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RESEARCH ARTICLE

ASSESSMENT OF MORPHOLOGICAL CHANGES IN PANCREATIC ISLETS OF DIABETIC WISTAR RATS BY ARTEMISIA HERBA ALBA TREATMENT

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ABSTRACT

The aim of this study is to evaluate the induced histological changes in the Islet of Langerhans and β-cells. The ethanolic extraction of Artemisia Herba Alba (AHA) as 20 mg has been injected intraperitoneally (I/P) in induced diabetic (*Injected rats intraperitoneal with a dose of 60mg/kg streptozotocin* STZ) and normal Wistar rats. Three diabetic groups were selected, each consists of eight rats. Group one injected (I/P) with distilled water only; used as diabetic control, group two was injected with insulin subcutaneously (S/C), group three injected with Ether-extract of AHA (I/P), and group four selected from non diabetic rats, used as normal control injected (I/P) with distilled water. All groups were fostered for 21 day; then weighted and executed. The pancreases were taken and the histological preparations for haematoxylin & eosin (H&E) and immunohistochemistry have been done. The analyzed results showed that: the induced diabetes reduced the rats' weight by 62.5% in average while the treatment by Insulin and AHA regain the weight up to 75% and 71.4% respectively. The induced diabetes reduced the β-cells% per islet 56.8% while the treatment by Insulin and AHA increase the β-cells% per islet 73.8%, and 73.4% respectively. The induced diabetes reduced the islet volumes from 1990 mm^3 to 766.8 mm^3, while the treatment by insulin and AHA regain the volumes up to 1730 and 1590 mm^3 respectively. The microscopic results confirmed that: AHA stimulate and regenerate new β-cells in islets of langerhans thus leading to increase the volume which enhancing the host weight gain.

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INTRODUCTION

Diabetes mellitus has been considered as endemic and chronic endocrine disease worldwide, with two types as: type-1(*Diabetes mellitus of insulin-dependent diabetes mellitus (IDDM)*) which is characterized by blood hyperglycemia due to deficiency in insulin production by the β-cells of the islets of the pancreas (Knip *et al*., 2005) and type-2 (*Non-insulin-dependent diabetes mellitus* NIDDM) which may be due to ineffective use of insulin by the body cells resulting in high levels of blood glucose (Kameswara Rao *et al*., 2003; Feldman *et al*., 1997). Many causative and induction factors could be blamed; for instance the changes into sedentary life- style, the high consumption of energy-rich diet (carbohydrates and fats), the poverty and the ignorance could led to increase incidences of the disease worldwide. It was reported that 382 million were diabetic worldwide and 80% were living in low and middle income countries (Guariguata *et al*., 2014). The worldwide prevalence of diabetes for all age groups was estimated to be 2.8% in 2000 (200 million) mostly were young adult (171 million) (WHO, 2015) and it is expected to increase to 5.4% by year 2025 (Moller and Flier, 1991; Rao *et al*., 2010; Sarah Wild

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et al., 2004) in addition to its morbidities and consequences that involving cardiovascular, urinary and nervous; diabetes has been estimated to be as the major killing diseases in the coming 25 years and as the 7th leading cause of death in 2030 (WHO, 2015; Sharma *et al*., 1993) Asia's and Africa's rural areas have been identified as greatest potential of the disease, which could rise up to two or three fold above the estimated level (Hwang *et al*., 2012; Muninarayana *et al*., 2010; Singh *et al*., 2012; Ulvi *et al*., 2009). The recent study by Mohammed *et al*. (2014) in Sudan, revealed that: diabetes has been as endemic disease in central Sudan (Khartoum & Jazeera) representing 55% and in the west of Sudan representing 38% relative to other regions. It represents 7% of all hospital admissions and 10% of general mortality (Ahmed and Ahmed, 2000) and it has been arising from 9.3% in 2010 to an expected 10.6 % in 2013, in four Sudan states (Balla *et al*., 2014; Siham Ahmed Balla *et al*., 2014). The formal utilizes drugs to treat diabetic patients are the *Insulin* for type-1 or the oral hypoglycemic drugs like *Alpha-glucosidase inhibitors* which inhibit glucose absorption from intestine (UBS Warburg Report, 2001), *Sulphonylureas* act via blocking ATP-dependent potassium channels that result in release of endogenous insulin from beta cells (Trube *et al*., 1986), *prandial glucose regulators* act via inactivating ATP-dependent potassium channels & affect other receptors (Owens, 1998), *Glitazones* that stimulate glucose uptake in muscle and liver via activation of Peroxisome Proliferator Activated Receptor-gamma (PPAR-gamma) (Kim *et al*., 2004) and *Biguanides* act by inhibition of glycolysis and gluconeogenesis in the liver, reduction of glucose absorption in intestine and increase of glucose sensitivity, uptake and utilization in the muscle (Gandhipuram *et al*., 2006). However all these types of drugs dealing with the signs and symptoms regardless to the inevitable occurring serious complications of the disease in addition to their own side effects, high cost and rarity (Kameswara Rao *et al*., 2003; Rao *et al*., 2010). The shortage of these medication open the trend of research to find and reveal other alternative drugs which deals with adjuvant treatment from nature, with low cost, greater potential, and no adverse side effects. A surveys conducted in Australia and the 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*., 1985). Based on the recommendation of WHO that deals with further evaluation for the folkloric methods of managing diabetic disease due to its high mortality and morbidity (Chattopadhyay, 1999), and the state that: 65-80% of world population in developing counties depends on plant for their primary health care due to scarcity and lack of access to modern medicine (Calixto, 2005), African traditional societies are highly valued and utilized phytotherapy which now is recommended for many diseases and for the treatment of diabetes throughout the world (Venkatesh *et al*, 2003), the researchers trend is to assess the morphological changes in pancreatic Islets of diabetic Wistar rats by Artemisia Herba Alba (AHA) (*Is medicinal and aromatic dwarf shrub known in Arabic as shih and in French as Armoise Blanche. This plant grows wild in arid areas of the Mediterranean basin, in North Africa and certain parts of Asia and Middle East extending into northwestern Himalayas and in Spain* (Salido *et al*., 2004; Dunn *et al*., 1996), in view of (*revealing the induced morphological changes in β-cells, determine the percentage of β-cells per islets, compare the weight gain for diabetic rats relative to Artemisia Herba Alba and compare between the volume of the islets of Langerhans gain for Artemisia Herba Alba*). AHA is one of the herbs traditionally used as an anti diabetic drug for its hypoglycemic effects and also for many other medical uses (Mehmet Iriadam *et al*., 2006; Seddiek *et al*., 2007; Mobarak *et al*., 2008; Mohamed *et al*., 2010). The general actions of anti diabetic herbal drugs have been revealed by some scholars, which are acting as; *α*-Glucisidase or *α*-Amylase inhibitor (Kim *et al*., 2011) or increases insulin secretion (Si *et al*., 2010; Lee *et al*., 2006) or stimulate *β*-cell regeneration by proliferation of its precursor or cells in the pancreatic duct (Si *et al*., 2010; Rosely *et al*., 2004). Scientists proposed that β-cell mass is in a dynamic process because of its significant capacity for adaptation to changes in insulin demand (Bonner-Weir, 2000). Increase in β-cells 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). Some anti-diabetic plant drugs are reported to have a regenerative capacity for the pancreatic β-cells. The extract of the plant Chard (Beta vulgaris *L. Var. Cicla*) which is used as a hypoglycemic agent by diabetic patients in Turkey it reduces blood glucose levels by the regeneration and increases the number of β-cells of Langerhans islets (Bolkent *et al*., 2000).

MATERILS AND METHODS

The following experimental work has been accepted and approved by the scientific research and ethical committees in Al-Neelain University in 2012.

The Drugs

- Insulin: long – acting insulin, ultralente humulin U (lily), 0.5 ml/rat, S/C.
- Herbs: Aretemisia Herba Alba was purchased from Khartoum medical herbs market. They were finely grinded, packed and subjected to 70% ethyl alcohol extraction. The extract was left to dry and dissolved as 20 mg/Rat.

WISTAR RATS: sixty four (64) wistar Rats, of the same ages (6 weeks old) were brought from the animal house of the faculty of medicine - Sudan. For adaptation, the rats (8 Rats i.e. 8 groups) were kept one week before commencing the experiment in a well prepared research room, with access to enough food and water. Then 48 of the rats were fasted for 6 hours before their intra peritoneal injection with 60 mg/ kg of streptozitocin (*STZ, has a chemical formula of $C_8H_{15}N_3O_7$, 265 g/mol which implies nitrosourea moiety with a methyl group attached to one end and a glucose molecule at the other end* (Dolan, 1997) to induce diabetes mellitus, 16 rats were left to select the normal control from them. Five days later, the blood glucose has been measured (*using - i care TD-4279 - Blood Glucose Monitoring System*) and the weight of the STZ injected rats, those rats with blood glucose level more than 320 mg/dl were considered as diabetic and randomly divided into three groups each with 8 Rats, and 8 rats from the non injected, non diabetic ones were also randomly selected and their measured blood glucose was (70-100mg/dl), then the weight for all groups was checked. The total number of the Rats that used to complete the experiment was (32), while the remaining

was removed to be used for other running experiment. The nominated groups were as follow: Group A: as diabetic treated with insulin subcutaneous, with a dose of 0.5 ml of insulin =1 iu, for 21 day. Group B: as diabetic treated with Artemesia Herba Alba intraperitoneal (I/P) with a dose of 20 mg/Rat, dissolved 2 ml distilled water, for 21 day. Group D: diabetic, injected (I/P) daily with 2 ml of distilled water (diabetic control) for 21 Day. Group E: non-diabetic, injected (I/P) daily with 2 ml of distilled water (normal control) for 21 day.

Five days later the blood glucose for all groups was estimated and it showed considerable drop, in the drug treated groups (group A & B) ranging from 120 to 160 mg/dl. While the diabetic control (group - D) was still hyperglycemic, blood glucose was 290 mg/dl and more relative to the normal control (group E - non diabetic, none treated) which was (70 – 110 mg/dl). In the last day (day 23rd.), all animal groups (A,B,D&E) were weighed, anesthetized by ether inhalations, then abdomens were operated, the pancreases were removed, and their volumes were measured by water displacement method and immersed in bottles of 10% buffered formalin (fixative) to be used for general histology (H&E) and immunohistochemistry.

Histological Techniques

Pieces from the upper part of all pancreatic heads were fixed in 10% buffered formalin. One day later all pieces were automatically processed, sectioned, 5 micron thickness, 6 slides from each piece were prepared for staining, using heat resistant slides.

HEAMATOXYLIN & EOSIN (H & E) STAINING

2 slides from each pancreatic piece were stained with H&E to study, the average densities, length, area and the volume of islets in equal field areas, using light transmitted microscope (LEICA DMD 108) attached to digital camera and computer.

IMMUNOHISTOCHEMICAL STAINING

- Primary anti insulin (G.pig primary anti body to insulin - Ab7842 500µL "0.2mg/ml,, - G.pig pAb to insulin – abcam) was used according to the manufacturer protocol, to detect the □- cell distribution, and percentage relative to the islets of Langerhans in the studied groups. To detect any new distribution of □- cells outside the islets
- Primary anti glucagon (Rabbit primary anti body to glucagon) (ab 80 55, 125µL, Rb. P Ab to glucagon- abcam) was used to detect the alpha cell distribution and percentage relative to the islets of Langerhans in the studied groups.

RESULTS

The results deal with the rats after close care and observation for 21 days and operated were the rat's weight after induction of diabetes and after administration of insulin drugs and AHA, the volume of islets in mm^3 and the β-cells% per islet as well as the confirmation section of the transmitted light microscope for the transformed pancreatic duct to β-cell and acinar cells transformed to β- cell.

DISCUSSION

Based on the accumulated knowledge that: diabetes used to reduce the host weight, hence as a confirming obtained data in this study that the induced diabetes in rats by STZ which raised their blood sugar greater than 320 mg/dl in average also reduced the rats weight from 100% down to 37.5% i.e. 62.5% as a missing weight as shown in Figure (1). However the treatment of rats by insulin administration resulted in body weight regain up to 75% while the foresee drug under estimation AHA regain the rats body weight from 37.5% up to 71.4%. Such decrement and increment in body weight could be ascribed to destruction or degeneration of β-cells (*factories of insulin*) by the act of STZ and the regeneration of same cells in case of insulin and AHA administration respectively (Ikebukuro *et al*., 2002).

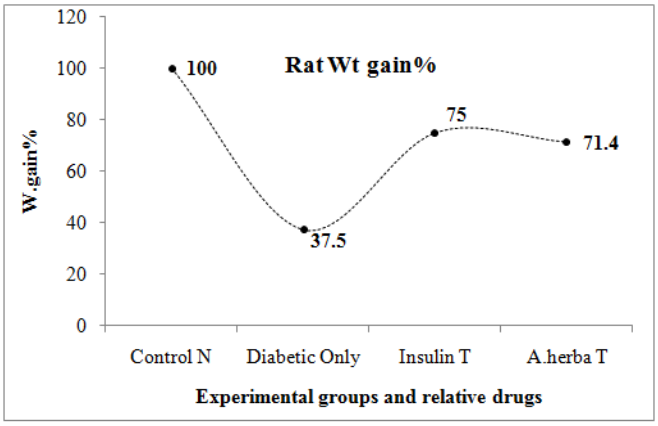

Figure 1. Shows the rats' weight after induction of diabetes by STZ and the relative weight after treatment by insulin and AHA

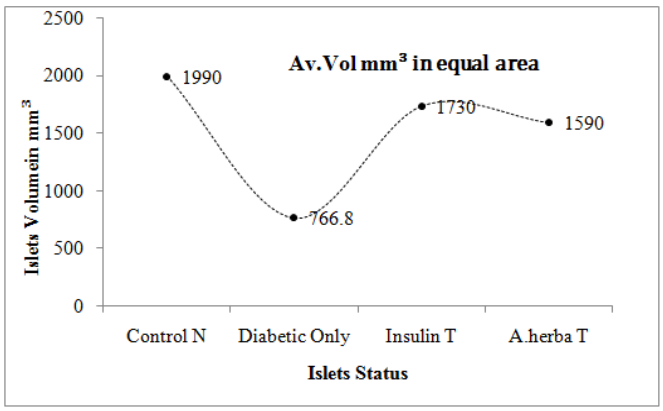

Figure 2. Shows the rats' islets volume in mm^3 after induction of diabetes by STZ and the relative volume after treatment by insulin and AHA

The increment of β-cells could be obviously confirmed by the increment in rat's islet volume which was 766.8 mm^3 (*diabetic non-treated rats*) that raised up to 1730mm^3 and 1590 mm^3 in case of insulin and AHA treatment relative to 1990 mm^3 for control group as shown in Figure (2). In comparison to this fact, only some other herbs have been confirmed as anti diabetes; while AHA is considered as new fact showing the anti diabetes potency acting specifically in regeneration of β-cells. In the same trend, Figure (3) shows the rats' β-cells% per islet after induction of diabetes by STZ and the relative β-cells% per

islet after treatment by insulin and AHA. Also it revealed that: β-cells% per islet have been reduced due to effect of STZ from 73.5% to 56.8%, while the effects of insulin and AHA treatment increased β-cells% per islet from 56.8% up to 73.8% and 73.4% respectively. This impressive fact has been confirmed by the immunohistochemistry section of diabetic rat pancreas treated with AHA, studied by lighted transmitted microscope, which revealed that: an obvious transformed pancreatic duct to β-cells (Figure 4-a) and high lymphocytic infiltration with β-cells between the pancreatic duct and the lymphocytes (arrows) (Figure 4-b).

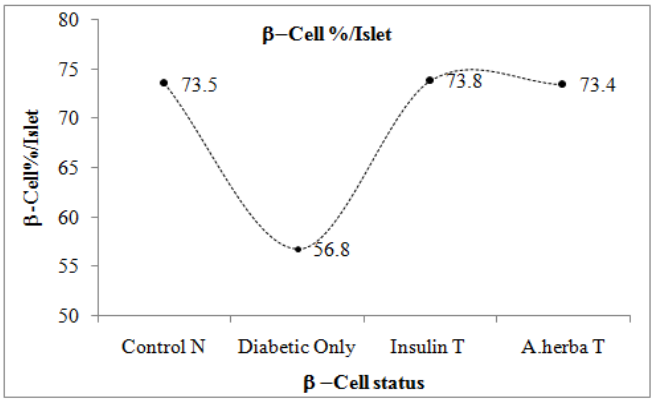

Figure 3. Shows the rats' β–χells% per islet after induction of diabetes by STZ and the relative β–cells% per islet after treatment by insulin and AHA

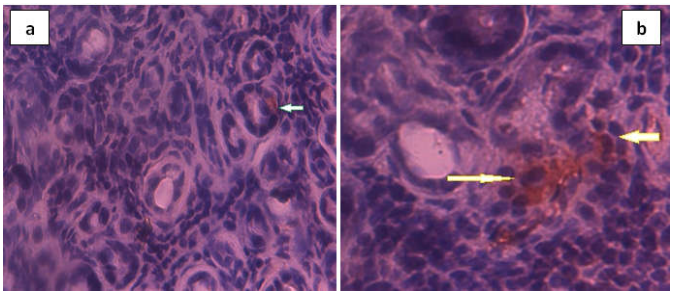

Figure 4. Shows light microscope of immune histo chemistry section of diabetic rat pancreas treated with AHA, (a) a transformed pancreatic duct to β-cell, (b) shows high lymphocytic infiltration with β-cell between the pancreatic duct and the lymphocytes (arrows)

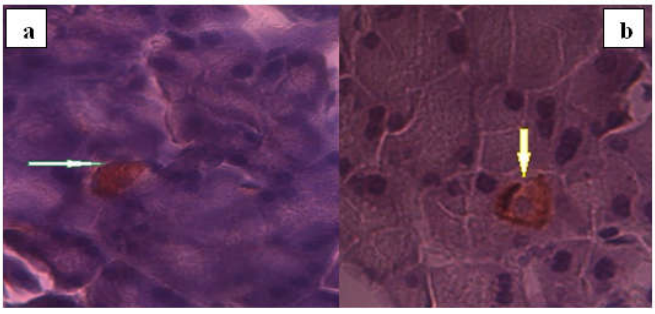

Figure 5. Shows light microscope of immunohisto chemistry section of diabetic rat pancreas treated with AHA that reveal (a) one β-cell between pancreatic acini and (b) one of the acinar cells transformed to β- cell

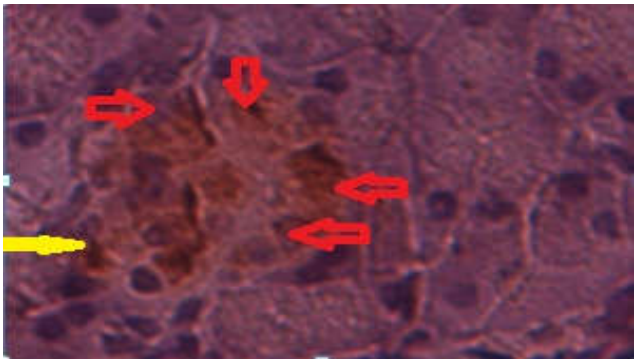

Figure 6. Shows light microscope of immunohisto chemistry section of diabetic rat pancreas treated by AHA which reveal the entire of the acinar cells transformed to β-cells as shown by red arrows and detached β-cell out of the acinus as shown by yellow arrow

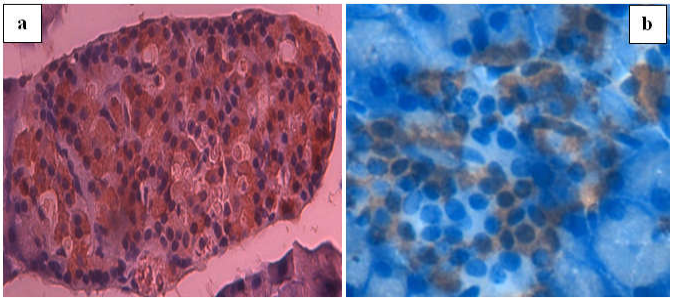

Figure 7. Shows light microscope of immunohistochemistry section of diabetic rat pancreas treated by AHA (a) large islet of langerhans with many β-cells compared with (b) non treated diabetic rat pancreatic section saturated by counter stain

As well Figure (5) shows a section of diabetic rat pancreas treated with AHA that reveal one β-cell between pancreatic acini (Figure 5-a) and one of the acinar cells have been transformed to β-cells (Figure 5-b). while in Figure (6) the entire of the acinar cells of diabetic rat's pancreas treated by AHA, have been transformed to β-cells as shown by red arrows and the detached β-cell out of the acinus as shown by yellow arrow. The increasing effect in the volume of islet is being ascribed to active transformation of acinar cells, as this process has been shown in Figure (7), where there is large islet of langerhans with many β-cells compared appeared in the section of diabetic rat's pancreas treated by AHA (Figure 7-a) in contrast with non treated diabetic rat pancreatic section which possess fewer number of β-cells (Figure 7-b). AHA showed great success in regeneration of β-cells which in turn deal with insulin production to fight against diabetes, however there is some adverse effects have been mention by Khataibeh and Daradka, (2007) which showed that: the exposure of Sprague-Dawley rats to AHA in duration of 12 weeks resulted in a reduction in the percentage of pregnancies and in the number of implantation sites as well the treated testicular of the rats showed a decrease in number of spermatocytes and spermatids ($p < 0.01$) (Almasad *et al*., 2007), such adverse effects open up the further studies in the herbal medicine extraction and economic commercialization for poor diabetic population worldwide.

Conclusion

As it has been proved that AHA has impressive potency to act against diabetes by formation of β-dell in the islets of langerhans, it is worth to be recommended for further studies so as to have well established utilizable anti-diabetic drug extracted from AHA and including any side effects related to administration of such herb.

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