Antihyperglycaemic and pancreatic regenerative effect of n-butanol extract of celery (*Apium graveolens*) seed in STZ-induced diabetic male rats.

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ABSTRACT

The antihyperglycemic potency of n-butanol extract of celery (*Apium graveolens*) seed was investigated in Streptozotocin-induced diabetic rats. Seventy two mature male rats were assigned to four groups, non-diabetic control and three diabetic groups. Diabetes was induced by single injection with STZ (60 mg/kg b.w., *i.p.*). Rats ≥ 200 mg/dl of blood glucose were used as diabetic. Diabetic groups (D, N, and I) were drenched with drinking water, n-butanol extract (60 mg/kg, b.w.), or injected with insulin (4 IU/animal), respectively, for 15, 25, and 35 days. Blood samples were obtained every 3 days for assessment of plasma glucose concentrations. On day 15, 25, and 35 days, body weight gain was registered and rats were sacrificed. Specimens from pancreases in all groups have been obtained and fixed in formalin (10%) for histopathological and immunohistochemical study. Diabetic rats (D) showed marked increased blood glucose and decreased weight gain, whereas n-butanol extract of celery seed (N) or insulin (I) therapy moderated blood glucose within normal range and enhanced body weight gain. Immunohistochemical results revealed that n-butanol extract of celery seed treatment caused higher scores of positive cells and intensity of staining compared with other diabetic groups (D and I groups). Histological sections of pancreas from non treated diabetic male rats showed necrosis of most cell population and negatively stained for the few remaining beta cells. These changes were time dependent during the studied experimental periods. It can be concluded that drenching of 60 mg/ kg of n-butanol extract of celery (*Apium graveolens*) seed has hypoglycemic effect and regenerate islets of Langerhans in experimentally-induced diabetic male rats.

Key words: Celery, *Apium graveolens,* diabetes mellitus, hyperglycemia.

INTRODUCTION

Diabetes is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. In type 1 diabetes, the cause is an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of autoimmune pathologic process occurring in the pancreatic islets and by genetic markers. In the other, much more prevalent category, type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response ***(American Diabetes Association, 2011).*** The incidence of type I diabetes mellitus increases rapidly, especially in the developed world, and the time of onset shifts towards a younger age. Type I diabetes mellitus most likely results from a combination of genetic susceptibility and exposure to an environmental trigger. Diabetes incidence has reached epidemic proportions, where it is estimated that some 150 million people worldwide are afflicted with diabetes. There are two major types of diabetes: type 1 (juvenile) diabetes is an autoimmune disease, caused by selective destruction of the insulin-producing beta cells of the pancreas, and type 2 (adult onset) diabetes results from a combination of impaired insulin production by beta cells and impaired insulin action ***(Van Belle et al., 2011).*** During the past few years, some of the new bioactive drugs that are isolated from plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy. The traditional medicine performed a good clinical practice and is showing a bright future in the therapy of diabetes mellitus ***(Malviya et al., 2010).*** Wild celery has a long history as natural medicine and food use. Celery (*Apium graveolens*) is an aromatic bitter tonic herb that reduces blood pressure, relieves indigestion, stimulates the uterus and anti-inflammatory. Celery is one of the most well known plants used in the history of mankind as a medicament or spice. The whole plant has a specific taste and aromatic smell, especially the leaves and roots ***(Teng et al., 1985).*** As of 2009, there are no studies with this herb either by itself or combined with other herbs or food extracts, the alcoholic extract of celery seed has been tested in alloxan-induced diabetic male rabbits ***(Al-Jabori, 2009)*** who found that crude extract of celery seed has a good hypoglycemic effect. In 2009 one study concluded that drenching of 60 mg/kg of n-butanol fraction of methanolic

extract of celery (*A. graveolens*) seed has an efficient hypoglycemic effect in experimentally induced diabetic mature male rats. As well as its positive role in elevating the expression level of *Reg3a, InsI* and *InsII* genes in pancreatic β-cell, when used for 21 days ***(Al-Shwilly, 2011).*** The present study focus on the signaling mechanisms that permit beta cells to couple the release of insulin to physiological needs. On the other hand, the second significance of our study concern with improved treatments and an eventual cure for diabetes which require better understanding of the basic mechanisms controlling beta cell function. Hence, the present experiment has been designed to study the hypoglycemic potential of n-butanol extract of celery (*Apium graveolens*) seed in STZ-induced diabetic male rats at regeneration and insulin genes expression level.

MATERIALS AND METHODS

1. Experimental rats: Mature male Sprague-Dawley rats have been used in the experiment. and were allowed one week to acclimatize to the animal house environment before beginning of experiment. Rats were fed on the standard chow and drinking water *ad libitum* throughout the experiment. Room temperature was maintained at 23 ± 2°C, the light-dark cycle was on a 12:12 h with light on at 06:00 a.m and off at 06:00 p.m throughout the experimental period.

2. **Preparation of n-butanol extract**: Celery (*A. graveolens*) seed was purchased from the local market and classified by State Board for Seed Testing and Classification, Agriculture Ministry, Iraq (SBSTC). N-butanol extract of celery seed has been prepared from methanolic extract according to ***Harborne (1984)*** using Soxhlet apparatus. Using 1 kg of celery seed, methanolic extract was prepared, rotavaporated (40°C and 50-60 rpm), and lyophilized by dry freezer. Dried extract was weighted and stored in deep freeze. According to the polarity, middle polar fraction of the crude extract of celery seed was prepared by using n-butanol. The extract has been evaporated, lyophilized, and kept at -4°C until use ***(Tsi and Benny, 1999).***

3. **Induction of diabetes in rats:** According to ***Mansford and Opie (1968),*** diabetes has been inducted in 54 male rats (weighting 129.2 to 146.8 g and aged 56 days) by injection of single dose of STZ; Sigma Aldrich, England (60 mg/kg b.w., i.p.). STZ was dissolved in 1 M of sodium citrate buffer (pH 4.5). STZ induces diabetes mellitus within 3-5 days by destroying the beta cells of Langerhans islets in the pancreas. The rats with plasma glucose ≥ 200 mg/dL were considered as DM rats and used for experiment ***(Cakatay and Kayali, 2006).***

4. Experimental design: Intact and STZ-induced male rats were

classified into four equal groups (18 rats, each); intact control (C), diabetic control (D), diabetic n-butanol treated (N), and diabetic insulin treated (I) groups**.** Rats of each group have been separated for three equal subgroups (6 per each). Intact and diabetic control rats were injected with normal saline (100µl, *sc*) and drenched with drinking water daily for 15, 25 and 35 days. Diabetic n-butanol treated rats were injected with normal saline (100µl, *s.c*) and drenched with n-butanol extract (60 mg/ kg, b.w.) daily for15, 25, and 35 days. Diabetic insulin treated were injected with insulin (4 IU, *s.c*) and drenched with drinking water daily for 15, 25, and 35 days. Daily body weights have been recorded during the experimental period. All overnight fasted animals were sacrificed after general anesthesia by combination of xylazine and ketamine (10mg and 90mg/kg, *i.p*, respectively). Blood samples were collected for determination of plasma glucose concentrations. Specimens from pancreases in all groups have been removed and fixed in formalin (10%) for histopathological and immunohistochemical study.

5. Blood glucose assessment: According to the manufacturer, blood glucose was measured by using GLUCOSE MR® kit, provided by Cromatest, Spain.

6. Histological study: histological sections were prepared and stained with eosin and haematoxylin according to ***Luna (1968).***

7. Immunohistochemistry assay: According to the manufacture instructions, IHC assay has been performed on rat pancreas by using rabbit anti-human monoclonal IgG, 0.2ml (200ug/ml) provided by Abcam, UK. After deparaffinization and rehydration of formalin-fixed paraffin-embedded tissue sections, enough drops of Hydrogen Peroxide Block were added to cover the sections and incubated for 10 minutes, then washed twice in buffer. Antigen retrieval was performed according to ***Mao et al., (2008)*** by heating the paraffin sections in citrate buffer (pH 6.0) in a 95 °C water bath for 20 minutes, then washed 3 times in buffer. Protein block was applied and incubated for 5 minutes at room temperature to block nonspecific background staining, then washed once in buffer. Primary antibody was applied and incubated at 4 °C overnight according to manufacturer's protocol, washing 4 times in buffer, applying rabbit anti-human monoclonal IgG, 0.2ml (200ug/ml) and incubating for 10 minutes at room temperature, then, it was washed for 4 times in buffer. Streptavidin Peroxidase was applied and incubated for 10 minutes at room temperature, then, it was rinsed for 4 times in buffer. Adding 20ul DAB Chromogen to 1 ml of DAB Substrate, mixing by swirling and applied to tissue, then, it was incubated for 10 minutes, and rinsed for 4 times in buffer. Enough drops of Hematoxylin were added

to cover the sections, and incubated for 1 minute. Rinsing for 7-8 times in tap water and adding Mounting Medium to cover the section.

8. Statistical analysis: Results were expressed as mean ± standard deviation of the mean (SDM). Comparisons were performed using one way analysis of variance (ANOVA1) and newman- keuls to test all groups unpaired values. Differences were considered to be significant at the level of $P<0.05$. All statistical analysis were carried out using the GraphPad Prism (SAS Institute, Inc., USA).

RESULTS

1. Body weight gain: Initial body weight of the rats was approximately similar in all groups. At the end of each treatment period (15, 25, and 35 days), all rats gained weight (Figure 1) but non-treated diabetic rats had a significantly ($P<0.05$) lesser weight gain in all stages of experiment when compared with other three groups.

2. Blood glucose concentration: After injection of STZ, blood glucose concentrations were monitored for five days. One day after, blood glucose has been detected to confirm the presence of diabetes mellitus, whose levels exceed 200 mg/dl was considered to be the zero day of the experiment. The results revealed sharp decrease in blood glucose concentrations of male rats treated with insulin (I group) started from day 3 of experiment and continued to reach the normal levels at sixth day, while n-butanol extract treated male rats (N group) stay six days to begin to decrease and reached the normal level after twelve days. Whereas diabetic non treated male rats (D group) registered significant higher levels ($P<0.05$) among the experimental groups throughout the experiment (figure 2).

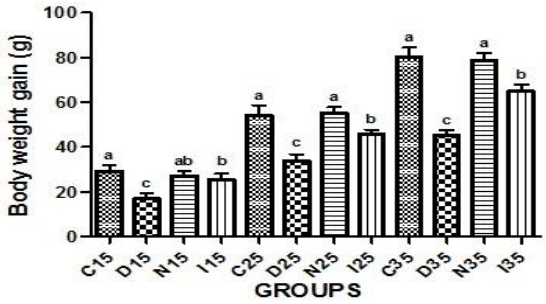

Figure (1): effect of n-butanol extract of celery (*Apium graveolens*) seed on body weight gain (g) at 15, 25, and 35 day stages in streptozotocin- induced diabetic male rats.

Numbers represent mean ± standard deviation.
Different small letters represent significance ($P<0.05$) between groups in each stage.
C: Intact control rats: drenched with D.W. and injected with N.S. (100 µl sc) daily for 15,25,and 35 d.
D: Diabetic control rats: drenched with D.W. and injected with N.S. (100 µl sc) daily for 15,25,and 35 d.
N: Diabetic rats: drenched with n-butanol extract of celery seed (60 mg/kg, dissolved in 500 µl) and injected with N.S. (100 µl s.c) daily for 15,25,and 35 d.
I: Diabetic rats: drenched with D.W. and injected with insulin (100 µl contain 4 IU) daily for 15,25,and 35 d.

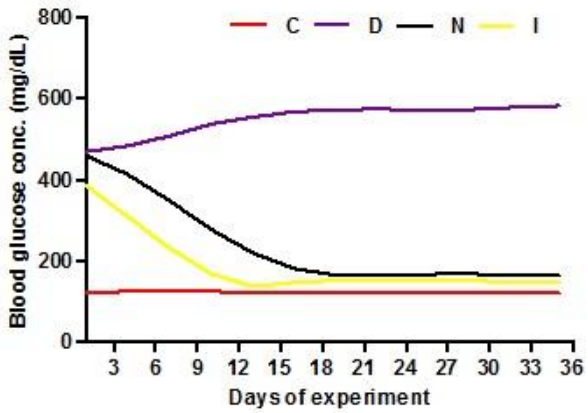

Figure (2): effect of n-butanol extract of celery (*Apium graveolens*) seed on blood glucose concentration (mg/dL) in streptozotocin- induced diabetic male rats.

C: Intact control rats: drenched with D.W. and injected with N.S. (100 µl sc) daily for 15,25,and 35 d.

D: Diabetic control rats: drenched with D.W. and injected with N.S. (100 µl sc) daily for 15,25,and 35 d.

N: Diabetic rats: drenched with n-butanol extract of celery seed (60 mg/kg, dissolved in 500 µl) and injected with N.S. (100 µl s.c) daily for 15,25,and 35 d.

I: Diabetic rats: drenched with D.W. and injected with insulin (100 µl contain 4 IU) daily for 15,25,and 35 d.

3. Results of immunohisto-chemistry: Hisological sections obtained from pancreas of control male rats, in the 15th, 25th, and 35th day stages of experiment, shows actively stained islets of Langerhans by immunohistochemistry with actively stained population of beta, alpha, and delta cells (figure 3-A, B, and C, respectively), whereas those obtained from pancreas of non treated diabetic male rats, during the same previous stages, showed necrosis of most cell population and less affinity to stain of the few remaining beta cells (figure 3-D, E, and F, respectively). On the other hand, sections obtained from pancreas of diabetic male rats treated with n-butanol extract of celery seed, in the 15th, 25th, and 35th day stages of experiment, showed actively stained islets of Langerhans by immunohistochemistry with actively stained population of beta, alpha, and delta cells (figures 3-G, H, and I, respectively). While those obtained from pancreas of diabetic male rats treated with insulin, in the same stages of experiment, showed less staining affinity beta cells and other cells of islets of Langerhans (figure 3-J, K, and L, respectively).

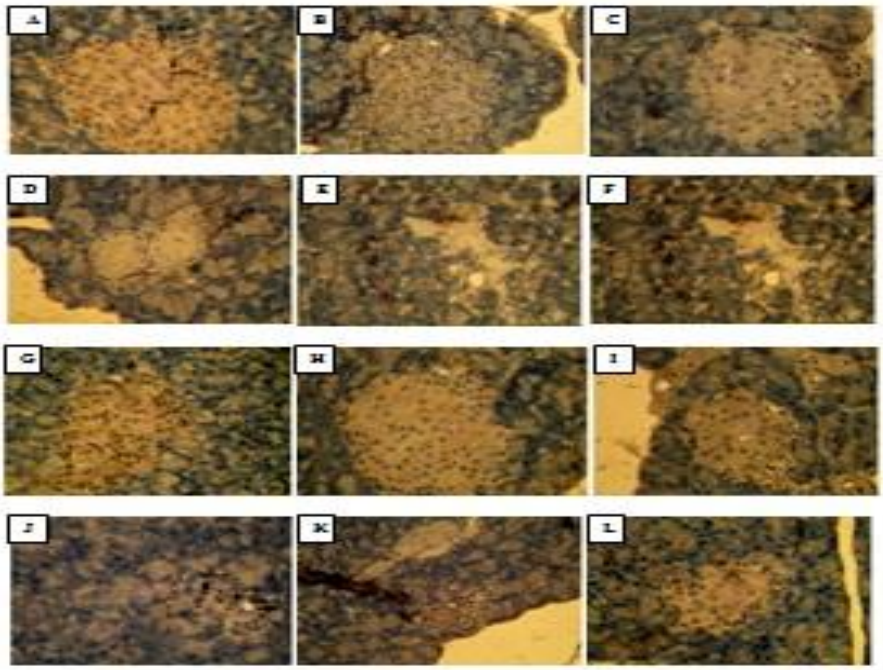

Figure (1): A, B, & C: pancreatic tissue obtained from control rat in the 15th , 25th , and 35th days of experiment, respectively, shows actively stained islet of Langerhan's by IHC with actively stained population beta, alpha, and delta cells. D, E, & F: pancreatic tissue obtained from diabetic rat in the 15th , 25th , and 35th days of experiment, respectively, shows damage of most cell population and less affinity stained for the few remaining beta cells. G, H, & I: pancreatic tissue obtained from diabetic rat treated with n-butanol extract of celery seed, in the 15th , 25th , and 35th days of experiment, respectively, shows actively stained islet of Langerhan's by IHC with actively stained population beta, alpha, and delta cells. J, K, & L: pancreatic tissue obtained from diabetic rat treated with insulin, in the 15th , 25th , and 35th days of experiment, respectively, shows negatively stained beta cells and other cells of islet of Langerhan's. (IHC stain, 40x).

Results of histopathological study: In comparison with pancreatic sections obtained from control rats, in the 15th , 25th, and 35th days of experiment (figure 4-A, E, and I, respectively), n-butanol treated group revealed normal exocrine and endocrine parts of pancreas (figure 4-C, G, and K, respectively) with mild necrosis. Insulin treated group revealed some normal exocrine glands and other are mildly necrotic (figure 4-D, H, and L, respectively). Sections obtained from Diabetic group (figure 4-B, F, and J, respectively) revealed completely or severely necrotic endocrine part with necrosis of exocrine part as well as presence of congestion of blood vessel, but few viable islets of Langerhans are still found in these sections. Other sections showed complete depletion of islets of Langerhans and the exocrine glands are necrotic with increase vascularity and congestion of glandular vessels, but some other exocrine glands are still viable.

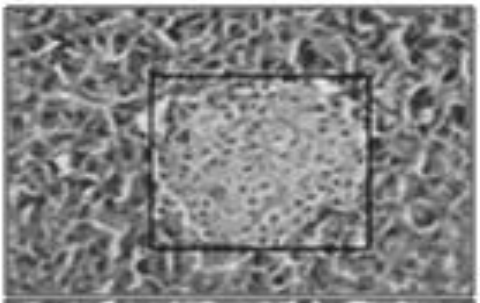

Figure (4-A): Pancreatic section obtained from control male rat, in the 15th day stage of experiment, showed normal endocrine part (1) and exocrine part (2). H&E (40x).

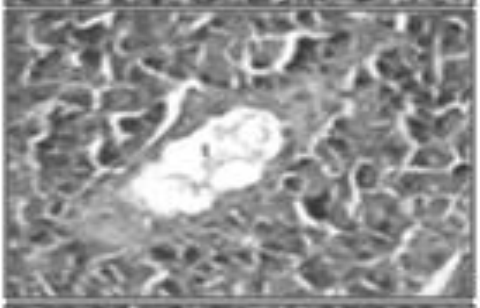

Figure (4-B): Pancreatic section obtained from non treated diabetic male rat, in the 15th day stage of experiment, showed necrotic endocrine part (1) with necrosis of exocrine part (2). H&E (40x).

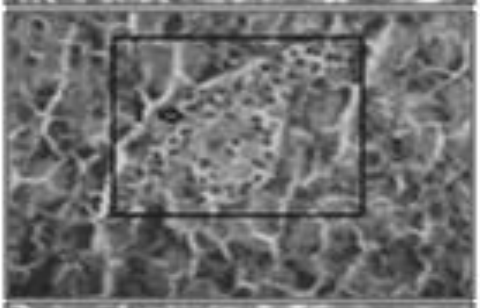

Figure (4-C): Pancreatic section obtained from diabetic male rat treated with n-butanol extract of celery seed, in the 15th day stage of experiment, showed normal exocrine (2) and endocrine parts (1) with mild necrosis. H&E (40x).

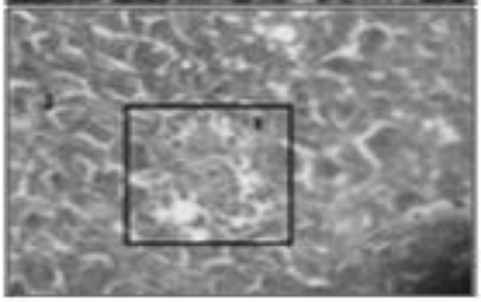

Figure (4-D): Pancreatic section obtained from insulin treated diabetic male rat, in the 15th day stage of experiment, showed some normal exocrine glands (2) and mildly necrotic with mildly degenerated islets of Langerhans (1). H&E (40x).

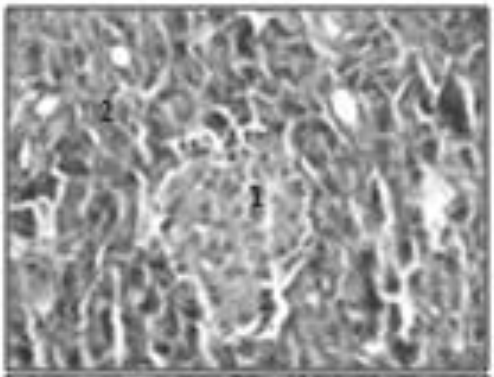

Figure (4-E): Pancreatic section obtained from control male rat, in the 25* day stage of experiment, showed normal endocrine part (1) and exocrine part (2). H&E (40x).

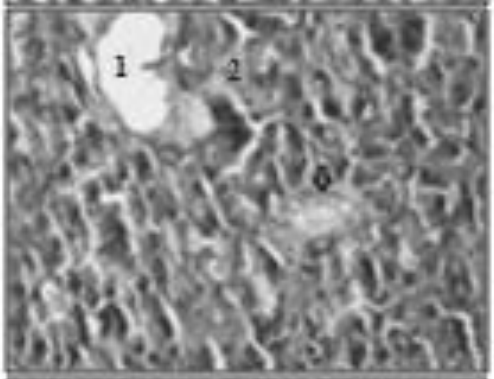

Figure (4-F): Pancreatic section obtained from non treated diabetic male rat, in the 25* day stage of experiment, showed severely necrotic endocrine part (arrow) and complete depletion of Langerhans islet (1). H&E (40x).

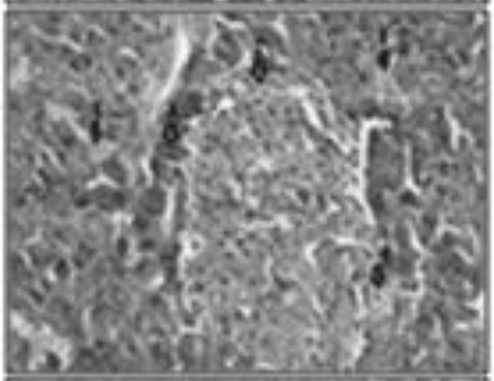

Figure (4-G): Pancreatic section obtained from diabetic male rat treated with n-butanol extract of celery seed, in the 25* day stage of experiment, showed normal exocrine (1) and endocrine parts (2) with mild necrosis (3). H&E (40x).

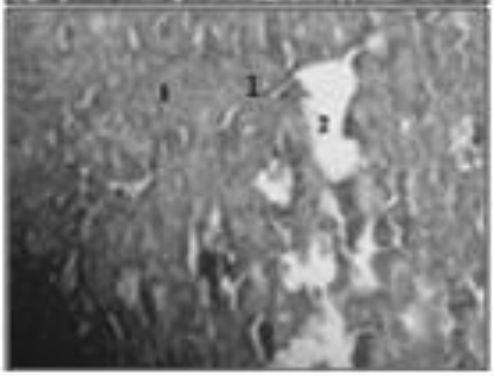

Figure (4-H): Pancreatic section obtained from insulin treated diabetic male rat, in the 25* day stage of experiment, showed some necrosis of exocrine part (1) and depletion of islets of Langerhans (2). H&E (40x).

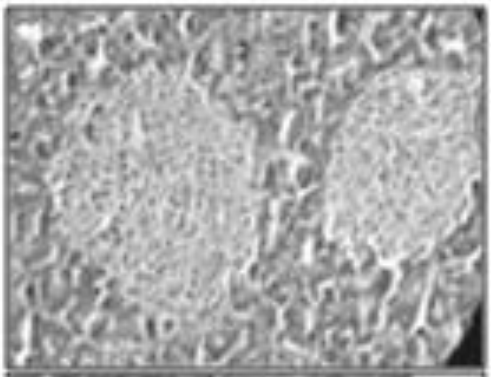

Figure (4-I): Pancreatic section obtained from control male rat, in the 35* day stage of experiment, showed normal endocrine part (1) and exocrine part (2). H&E (40x).

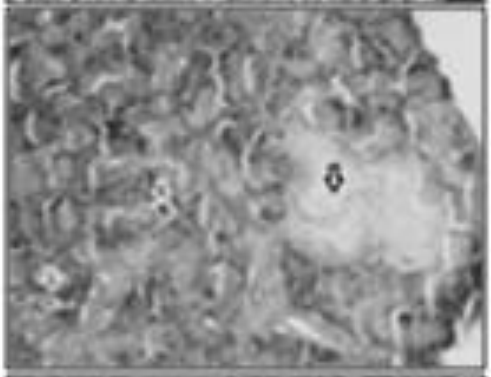

Figure (4-J): Pancreatic section obtained from non treated diabetic male rat, in the 35* day stage of experiment, showed severely necrotic endocrine part (arrow) with sever necrosis of exocrine part (yellow arrow). H&E (40x).

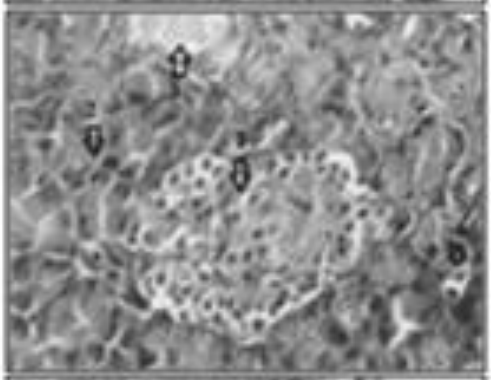

Figure (4-K): Pancreatic section obtained from diabetic male rat treated with n-[illegible] extract of celery seed, in the 35* day stage of experiment, showed normal exocrine (2) and endocrine parts (1) with mild necrosis (3). H&E (40x).

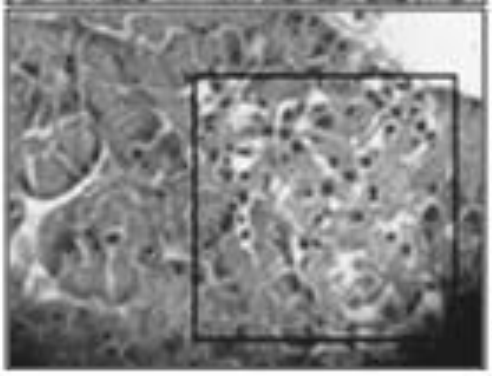

Figure (4-L): Pancreatic section obtained from insulin treated diabetic male rat, in the 35* day stage of experiment, showed some necrosis of exocrine part (2) and necrotic islets of Langerhans (1). H&E (40x).

DISCUSSION

The normal activity and body health of all male rats treated with n-butanol extract of celery seed (*Apium graveolens*) throughout the three stages of the experimental period in the present study, revealed no harmful effect of this extract at the given dose (60 mg/kg b.w.) on general body functions in normal rats, instead, it has a positive beneficial effect in ameliorating side effects of STZ-induced diabetic male rats. Maintenance of body weight within normal range can be considered as a good indicator to the general body health. The increment of body weight in male rats treated with n-butanol extract of celery seed compared with untreated diabetic male rats may results from the beneficial effect of n- butanol fraction of celery (*A. graveolens*) seed extract on the metabolic processes, as a result of increased insulin level and glucose utilization by body cells. ***Marles and Fransworth (1996)*** found that the active compound, cumarine, of celery seed caused decreased blood glucose which may be the main reason of body weight improvement. Also it can be attributed to the effect of the extract on thyroid hormone levels, as it has been found that improvement of metabolic processes and providing energy in available amount for the body to perform its activities, is another cause for retention of additional amount of the hormones responsible for increasing basal metabolic rate, like T3 and T4 ***(Moses, 2001).***

On the other hand, the hypoglycemic effect of this extract, shown in the present study, can be safely concluded that *Apium graveolens* seed possess antidiabetic activity, which at least partly, is mediated by stimulated glucose induced insulin release from beta cells, reduced gluconeogenesis in the liver, and antioxidant activity. Animal experiments have found that the use of *Apium graveolens* seed was safe in appropriate doses ***(Ismael, 2011; Al-Shwilly, 2011; Al-Sa'aidi et al., 2012).*** The failure of hypoglycemic effect of n-butanol fraction of celery seed extract and insulin to reach the control level may be related to the severe cytotoxicity of STZ on the β-cells of the pancreas, which resulted in severe damage of islets cells ***(Ikebukuro et al., 2002; Abu Abeeleh et al., 2009),*** therefore there was no complete response to insulinotropic agents in this model of diabetes mellitus. The hypoglycemic action of n-butanol fraction of celery seed extract may be mediated through a combined action of decreasing hepatic glconeogenesis and activating pancreatic β-cells with increase in serum insulin level. Therefore the observed decrease in blood glucose after two weeks of treatment may be due to decrease hepatic gluconeogenesis, but after activating pancreatic β-cells with increase in insulin level which

become significant after 35 days of treatment, a significant decrease in blood glucose level was observed. So n-butanol extract of celery (*Apium graveolens*) seed showed potent effect in decreasing blood glucose concentration as efficiently as insulin (4 IU/ rat).

Oral administration of 60 mg/kg n-butanol extract of celery (*Apium graveolens*) seed for 15 days in the rats, caused gradual partial regeneration/ proliferation of pancreatic beta-cells and decrease in the elevated serum glucose. Similar effects were observed in STZ induced diabetic rats when given for 21 days ***(Al-Shwilly, 2011).*** In his study, the results were same with similar dose of n-butanol extract of *Apium graveolens*. It seems that *Apium graveolens* seed protect beta-cells against oxidative stress. STZ induces an increase in lipid peroxidation and serum nitric oxide (NO) concentrations, and decreases antioxidant enzyme activity in rats ***(Al-Sa'aidi et al, 2012).*** Islet cell degeneration and weak insulin immunohistochemical staining are observed in rats with STZ-induced diabetes. Increased intensity of staining for insulin, and preservation of beta-cell numbers are apparent in the *Apium graveolens* seed treated diabetic rats. *Apium graveolens* seed treatment exerts a therapeutic protective effect in diabetes by decreasing oxidative stress and preserving pancreatic beta-cell integrity in STZ induced diabetes in rats ***(Ismael, 2011; Al-Sa'aidi et al, 2012).*** In diabetes, *Apium graveolens* seed prevents lipid peroxidation and increases anti-oxidant defence system activity ***(Al-Sa'aidi et al., 2012). Ismael (2011)*** induced diabetes in rats using 60 mg/kg of SZT. Oral n-butanol extract of *Apium graveolens* seed treatment for 21 days in these rats, decreased the elevated glucose and malondialdehyde (MDA) concentrations, increased the lowered glutathione (GSH) concentration, and prevented lipid-peroxidation-induced liver damage in diabetic rats.

In the present investigation no pathological changes were detected in the islets of control rats. In contrast, in STZ diabetic rats. light micrographs revealed many changes in pancreatic islets. Relatively small and atrophied islets were noted. Vacoulation of the cytoplasm and degranulation were observed. These findings were consistent with the results of immunohistochemistry which detected the precise histological findings.

In STZ diabetic rats, microscopical examination showed that α cell secretory vesicles showed intact morphology without significant alterations. However, vacoulation of the cytoplasm was noted in some cells. Some α cells showed cytoplasmic fragmentation when treated with 60 mg/kg STZ. On the other hand, B cells showed several pathological changes. The nuclei showed chromatin aggregation and

reduced heterochromatin indicating DNA damage. It was suggested that these could be due to shrinkage of the nuclear material as a results of accumulation of secretory granules in acinar cells that pushed these nuclei to the periphery. In addition some cells possessed irregular outline nuclei and some nuclei possessed inclusions.

These changes can be interpreted as consequences of long term metabolic impairment. Degranulation was noted in several cells and most probably due to the decreased insulin synthesis. It has been stated that B cell degranulation is encountered in insulin dependent diabetes due to depletion of insulin stores ***(Cotran et al., 1994).*** Administration of *Apium graveolens* seed n-butanol extract decreased some observations noted in the diabetic group. The islets appeared small in size and with reduced number of cells but of normal shape and architecture. The α cells showed normal morphology.

In conclusion, the present study propose that n-butanol extract of *Apium graveolens* seed in a dose of 60 mg/kg of body weight supplemented to diabetes mellitus rats experimentally induced by STZ improves significantly the laboratory parameters of glycemia and diabetes control. However, further randomized placebo controlled clinical trials are needed to prove the promising findings reported in this study.

REFERENCES

Abu Abeeleh M, Ismail Z, Alzaben K, Abu-Halawah S, Al-Essa M, Abuabeeleh J, and Alsmady M., (2009): Induction of diabetes mellitus in rats using intraperitoneal STZ: A comparison between 2 strain Rats. *European Journal of scientific Research*. 32:398-402.

Al-Jabori A., (2009): A comparative study of the effect of insulin and alcoholic extract of *Apium graveolens* seed on the treatment of experimentally-induced diabetes mellitus in mature male rabbit. M Sc. thesis, College of Vet. Med., Al-Qadisiya Univ., Iraq.

Al-Sa'aidi, J.A.A.; Alrhodan, M.N.A.; Ismael, A.K., (2012): Antioxidant activity of n-butanol extract of celery (*Apium graveolens*) seed in STZ-induced diabetic male rats. Research in Pharmaceutical Biotechnology, 4(2): 24-29.

Al-Shwily, H.A.J., (2011): Antihyperglycemic and regenerative effect of n-butanol fraction of celery seed in SZT-induced diabetic male rats. MSc thesis, College of Veterinary Medicine, Al-Qadisiya University, Iraq.

American Diabetes Association (2011): Diagnosis and classification of Diabetes Mellitus: diabetes care, volume 34, supplement 1, January.

Cakatay, U., and Kayali, R. (2006): The evaluation of altered redox status in plasma and

mitochondria of acute and chronic diabetic rats. Clin Biochem; 39: 907-12.

Cotran, R.S.; Kumar, V.; Robbins, S.L.; Schoen, F.J., (1994): Robbins pathologic basis of disease. W.B. Saunders. pp 1-35.

Harborne, J.B. (1984): Phytochemical Methods: A Guide to Modern Techniques of plant Analysis. Chapman and Hall, London, UK., pp: 1-34.

Ikebukuro K, Adachi Y, Yamada Y, Fujimoto S, Seino Y, Oyaizu H, Hioki K, and Ikehara S., (2002): Treatment of STZ-induced diabetes mellitus by transplantation of islet cells plus bone marrow cells via portal vein in rats. *Transplantation* **73** 512-8.

Ismael, A.K., (2011): Antioxidant and Hypolipidemic Effects of n-butanol Fraction of Celery (*Apium graveolens*) Seed Extract in Intact and STZ-Induced Diabetic Mature Male Rats. MSc thesis, College of Vet. Med., Al-Qadisiya Univ., Iraq.

Luna, L. G., Armed Forces Institute of Pathology (U.S.), et al. (1968): Manual of histologic staining methods of the Armed Forces Institute of Pathology. New York,, Blakiston Division.

Malviya, N., Jain, S., and Malviya, S. (2010): Antidiabetic potential of medicinal plants. Acta Poloniae Pharmaceutica- Drug Research, 67 (2): 113-118, 2010.

Mansford, K.R., and Opie, L. (1968): Comparison of metabolic abnormalities in diabetes mellitus induced by STZ or by alloxan. Lancet 1, 670–671.

Mao, M.D., Robert ,J., Kurman, M.D. and IE-Ming ,S. (2008): HSD3B1 as a novel trophoblast-associated marker that assists in the differential diagnosis of trophoblastic tumors and tumorlike lesions. In The American journal of surgical pathology, 32 (2), 236-42.

Marles, R.J.; Fransworth, N.R., (1994): Plants as source of antidiabetic agents. Econ. Med. Plant Res., 6: 149-187.

Moses, G. (2001): Thyroxin interacts with celery seed tablets. Aust. Prescr., 24: 6-7.

Teng CM, Lee. L.G., Ko S.N. (1985): Inhibition of platelet aggregation by apigenin from Apium graveolens Asia Pac. *J. Pharmacol*(3), 85-88.

Tsi, D. and Benny, K.H. (1999): The mechanism underlying the hypocholesterolemic activity of aqueous celery extract, its butanol and aqueous fraction in genetically hypercholesterolemic RICO rats. Life Sci., 66(8): 755-767.

Van Belle T L, Coppieters K T, and Von Herrath MG., (2011): Type 1 Diabetes: Etiology, Immunology, and Therapeutic Strategies. *Physiol. Rev.* 91:79-118.

التأثير الخافض للسكر والمجدد للبنكرياس للمستخلص البيوتانولي لبذور الكرفس (*Apium graveolens*) في ذكور الجرذان المستحدث فيها داء السكري تجريبيا باستخدام عقار الستربتوزوتوسين

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الملخص العربي

أجريت الدراسة الحالية لتقييم فعالية المستخلص البيوتانولي لبذور الكرفس (*Apium graveolens*) في الجرذان المستحدث فيها داء السكري تجريبيا باستخدام عقار الستربتوزوتوسين. تم توزيع ٧٢ جرذا ذكرا بالغا على أربع مجموعات (الضابطة السليمة و ثلاث مجموعات مصابة بداء السكري). تم استحداث داء السكري باستخدام حقنة مفردة من الستربتوزوتوسين (٦٠ ملغم/ كغم من وزن الجسم في البريتون). تم استخدام الجرذان التي بلغ فيها مستوى سكر الدم مساوي أو أعلى من ٢٠٠ ملغم/١٠٠ مل بوصفها مصابة بالسكري. تم تجريع الجرذان المصابة بداء السكري (D و N و I) بماء الشرب والمستخلص البيوتانولي لبذور الكرفس وحقن الثالثة بالانسولين، على التوالي، لمدة ١٥ و ٢٥ و ٣٥ يوما. تم تسجيل وزن الجسم في بداية و نهاية كل مرحلة من مراحل الدراسة كما تم أخذ عينات دم كل ٣ أيام لغرض قياس تركيز الكلوكوز. تمت التضحية بالجرذان في نهاية لكل مرحلة وأخذت منها عينات من البنكرياس وثبتت في الفورمالين لغرض اجراء الدراسة النسيجية- المرضية والدراسة المناعية-النسيجية- الكيميائية.

أظهرت مجموعة D زيادة معنوية في تركيز كلوكوز الدم وانخفاض الكسب الوزني عن مجاميع التجربة الأخرى، بينما أدت المعالجة بالمستخلص (N) والانسولين (I) الى تعديل تركيز كلوكوز الدم ضمن المستوى الطبيعي مع رفع الكسب الوزني قريبا من السيطرة. أشارت النتائج النسيجية بنوعيها الى فعل المستخلص في رفع أعداد الخلايا الموجبة للصبغة مع كثافة الصبغة بالمقارنة مع المجموعات المصابة (D و I). أظهرت مقاطع أنسجة البنكرياس لمجموعة D تلف لمعظم التجمعات الخلوية وكانت سالبة الصبغة لبقايا الخلايا السليمة منها. أن التغيرات الحاصلة ، ايجابية كانت أم سلبية، كانت تتطور مع تقدم الوقت خلال مدد الدراسة.

يستنتج من الدراسة الحالية أن تجريع المستخلص البيوتانولي لبذور الكرفس (*Apium graveolens*) فعال في خفض تركيز سكر الدم وتجديد أنسجة البنكرياس (جزر لانجرهانس) في ذكور الجرذان المستحدث فيها داء السكر تجريبيا.