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

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ABSTRACT

An change of anti-hyperglycemia 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 albinos 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 greater value reported by CAR2 genes, also showed 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.

INTRODUCTION

Gene knockout studies have proved invaluable in 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 (Chenet al., 1997). The rodent seems to be the only form or paradigm as not been successful. 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

Keywords:
Celery, Car2 gene, streptozotocin, fetus.

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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.

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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 
fractons of the crude extract; ethyl acetate, n-butanol, and 
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, 1960). In 1 M sodium citrate buffer 
(pH 4.5), rats were injected with liquid STZ (60 mg / kg 
bw.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 
iuced 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 
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 
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 RNase-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 MgCl₂ at 37°C, 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 
Magnesium·0.5 mM and 25 microliters of DNase-treated RNA 
solution are used. 30 RNaccord deoxynucletotide 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 300 units of reverse transcriptase 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-
AGAGAATCTGGCAAGAGACTT -30 , R 50-
CTCTCTTTTACGACTGCAATTGT -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 ;r>ducil was controlled by a separate 
PCR with primers specific forthehousekeeping mRNA 
GAPDH (fw:5'TGAAACGATTTGCGGTATTGCCG;rv:5'-
CTCTGGGGTGGGAGTGATGGA -3'). Electrophorosis 
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 (M Research), 
White 965 < PCR and Easy PCR caps (M 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) between diabetic 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.
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 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.

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 celery 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 results of relative expression of car2 gene.

<table>
<thead>
<tr>
<th>Group</th>
<th>14 th</th>
<th>16 th</th>
<th>18th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.519±0.27</td>
<td>1.515±0.13</td>
<td>1.519±0.275</td>
</tr>
<tr>
<td>Celery</td>
<td>6.663±2.39</td>
<td>6.663±2.39</td>
<td>10.829±6.31</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1.814±1.20</td>
<td>1.814±1.20</td>
<td>0.036±0.024</td>
</tr>
<tr>
<td>Diabetic and Celery</td>
<td>4.184±1.64</td>
<td>4.184±1.64</td>
<td>4.778±1.657</td>
</tr>
</tbody>
</table>

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 hypoglycemic impact of n-celery seed extract, which can be due to phenolic seed strong antihyperglycemics (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 by CAR2 genes (Maggan et al., 2020), also showed 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 tcalc 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 antihyperglycemic by either clearing free radicals by increasing the gene express for car2 responsible for reducing inflammation in fetus female rat.

REFERENCES


