

Effect of Aqueous Olive Leaves Extract on the Pancreatic Islets in Rats

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ABSTRACT

The objectives of this study were to investigate the effects of the aqueous crude extract of *Olea europaea* serum glucose level and histopathological changes in islets of Langerhans in an induced-diabetic mellitus in rats. The hypoglycemic activity of Olive leaf extract was investigated in fasting health rats and alloxan–diabetic rats. Blood samples were collected from the tail of rats and serum glucose measured on the 1st, 15th, 30th days of the experiment. A significant reduction in blood glucose of diabetic animal's (350.5 ± 0.0348^c) mg/dl in case of administration of Olive leaves in comparison with diabetic control (373.4 ± 0.0562^a) mg/dl. The present study clearly indicated a significant antidiabetic activity with the Olive leaves and supports the traditional usage for the control of diabetes (Nadar *et al.*, 2003), Aqueous crude extract considered as hypoglycemic agent reduce oxidative stress, indirectly by lowering blood glucose and preventing hyperinsulinemia (Casalino *et al.*, 2002). The study showed islets of Langerhans continuous infarctions and lymphocyte infiltration in alloxan–diabetic rats while the treatment group showed the islets irregularity in size, but they appeared normal with decreased granulation and some islets appeared very small, all of islets appeared normal as of the control animals.

Keywords: Alloxan, Diabetes Mellitus, hypoglycemic plant, Olive leaves, anti- hyperglycemic, antioxidant.

تأثير المستخلص المائي لأوراق الزيتون على جزر لنكرهانس في الجرذان

الملخص

Olea europaea

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30 15

(373.4 ± 0.0562^a)

/100

(350.5 ± 0.0348^c)

/100

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia due to defective insulin action, insulin secretion or both. It is a complex and multifarious group of disorders characterized by hyperglycemia that has reached epidemic proportions in the present century (Noor *et al.*, 2008).

Diabetes Type 2 is characterized by hyperglycemia, peripheral resistance to the action of insulin, and are eventual destruction of insulin producing β -cells in the pancreas (Abu-Zaiton and

Mariam, 2012). Diabetic patients are at an increased risk for cardiovascular disease, blindness, nerve and kidney damage, and limb amputations. Diabetics are also at a greater risk for developing different cancers due to the immunological disturbances induced by aberrant metabolism. Obesity is a major predisposing factor in the development of diabetes. In obese populations, inflammatory molecules produced by adipose tissue and increased circulation of free fatty acid (FFA) play an important role in producing peripheral insulin resistance as well as increasing damage to the insulin-producing β -cells. The main treatments for type 2 diabetes include diet, exercise, and several medicinal plants are used in the management of DM (Akah *et al.*, 2002; Verspohl, 2002 ; Colca, 2006).

Currently, many herbal medicines therapeutic available have been recommended for the treatment of diabetes. Herbal drugs are prescribed widely because of their effectiveness, less side effects and a relatively low cost (Venkatesh *et al.*, 2003).

Olive (*Olea europaea* L.) phenolics are known as powerful antioxidants, both in vitro and in vivo and it is known that olive oil represents a key healthy component of Mediterranean diet (Karakaya, 2009). Not only olive oil, but olive leaf also has different beneficial effects on human health. Compounds obtained from olive leaf extracts (OLE) have been subject to numerous investigations (Nadar *et al.*, 2003), OLE from Mediterranean olive has been commercialized as a food supplement, which can be consumed in the form of tea, syrup and capsules. OLE is well-known for its hypotensive effects and its antioxidant properties, hypoglycemic, cardio protective antimicrobial, radio protective, antiatherogenic, antitumoral, anti-inflammatory, hypocholesterolemic, hepato-protective and anti-viral properties. The main constituent of the olive leaf is the iridoid glycoside oleuropein, which is metabolized in the body to calcium elenolate, which is apparently responsible for many of the pharmacological actions of the olive leaf. Furthermore, the olive leaf contains triterpenes (oleanolic and maslinic acid), flavonoids (luteolin, apigenine, rutin), and chalcones (olivin, olivin-diglucoside). It has been traditionally used in hypertension, arteriosclerosis, rheumatism, gout, diabetes mellitus, and fever. OLE is antibacterial, antifungal, and antiviral. Clearing up systemic yeast is another good start (Abdel-Sattar *et al.*, 2012).

Finally among the phytochemicals that have interest is oleuropein a substance found in the olive leaf, olive oil and olive fruit, a bitter principle of the leaves which was identified as oleuropeoside later designated oleuropein a polyphenolic secoiridoid glucoside. Biophenols are efficient components for the low incidence of cardiovascular diseases. It is potentially a rich source of a diverse range of biophenols with a wide array of biological activities (Obeid *et al.*, 2005). The beneficial health effects of OLE have been mainly attributed to its elevated phenols content (Casalino *et al.*, 2002). Among them, hydroxytyrosol (3,4-dihydroxyphenylethanol) stands out as a compound of high added-value, due to its interesting antioxidant and potential beneficial human health properties (Dudley *et al.*, 2008; Hamden *et al.*, 2009). Preventing human erythrocytes from oxidative damage induced by hydrogen peroxide (Zhang *et al.*, 2008), anti-inflammatory antithrombotic and hypocholesterolemic effects in rats (Covas *et al.*, 2006).

MATERIALS AND METHODS

A. Animal Experiments

Rats of original Wistar strain bred in the central animal house, Department of Biology, College of Education, Salahadden University were used in this study. Experiments were carried out in male rats weighing between 200 and 225 g. Rats provided with a standard diet and water ad libitum. All were kept in cages with wide square mesh at the bottom and maintained in a well-ventilated animal house with 12 h light and dark cycle. Rats were divided into four equal groups of 10 rats each. The first was group used as a control group and this received 1.0 ml of physiological saline orally daily. The second group was injected Intra peritoneal (i.p) by alloxan at a single dosage of 150 mg/kg body weight. The third group was gastro-gavaged with 0.5 g/kg body weight of OLE orally on daily basis. The fourth group was injected i.p by alloxan at a single dosage of 150 mg/kg body weight and

gastro-gavaged with 0.5 g/kg of OLE orally on daily basis after 5 days of alloxan injection (after induction of experimental diabetes). The experimental period was extended to thirty days. Fasting blood glucose (FBG) levels were determined weekly during the treatment period (Aruna *et al.*, 1999).

B. Induction of experimental diabetes

Animals were rendered diabetic by treatment with alloxan intravenously in a daily dose of 150 mg/ kg dissolved in normal saline and injected intravenously by 1.0 ml disposable syringe, alternatively a 20 % solution of glucose injected intraperitoneal after 4-6 hr. repeat this process 5 days. After 7 days when the condition of diabetes was stabilized rats with blood glucose range of 200-300 mg/dl were selected for the study (Tamara *et al.* 2010).

C. Histopathological study

A portion of pancreatic tissue was dissected out and fixed in 10% buffered neutral formal saline and processed. After fixation tissues were cut at 5 μ m and stained with hematoxylin and eosin. The sections were examined under optical microscope (Olympus microscope, Letiz Watzler, Germany) and photomicrographs were taken by digital camera (Sony DSC-W30, Korea). The total magnification of the microscope is calculated from the magnifying power of the objective multiplied by the magnification of the eyepiece and, where applicable, multiplied by original magnifications (Sanarya *et al.*, 2011).

D. *Olea europaea* Leaves

Leaves of *Olea europaea* were collected from olive farms in Erbil city. The leaves were cleaned and washed with distilled water and dried at room temperature of 25 C⁰ in the laboratory, the dried leaves were powdered in an electrical grinder and the powder was kept in plastic bags until used (Sanarya *et al.*, 2011).

E. Preparation of the Aqueous Extract

The aqueous extract of olive (AEO) was prepared by soaked 50gm of the powdered material in one liter of distilled water and kept overnight in shaking incubator at room temperature. The extract was filtrated after 24 hours and the filtrate was evaporate at a 40 C⁰ under reduced pressure in a rotary evaporator and the aqueous preparation of the residue was kept in the refrigerator until used in the experiment. The OLE was suspended in distilled water and administered orally (0.5 g/kg) body weight (Yuanfeng *et al.*, 1996).

F. Statistical Analysis

A statistical analysis was carried out by ANOVA after multiple comparisons Duncan's test. The data were expressed as mean \pm S.E, P < 0.05 considered a statistical difference.

RESULTS

In the present study, the table (1) shows mean values of changes in body weight in the treatment and control groups. Administration of olive leaves extract had increased the body weight.

Table 1: Mean values of body weight of the rats groups (G)

groups	Initial body weight (g)	Final Body weight (g)
Normal control	224.7 \pm 0.8570 ^d	276.8 \pm 0.4163 ^a
Diabetic control	205.6 \pm 0.0875 ^b	202.2 \pm 0.2802 ^c
Normal+ Olive leaves	206.9 \pm 0.1828 ^c	255.2 \pm 0.4158 ^d
Diabetic+ Olive leaves	163.6 \pm 0.1045 ^a	172.4 \pm 0.4010 ^b

Table 2: Blood glucose value(mg/dl) of normal and experimental animals.

groups	0 Day	15 Day	30 Day
Normal control	118.5 \pm 0.3601 ^d	121.5 \pm 0.1374 ^b	109.7 \pm 0.0395 ^c
Diabetic control	216.4 \pm 0.3173 ^c	413.3 \pm 0.1552 ^d	373.4 \pm 0.0562 ^a
Normal+ Olive leaves	197.9 \pm 0.1390 ^b	107.0 \pm 0.4582 ^a	103.0 \pm 0.1425 ^d
Diabetic+ Olive leaves	104.1 \pm 0.0258 ^a	369.3 \pm 0.2148 ^c	350.5 \pm 0.0348 ^b

The same letters mean no significant differences.

The different letters mean significant differences at p < 0.05.

Values are given as mean \pm S.D. for ten rats in each group. OLE produced a dose-maximum reduction in blood glucose at the 15th day of experiment in comparison with normal control (109.7 ± 0.0395^c) mg/dl Table (2). Dose-dependent reduction in blood glucose was also observed in alloxan-induced diabetic rats treated with OLE. The percent reduction in blood glucose tended to be higher in the diabetic condition compared to the normal state.

A significant reduction in blood glucose of diabetic rats (350.5 ± 0.0348^b) mg/dl in case of administration of OLE in comparison with diabetic control (373.4 ± 0.0562^a) mg/dl Table (2).

HISTOLOGICAL OBSERVATIONS

The pancreatic islets histopathology in alloxan-OLE group showed variation in size and shape of cells associated in filtrations mononuclear inflammatory cells pancreatitis associated necrosis of epithelium lining acini with sever congestion of blood vessels (Fig. 3,4) in comparison to the animals of the control group showed normal structures during the stages of experiment (Fig. 1). Also the alloxan -OLE group on 15 day showed mild to moderate expansion of pancreatic islets and absence of dilations with prominent hyperplastic islet (Fig. 5,6). On the day 30, the alloxan-OLE islets irregularity in size, but they appeared normal with decreased granulation group and showed that some islets appeared as very small, few cells (Fig. 7,8).

At the end of the experiment, the normal groups and some of alloxan groups showed the regular of the pancreatic islets shape associated expansion and dilated respectively (Fig. 1, 9).

DISCUSSION

The present study illustrates the blood glucose lowering properties of the leaves of *Olea europaea* that focused onto the hypoglycemic property of this traditional plant. alloxan causes massive reduction in insulin release, through the destruction of the β -cells of the islets of Langerhans, it is interesting to note that the aqueous extract of *Olea europaea* at the dose (0.5 g/kg) effectively prevented the increase in blood glucose levels. Since Alloxan induces diabetes by destroying β -cells, in the present study on OLE showed a marked antihyperglycemic effect. It is important to notice the significant increase in blood glucose levels of the alloxan diabetic rats and blood glucose levels of the alloxan treated daily with aqueous extract animals showed a significant decrease compared to the alloxan diabetic rats. This decrement was seen significantly on the second and fourth week. The results of this study reveal that a continuous administration of OLE for 30 days prevents elevation of the glucose level in blood. These results are in agreement with those of (Cardinal *et al.*, 2001) since they showed that there was a significant decline in blood glucose levels on day 21 of the STZ diabetic rats daily treated with blue berry in Italy. The present study is in agreement with the hypoglycemic effect of the *Ziryphusspina*-Christi, a plant used in Egyptian folk medicine, this plant reduced blood glucose levels in STZ diabetic rats (Nesseem *et al.*, 2009). The persistent hyperglycemia can be explained by the absence of insulin production in the destroyed β -cells. The significant increase in alloxan diabetic animals compared to control animals agrees with the results that are similar to those obtained by (Kumar *et al.*, 2009) who used the aqueous extract of *Artemisia herbal alba* which is widely used in folk medicine at a dose of 0.39g/kg body weight for 2-4 weeks where found a significant reduction in blood glucose levels of the diabetic animals. This study reported a further reduction in blood glucose level at $p < 0.05$ during the treatment with plant extract for four weeks. The present work had indicated that the aqueous crude extract of OLE at the dose used, significantly reduced blood glucose level in Alloxan diabetic rats. It is still unclear by which mechanism does OLE produce its effect on blood glucose. It is possible that the plant may reverse the catabolic features of insulin deficiency, decrease the release of glucagon or increase that of insulin, stimulate directly glycolysis in peripheral tissues increase glucose removal from blood or reduce glucose absorption from the gastrointestinal tract.

During the first week, the alloxan diabetic group showed β -cell degranulation, which might occurred as a rapid event following alloxan administration which agrees with the study of

(Guillausseau *et al.*, 2008). β -cell degranulation is caused mostly by the deposition of glycogen, the presence of glycogen seems to be related to the height and duration of hyperglycemia (Zunino, 2009) while islets of the alloxan – OLE group showed regular shape islets with less degranulation which means that not all islets or all β -cells are affected at the same rate since small area of the islet showed almost normal picture. Eventually, islets of hyperglycemic rats were significantly altered with their overall size gradually increasing with the duration and severity of the hyperglycemia. By the end of the second week, islets of the alloxan diabetic animals showed absence of dilations with prominent hyperplastic islet and this continued to the last week, those islets appeared regular in shape in comparison with the control group.

In a study of Juvenile diabetes (Okoli *et al.*, 2010) elucidated that islets appear hypertrophied, therefore they showed regularity in shape with hypertrophied cells and no regular shape nuclei, or appear with atrophy, which shows irregularity in shape with atrophied cells and dense nuclei, followed by small thickening of the connectivetissue between cells. Those islets were more active than hypertrophied islets, also in the alloxan -OLE group islets showed normal shape similar to control islets. In the last week, alloxan diabetic islets showed insulinitis associated mostly with lymphocyte infiltration which has been noticed in chronic juvenile diabetes similar to those of (Selvan *et al.*, 2008).The increase in lymphocyte cells has been produced by a simple immune response which causes necrosis of islets or anti insulin serum injection, and small doses of the drug alloxan. The present study has illustrated the effect of OLE on the pancreatic islets and correlated changes observed to the levels of blood glucose. It is possible that OLE may have a direct or an indirect effect on insulin release it may be able to help diminish the complications of diabetes and support the traditional usage to control of diabetes.

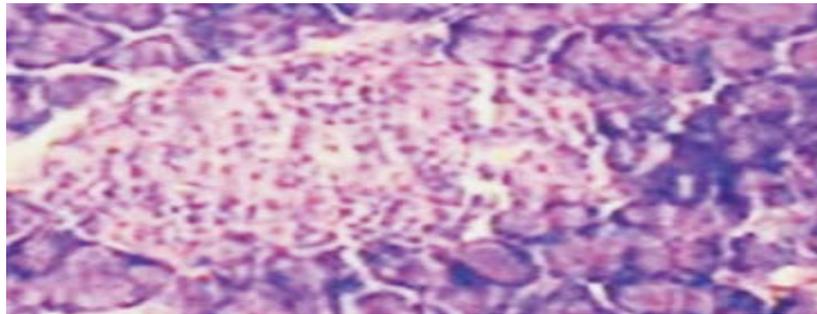


Fig. 1: Normal control rat showed presence of normal pancreatic islet cells (H&E 400X).

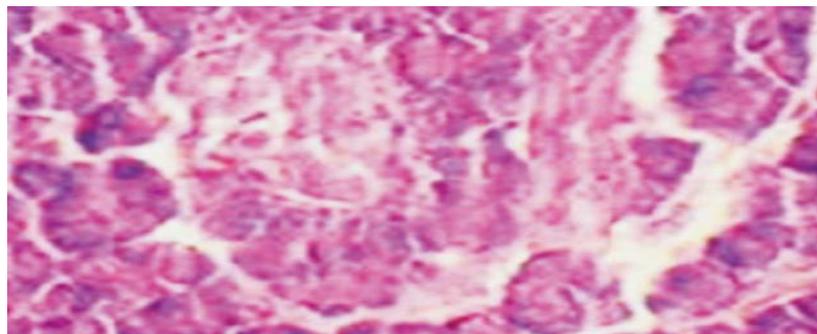


Fig. 2 : Diabetic control rats showed expansion and dilated islet cells (H&E 400X).

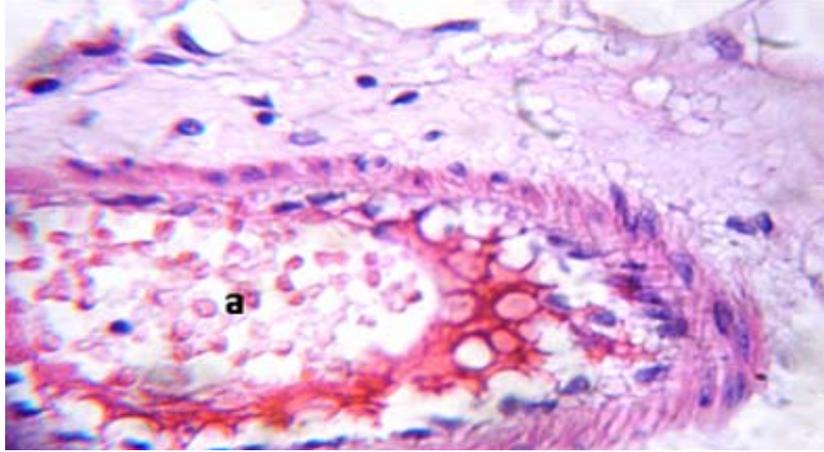


Fig. 3 : Diabetic + OLE (0.5 mg/kg) after two weeks of treated rat, showed congestive of pancreatic artery (a), (H&E 165X).

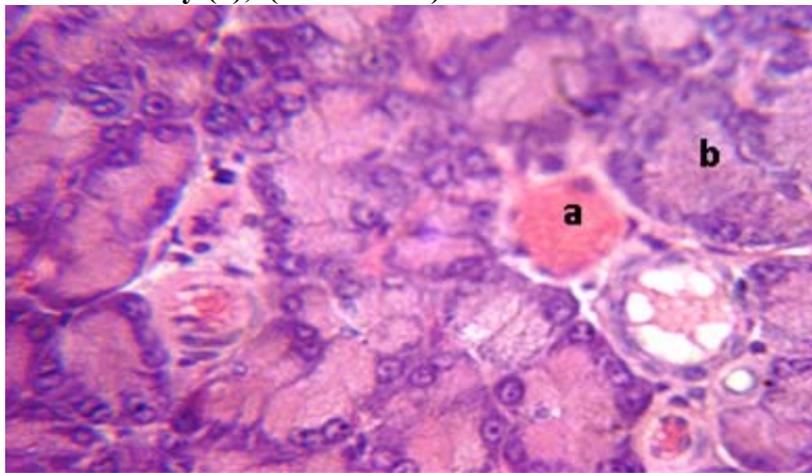


Fig. 4: Diabetic+OLE (0.5mg/kg) after two weeksof treated rat, showed congestive of pancreatic artery (a), associated with accumulation of zymogene granule in acini (b), (H&E 165X).

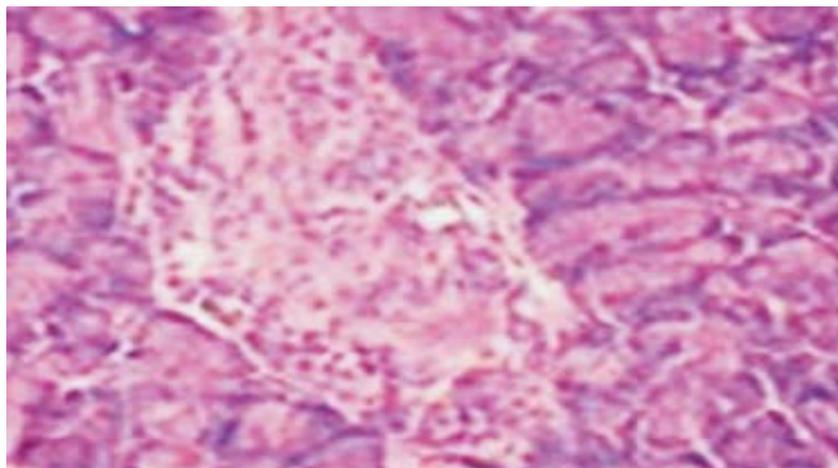


Fig. 5: Diabetic + OLE (0.5 mg/kg) after two weeks of treatment showed mild expansion and absence of dilations (H&E 400X).

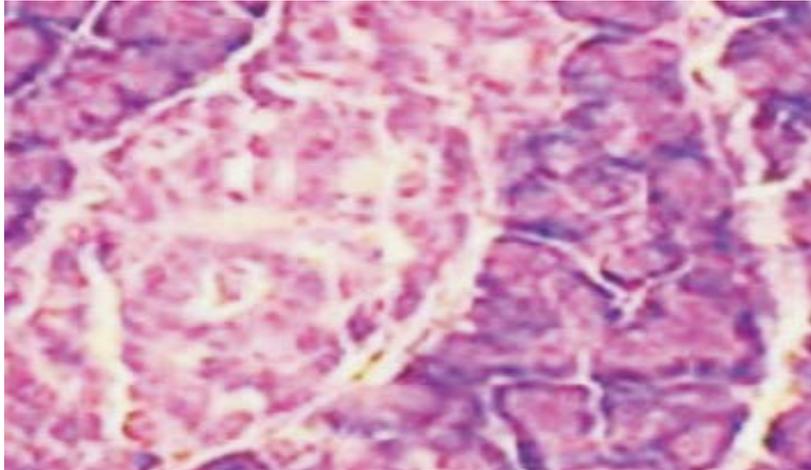


Fig. 6: Diabetic + OLE (0.5 mg/kg) after two weeks of treatment showed a moderate expansion of pancreatic islets, with a prominent hyperplastic islet (H&E 400X).

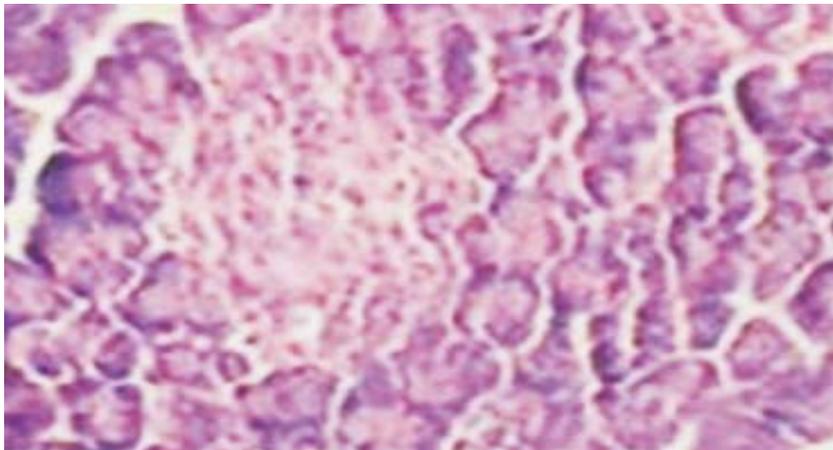


Fig. 7: Diabetic + OLE (0.5 mg/kg) after four weeks of treatment showed an absence of dilation and prominent hyperplastic of islets (H&E 400X).

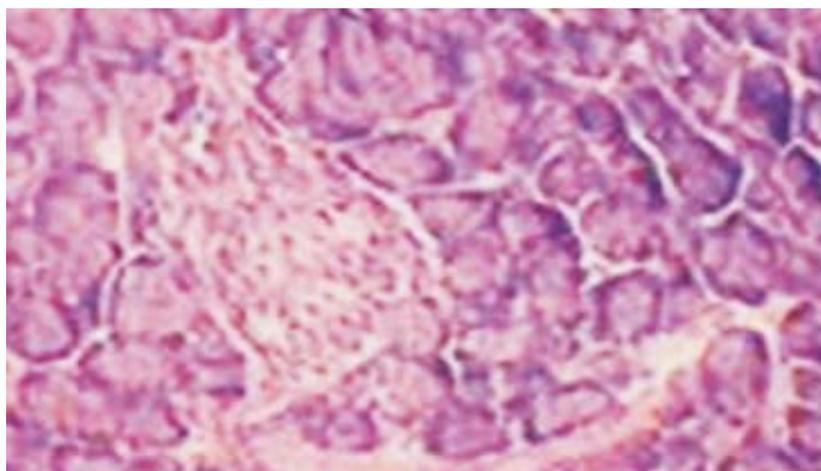


Fig. 8: Diabetic + OLE (0.5 mg/kg) after four weeks of treatment showed an absence of dilation and prominent hyperplastic of islets (H&E 400X).

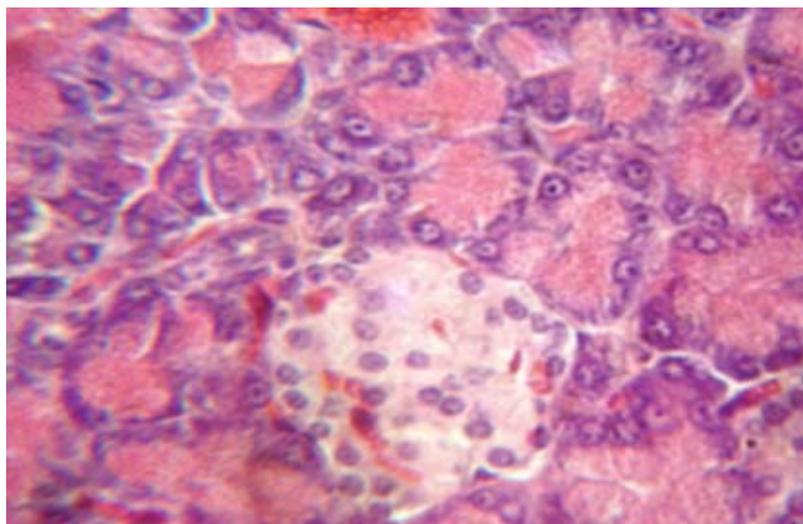


Fig. 9: Diabetic + OLE (0. 5 mg/kg) after four weeks of treatment showed irregularity in size, with a decreased granulation (H&E 165X).

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