volume of citrate buffer. Daily intra peritoneal injection of 100mg/kg A. muricata was administered to group C rats for two weeks. The rats were sacrificed and the pancreas were removed and fixed in Bouins fluid. The tissues were processed for paraffin embedding and sections of 5µm thickness were produced and stained. Histopathological examination of the stained sections showed regeneration β-cells of islets of pancreas of A. muricata-treated rats when compared to untreated diabetic group of rats.

Introduction

For a long time, it was believed that the endocrine pancreas belonged to a category of tissues that were finally differentiated and irreplaceable in the adult. This was mainly supported by the low replication rate of endocrine cells in adulthood (Swenne, 1992). In the light of many recent data, this point of view has been drastically changed, and nobody disputes today that that endocrine pancreas is a plastic organ and that β-cell mass is dynamic especially because of its significant capacity for adaptation to changes in insulin demand (Bonner-Weir, 2000). This property has been demonstrated in physiological as well pathophysiological conditions such as pregnancy (Scaglia et al., 1995) and obesity (Klöppel et al., 1985). Increase in β-cell mass may occur through increased β-cell replication, increased β-cell size, decreased β-cell death, and differentiation of β-cell progenitors (neogenesis) (Finegood et al., 1995).

Diabetes Mellitus (DM) is one of the most common metabolic disorders with a worldwide prevalence estimated to be between 1% and 5% of the world population (Kameswararao et al., 2003). It is estimated that in the year 2000, 171 million people had diabetes, and this is expected to double by year 2030 (Boon et al., 2006). Conventionally, insulin-dependant diabetes mellitus is treated with exogenous insulin (Felig et al., 1995) and non insulin-dependant diabetes mellitus with synthetic oral hypoglycemic agents like sulphonylureas and biguanides (Rosac et al., 2002). However the hormone fails as a curative agent for complications of diabetes (Mukherjee et al., 1966) and synthetic oral drugs produce adverse health

effects (Raheja, 1977). Different medicinal systems are using the active plant constituents, which discovered as natural hypoglycemic medicine, came from the virtue of traditional knowledge. Annona muricata has been found to contain numerous bioactive compounds useful for the management of various ailments including diabetes mellitus in folkloric medicine. The management of Diabetes mellitus depends on continuous hypoglycemic therapy, which may not be consistently adhere to by the patient. This research therefore investigates whether or not extracts of Annona muricata could provide lasting hypoglycemic control through regeneration of the destroyed β- cells of the pancreatic islets of experimentally induced diabetic Wistar rats.

Annona muricata is a plant, which belongs to the family Annonaceae. It is a medicinal plant that has been used as a natural remedy for a variety of illnesses. Several studies by different researchers demonstrated that the bark as well as the leaves had anti-hypertensive, vasodilator, anti-spasmodic (smooth muscle relaxant) and cardio depressant (slowing of heart rate) activities in animals (Feng, 1962, Meyer 1941). Researchers had re-verifiedA. muricata leaf's hypotensive properties in rats (Carbajal et al., 1991). Other properties and actions of A. muricata documented by traditional uses include its use as anti-cancerous, (Oberlies et al., 1995; Tormo et al., 2003), anti-diabetes (Vasquez, 1990); anti-bacterial, (Takahashi et al., 2006); anti-fungal (Heinrich et al., 1992, Lopez-Abraham, 1979); anti-malarial, anti-mutagenic (cellular protector), emetic (induce vomiting), anti-convulsant (N'gouemo, 1997), sedative, insecticidal and uterine stimulant. It is also believed to be a digestive stimulant, antiviral cardio tonic (tones, balances and strengthens the heart), febrifuge (cures fever), nerviness (balances/calms the nerves), vermifuge (expels worms), pediculocide (kills lice), and as an analgesic. Padma et al., (1998) confirmed the anti-viral activity of ethanolic extracts of A. muricata against Herpes simplex virus. Extracts of A. muricata have been shown to have anti-parasitic (Bories et al., 1991), anti-rheumatic, astringent, (dos Santos and Sant'Ana, 2001), anti-leishmanial and cytotoxic effects (Jaramillo et al., 2000; Liaw et.al. 2002). A. muricata has also been shown to be effective against multi-drug resistant (MDR) cancer cell lines (Oberlies et al., 1997; Liaw et al., 2002). Extracts of A. muricata were also shown to be effective against the cancer cell line U973 (Jaramillo et al., 2000), and hematoma cell lines in-vitro (Chen et al., 2000). Extracts were also shown to be lethal to the fresh water mollusk, Biomphalaria glabrata, which act as a host for the parasitic worm Schistosoma mansoni (dos Santos and Sant'Ana, 2000; Luna et al., 2006).

Streptozotocin-induced hyperglycemia in rats is considered a good model for the preliminary screening of agents active against type II diabetes (Ivorra et al., 1989) and is widely used. Generally, destruction of β-cells starts three days after STZ administration and reaches its peak at three to four weeks in rats (Adeghate, E., and Ponery, 2002). Streptozotocin-induced diabetes in laboratory animals has been widely used for research on diabetes and its long-term complications. Control animals in these studies are usually injected with citrate buffer solution. Streptozotocin is a potent DNA methylating agent and act as a nitric oxide donor in pancreatic islet cells. Although, the β-cell cytotoxic action of STZ is not fully understood, it is thought to be mediated by the inhibition of free radical scavenger enzyme thereby enhancing the production of superoxide. The latter has been implicated in lipid oxidation, DNA damage and sulfhydryl oxidation.

Materials And Methods

Plant Material

Annona muricata leaves were collected from Mowe, Ogun State, Nigeria in February 2006. The plant was identified by Dr. Folorunso of the Department of Botany, Obafemi Awolowo University, Ile Ife and a voucher specimen was deposited in the Herbarium of the Department

Preparation of Extracts

The leaves were air dried at room temperature for four weeks. The air-dried leaves were ground into smaller bits in a warring blender at the department of pharmacognosy Obafemi Awolowo University Ile Ife. 600g 0f the ground leaves was soaked in 5000mls of 70% methanol for 72 hours at room temperature and pressure for extraction to take place normally. The crude methanol extracts obtained was separated from the remaining shaft of the leaves by simple filtration technique. The filtrate (extracts containing methanol) was evaporated at 600C and reduced pressure in a vacuum rotary evaporator (RE 100B, Bibby Sterilin Ltd, UK.) to remove methanol following which it was freeze-dried in a vacuum freeze drier (FT33-

Armfield, England) to obtain a dried crude extract.

Care and Management of Animals

Thirty healthy adult Wistar rats (Rattus norvegicus) of both sexes, weighing between 150g and 250g were used for the experiment. The rats were bred in the animal holding of department of anatomy and cell biology Obafemi Awolowo University Ile Ife, were maintained on standard rat pellets (Ladokun feeds, Ibadan, Nigeria), and were given water ad libitum. The animals were randomly assigned into three groups A, B, and C of ten rats each. Group A was the control, non-diabetic group of rats, group B was the experimentally induced diabetic group without A. muricata treatment while group C was the experimentally induced diabetic group treated with methanolic extracts of A. muricata. There was a pre-experimental period of four weeks during which the body weight and blood glucose level were monitored in the animals before the commencement of the experiment

Administration of Streptozotocin and

Diabetes mellitus was experimentally induced in groups B and C by a single intraperitoneal injection of 80mg/kg b.w. streptozotocin (Sigma, St. Louis, USA) dissolved in 0.1M sodium citrate buffer pH 6.3. The control (group A animals) were injected intraperitoneally with equivalent volume of the citrate buffer. The rats were fasted overnight before STZ administration. After four weeks of experimental-induction of diabetes, group C rats were given daily intraperitoneal injection of 100mg/kg of extracts of A.muricata dissolved in distilled water for two weeks and the animals were monitored for another four weeks.

Histological Procedure

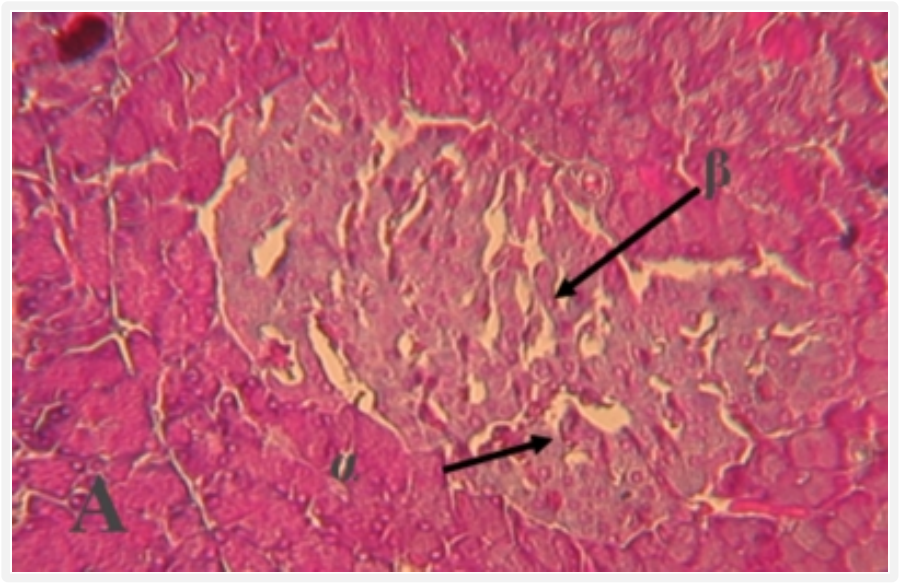
The animals were sacrificed by cervical dislocation and the pancreas of each of the animals was dissected out. The splenic part of the pancreas was fixed in Bouin's fluid by total immersion for 24 hours after which it was processed via paraffin wax embedding method of Drury and Wallington (1980). Sections of 5µm thickness were produced from the tissue blocks and stained with hematoxylin and eosin, Gomori aldehyde fuchsin and Gomori chrome alum hematoxylin phloxine for light microscopic examination of the pancreatic islets architecture. The sections were examined under a Carl Zeiss research microscope (Axioskope 40, Germany) with a digital camera attached. Digital photomicrographs of the pancreatic sections were taken at various magnifications.

Results

Histopathological examination of the pancreas.

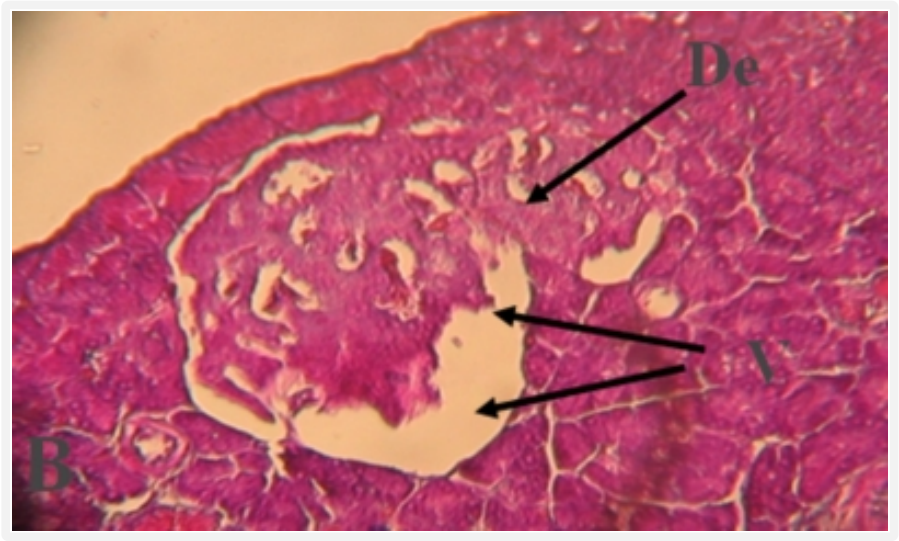
Photomicrograph of a normal (control) pancreatic islet showing cluster of β-cells which are centrally placed and peripherally placed α-cells. (Hematoxylin and Eosin X 2200)

Figure 1



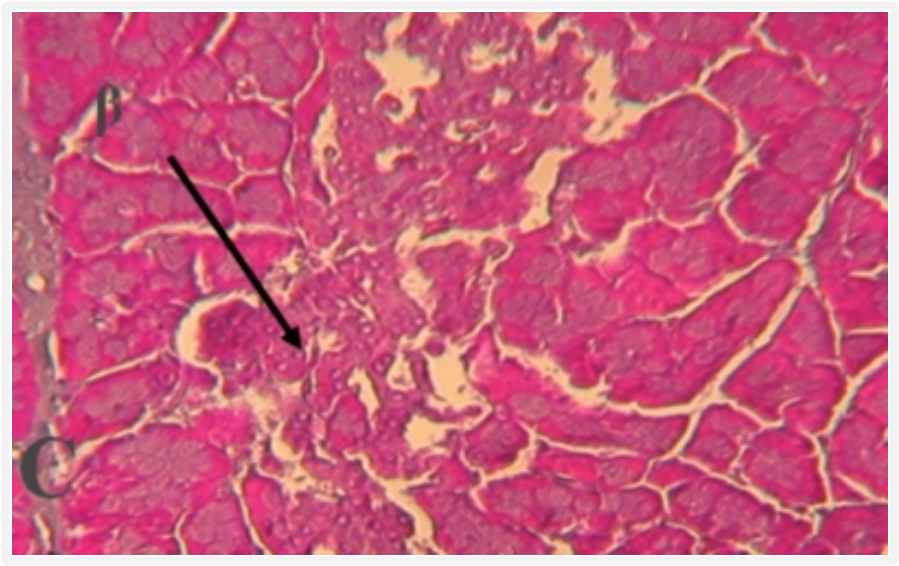
Photomicrograph of pancreatic islet of streptozotocin-induced diabetic rats showing degranulation (De) of β-cells and severe vacuolation (V) of the pancreatic islets. (Hematoxylin and Eosin X 2200)

Figure 2



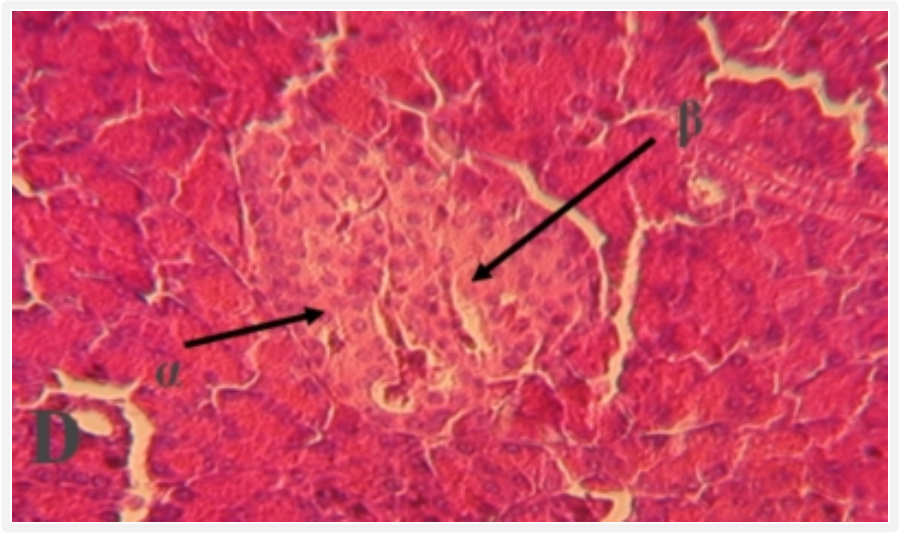
Photomicrograph of pancreatic islets of A.muricata treated diabetic rat showing recovery of the β- cells. As it is evident, the islet cells are regenerated, the inflammatory infiltration has disappeared and there is reduction in the vacuolation cased by administration of STZ (Hematoxylin and Eosin X2200)

Figure 3



Photomicrograph of a normal (control) pancreatic islet showing cluster of β-cells which are centrally placed and peripherally placed α-cells. (Gomori Chrome Alum hematoxylin phloxine X2200)

Figure 4



Photomicrograph of pancreatic islet of streptozotocin-induced diabetic rats showing degranulation (De) of β-cells and severe vacuolation (V) of the pancreatic islets Insulitis (In) is also detectable in the islets. (Gomori Chrome Alum hematoxylin phloxine X2200)

Figure 5

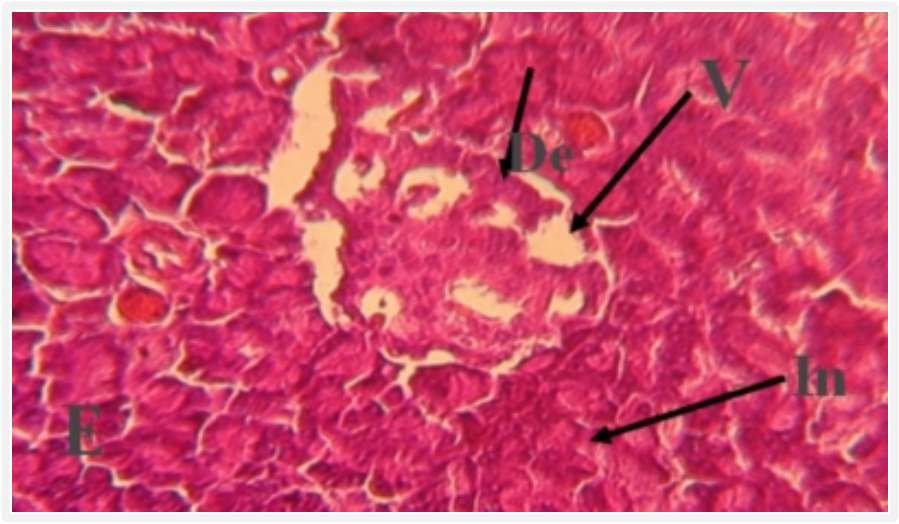


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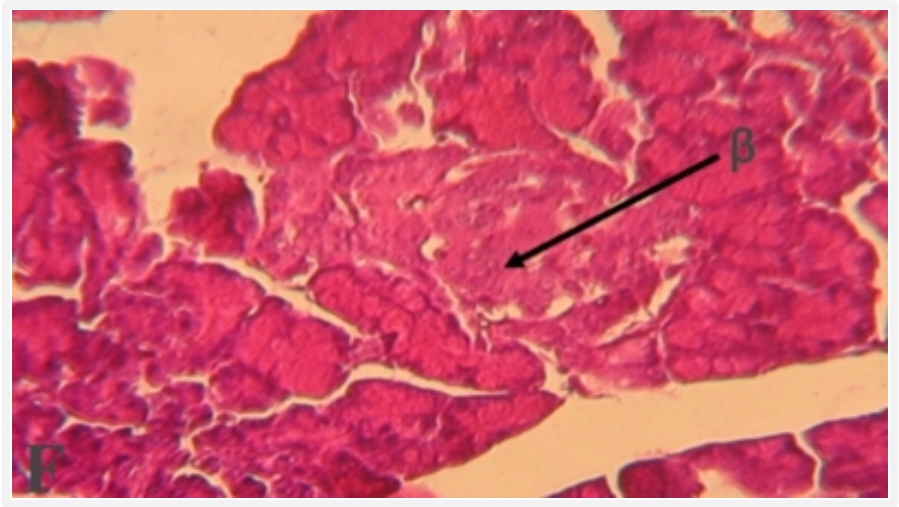
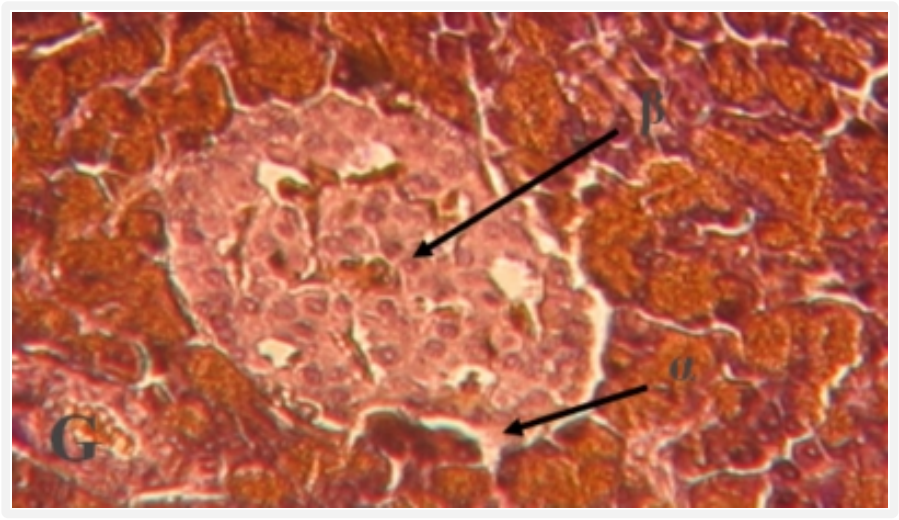
Figure 6



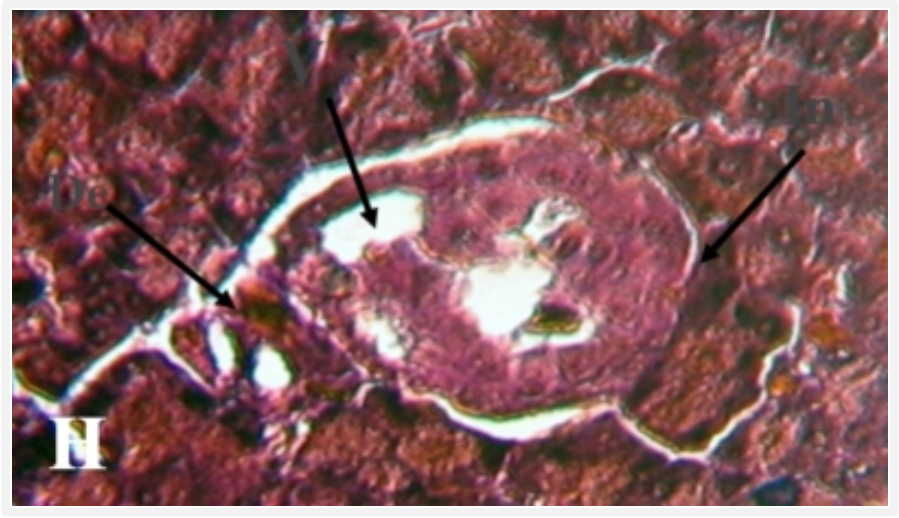
Photomicrograph of a normal (control) pancreatic islet showing cluster of β-cells which are centrally placed and peripherally placed α-cells. (Gomori Aldehyde Fuchsin X2200)

Figure 7



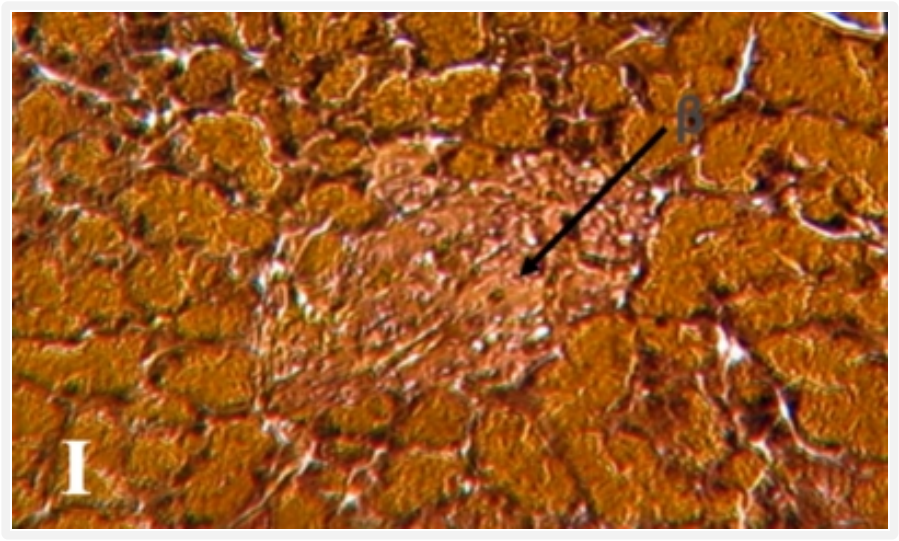
Photomicrograph of pancreatic islet of streptozotocin-induced diabetic rats showing degranulation (De) of β-cells and severe vacuolation (V) of the pancreatic islets Insulitis (In) is also detectable in the islets. (Gomori Aldehyde Fuchsin X2200)

Figure 8



Photomicrograph of pancreatic islets of A.muricata treated diabetic rat showing recovery of the β-cells. As it is evident, the islet cells are regenerated, the inflammatory infiltration has disappeared and there is reduction in the vacuolation cased by administration of STZ (Gomori Aldehyde Fuchsin X2200)

Figure 9



Plates A, D, G and B, E, H represent three islets of Langerhans from normal and STZ-induced diabetic rats, respectively. Comparison of these two groups of plates clearly indicates the reduction in the number of pancreatic islets as well as their number of β-cells in the diabetic rats. As it is evident from plates B, E and H, the islets are irregularly shaped, relatively small and atrophic. Most cells of the islets are small, degranulated and dark with scanty cytoplasm.

Severe vacuolation and degranulation are present in the β-cells of a number of islets. Insulitis (inflammation of the islets) is also detectable in the islets (plates B, E and H). An exudate predominantly of lymphocytes, with a few macrophages and neutrophils is evident within and around the affected islets. However, compared to the untreated diabetic rats, histophatological examination of the plant A. muricata extract- treated diabetic rats revealed an increase in the number of pancreatic islets and the number of β-cells, along with a reduction in the number of initiated lymphocytes and macrophages (Plates C, F, and I). In other words, the plant extract treated diabetic samples histopathologically approach the corresponding healthy pancreatic samples. The regeneration of the β-cells of the STZ-destructed islets is probably due to the fact that pancreas contains stable (Quiescent) cells which have the capacity of regeneration. Therefore, the surviving cells can proliferate to replace the lost cells (Kumar et al, .1992, Govan et al, .1986)

Discussion

Diabetes is a metabolic disorder affecting carbohydrate, fats and protein metabolism. A worldwide survey reported that the diabetes affect nearly 10% of the word population (Kar et al., 1999). It is likely to remain a significant threat to public health in the years to come. In the absence of effective and affordable intervention for either type of diabetes, the frequency of the disease will escalate worldwide, with a major impact on the populations of the developing countries (Marix, 2002). In modern medicine, the beneficial effects of standard medications on glycemic levels are well documented; the preventive activity of medications against the progressive nature of diabetes and its complications was the modest but not always effective. Insulin therapy affords glycemic control in type 1 diabetes, yet its shortcomings such as ineffectiveness on oral administration, short shelf life, the requirement of constant refrigeration, fatal hypoglycemia in event of excess dosage, reluctance of patients to take insulin injection and above all the resistance due to prolonged administration limits its usage (Kasiviswanath et al., 2005). Similarly, treatment of type 2 diabetes patients with sulfonylureas and biguanides is always associated with side effects (Grandhipuram et al., 2006). Hence, search for a drug with low cost, more potential and without adverse side effect is being pursued in several laboratories around the world.

For various reasons in recent years, the popularity of alternative medicine has increased. Surveys conducted in Australia and United States indicate that almost 48.5% and 34% of the respondents respectively had used at least one form of unconventional therapy, including herbal medicine (Eisenberg et al., 1993). The World Health Organisation has also recommended evaluation of effective plants for conditions save modern drugs (Chattopadhyay et al., 1999). This has led to the increasing demand for herbal products with anti diabetic activity and fewer side effects (Kim et al., 2007).

Streptozotocin-induced hyperglycemia in rats is considered a good model for the preliminary screening of agents active against type II diabetes (Ivorra et al., 1989) and is widely used. Generally, destruction of β-cells starts three days after STZ administration and reaches its peak at three to four weeks in rats (Adeghate, E., and Ponery, 2002). Streptozotocin-induced diabetes in laboratory animals has been widely used for research on diabetes and its long-term complications. Control animals in these studies are usually injected with citrate buffer solution. However, STZ is known to possess pharmacological effects other than its diabetogenic properties (Schein et al.,1974), and extra pancreatic actions of streptozotocin cannot be excluded. The presence of GLUT2 in liver and kidney might explain the long-term complications seen with hepatic and renal tumors in rats treated with streptozotocin (Simon and West, 1991; Delahunt et al., 1995). Because of the extra pancreatic effects of streptozotocin, it may be difficult to distinguish effect secondary to diabetes from those secondary to streptozotocin per se. Streptozotocin is a potent DNA methylating agent and act as a nitric oxide donor in pancreatic islet cells. Although, the β-cell cytotoxic action of STZ is not fully understood, it is thought to be mediated by the inhibition of free radical scavenger enzyme thereby enhancing the production of superoxide. The latter has been implicated in lipid oxidation, DNA damage and sulfhydryl oxidation.

β-cells are particularly sensitive to damage by nitric oxide and free radicals because of their low levels of free radical scavenging enzymes (Spinas, 1999). In this present study, almost all the insulin-producing β-cells were degranulated, degenerated or necrosed in the streptozotocin treated rats leading to a decreased in insulin secretion and an increase in the blood glucose concentration. However, treatment with extracts of A. muricata shows a significant antihyperglycemic activity in STZ-induced diabetic rats at the end of the experiment. It has been suggested that bioactive compounds from plants sources having antihyperglycemic activities might act by several mechanisms such as stimulating insulin secretion, increasing repair or proliferation of β-cells and enhancing the effects of insulin and adrenalin (Shanmugasundaram et al., 1990; Fayed et al., 1998). The result of this present study indicated that decreased in the blood glucose concentration of diabetic rats by A. muricata treatment is due to the regeneration/proliferation in the pancreatic β-cells.

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