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Gene expression analysis of CAR2 Effects of n-butanol extract of celery STZ-Indused Diabetic female Rat

Zahida Miran Hussein Alqayyim

Department of Basic Science, Faculty of Dentistry, Al-Qadisyah University, Iraq
E.mail:zahida16.mh@gmail.com, Tel: 009647823502501

ABSTRACT

An change of anti-hyperglycemiain streptozotocin-induced diabetic rats and gene expression (car2) as investigated for the active celery n-butanol extraction (Apium graveolens) crop. Diabetes mellitus induced on pregnant rats, their fetus and organogenesis of the pancreas at each time (14, 16, and 18) days post gestation. This research is performed in (75) healthy as adult virgin females Wistar albino rats (Rattus norvegicus), diabetes mellitus induced one intraperitoneal injection in (36) females before mating using streptozotocin (60 mg / kg animal body weight), instead divided in two classes one intraperitoneal injection. of them treated with n-butanolic celery seeds extract (60 mg/kg of body weight daily), while the other drink tap water, both of them included (18) females. Other (36) females use as control groups, which also divided in to two groups first one treated with the same dose of the extract and the second one drink tap water, both of them included (18) females With respect of molecular study on gene expression of fetus was referred to highly decrease in level of gene expression for genes (CAR2) of diabetic groups with a great increased of it in pregnant rats that treated with extract,whereas the greater value reported byCAR2 genes, alsoshowed clearly increase in diabetic rats that treated with extract, all this changes of the level of gene expression referred to increased through gestation age. Data on gene expression of (CAR2) in fetuses showed great increase in pregnant rats treated with extract, while the level of gene expression reported highly decrease in diabetic rats and clearly increase in diabetic pregnant rats treated with extract. All these changes increased with the time of gestation and reported higher value in 18th day postgestation.The study also included the identification of effective compounds of extract that used as a possible treatment for diabetes in pregnant mothers,included medical important compounds.

Keywords: Celery, Car2 gene, streptozotocin, fetus.

Correspondence:
Zahida Miran Hussein Alqayyim
Department of Basic Science, Faculty of Dentistry, Al-Qadisyah University, Iraq
E.mail:zahida16.mh@gmail.com, Tel: 009647823502501

INTRODUCTION

Gene knockout studies have proved invaluable to recent years in the study of gene control using homologous recombination in embryonic stem (ES) cells (Wyde et al., 2005). Among other mammals, especially the rat, though routine among the dogs, homologous recombination as just an approach to producing loss of function mutations was not feasible. The rat provides a major human disease model, with multiple inbred strains, large numbers of phenotypic data and several transgenic strains (Chen et al., 1997). The rodent seems to be the only form or paradigm of choice for a large range of physiological, behavioral sciences Study, Reading and understanding studies, neuropharmacology and substance addiction, asthma, neurological or respiratory diseases; a large genomic network promotes genetic experiments using the rat as a Ture model (Xu et al., 2018), providing high-density markers, hybrid radiation charts and an EST rat project generating about 60,000 UniGene NCBI clusters; An addition, one big downside in rat model genetics research was the current inability to induce loss of fun due to homologous recombination (gene knock-outs) Rat ES cells have not been able to produce germ line chimeras to date, and this knock-out approach has not been successful. The scientists are curious with flavonoids, because they tend to thrive. Antioxidants help to defend the body from free radicals and oxidative stress (Robillard et al., 1995). There were no flavonoids in the human body and daily meals carry them up. A evidence indicates that taste noids exercise a crucial biological function by the cycle of scavenging reactive oxygen species (Seckl, 1997).Phenolics are also important antioxidants edible to plants or vegetables (Al-Sanabra et al., 2013). Phenolics ' antioxidant abilities arise by their strong reactivity as donors of hydrogen or electrons to the capacity to regulate and move radicals produced by polyphenols.(Hussein, 2017). Therefore the acquisition of possible antioxidants derived from natural sources is of special significance. A number of plants also taught free radical scavenging actions with experimental animals and one of these is the celery. Celery fruit (seed) extracts are widely in many food foods as flavoring ingredients, including meat items, soups, frozen dairy desserts, biscuits, baked goods, gelatins, puddings, condiments and delicacies, sweets, alcoholic and non-alcoholic drinks, among others (AlMalaak et al., 2018). Throughout this analysis, the actions of the n-butanol extract will be studied.

METHODOLOGY

Experimental animal

Total females (75) Albino rats (Rattus norvegicus) classified into two groups (non-diabetic and diabetic), with approximately (36) females in each group,mean (6) pregnant rats per period (14, 16 and 18) days of gestation.

Preparation of n-butanol

After one week of diabetes mellitus adaptation and five days before the mating cycle, the population subdivided into two subgroups (one from the control community and the other from the diabetic party) treated with n-butanolic fraction of celery seed extract in effective dose (60 mg / k of body weight) regularly and persisted until the end of the trial. Celery (A. graveolens) seeds were obtained from the local market and classified by the State Department of Agriculture, Iraq (SBSTC), Seed Testing and Classification Committee. As per (Harborne, 1984)N-butanol extract from celery seeds prepared using Soxhlet Methanol Extracts. Methanol extract was made from 1 kg of celery

seed. Rotavaporated (40 ° C and 50 to 60 rpm), then dry-freezer lyophilized. Dry extract was measured and placed into extreme freezing. The polarity suggests that three types of solvent were used to distinguish different fractions of the crude extract; ethyl acetate, n-butanol, and purified water, utilizing a specific funnel to collect high, medium, and small polar fractions of seed. The n-butanol seed celery fraction was evaporated, lyophilized, and stored at-4 ° C until usage (Tsi & Tan, 2000).

Induction of diabetes in rats

36 Albino rats (Rattus norvegicus), Registered for injection of diabetes from 225-250 g (6-7 week old)(Mansford & Opie, 1968). In 1 M sodium citrate buffer (pH 4.5), rats were injected with liquid STZ (60 mg / kg b.w.\i.p.) STZ causes DM within the Langerhans islet pancreas by destroying beta cells for around 3 to 5 days(Tomlin et al., 2006).

The design of Experimental

Developed to illustrate the impact of diabetes mellitus induced on pregnant rats, their pregnancy and pancreatic organogenesis at each point (14, 16, and 18) days post gestation. The present work is carried out on (75) healthy adult virgin females Wistar albino rats (Rattus norvegicus), diabetes mellitus was induced in (36) pre-mating females utilizing streptozotocin (60 mg / kg of animal body weight) in one intraperitoneal injection dosage,then divided into two sets, one of which was handled with n-butanolic celery seed extract (60 mg / kg of body weight daily), while the other drink tap Certain (36) females are used as monitoring classes, split into two categories. The first was handled with the same extract dose and the second was handled with tap water, both including (18) females, instead mating rats and evaluating zero-day gestation, after which all classes were split and subdivided into three subgroups, namely (6) females for both the period of (14, 16 and 18) days postgestation. That animals were eventually slaughtered, and the embryo was washed in ice-cold natural saline for gene expression analysis.

Preparation Subcellular fluid

Pregnant rats from all classes were anesthetized and dissected by abdominal opening at (14, 16, and 18) days of gestation, fetuses (after the embryonic membranes had been removed) Were perfused by distilled water, until a pink color. Tissues in a ground-glass tissue grinder were homogenized by about 20 strokes up and down. Sucrose (0.88 M) was used to homogenize the particulate fractions, wash them and re-suspend them. Homogenates is fractionated to obtain subcellular fluid using cooled ultracentrifuge (Ayako & Fridovich, 2002).

Determination of blood glucose

The blood glucose content was measured using the test of glucose oxidase (Braham & Trinder, 1972)using a Randox portable industrial testing package, USA.

Realtime-polymerase chain reaction.

Using the RNeasy Micro, RNA protection kit based on the manufacturer's protocol (Qiagen, Courtaboeuf, France), complete cellular RNA was extracted from the pancreas. Total RNA was eluted from the 35 foot RNasc-free water matrix. Residual genomic DNA were extracted for 10 minutes by incubating RNA solution with 15 RNase-free units of DNase I in 2 mM MgCE at 37X, followed by 5 min at 90*^C to inactivate DNase. A reaction consisting of 50 mM Tris-HCl (pH 8.3), 10 mM dithiothreitol, 100 ng random hexamers, 3.5 pg bovine serum albumin, 3 mM MgCb.0.5 mM and 25 microliters of DNase-treated RNA solution are used. 30 RNAccord deoxynucicotide units triphosphates RNaseinhibitor(Promega, Madison, WI), 200 units of the Moloney murine leukemia virus reverse transcriptase (M-MLV RT), and 50 pi of RNase-free water. To order to produce equivalent amounts of overall RNA, the reverse transcription reactions were not standardised. The reactions were incubated for 10 minutes at 26 ® C and then 42 ° C for 45 minutes, accompanied by 3 minutes of incubation at 90 ° C to denature secondary RNA structure. Additional 300unitsofreversetranscriptase was applied, incubating the reactions at 42 ° C for 45 minutes, followed by 75 ° C for 10 minutes to inactivate th. Negative checks were conducted on RT procedures and DNA exposure testing by omitting the reverse transcriptase in parallel samples. The cDNA samples were aliquoted and stored at -80°C. The identical cDNA samples were used throughout the study. Primers for rat car2 were forward(fw), F:50-AGAGAACTGGCACAAGGACTT -30 , R 50-CCTCCTTTCAGCACTGCATTGT -30. PCR conditions were 35 cycles of initial denaturation y'"C for 5 rain, 95°C for 45 s, 60°C for 30 s, 72°C for 1min, and, finally, 72°C for 5 min; viability of the RT ;>rr>duci was controlled by a separate PCR with primers specific forthehousekeeping mRNA GAPDH) (fw:5'-TGAACGGATTTGGCCGTATTGGGC3';rv:5'-CTTCTGGGTGGCAGTGATGGCAT-3').Electrophoresis analyzed the PCR products on even a 1.5 per cent agarose gel. Realtime PCR was conducted use the sets QuantiTect SYBR Green PCR (Qiagen), Opticon-2 PCR (MJ Research), White 965 < PCR and Easy PCR caps (MJ Research). Both primers for both the correct annealing temperature have been checked by gradient PCRs.

Statistical analysis

All the clustered data were analyzed using one-way variance analysis accompanied by Duncan's multi-range research using the SPSS software package, version 9.05. Mean values are about ±S.D. To every party of eight rats. P-value < 0.05 was regarded as significant and included in the analysis.

RESULTS

Bodyweight gain

Results of daily body weight clarified in (Fig.1). revealed significant differences (P<0.05) betweendiabetic groups, normal control group and celery onlygroup starting on the third day and continue throughout the following days of the experiment. On the other hand,the statistical comparison between the three diabetic groups showed that the overall body weight recorded insignificant changes (P>0.05) throughout the experimental period.

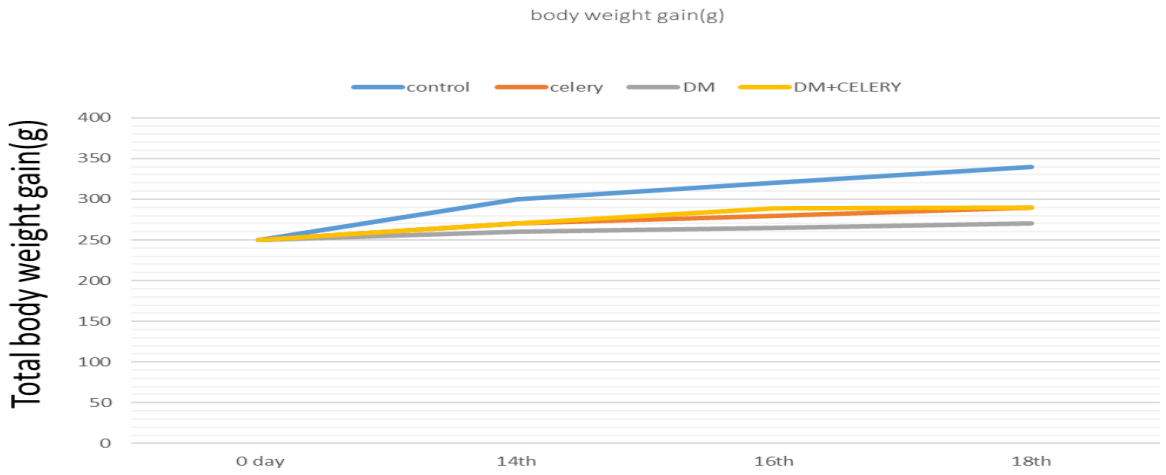

Fig. 1. Effect of n-butanolic extract of celery seeds on body weight gain (g) in strcptozotocin.

Blood glucose

On day five, blood glucose has been measured to select the diabetic rats, whose levels exceed 200 mg/dl. The results revealed that male rats treated with celery recorded the best hypoglycemic effects compared with diabetic control rats. However, their blood glucose concentrations are still higher than that of normal control rats as (Fig.2). On other hand, blood glucose of celery only treated rats showed significant lower concentration ($p<0.05$) and reached to that of normal control rats. groups I and IV were found, but the group III was significantly lower among groups II.

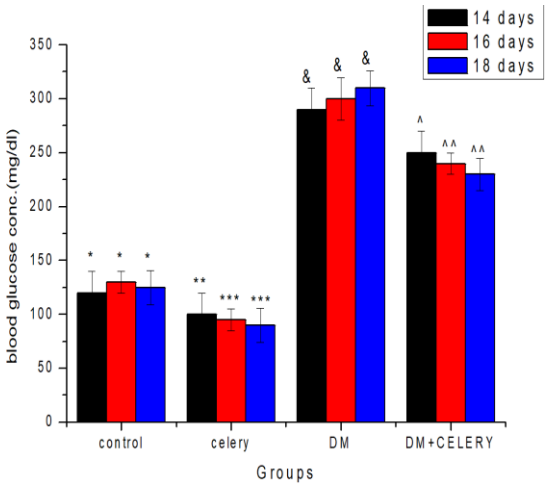

Fig. 2. Effect of n-butanolic extract of celery seeds on blood glucose concentration.(mg/dl). The means P- values are ±S.D. ($p < 0.05$) .

Gene expression analysis

Results of car2 gene expression levels quantification clarified in (table.1) showed that gene Expression levels in embryo tissue derived from usual control group rats and cerley treated group only improved dramatically in accordance with other classes in this experiment. On the other side, Car2 gene expression rates were dramatically improved in community celery treated diabetic rats relative to diabetic control rats, though it was also significantly decreased compared to standard control rats. But, in embryo tissue collected from diabetic male rats treated with celery, the rates of car2 gene expression decreased insignificantly relative to diabetic male rats treated with celery.

Table 1. Data analysis resuls of relative expression of car2 gene. The

Car2 gene expression			
Day / Groups	14 th	16 th	18th
Control	1.519±0.275	1.515±0.135	1.519±0.275
Celery	6.663±2.391	6.663±2.391	10.829±6.316
Diabetic	1.814±1.200	1.814±1.200	0.036±0.024
Diabetic and Celery	4.184±1.648	4.184±1.648	4.778±1.657

mean P- values are ±S.D. ($p < 0.05$) .

DISCUSSION

The objective of this experiment was to determine the antioxidant efficacy of n-butanol (A. graveolens) celery extract seed in mature male rats induced by STZ. This compound was Widespread usage in laboratory animals for development of diabetes (Bianchi et al., 2017)and oxidative stress (Chou et al., 2018), as pancreatic beta-cell death has been discovered that STZ has the characteristics of substance diabetes. Latest experiments have shown that celery seed extract acts as an antihyperglycemic, because it has a free radical scavenger (Fahrenkrug et al., 2017). Our results revealed a hypoglycaemic impact of n-celery seed extract, which can be due to phenolic seed strong ntihyperglycemics (Ahmed, 2017), or high alkaloid and flavonoid concentrations can be suggested are present in n-B celery seed extract (Lotteau et al., 2020). The oral hypoglycaemic effect as found in this work may be responsible. It has been suggested that lipid peroxidation may be a link between tissue injury and liver fibrosis (Parola) by the expression of modulatory collagen genes. As regards genetic research on the expression of fetal genes, the level of gene expression for diabetic genes (CAR2) was significantly decreased groups with a great increased of it in pregnant rats that treated with extract,whereas the greater value reported byCAR2 genes (Maggen et al., 2020), alsoshowed clearly increase in diabetic rats that treated with extract, all this changes in the level of gene expression referred to increased through

gestation age. Data on gene expression of (CAR2) in fetuses showed great increase in pregnant rats treated with extract, while the level of gene expression reported highly decrease in diabetic rats and clearly increase in diabetic pregnant rats treated with extract. All these changes increased with the time of gestation and reported higher value in 18th day postgestation (Hassani & AL-Mallak, 2019).The study also included the identification of effective compounds of extract that used as a possible treatment for diabetes in pregnant mothers,included medical important compounds.. Hyperglycemia may lead to an increase in oxygen-free radicals in diabetes, The data obtained in our study showed that CAR had It plays a positive role in the health of the fetus (Ye et al., 2019). A model for postmenopausal loss (Yang, 2019). Estrogen has the potential to suppress production Inflammatory cytokines, and estrogen-related postmenopausal withdrawal that leads to stimulation The process of chronic inflammation is disorganized by increasing Domestic production of various cytokines rat .In our study, we investigated the celery stimulation process caused by chronic inflammation That was caused by talc in mice. Diabetes decreased significantly in diabetic and celery-treated mice Compared to the control group. Diabetic rat levels were elevated in car2 Inflammation effect of magnesium silicate. The inBMD reduction can be associated with inflammation caused by magnesium silicate in mice, which is very similar to chronic (Mustafa et al., 2016).

Conclusion

It can be concluded that Flavonoids can work in the starting stage of peroxide that interferes with the oxidative metabolism and antihyperglysimic by either clearing free radicals by increasing the gene express for car2 responsible for reducing inflammation in fetus female rat.

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