Histopathological study on maternal pancreas, placenta and fetal pancreas at 18th day postgestation of streptozotocin induced maternal diabetic rats (*Rattus norvegicus*), and the effect of n-butanolic extract of celery seeds (*Apium graveolens*)

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Abstract

The study is designed to clarify the influence of induced maternal diabetes mellitus on placenta and the fetus at 18th day of gestation. Diabetes mellitus was induced in (24) female's rats (*Rattus norvegicus*) before mating by streptozotocin (60 mg/kg of animal body weight) one dose intrapersonal injection, all animals were isolated and divided into two groups (non-diabetic and diabetic group), each group was composed of (12) female, also each group was subdivided into two groups, healthy females group as control and the subgroup treated with n-butanolic extract of celery seeds (60 mg/kg of body weight daily). The diabetic group also had been subdivided into two groups, another one of them had been treated with celery seeds extraction, and one had diabetes mellitus without treatment. There were many macroscopic observations reported by the current study, include the still birth in addition to differences in size between the fetuses which was tend to increased (macrosomia) in the diabetic groups resulted from hyperglycemic mother. Microscopic study detected harmful effects of diabetes mellitus on the tissues, while in the other hand there are a regeneration in islets of Langerhans when treated the animals with n-butanolic extraction of celery seeds with slightly heal in certain points in tissues, also had seen some of the side effects of treated with extract.

Introduction

The term ‘diabetes’ was first used by Aretaeus of Cappadocia in the second century AC. Aretaeus gave a clinical description of disease, noting the increased urine flow, thirst and weight loss, features that are instantly recognizable today (Bilous and Donnelly, 2010). Diabetes mellitus is a group of heterogeneous disorders with distinct genetic, etiologic, and pathophysiologic mechanisms with the common elements of glucose intolerance and hyperglycemia, due to insulin deficiency, impaired insulin action, or both, the world health organization (WHO) estimates that more than 220 million people worldwide have diabetes mellitus (Gonzalez and Adi, 2012). There are two main categories of diabetes Type 1 diabetes resulting from insulin deficiency encompasses diabetes that is primarily a result of pancreatic beta cell destruction and is prone to ketoacidosis, this form includes cases due to an autoimmune process and those for which the etiology of beta cells destruction is unknown, while type 2 diabetes may range from predominant insulin resistance with relative insulin deficiency to a predominant secretory defect with insulin resistance, in addition to other categories includes gestational diabetes mellitus refers to glucose intolerance with onset or first recognition during pregnancy, moreover there are other specific types

include a wide variety of relatively uncommon conditions, primarily specific genetically defined forms of diabetes or diabetes associated with other diseases or drug use (Goldenberg and Punthakee, 2013; American Diabetes Association, 2010). Researchers Murthy *et al.* (2002) noted that the impact of diabetes in pregnant mothers on fuel metabolism is one of under utilization of exogenous fuel in the fed state (facilitated anabolism reduced) and over production from endogenous source in the fasted state (particularly in type 1 diabetes) as early as the first week of gestation and even before nausea or vomiting sets in may be early morning fasting ketonuria, but, a minor proportion of women lack the necessary beta cell reserve to maintain euglycemia during pregnancy, and develop impaired glucose tolerance (IGT), so they have significantly lower insulin responses at 30 and 60 min after oral glucose load compared with glucose tolerant controls, while insulin sensitivity is similarly reduced in the second trimester, as well the C-peptide response to intravenous glucagon is also significantly reduced in women with (IGT) in pregnancy, but serum proinsulin concentrations are increased (Wei *et al.*, 2014; Yang *et al.*, 2002). Management of diabetes without any side effect is still a challenge to the medical community, as well there is continuous search for alternative drugs, therefore it is prudent to look for options in herbal medicines for diabetes, although, herbal medicines have long been used effectively in treating diseases in throughout the world (Grover *et al.*, 2002). Abundant of plants that have hypoglycemic effect on mammals, but, many studies referred to the hypoglycemic effect of celery (*Apium graveolens*), which is a well known medicinal plant that has been used in the middle east traditional medicine for treating various diseases according to its action as: Anti-inflammatory (used in rheumatic disorders, inflammation of the urinary tract), diuretic, carminative, nervine, sedative, antiemetic, antispasmodic, antiseptic (used in bronchitis, asthma, as well as liver and spleen diseases), emmenagogue. Essential oil from seeds – tranquilizer, anticonvulsant, antifungal. Seeds are used in the treatment of chronic skin disorders including psoriasis (Khare, 2007), in addition to hypoglycemic activity (Al-Sa'aidi and Al-Shihmani, 2013; Niaz *et al*., 2013; Hassan, 2007).

Materials and Methods

Experimental Animals: The present study is done on healthy adult virgin females Wistar albino rats (*Rattus norvegicus*) age (10-12) weeks and the average weight (225±25) g, which are breed at the animal house of the College of Veterinary Medicine, Al-Qadissiya University. The animals are housed under controlled standard conditions in a temperature (20-23) C°, controlled room on a (12: 12) Light: Dark schedule, they are isolated in plastic cages with hygienic bed and were fed on standard laboratory food, whereas each (10) kg of it composed of milk (20%)/ 2 kg, heat particles (17%)/1.7 kg, wheat powder (17%)/ 1.7 kg, barley particles (20%)/2 kg, corn particles (25%)/ 2.5 kg, and food salt (1%)/ 0.1 kg, also supplemented by multivitamins, minerals, and amino acid, in addition to drinking water ad libitum (AlTameemi, 2014). For the purpose of mating and determining the gestation date, the researcher has chosen (24) virgin female rats have age range (10-12) weeks old and weights (225±25) g, after making sure of the rats health and safe, the adult females that predisposing to fertilize are isolated with males by (2 females : 1 male) in each cage and left to overnight, then had confirmed of the mating in the next morning by observing the presence of

vaginal plug that indicates the occurrence of mating, then isolated the females which have vaginal plug in separate cages and regarded the day on which the vaginal plug was observed zero-day of gestation (G0) and the next day is first day of gestation (Hamid and Zakaria, 2013).

Induction of Diabetes Mellitus by Streptozotocin: The animals are allowed to acclimatize for one week, before the experiment, the important point that the diabetes mellitus was induced in the overnight fasting (36) female virgin rats by a single intraperitoneal injection of streptozotocin (STZ) at a dose of (60 mg/kg of body weight) (Cakatay and Kayali, 2006), while other groups are injected with normal saline intraperitoneal, the suitable amount of STZ (600) mg is dissolved in (40) ml of citrate buffer (pH 4.5) to inject (1) ml of solution for each animal, the citrate buffer was freshly prepared about (20 minutes) before injection, and avoid direct light by covered the container with aluminium foil (Akbarzadeh *et al*. 2007), it should be noted that to prepare 1 molar of citrate buffer, 2.1024g of it is dissolved in 50 ml of distilled water and pH was adjusted to 4.5, then the volume is completed to 100ml (AlHisnawy, 2013; and AbuAbeeleh *et al*.,2009). Subsequently, hyperglycemia in female rats followed up (72) hours, then glucose level was measured in animals blood by using the method of the capillary blood glucose strips, the glucose was determined in the capillary blood samples from the tail vein, female rats with blood glucose concentration more than (200 mg/ dl or 11.1 mmol/ L) were considered as diabetic (Rushita *et al.* 2013; and Deeds *et al.*, 2011).

Treated with Celery Seeds Extract: After one week of adaptation of diabetes mellitus and five days before the mating process the group subdivided into two subgroups (one from the control and another from the diabetic groups) treated with n-butanolic fraction of n-butanolic extract of celery seeds shown in (image 1) in effective dose (60 mg/k of body weight) daily and continue to the end of experiment, it is important to know that the preparation of extract suspension had done by drenching solution (500 µl per each 100g of body weight), for each dose has been prepared by dissolving 12mg/ ml of extract in drinking water and shaking well in water bath at 45C° (for example: rat weighted 100g need to be drenched with 50 µl contains 6mg of extract, if the dose is 60 mg/ kg of body weight), thereafter, all virgin female rats were left for mating in 1:2 ratio (male: female) overnight and determine the starting point of gestation.

Image 1: Photography of n-butanolic extract of celery seeds.

Pregnant Rats Dissection: On the days 14th, 16th, and 18th of gestation, the fasting overnight experimental animals of all groups were sacrifice after general anesthesia by combination of Ketamine: Xylazine (90mg/ kg: 10mg/ kg intraperitoneal) (AlTameemi, 2014), also reported the abnormal changes and specially the presence of death fetuses.

Tissues and Fetuses Samples: Pregnant rats from all groups are anesthesia and dissection at 18^{th} day of gestation, by abdominal opened, the placenta and fetuses (after removed the embryonic membranes) are immediately and carefully removed, put part of all sample into suitable fixative for histological and embryological studies.

Celery Extraction: (By method adopted by AlShihmani (2013)

Preparation of n-butanolic Fraction of Celery (*A. graveolens*) seeds: Celery (*A. graveolens*) seeds is purchased from the local market and classified by SBSTC (State Board for Seed Testing and Classification, Agriculture Ministry, Iraq), N-butanolic fraction of celery seeds extract has been prepared in two steps According to AlShihmani (2013).

Preparation of Methanolic Extract: Methanolic extract has been prepared according to Harborne (1984) using Soxhlet apparatus as follow: **(1)** Celery seeds (0.5) kg was put in a cellulose bag of Soxhlet container, **(2)** Adding half liter of 99.9% methanol in the cellulose bag, **(3)** Adding 2 liter of 99.9% methanol in the round bottom flask of the apparatus, **(4)** After setting up the equipments and adjustment of heater temperature (45 C°), the extraction allowed to progress for 10-12 hrs each time according to the clearance of column inside the soxhlet container, **(5)** Adding the total extraction content in the big round container of the Rotary evaporate, **(6)** Rotavaporator adjusted on (40) C° and (50-60) rpm, the device turned on and allowed to continue evaporation for at least 2 hrs, **(7)** Dried extract is weighted and stored in deep freeze until used.

Preparation of n-butanolic Fraction of Celery Seeds: According to the polarity, three types of solvent have been used to separate different fractions of the crude extract; ethyl acetate, n-butanolic, and distilled water, using a separating funnel, in order to obtain the high, middle, and low polar fractions of the extract. n-butanolic fraction of the celery seeds has been evaporated, lyophilized, and kept at -4 C^{o} until use (Tsi and Tan, 1999).

Preparation of n-butanolic Solution for Oral Administration: One dose of n-butanolic fraction of celery (*A. graveolens*) seeds extract (effective dose; 60 mg/ kg of body weight) (Al-Sa'aidi *et al.*, 2012), has been used in the present study. Drenching solution (500 µl per each 100 g of body weight) for each dose has been prepared by dissolving (12mg/ml of drinking water) in shaking water bath at (45) C^{o}, respectively. For example: rat weighted (100) g need to be drenched with (50) µl contains (6) mg of extract, if the dose is (60 mg/ kg of body weight).

Histological Study: All the samples from maternal tissues and fetuses has been prepared for light microscopic study according to (Suvarna *et al.* 2013).

Preparation of Histological Sections: **(1)** Fixation of the samples: histological samples of mother (placenta and the fetuses) had been fixed by neutral buffered formalin (10%) for (24-48) hrs. **(2)** Washing and dehydration: washing the samples in water for (3) hrs, remove the formalin residue, then the samples transferred to graded series of ethanol

concentration which (70, 80, 90, 95, and 100%) for about (1-2) hrs for each concentration. **(3)** Clearing: the samples cleared with (xylene) for (3-5) min (3) times**. (4)** The samples infiltrated in liquid paraffin (56-58) C° overnight. **(5)** The samples are embedded in paraffin within special containers (blocks) and all the specimens oriented and left in room temperature to be harden and put it in refrigerator until sectioning. **(6)** Sectioning: the process had done by the rotary microtome the tissue sectioned to 5 micron thickness and floated in water bath (40-45C°) then picked to slides which coated with mayer's albumin.

Staining: Deparaffinized slides is done by putting it in xylene for (5) min, then dry it from xylene and rehydration in graded series of alcohol (100, 90, 80, 70, and 50%) for about (2-3) min in every concentration, after that washed with water for (1) min, stained the slides in alum hematoxylin stain for (15) min, washed with water, transferred the slides to (1%) acid alcohol (1% HCL in 70% alcohol) for few second, washed with water for (1) min, then stained with eosin stain for (10) min, washed with water and then dehydrated in a graded series of alcohol (70, 80, 90, and 100%) for (2-3) second in each of them, put the slides in xylene for (5) min, and finally, mounted the slides with the sticky material D.P.X, covered, and leaved it to dry.

Results

Histological Study on Maternal Pancreas: Pancreatic tissue of normal control showed normal distribution of islet of Langerhans within the exocrine part, islets are regular with well defined boundaries, the normal histological structure of pancreatic tissue composed of a mixed gland made up mostly of an exocrine component, consist of clusters of cells that make up the islets of Langerhans. The islets make up about 1% of the gland, a very thin layer of connective tissue surrounds the organ, connective tissue septa divided it into lobules, the exocrine parenchyma consists of acini that are joined to intercalated ducts, these ducts empty into intralobular ducts, their contents then flow into the larger interlobular ducts located in the connective tissue septa that divide the gland into lobules, the interlobular ducts finally empty into the much larger pancreatic duct, the section of pancreatic tissue of normal control have large interlobular duct lined by columnar epithelium, at its entry to the duodenum, the cluster of islet cells are secrete a number of polypeptide and protein hormones, the islet cell population consists of several cell types, include beta cells that insulin secreting calls, alpha cells that glucagon secreting cells and delta cells which secrete somatostatine, furthermore, other hormones are also produced though the specific cell type, the islets appeared lightly stained and always surrounded with pancreatic acini with interlobular dense connective tissue with blood vessels extend within the pancreas stroma, each acinus showed the centroacinar cell inserted into the acinar lumen also the acinar cells showed its basophilic staining and the apical with acidophillia (figure 1).

The pancreatic tissue which related to rats treated with n-butanolic extract of celery seeds group mostly revealed normal appearance of exocrine part, while there are mild necrosis and diffuse of inflammatory cells in endocrine part, also most sections showed enlargment islets of Langerhans but greater in size (hyperplasia) and increased in number compared to control, other regions showed exocrine portion with edema, mild degeneration with slightly

congested islet and mild necrosis and irregularity as shown (figure 2). Sections from pancreas related to induced group with diabetes referred to shrinkage of islets Langerhans in size, signs of necrosis (destruction) of beta cells and the size and number of islets were reduced which have been described as atrophy and replaced by fibrous or adipose connective tissue.

The microscopic observations on sections from pancreas of streptozotocin treated rats (diabetic group), showed marked cellular changes, that the regions between pancreatic acini and endocrine region appeared indistinct, also heavy infiltration of inflammatory cells in connective stroma and around the peripheral zone of islets of Langerhans, in addition to sever degeneration and necrosis in pancreatic tissue, particularly around the larger vessels, and revealed either pancreatitis or hemorrhagic pancreatitis that characterized by severely necrotic endocrine part with deposition of fibrinoid material (amyloid) and necrosis of exocrine part with occurrence of fibrosis and extend of adipose tissue, congested of blood vessels is clear, but few viable islet of Langerhans are still found in these fields (figure 3).

In diabetic pancreatic tissue treated with n-butanolic extract of celery seeds showed islets cell looks like normal with increase in size (figure 22), also the numbers of islets per field are found to be normal, however, some normal acinus with extravessel RBCs, presence of few degenerated acins as well as deposition of extracellular amyloid, other sections showed normal exocrine part, congested vessels with leakage of blood into the neighboring tissue (figure 4).

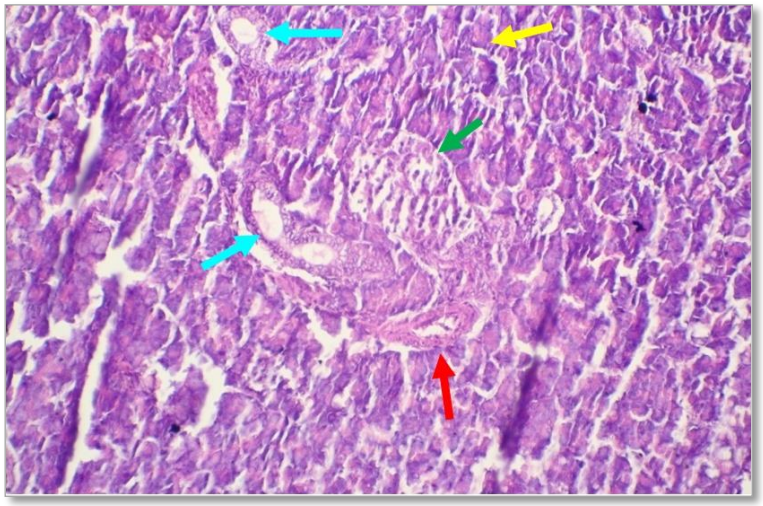

Figure 1: Section in normal pancreas from pregnant rat in 18th day postgestation, shown islet of Langerhans (➡), pancreatic duct (➡), blood vessel (➡), and exocrine acini (➡). H&E, (498 X)

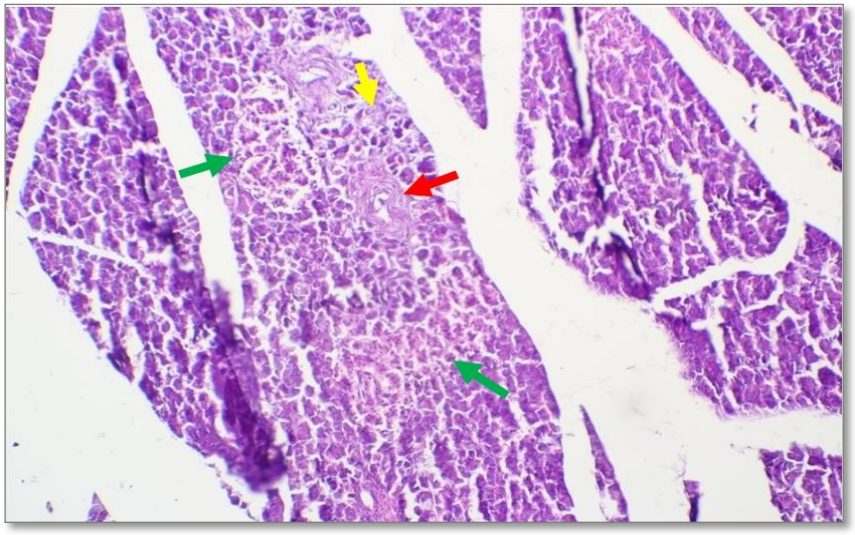

Figure 2: Section in pancreas gland from pregnant rat at 18th day postgestation treated with extract, shown enlargment islets of Langerhans (➡), blood vessel (➡), and degeneration or necrosis in exocrine acini (➡). H&E (498X).

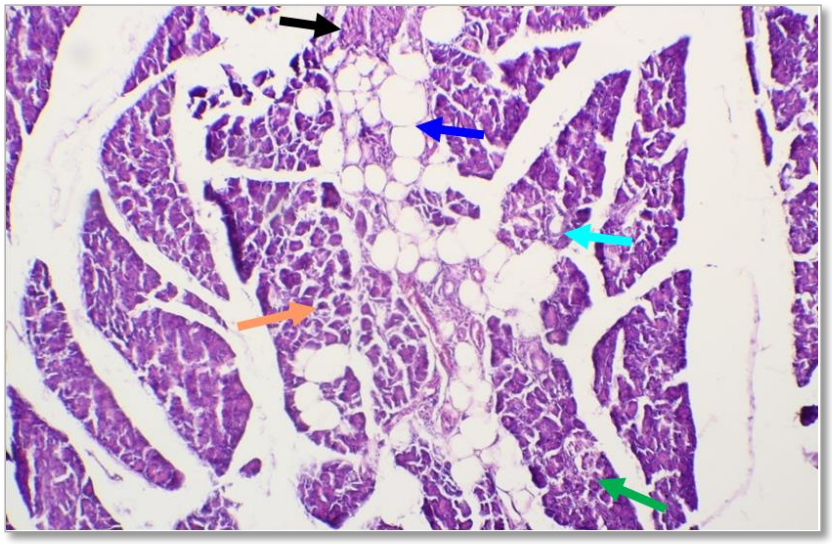

Figure 3: Section in pancreas gland from diabetic pregnant rat at 18th day of gestation, shown reduced islet of Langerhans (➡), fibrosis (➡), deposition of adipose tissue in exocrine tissue (➡), degenerated and necrosis with irregular pancreatic acini (➡), and pancreatic duct (➡). H&E (498 X)

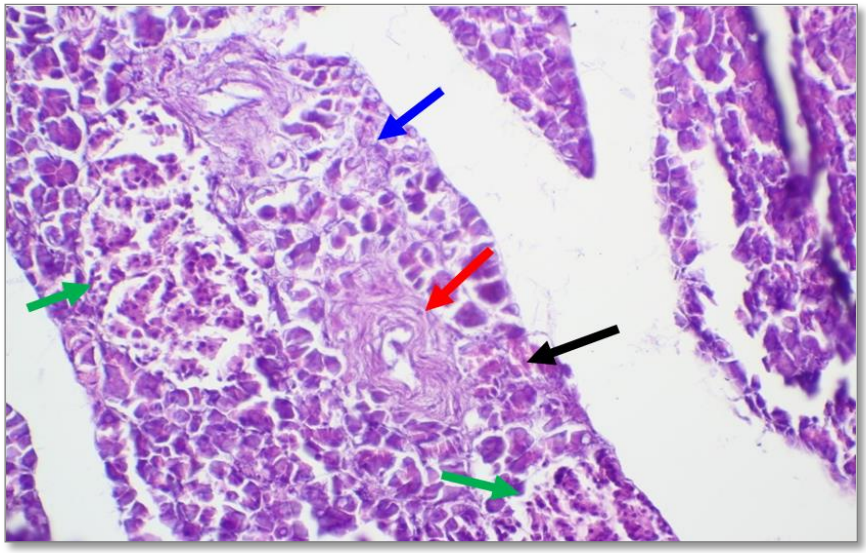

Figure 4: Section in pancreas from diabetic pregnant rat at 18th day of gestation treated with n-butanolic extract of celery seed, shown enlargement and increased in number in islets of Langerhans (➡), degeneration in exocrine tissue (➡), and congestion in blood vessel (➡). H&E (498 X).

Histological Study on Placenta: The microscopic observations on sections from normal placenta have shown three distinct histological regions the labyrinth zone, the basal zone, and the decidua, also there is additional layer called maternal glands. Moreover it should be know that the rats have a hemotrichorial and discoid type of placenta. In the labyrinth zone is composed of three layers of trophoblasts, separating the maternal blood spaces from the fetal blood vessels, first, the outer trophectoderm, which comes into direct contact with the maternal blood, is referred to as cytotrophoblasts with a microvillus surface, then under this trophectoderm, there are two layers of syncytiotriphoblasts (figure 5). As for the basal zone (junction zone) is comprised of three types of differentiated cells: spongiotrophoblasts, trophoblastic giant cells, and glycogen cells (figure 7), the spongiotrophoblasts were present immediately above the trophoblasic giant cell layer located at the materno-fetal placental interface, then the glycogen cells. Thereafter, the decidua is comprised of the mesomaterial decidual cells ultimately. Finally, the maternal gland zone is located in the mesomaterial triangle of the pregnant uterus (figure 6). Generally the histological structure of placenta in the group that treated with celery seeds extract is observed normal as compared to control (figures 8 and 9), but there are several histological changes in diabetic groups, as enlarged in basal zone, with increased the number of spongiotrophoblast cells (figures 11, 12, and 13), normal to mild degradation in glycogen cells layer (except the group that treated with extract), although the hemorrhagic nature of placenta, it showed increased of hemorrhage which be greater in severity in diabetic groups (with and without treated with extract) as shown in (figure 14, 15 and 16), and revealed slightly increased in glycogen cell layer in the groups that treated with extract as in (figures 10 and 17).

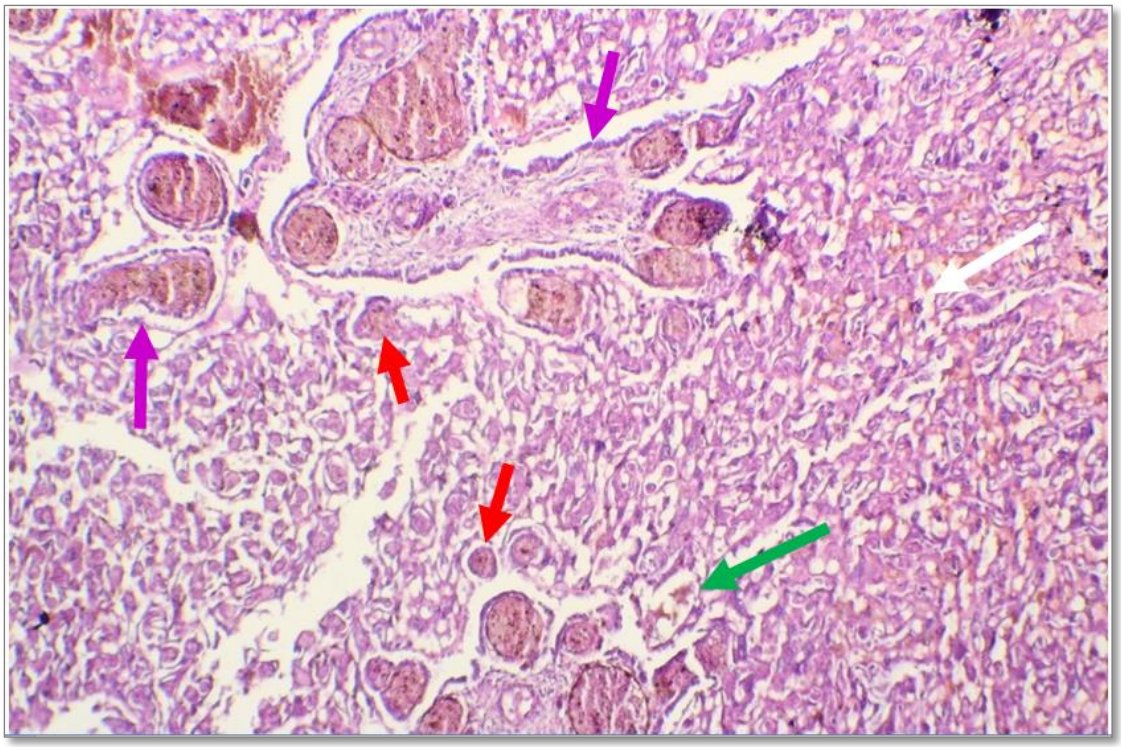

Figure 5: Section in labyrinth zone of normal placenta from pregnant rat at 18th day of gestation, shown syncytiotrophoblast on microvillus surface (➡), fetal blood capillary (➡), and maternal sinusoid (➡). H&E (498 X)

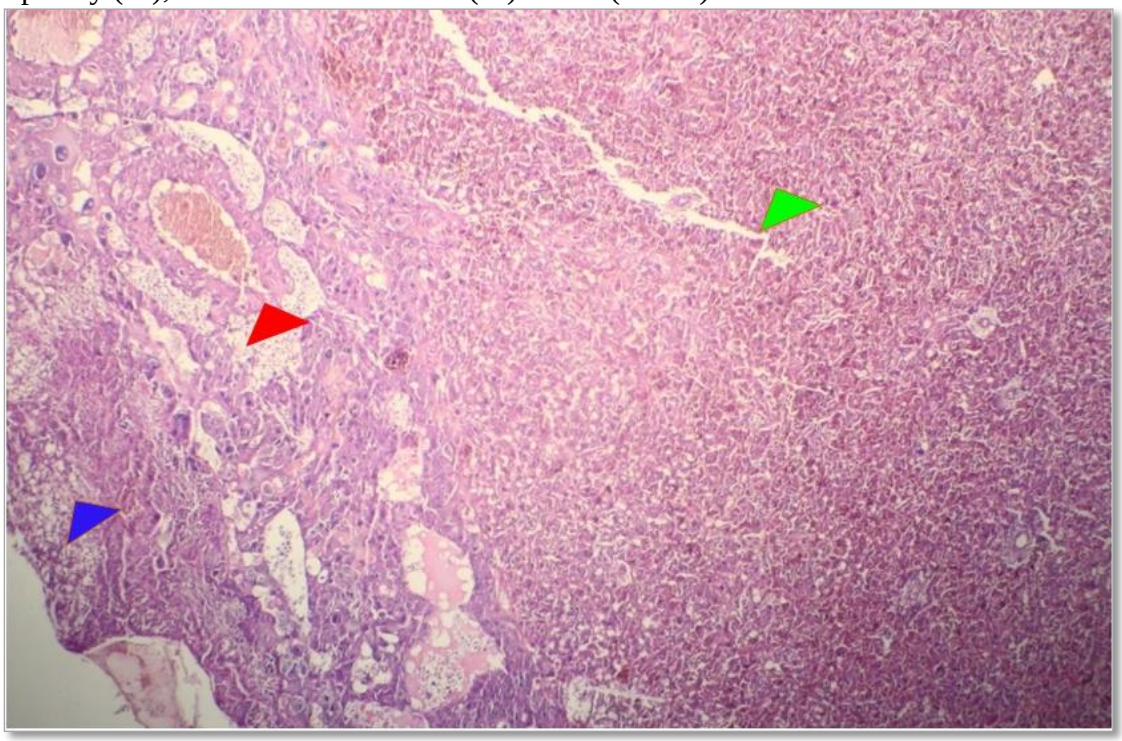

Figure 6: Section in normal placenta from pregnant rat at 18th day of gestation, shown labyrinth zone (▶), basal (junction) zone (▶), and decidual zone (▶). H&E (123 X)

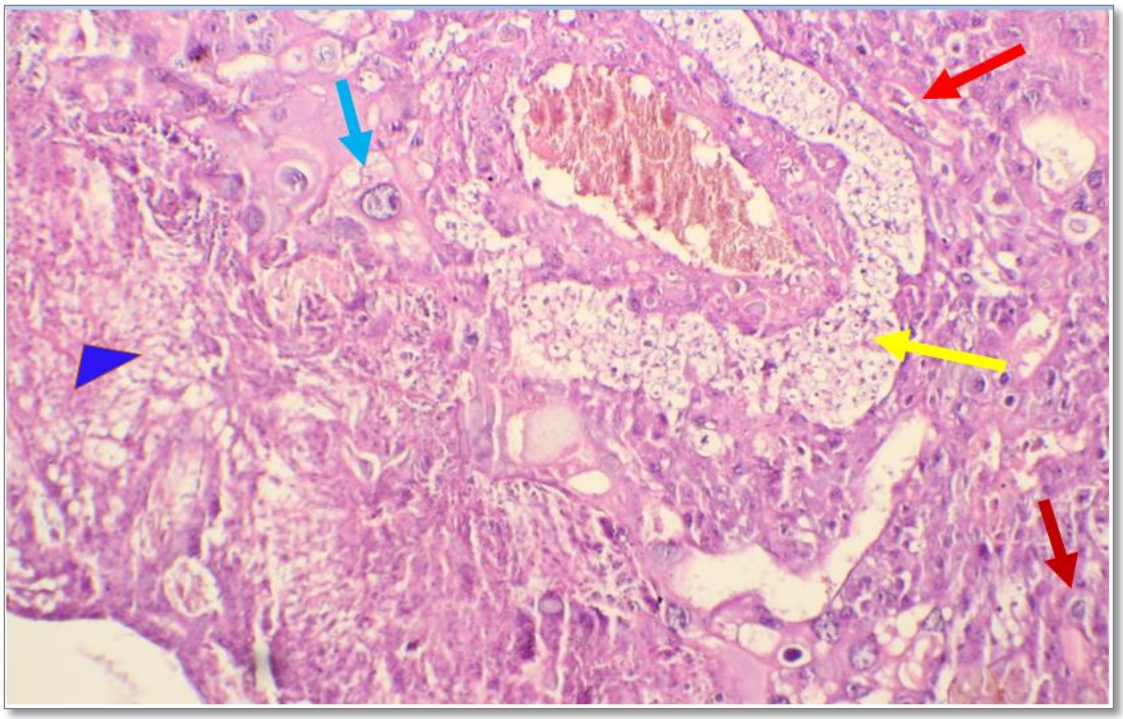

Figure 7: Section in normal placenta from pregnant rat at 18th day of gestation, shown sponyiotrophoblast cell (➡), glycogen cell (➡), and trophoblastic giant cell (➡) of basal junction zone and decidual zone (▶). H&E (498 X)

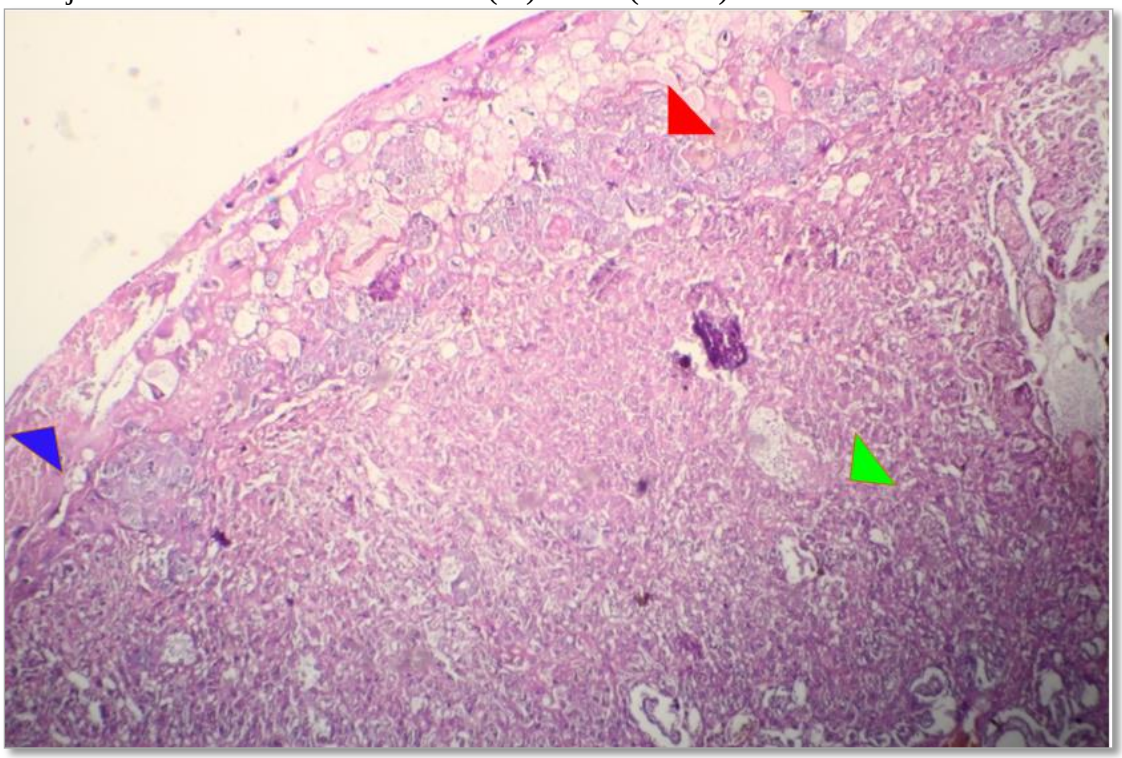

Figure 8: Section in pancreas from pregnant rat at 18th day of gestation treated with n-butanolic extract of celery seeds, shown labyrinth zone (▶), basal (junction) zone (▶), and decidual zone (▶). H&E (123 X).

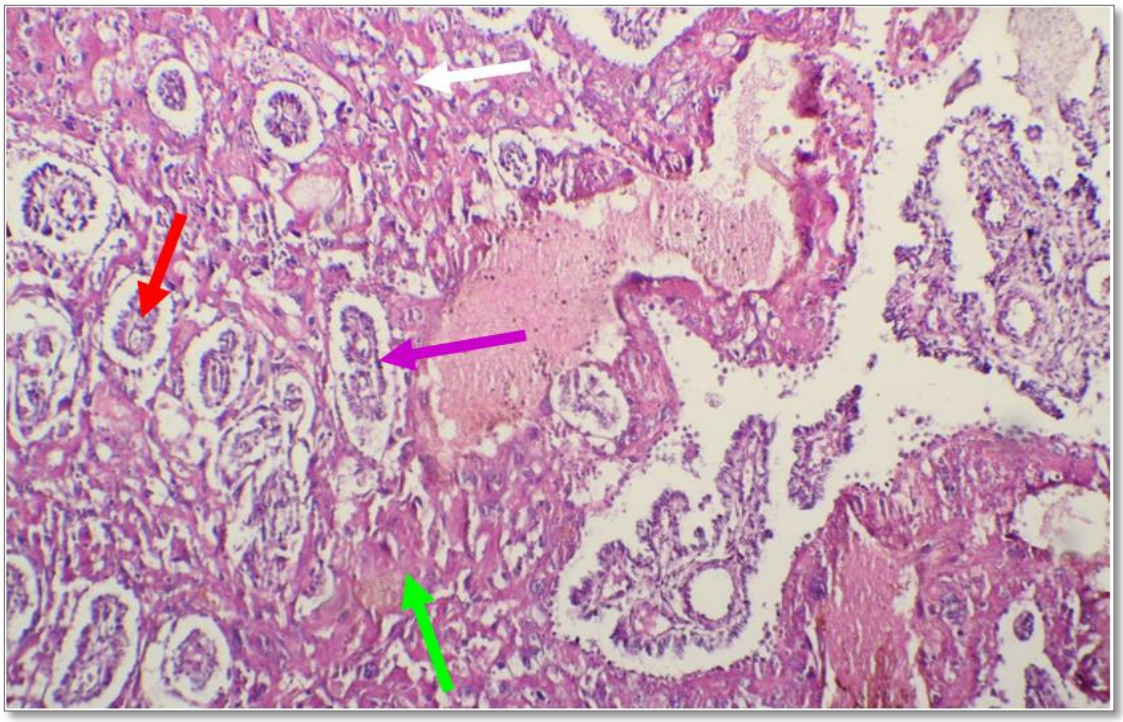

Figure 9: Section in labyrinth zone of placenta from pregnant rat at 18th day of gestation treated with n-butanolic extract of celery seed, shown microvillus (➡), fetal blood capillary (➡), inflammatory cells (white ➡), and fibrosis (➡). H&E (498 X)

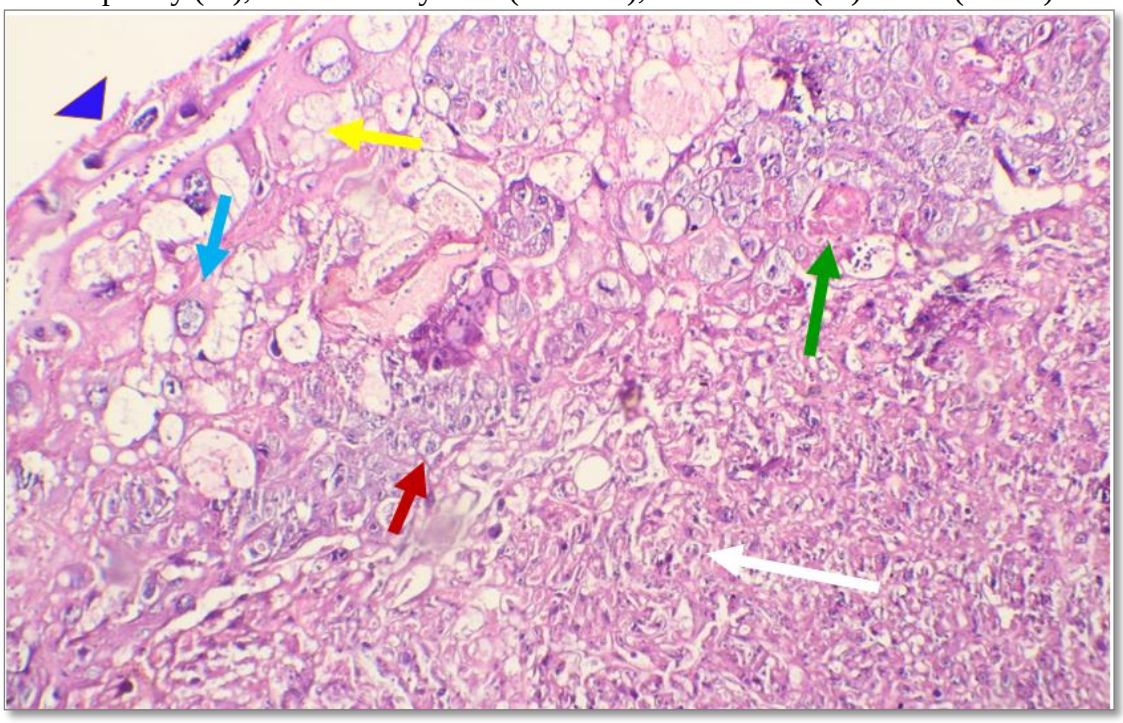

Figure 10: Section in basal (junction) zone of placenta from pregnant rat at 18th day of gestation treated with n-butanolic extract of celery seed, shown spongiotrophoblast cell (➡), increased accumulation in glycogen cell (➡), trophoblast giant cell (➡), congestion in blood capillary (➡), also decidual zone (▶), inflammatory cells (white ➡) in labyrinth zone. H&E (498 X)

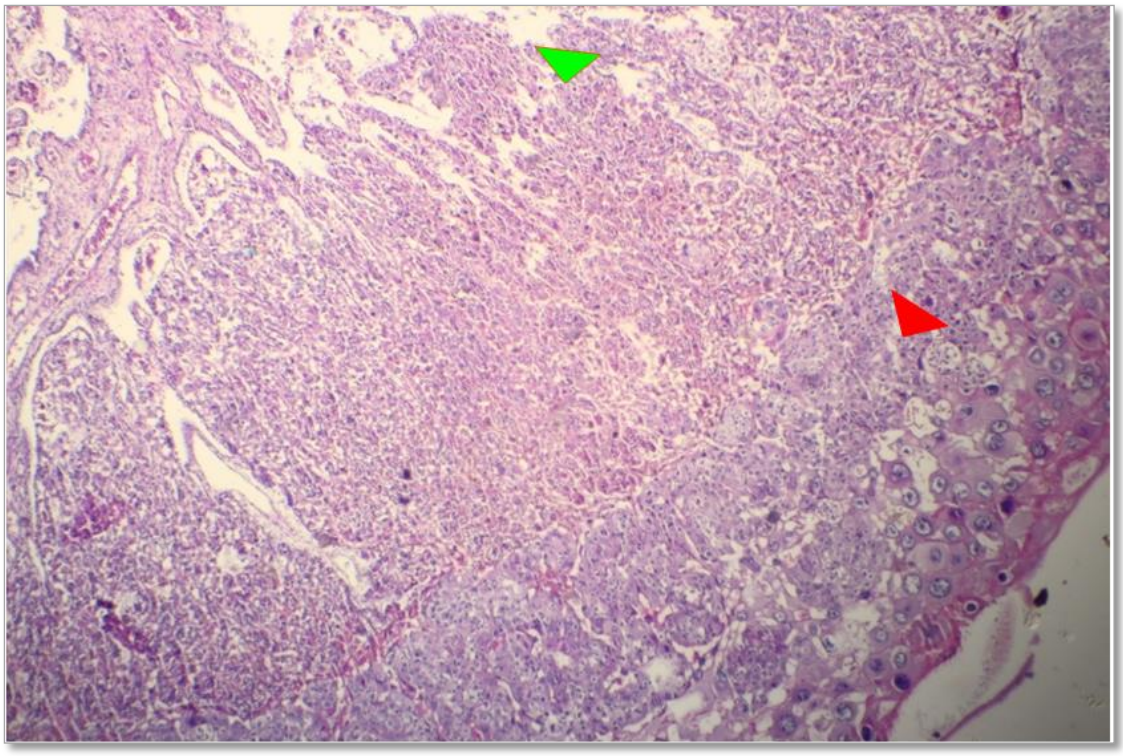

Figure 11: Section in placenta from diabetic pregnant rat at 18th day of gestation, shown labyrinth zone (▶) and basal zone (▶). H&E (123 X)

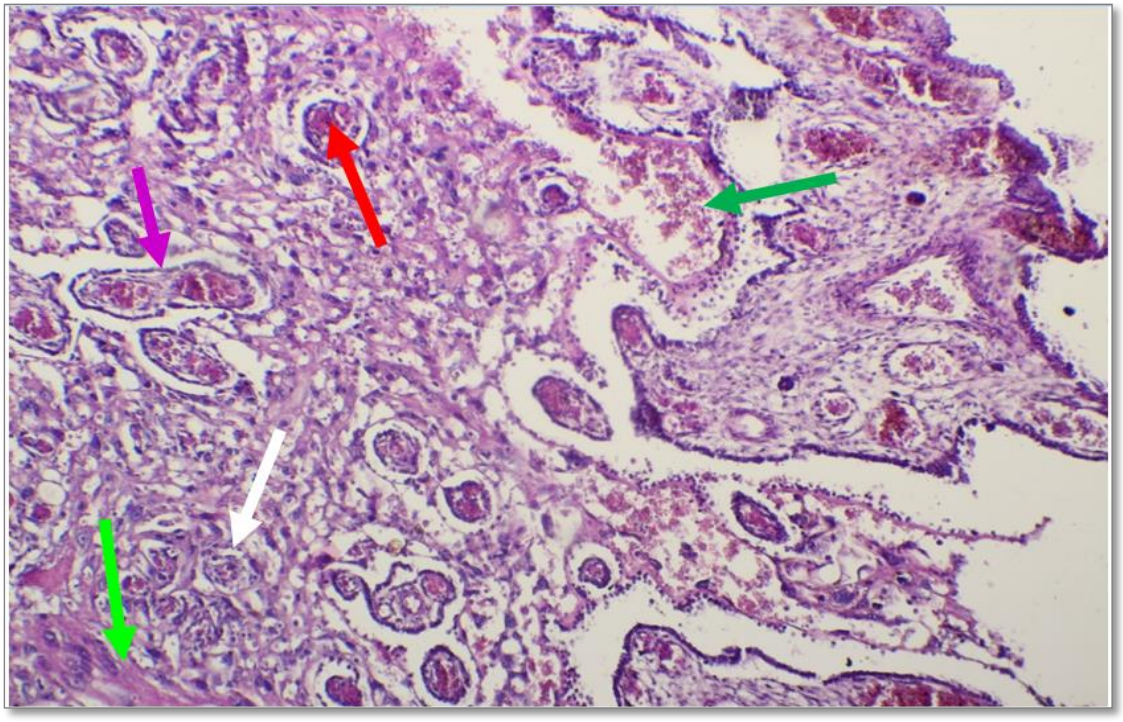

Figure 12: Section in labyrinth of placenta from diabetic pregnant rat at 18th day of gestation, shown syncytiotrophoblast on the microvillus surface (➡), fetal capillary (➡),extended maternal sinusoid (➡), fibrosis (➡), and inflammatory cell (white ➡). H&E (498 X)

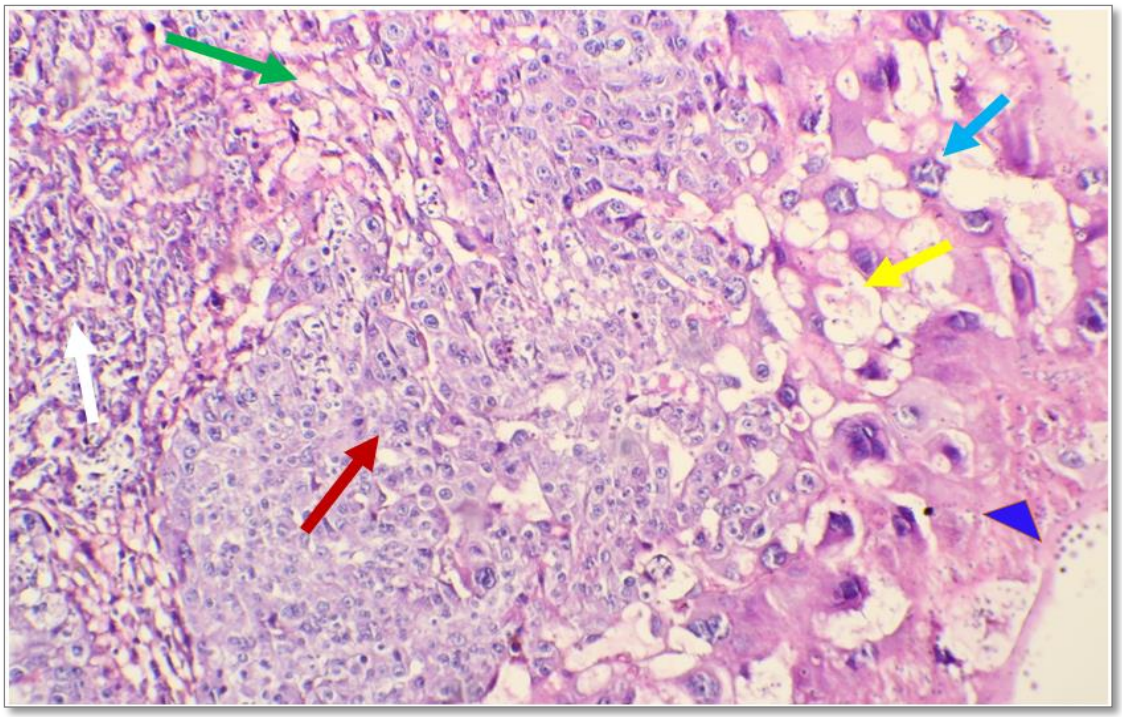

Figure 13: Section in basal (junction) zone of placenta from diabetic pregnant rat at 18th day of gestation , shown great accumulation of spongiotrophoblast cell (➡), atrophy in glycogen cell (➡), trophoblast giant cells (➡), also can see the fibrosis (➡) and inflammatory cell (white ➡) in labyrinth zone, and decidual zone (▶). H&E (498 X)

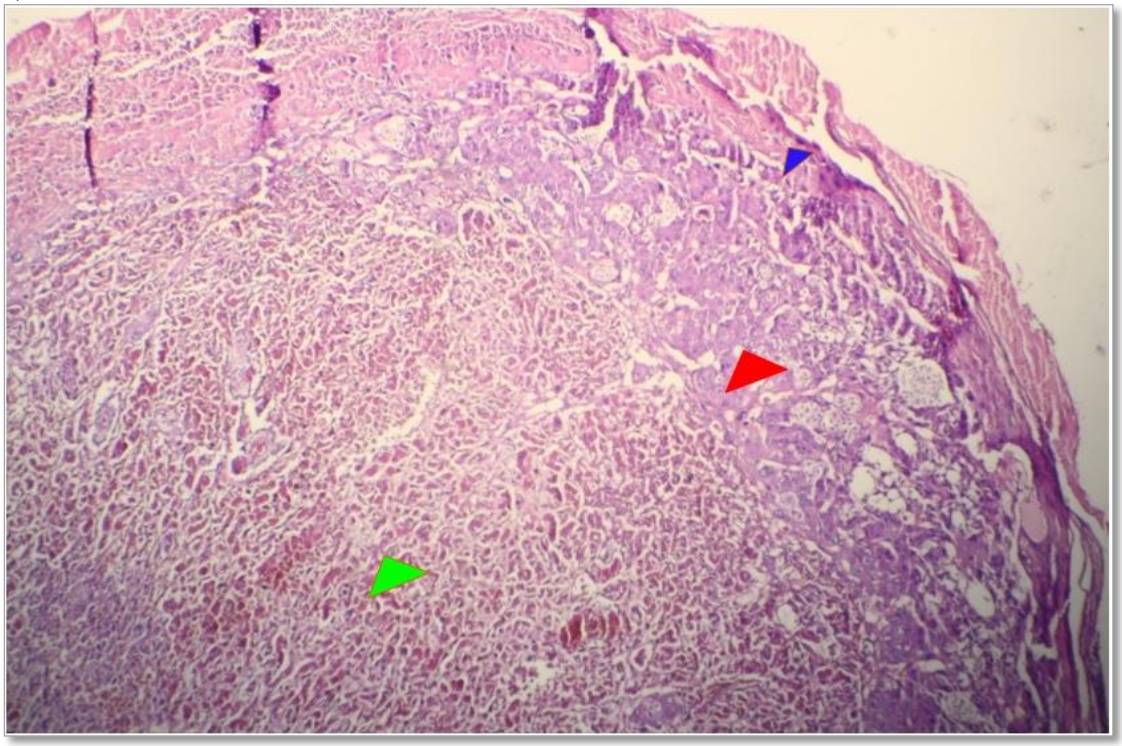

Figure 14: Section in placenta from diabetic pregnant rat at 18th day of gestation treated with celery seeds extract, shown sever hemorrhagic labyrinth zone (▶), basal (junction) zone (▶), decidual zone (▶). H&E (123 X)

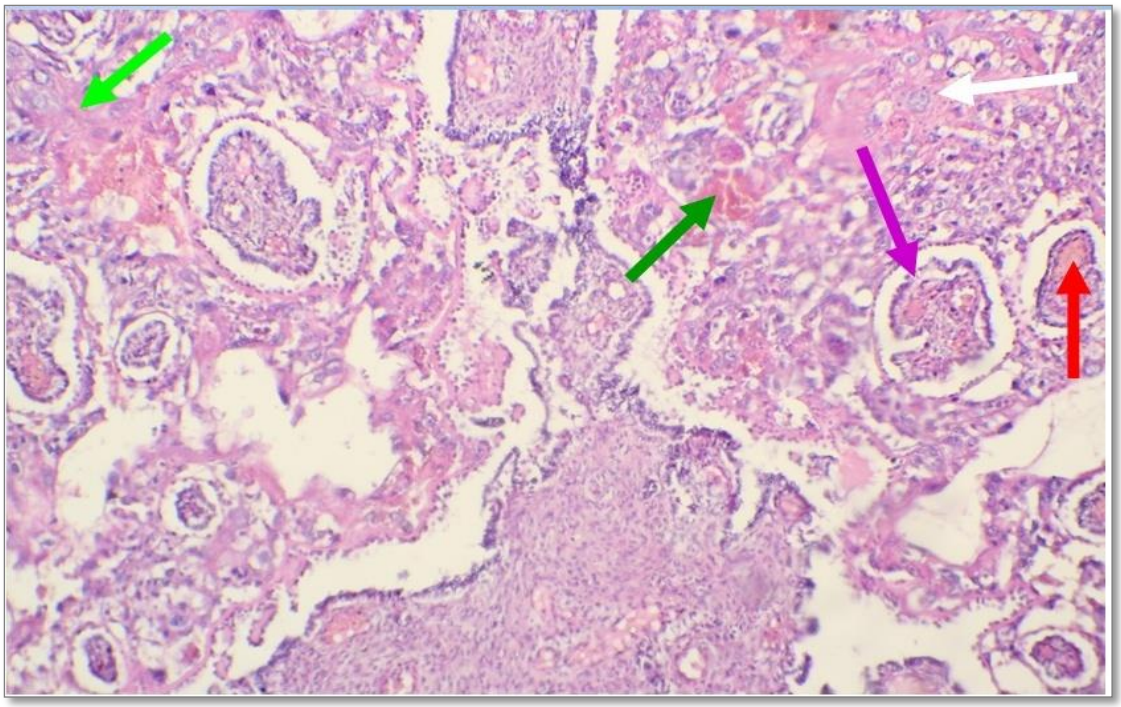

Figure 15: Section in labyrinth of placenta from diabetic pregnant rat at 18th day of gestation treated with celery seeds extract, shown syncytiotrophoblast on microvillus surface (➡), fetal capillary (➡), fibrosis (➡), and congestion in maternal sinusoid (➡). H&E (498 X)

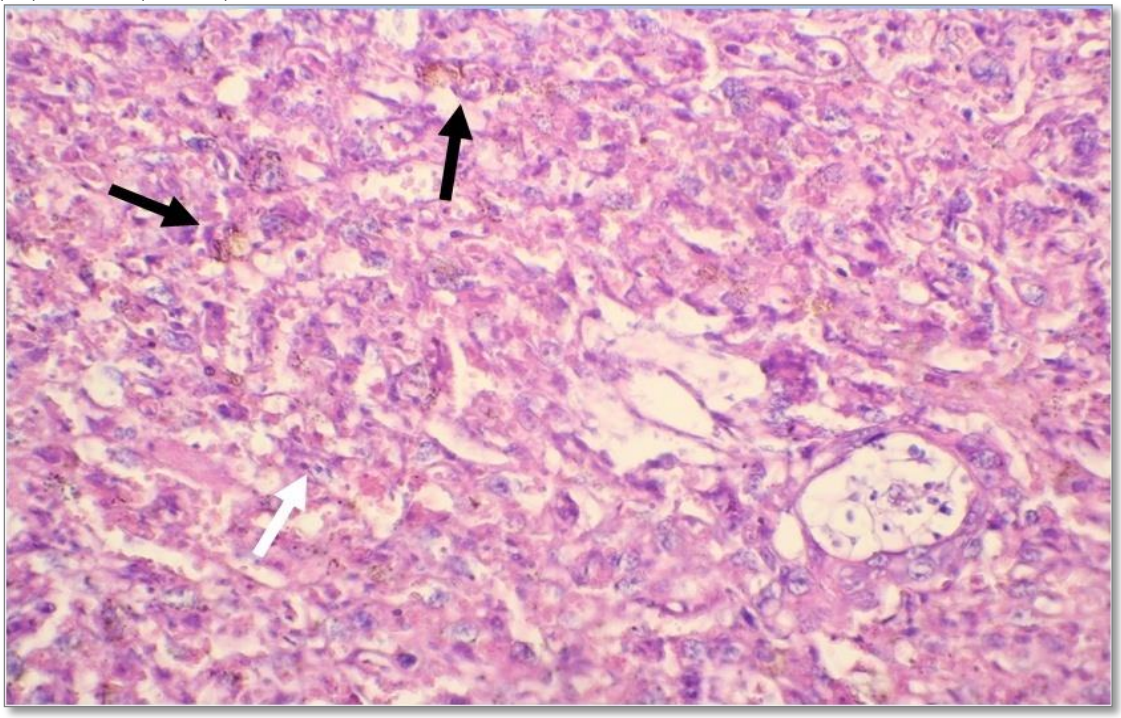

Figure 16: Section in labyrinth of placenta from pregnant rat at 18th day of gestation treated with celery seeds extract, shown hemorrhage in stroma (➡), and inflammatory cell (white ➡). H&E (498 X)

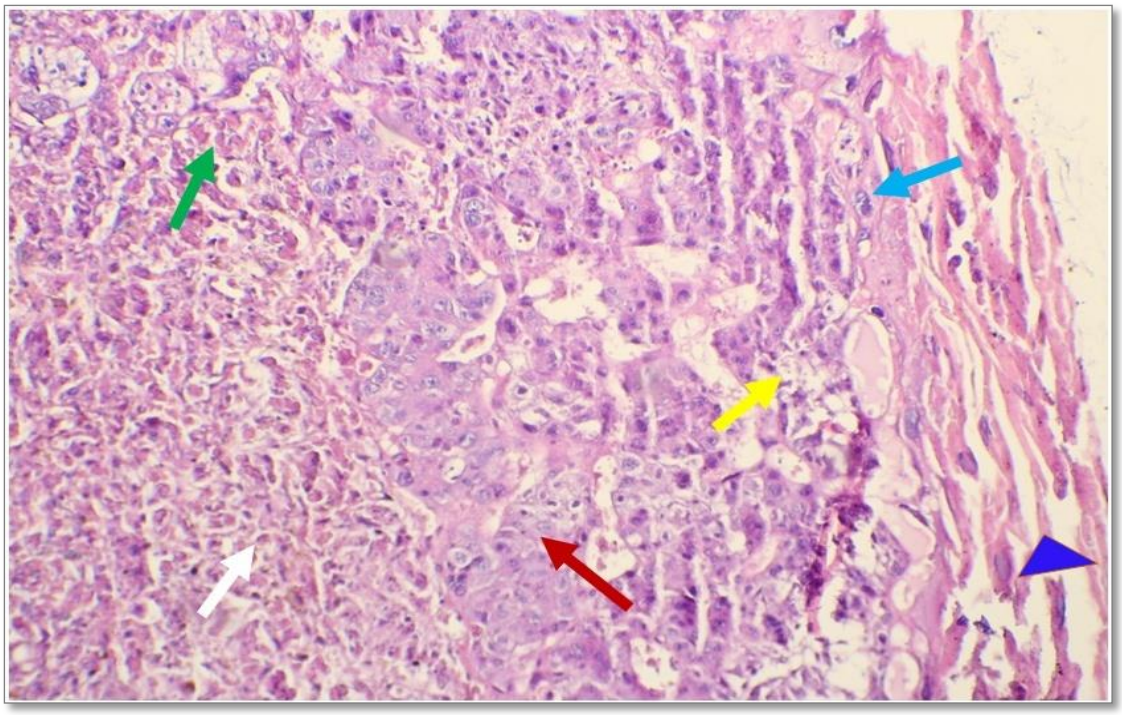

Figure 17: Section in basal (junction) zone of placenta from diabetic pregnant rat at 18th day of gestation treated with n-butanolic extract of celery seeds, shown spongiotrophoblast cell (➡), trophoblast giant cell (➡), reduce the accumulation of glycogen cells (➡), mild hemorrhrage (➡) and infammatory cell (white ➡) in stroma, and decidual zone (▶). H&E (498 X).

Histological Study on The Pancreas of Fetuses: Pancreatic tissue of fetuses at 18th days of gestation in normal control showed normal distribution of islet of Langerhans within the normal exocrine part, islets are shown regular with well defined boundaries (figure 18), this histological structure similar to adult pancreatic histological structure which have a normal exocrine and endocrine tissues with clear septa (figure 19). Also, the group of experimental animals that treated with celery seeds extract showed normal distribution in pancreatic exocrine tissue with great accumulation of adipose tissue, whereas there is a hyperplasia and increase the number of islets of Langerhans, which appear larger in size compared to control (figure 20). While diabetic rats group reveal hyperplasia of islets of Langerhans with increases in number, also the endocrine cells shown dark staining and surrounded with thickener layer of connective tissue compared to control with normal exocrine part of pancreas (figure 21). Whereas the diabetic rats group that treated with celery seeds extract reveal normal exocrine tissue with a mild accumulation of adipose tissue also there are increasing in the islets size and number compared to control (figure 22).

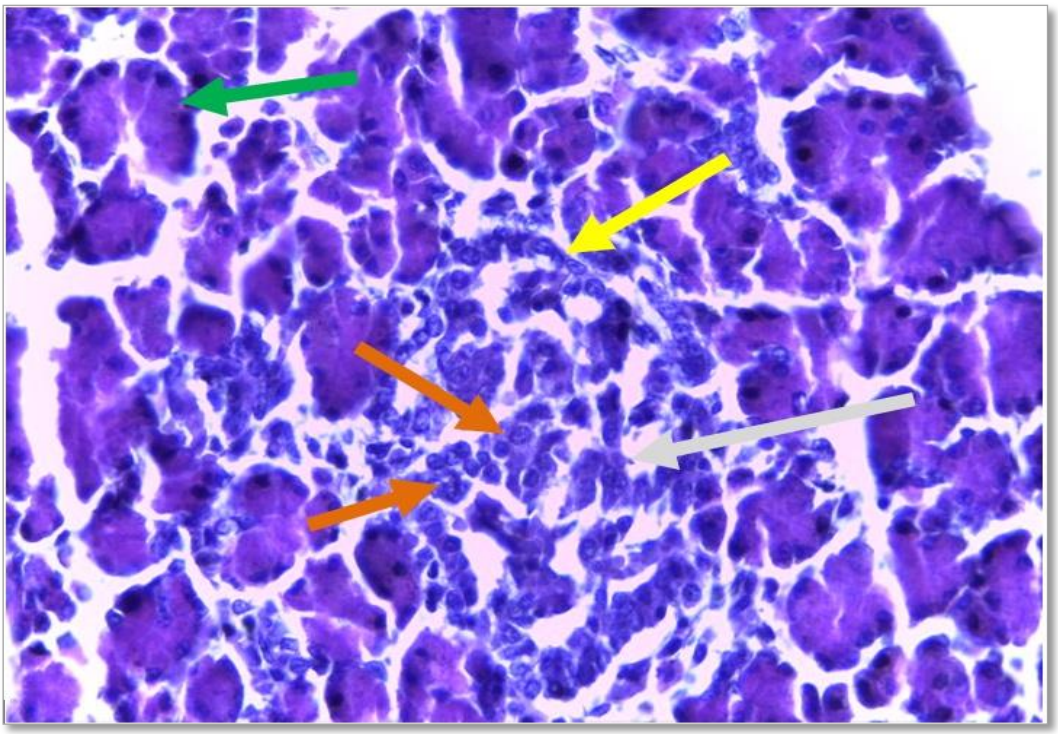

Figure 18: Section in normal pancreas from fetus rat at 18th day of gestation, shown histological structure, of islet of Langerhans (➡), well boundaries of islet (➡), dark staining endocrine cells (➡), and exocrine acini (➡). H&E (498 X).

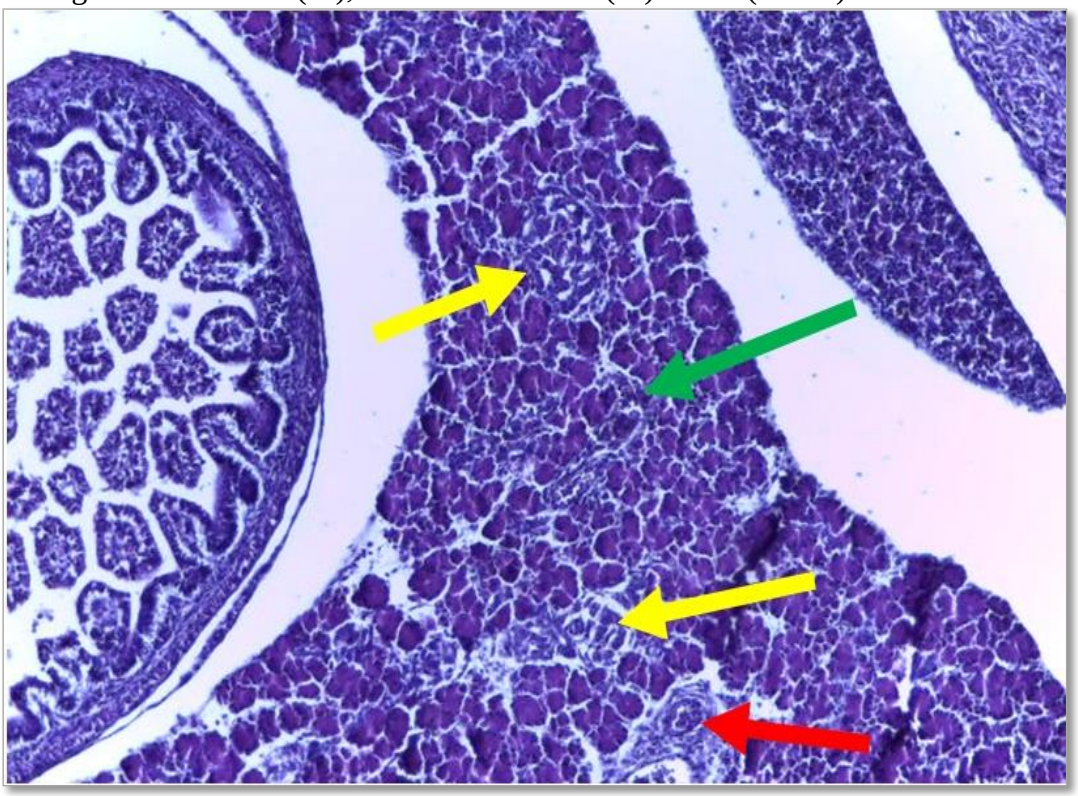

Figure 19: Section in normal pancreas from fetus rat at 18th day of gestation, shown histological structure, of islet of Langerhans (➡), pancreatic septa (➡), normal exocrine acini (➡), and blood capillary (➡). H&E (498 X)

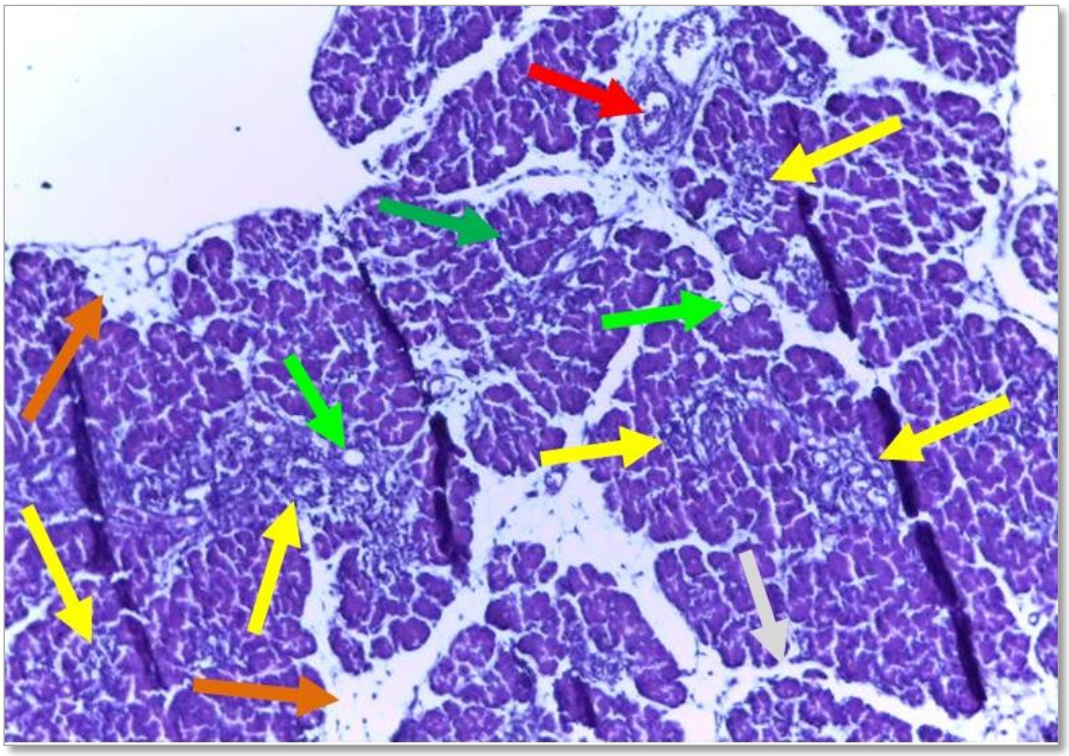

Figure 20: Section in pancreas from fetus of normal mother treated with celery seeds rat at 18th day of gestation treated with celery seeds extract, shown histological structure, of islet of Langerhans (➡), normal exocrine acini (➡), accumulation of adipose tissues(➡), pancreatic duct (➡), and blood capillary (➡). H&E (498 X)

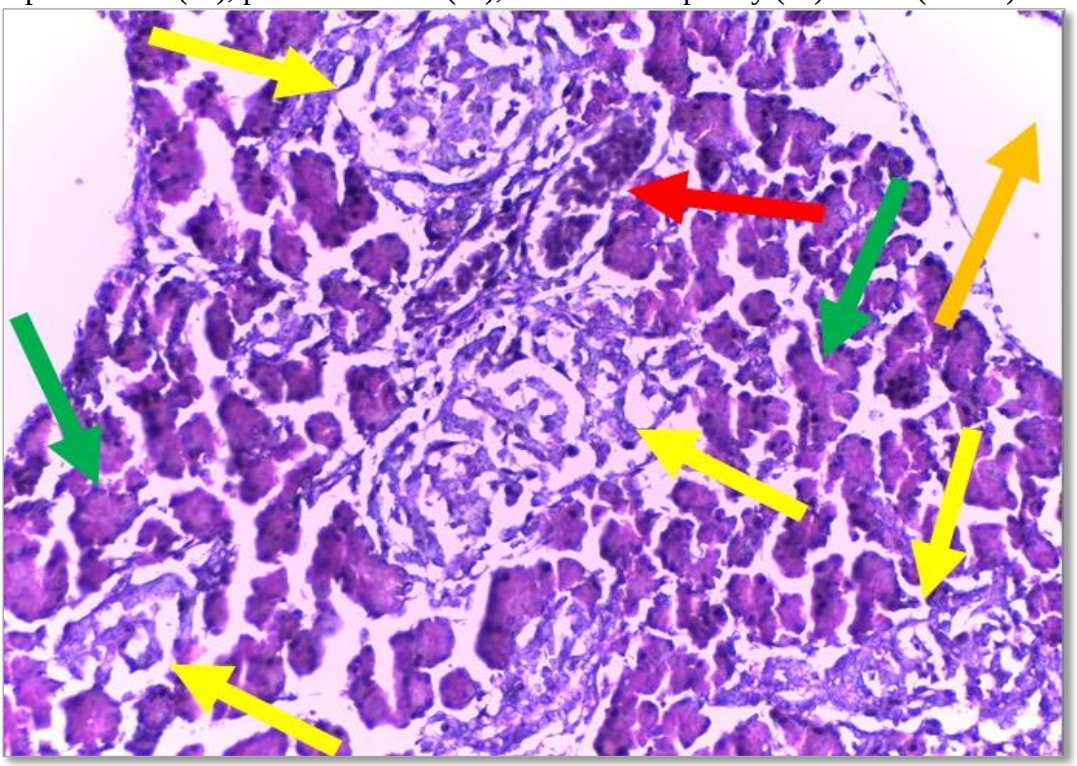

Figure 21: Section in pancreas from fetus of diabetic mother rat at 18th day of gestation, shown histological structure, of islet of Langerhans surrounded with thickening layer of connective tissue (➡), pancreatic septa (➡), normal distribution exocrine acini (➡), and blood capillary (➡). H&E (498 X)

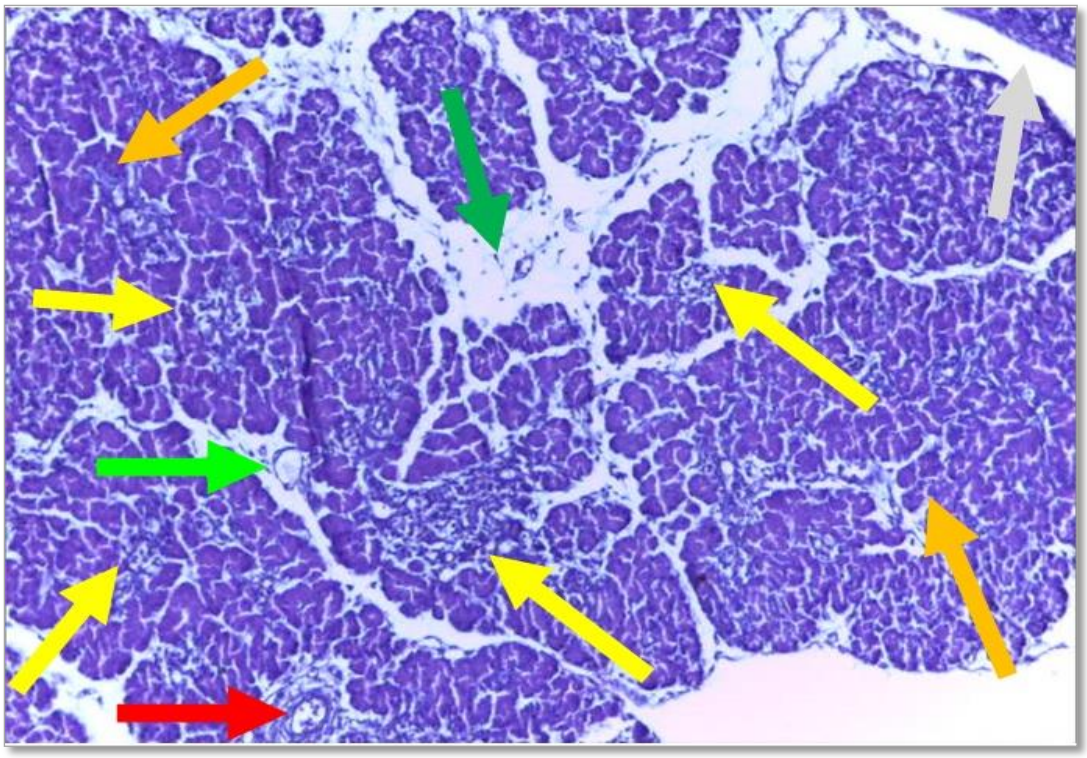

Figure 22: Section in normal pancreas from fetus rat at 18th day of gestation, shown histological, enlargement islet of Langerhans (➡), pancreatic septa (➡), pancreatic duct (➡), normal exocrine acini (➡), accumulation of adipose tissues (➡), and blood capillary (➡). H&E (498 X).

Discussion

Placenta cellularity was unaffected by diabetes in the present study in agreement with the finding of (Husain *et al*., 2001), there was no effect on glycogen content of the placenta which is similar to the finding of (Bueno *et al*., 2010). Although there is deficiency of insulin in diabetic group which should lead to retarded glycogen breakdown, the fetal hyperinsulinemia in response to hyperglycemia will prevent these metabolic events. Increased proliferation of placental spongiotrophoblast cells was observed in diabetic group in the current study and this was in agreement with the result of (Zorn et al., 2011). The proposed mechanism for this phenomenon may be explained by initially, increased expression of proliferative markers in junctional and labyrinth zones of rat placentas and villous cytotrophoblast, syncytiotrophoblast, stromal cells and fetal endothelial cells in human placentas is reported among diabetics. Moreover, reduced apoptotic index and expression of some apoptotic genes are described in placentas of GDM women. In addition, cell cycle regulators including cyclins and cyclin-dependent kinase inhibitors seem to be affected by the hyperglycemic environment. More studies are necessary to check the balance between proliferation, apoptosis and differentiation in trophoblasts cells during maternal diabetes (Aires and dos Santos, 2015). The present study showed that addition of celery seeds extracts resulted in hyperplasia and regeneration of islets of

Langerhans beside elevation of serum insulin hormone level, hence we propose that addition of celery, which contains apigenin and luteolin, stimulated the up regulation of Sox17 gene within pancreatic tissue, by certain unknown mechanism, and subsequently resulted in islets regeneration and increased insulin production. Celery has proved to be effective in causing regeneration and hyperplasia of islets of Langerhans and this may be due to the antioxidant activity of n-butanolic extract of celery (*Apium graveolens*) seeds on pancreatic tissue. This observation was similar to the finding of (Al-Sa'aidi et al., 2012). Increase in glycogen layer was observed in the present study after addition of the n-butanolic extract of celery (*Apium graveolens*) seeds to diabetic group indicating that the improvement in insulin following beta cell regeneration has led to the accumulation of extra glycogen in the placenta. The increment of glycogen in the non-diabetic group also may be secondary to the hyperinsulinemia cause by bet cell proliferation.

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