Antihyperglycemic effect of n-butanol extract of celery (*Apium graveolens*) seeds and expression level of pancreatic, placental and fetal Sox17, Pax6, Ins1, Ins2 and Glucagon genes in STZ-induced diabetic female rats (*Rattus norvegicus*)

Maha Khalil AlMalaak[1] , Nasir Abd Ali Almansour[1], Zahida Miran Hussein[2]
[1]Biology Department, College of sience, University of Basrah
[2]College of Dentistry, University of Qadisiyah

Abstract

The study is designed to clarify the influence of induced diabetes mellitus on maternal rats and their fetus at different stages (14, 16, and 18) days of gestation. Diabetes mellitus was induce in (75) 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 (36) female, also each group subdivided into two groups, health females group as control and the subgroup that treated with n-butanolic extract of celery seeds (60 mg/kg of body weight daily). While, the diabetic group had been subdivided into two groups too, one of them had been treated with celery seeds extraction, but another one had diabetes mellitus without treated, each of the four groups contains (18) females which had been separated as three equal groups (6) at each period (14, 16, and 18) days of gestation. 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 mothers, furthermore there are a difference in the number of fetuses in the horns of uterus. Molecular study of (Sox17, Pax6, Insulin1, Insulin2, and Glucagon genes) in the tissues of (pancreas of mothers, placentas, and fetuses) was refer to the decrease in the level of gene expression in diabetic groups but there are a great increased of it in the groups that treated with celery seeds extract, for all genes of all tissues and in all stages of gestation. It has been concluded that there is an effect of the extract at the genetic level in the tissues studied

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). However, secretion of insulin by in response to glucose, is central for glucose homeostasis, therefore the

dysregulation of this process is a major cause of the early stage of diabetes, so some genes related to glucose level in blood and diabetes directly or indirectly, that genes associated with embryonic development of the pancreas, as well as some of the genes responsible for the secretion of insulin and glucagon hormones, which responsible for regulation of the glucose value in blood, this genes are: Sox17 is the key regulator for giving instruction to cells in early mouse embryos to become either a ventral pancreatic cell or part of the biliary system (Spence *et al.* 2009), also Sox17 gene has a novel role in regulating insulin trafficking and secretion in adult beta cells both in normal and diabetic contexts (Jonatan *et al.* 2014). Other gene was Pax6 which the essential for pancreatic development, particularly in islet cell development (Yasuda *et al*. 2002), moreover, its expression in the pancreas is initiated in the pancreatic progenitors, concomitant with the onset of hormone expression, after which the expression persists in all endocrine cells throughout development and in the adult pancreas (AsheryPadan *et al.* 2004). In the other hand, the treatment agent for diabetes, test the effect of celery seeds extract. Celery *Apium graveolens* belongs to the Spermatophytes division, Angiospermae sub-division, Mangnolisisa class, Rosidace sub-class, Apicedes order, Apiceae family, Apium genus, and graveolens species (Fazal and Singla, 2012). The dry food of *Apium graveolens* family Apiaceae is known as celery, this is popularly known as karnaulli or ajmod, there are four known horticultural type of celery: *Apium graveolens*, *Apium rapaceum*, *Apium secalinum*, and *Apium smallege*, moreover, commercially celery is available as celery seed, celery flaks, vegetable, and celery seed oleoresin, despite the celery seed is one of the lesser known herbs in western herbal medicine, it has been used for thousands of years (David, 2006). Moreover the native habitual of celery is the lowland of Italy from where it spread to Sweden, Egypt, Algeria and Ethiopia in Asia to India, also the celery has been used as an aphrodisiac, anthelmintic, antispasmodic, carminative, diuretic, emmenagogue, laxative, sedative stimulant, and toxic, celery is known as mild diuretic, urinary antiseptic and has been in the relief of flatulence and griping pains, furthermore, Ayurvedic physician (Vaidyas) used celery seed to treat people with cold, flu, water retention, poor digestion, various type of arthritis and certain disease of liver and spleen (Fazal and Singla, 2012). Additionally previous study of AlSa'aidi and AlShihmani (2013), which proved the hypoglycemic activity of celery *Apium graveolens* seed extract, in addition to the same facts that reported by Niaz *et al.* (2013) which proved the hypoglycemic activity of celery too.

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 18th 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 Cº 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) Cº, 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).

Primers: The primers are used in this study, by using NCBI- Gene Bank data base and Primer 3 design online, the primers used in quantification of gene expression analysis using qRT-PCR techniques based SYBER Green DNA binding dye, and supported from (Bioneer, Korea) company as follow table (1):

Genes	Primer sequences		Amplicon
Sox17	F	TTATGGTGTGGGCCAAAGAC	105bp
	R	TCAACGCCTTCCAGGATTTG	
Pax6	F	TGGTGGTGTCTTTGTCAACG	117bp
	R	TTGGACACCTGCAGAATTCG	
Insulin 1	F	AGCAAGCAGGTCATTGTTCC	111bp

	R	GGTGCTGTTTGACAAAAGCC	
Insulin 2	F	TGCCCAGGCTTTTGTCAAAC	139bp
	R	CTCCAGTTGTGCCACTTGTG	
Glucagon	F	AGACAAACGCCATTCACAGG	122bp
	R	TGGCAATGTTGTTCCGGTTC	
B-actin	F	CTAGGCACCAGGGTGTGATG	85bp
	R	GTCAGGATGCCTCTCTTGCTC	

Quantitative Reverse Transcription Real-Time PCR: Real-Time PCR technique is performed for estimation of relative quantification (gene expression analysis). This technique was done according to protocol described by TRIzol® reagent manufacturer with some modification.

Statistics Analysis: Data were summarized analyses and presented using IBM ® SPSS statistic program version 20 and microsoft office excel 2010. Numeric variables were expressed as (mean + SD), whereas categorical variables are expressed as number and percentage. Kruskal wallis test is used to study difference in mean among groups while post hoc LSD test is used to study difference between any two groups. ($P \leq 0.05$) is considered as a level of significant.

Results

Mean Gene Expression in Pancreas of mothers: The results are summarized in table (2) and figure (1), they are as followed: **(1)** Significant difference is observed in Sox17gene expression among groups throughout the (14, 16 and 18) days of gestation that the gene expression is (P=0.024, 0.015 and 0.016) respectively, the highest gene expression is recorded by celery group at day (18) of gestation which is (38.41±6.20), whereas the lowest gene expression is seen in diabetic group at day (18) of gestation which is (0.01±0.00). No significant difference is noticed within each group during days of gestation that the gene expression is (P=0.705, 0.197, 0.086 and 0.087) respectively. **(2)** Data also referred to significant difference is observed in Pax6gene expression among groups throughout the (14, 16 and 18) days of gestation that the gene expression is (P=0.022, 0.019 and 0.016) respectively, the highest gene expression is recorded by celery group at day (18) of gestation which is (31.37±15.71), whereas, the lowest gene expression has seen in diabetic group at day (18) of gestation which is

(0.01±0.00). No significant difference is noticed within each group during days of gestation (14, 16, and 18) days that the gene expression is (P=0.368, 0.058, 0.151 and 0.105) respectively. **(3)** Also significant difference is recognized in Insulin1gene expression among groups throughout the (14, 16 and 18) days of gestation that the gene expression is (P=0.037, 0.026 and 0.019) respectively, the highest gene expression was recorded by celery group at day (18) of gestation which is (51.39±8.30), whereas the lowest gene expression is seen in diabetic group at day (18) of gestation which was (0.01±0.01). No significant difference is noticed within each group during days of gestation that the gene expression is (P=0.57, 0.103, 0.080 and 0.112) respectively. **(4)** Recent study clarified that there is significant difference is observed in Insulin2 gene expression among groups throughout the (14, 16 and 18) days of gestation that the gene expression is (P=0.021, 0.032 and 0.009) respectively, the highest gene expression is recorded by celery group at day (18) of gestation which is (50.69±8.18), whereas the lowest gene expression is seen in diabetic group at day (18) of gestation which is (0.012±0.01). No significant difference is noticed within each group during days of gestation that the gene expression is (P=0.87, 0.137, 0.108 and 0.264) respectively. **(5)** Furthermore there is significant difference in Glucagon gene expression among groups throughout the (14, 16 and 18) days of gestation that the gene expression is (P=0.004, 0.006 and 0.009) respectively, the highest gene expression is recorded by celery group at day (18) of gestation (38.68±6.25), whereas the lowest gene expression has seen in diabetic group at day (18) of gestation which is (0.01±0.00). No significant difference is noticed within each group during days of gestation that the gene expression is (P=0.059, 0.084, 0.058 and 0.103) respectively.

Table (2): Illustrate the gene expression on rats pancreas at each period (14, 16, and 18) days of gestation, values expressed as (mean±SD).

Organ	Gene	Day	Control	Celery	Diabetic	Diabetic and Celery	P
			Mean± SD	Mean± SD	Mean± SD	Mean± SD	
Pancreas	Sox17 gene	14th	1.04±0.16	11.23±6.59	0.20±0.03	7.81±0.63	0.024
		16th	1.09±0.03	12.94±2.63	0.06±0.00	9.33±1.09	0.015
		18th	0.98±0.09	38.41±6.20	0.01±0.00	9.33±1.32	0.016
	P		0.705	0.197	0.086	0.087	
	Pax6 gene	14th	1.01±0.16	7.93±2.46	0.12±0.04	6.38±2.46	0.022
		16th	1.43±0.66	13.46±1.92	0.04±0.02	3.88±1.63	0.019
		18th	1.05±0.10	31.37±15.71	0.01±0.00	8.13±2.36	0.016
	P		0.368	0.058	0.151	0.105	
	Insulin1 gene	14th	0.91±0.14	9.89±5.80	0.17±0.03	6.88±0.56	0.037
		16th	1.60±0.04	18.98±3.87	0.09±0.01	13.69±1.59	0.026
		18th	1.31±0.13	51.39±8.30	0.01±0.01	12.49±1.77	0.014

	P		0.057	0.103	0.080	0.112	
	Insulin2 gene	14th	1.54±0.24	16.67±9.78	0.29±0.05	11.59±0.94	0.021
		16th	1.58±0.04	18.72±3.81	0.09±0.01	13.50±1.57	0.032
		18th	1.29±0.12	50.69±8.18	0.01±0.01	12.32±1.74	0.009
	P		0.087	0.137	0.108	0.264	
	Glucagon gene	14th	1.28±0.20	13.92±8.17	0.25±0.04	9.68±0.78	0.004
		16th	1.63±0.05	19.38±3.95	0.10±0.01	13.98±1.63	0.006
		18th	0.99±0.09	38.68±6.25	0.01±0.00	9.40±	0.009
	P		0.059	0.084	0.058	0.103	

Mean Gene Expression in Placenta: The results are summarized in table (3) and figure (2), they are as followed: **(1)** Significant difference is observed in Sox17 gene expression among groups throughout the (14, 16 and 18) days of gestation that the gene expression is (P=0.008, 0.026 and 0.011) respectively, the highest gene expression is recorded by celery group at day (18) of gestation which is (18.92±6.55), whereas the lowest gene expression has seen in diabetic group at day (18) of gestation which was (0.02±0.02). No significant difference is noticed within each group during days of gestation that the gene expression is (P=0.368, 0.264, 0.097 and 0.970) respectively. **(2)** Recent study clarified that there is significant difference in Pax6gene expression among groups throughout the (14, 16 and 18) days of gestation that the gene expression is (P=0.028, 0.013 and 0.006) respectively, the highest gene expression was recorded by celery group at day (18) of gestation which is (20.27±7.02), whereas the lowest gene expression has seen in diabetic group at day (18) of gestation which is (0.03±0.02). No significant difference is noticed within each group during days of gestation that the gene expression is (P=0.398, 0.274, 0.087 and 0.107) respectively. **(3)** Furthermore there is significant difference in Insulin1gene expression among groups throughout the (14, 16 and 18) days of gestation that the gene expression is (P=0.015, 0.007 and 0.014) respectively, the highest gene expression is recorded by celery group at day (18) of gestation which is (25.31±8.76), whereas the lowest gene expression has seen in diabetic group at day (18) of gestation which is (0.03±0.02). No significant difference is noticed within each group during days of gestation that the gene expression is (P=0.125, 0.872, 0.775 and 0.059) respectively. **(4)** Also significant difference is observed in Insulin2 gene expression among groups throughout the (14, 16 and 18) days of gestation that the gene expression is (P=0.018, 0.026 and 0.035) respectively, the highest gene expression is recorded by celery group at day (18) of gestation which is (24.96±8.64), whereas the lowest gene expression was seen in diabetic group at day (18) of gestation which is (0.03±0.02). No significant difference is noticed within each group during days of gestation that the gene expression is (P=0.815, 0.254, 0.873

and 0.901) respectively. **(5)** Data also referred to significant difference in Glucagon gene expression among groups throughout the (14, 16 and 18) days of gestation that the gene expression is (P=0.009, 0.024 and 0.011) respectively, the highest gene expression is recorded by celery group at day (18) of gestation which is (18.53±6.41), whereas the lowest gene expression has seen in diabetic group at day (18) of gestation which is (0.02±0.02). No significant difference is noticed within each group during days of gestation that the gene expression is (P=0.117, 0.122, 0.068 and 0.802) respectively.

Table (3): Illustrate the gene expression on rats placenta at each period (14, 16, and 18) days of gestation, values expressed as (mean±SD).

Organ	Gene	Day	Control	Celery	Diabetic	Diabetic&Celery	P
			Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Placenta	Sox17gene	14th	0.98±0.16	6.46±2.54	0.24±0.00	4.23±2.87	0.008
		16th	1.00±0.08	11.72±3.27	0.03±0.00	4.26±1.69	0.026
		18th	1.05±0.06	18.92±6.55	0.02±0.02	7.80±2.19	0.011
	P		**0.368**	**0.264**	**0.097**	**0.970**	
	Pax6gene	14th	0.95±0.16	6.27±2.46	0.23±0.00	4.11±2.79	0.028
		16th	0.97±0.08	11.45±3.19	0.03±0.00	4.16±1.65	0.013
		18th	1.13±0.07	20.27±7.02	0.03±0.02	8.36±2.35	0.006
	P		**0.398**	**0.274**	**0.087**	**0.107**	
	Insulin1gene	14th	0.86±0.14	5.69±2.24	0.21±0.00	3.73±2.53	0.015
		16th	1.46±0.12	17.19±4.80	0.05±0.00	6.25±2.48	0.007
		18th	1.41±0.09	25.31±8.76	0.03±0.02	10.43±2.94	0.014
	P		**0.125**	**0.872**	**0.775**	**0.059**	
	Insulin2gene	14th	1.45±0.24	9.59±3.77	0.36±0.01	6.28±4.27	0.018
		16th	1.44±0.12	16.96±v	0.05±0.00	6.17±2.45	0.026
		18th	1.39±0.08	24.96±8.64	0.03±0.02	10.29±2.90	0.035

	P		**0.815**	**0.254**	**0.873**	**0.901**	
	Glucagong ene	14th	1.27±0.21	8.35±3.28	0.31±0.00	5.47±3.71	0.009
		16th	1.38±0.11	16.27±4.54	0.04±0.00	5.92±2.35	0.024
		18th	1.03±0.06	18.53±6.41	0.02±0.02	7.72±2.20	0.011
	P		**0.117**	**0.122**	**0.068**	**0.802**	

Mean Gene Expression in Embryo: The results are summarized in table (4) and figure (3), they are as followed: **(1)** Significant difference is observed in Sox17gene expression among groups throughout the period of gestation (14, 16 and 18) days of experiment (P=0.041, 0.022 and 0.019) respectively, the highest gene expression is recorded by celery group at day (18) which is (7.06±4.12), whereas, the lowest gene expression has seen in diabetic group at day (18) of gestation that the mean of gene expression is (0.02±0.02). No significant difference is noticed within each group during period of gestation (P=0.717, 0.667, 0.097 and 0.097) respectively. **(2)** Significant difference is observed in Pax6gene expression among groups throughout the (14, 16 and 18) days of gestation in experiment which is (P=0.044, 0.024 and 0.017) respectively, the highest gene expression is recorded by celery group at day (18) that the gene expression is (7.57±4.41), whereas the lowest gene expression has seen in diabetic group at day (18) which is (0.03±0.02). No significant difference is noticed within each group in all days of the gestation (P=0.727, 0.717, 0.087 and 0.079) respectively. **(3)** Also significant difference is observed in Insulin1 gene expression among groups throughout the (14, 16 and 18) days of gestation that the gene expression is (P=0.040, 0.027 and 0.016) respectively, the highest gene expression is recorded by celery group at day (18) which is (9.45±5.51), whereas the lowest gene expression has seen in diabetic group at day (18) of gestation which is (0.03±0.02). No significant difference is noticed within each group during all days of gestation (14, 16, and 18) that the gene expression is (P=0.077, 0.264, 0.085 and 0.107) respectively. **(4)** Data also referred to significant difference in Insulin2 gene expression among groups throughout the (14, 16 and 18) days of gestation that the gene expression is (P=0.031, 0.028 and 0.019) respectively, the highest gene expression was recorded by celery group at day (18) which is (9.32±5.43), whereas, the lowest gene expression has seen in diabetic group at day (18) of gestation which was (0.03±0.02). No significant difference is noticed within each group during days of gestation that the gene expression is (P=0.664, 0.737, 0.105 and 0.361) respectively. **(5)** Recent study clarified that there is significant difference in Glucagon gene expression among groups throughout the (14, 16 and 18)

days of gestation that the gene expression is (P=0.040, 0.025 and 0.029) respectively, the highest gene expression is recorded by celery group at day (16) which is (8.58±2.65), whereas the lowest gene expression has seen in diabetic group at day (18) of gestation which is (0.02±0.02). No significant difference is noticed within each group during days of gestation (14, 16, and 18) that the gene expression is (P=0.366, 0.717, 0.097 and 0.308) respectively.

Table (4): Illustrate the gene expression on rats embryos at each period (14, 16, and 18) days of gestation, values expressed as (mean±SD).

Organ	Gene	Day	Control	Celery	Diabetic	Diabetic and Celery	P
			Mean± SD	Mean± SD	Mean± SD	Mean± SD	
Embryo	Sox17gene	14th	1.00±0.02	4.10±1.47	1.12±0.74	2.58±1.01	0.041
		16th	1.01±0.60	6.18±1.91	0.67±0.23	2.37±0.61	0.022
		18th	0.99±0.18	7.06±4.12	0.02±0.02	3.12±1.08	0.019
	P		**0.717**	**0.667**	**0.097**	**0.097**	
	Pax6gene	14th	0.90±0.08	3.98±1.43	1.08±0.72	2.50±0.98	0.043
		16th	0.99±0.59	6.04±1.87	0.66±0.23	2.32±0.60	0.024
		18th	1.06±0.19	7.57±4.41	0.03±0.02	3.34±1.16	0.017
	P		**0.727**	**0.717**	**0.087**	**0.079**	
	Insulin1gene	14th	0.82±0.07	3.61±1.30	0.98±0.65	2.27±0.89	0.040
		16th	1.48±0.89	9.07±2.80	0.98±0.34	3.48±0.89	0.027
		18th	1.33±0.24	9.45±5.51	0.03±0.02	4.17±1.45	0.016
	P		**0.077**	**0.264**	**0.085**	**0.107**	
	Insulin2gene	14th	1.38±0.12	6.09±2.18	1.66±1.10	3.82±1.51	0.031
		16th	1.46±0.87	8.95±2.76	0.97±0.34	3.43±0.88	0.028
		18th	1.31±0.24	9.32±5.43	0.03±0.02	4.11±1.43	0.019
	P		**0.664**	**0.737**	**0.105**	**0.361**	
	Glucagon gene	14th	1.20±0.11	5.30±1.90	1.44±0.95	3.33±1.31	0.040
		16th	1.40±0.84	8.58±2.65	0.93±0.32	3.29±0.85	0.025
		18th	0.97±0.18	6.92±4.03	0.02±0.02	3.09±1.13	0.029
	P		**0.366**	**0.717**	**0.097**	**0.308**	

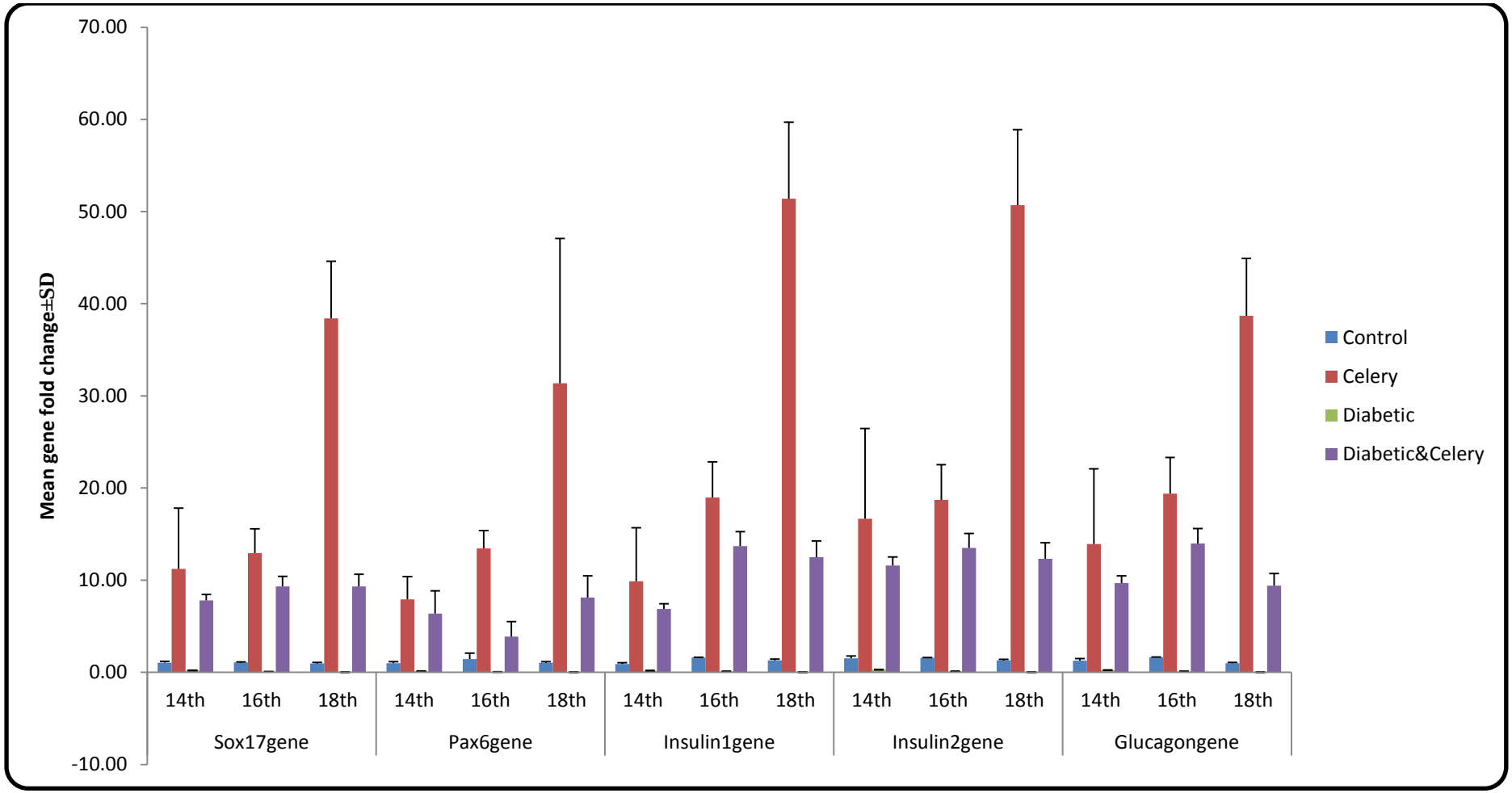

Figure (1): Mean Gene expression levels in Pancreas

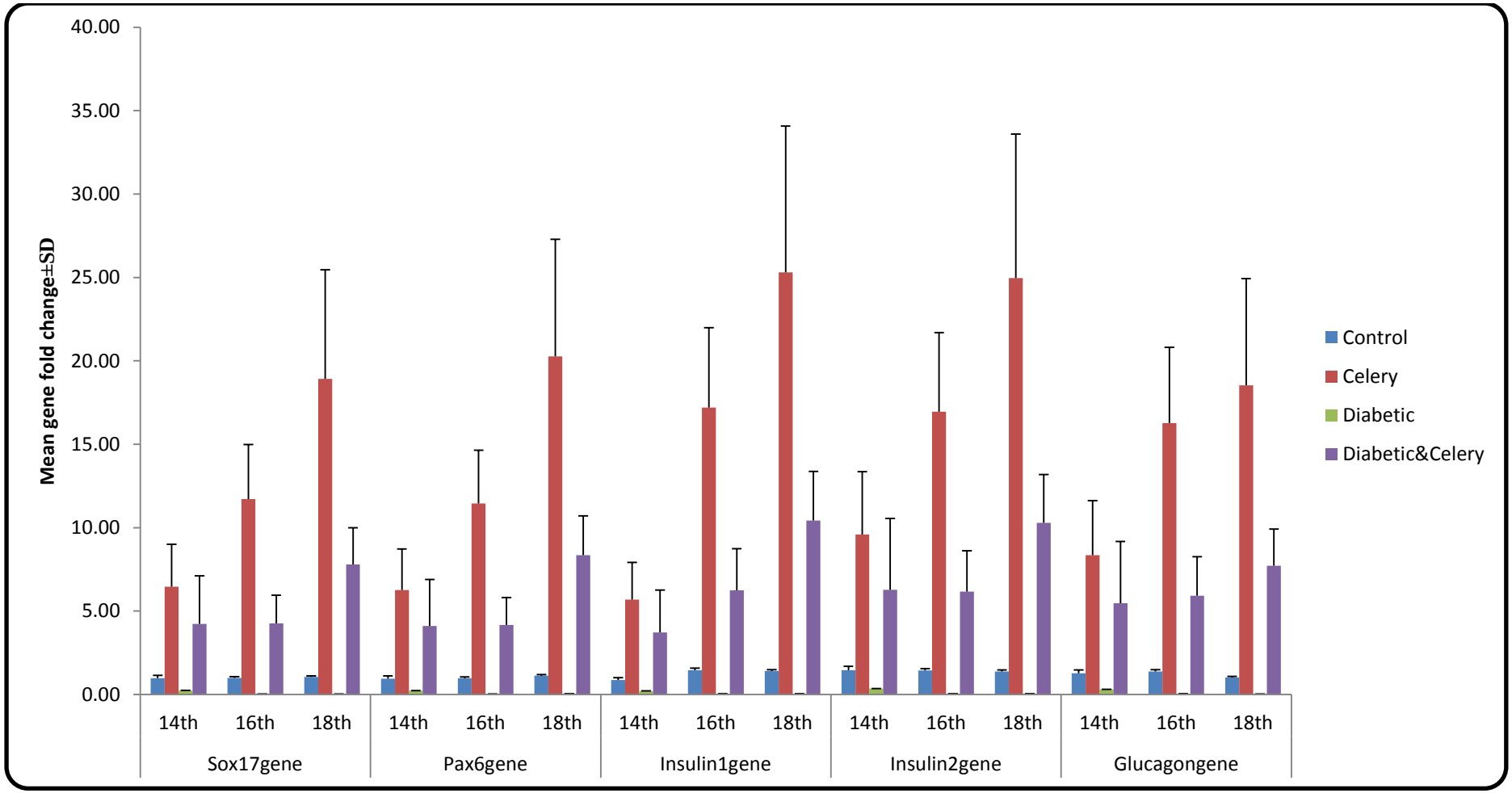

Figure (2): Mean Gene expression levels in Placenta

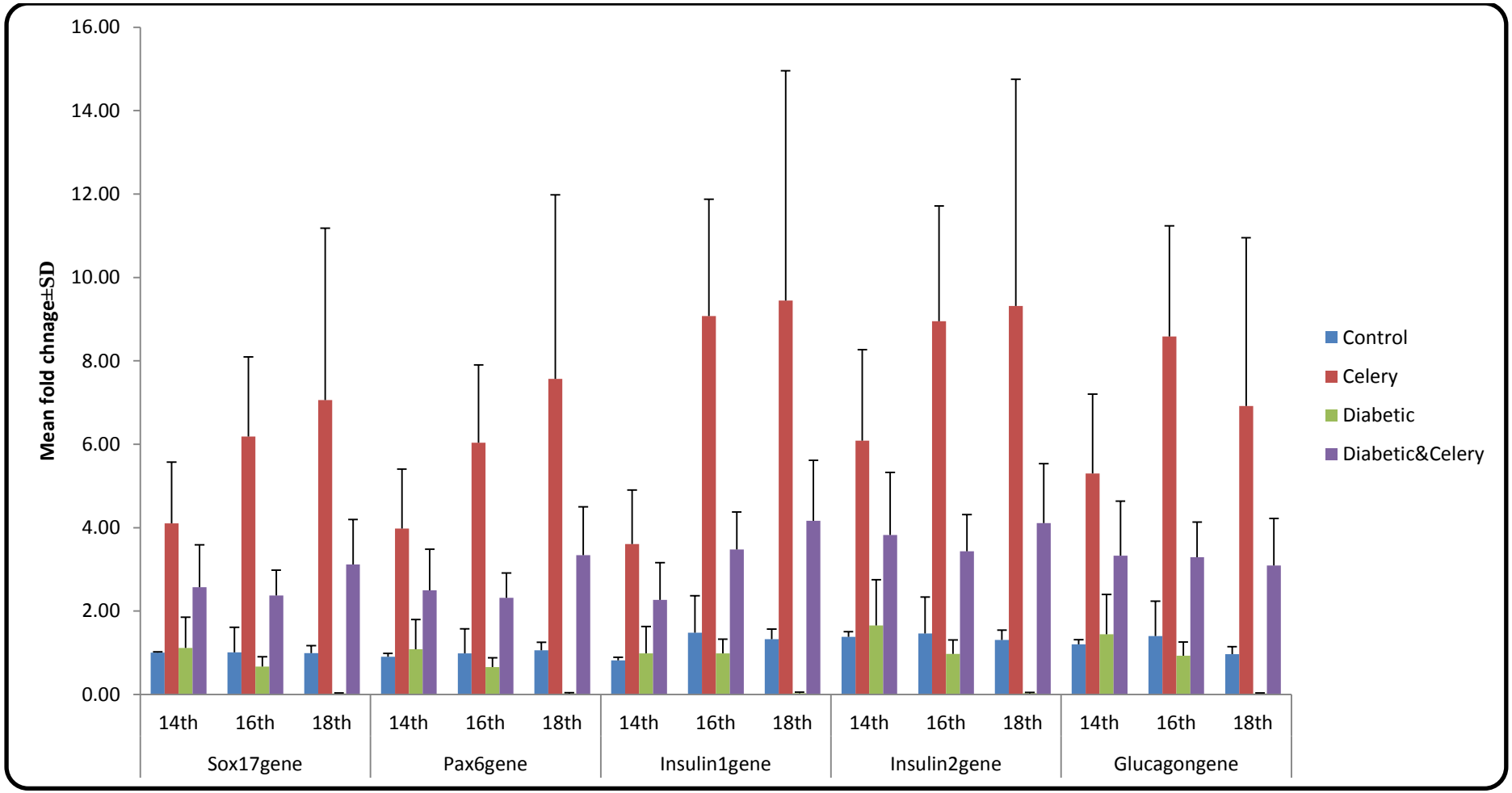

Figure (3): Mean Gene expression levels in embryo.

Discussion

The SRY/HMG box (Sox) family includes several genes that have been extensively studies and their role in developmental process has established. Of the major roles is their involvement in endodermal development, about 11 of these genes and specifically Sox17 have been indentified in fetal pancreatic tissues and plays a major role in pancreatic development (Domínguez-Bendala, 2009). It has been observed by recent authors that certain compounds in the celery seed extracts, namely apigenin and luteolin, have the potential of activating and up regulation of Sox genes (Liu et al., 2016). 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. It has been found that several members of Sox family of genes are expressed within placental tissues (Brunton and Russell, 2011) and also that Sox17 member plays a major role in uterine response to progesterone hormone (Rubel et al., 2012).

Accordingly we suppose that the raised progesterone level, caused by celery, has resulted in stimulation of PR receptors in uterine, ovarian and placental tissues and resulted in increased Sox17 gene expression. We also noticed improvement in the growth of uterus, placenta and ovary following addition of celery, provided with these bits of information we can conclude that growth and envelopment of uterus, ovary and placenta is connected to Sox17 gene expressed somehow. Pax6 gene encodes a transcription factor that is essential for neurodevelopment (Sakurai and Osumi, 2008), eye and the pancreas (Gosmain et al., 2010). Recent researches have extensively investigated the relation between Pax6 gene and α and β cell development and growth in mouse models, these researches showed that deactivation of Pax6 gene in developing rat embryos has resulted in absence of α and β cells from pancreatic tissue (Gosmain et al., 2010), furthermore, researches showed that deactivation of Pax6 gene in adult mouse has resulted in profound atrophy of both α and β (Ashery-Padan et al., 2004).

It was found that high fiber diet increases the expression of certain genes and Pax6 is among them (Berná et al., 2014). The finding of the present study showed increased expression Pax6 gene in fetal tissue which expected for the development of nervous system. But also it has been shown in the current study that Pax6, Insulin1, Insulin 2 and Glucagon genes were all over expressed following celery administration. According to above mention literature we may propose the following mechanism: celery is a high fiber diet and caused, by some obscure mechanism, increased

Pax6 gene expression which in turn caused up regulation of Insulin1, Insulin 2 and Glucagon genes and this may explain the rise of both glucagon and insulin genes expression at the same. The increased expression of glucagon and insulin genes is believed to be directly related to regeneration of both α and β cells through the action of Pax6 gene.

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