Evaluated the Up –regulation in Gene Expression of Hepatic Insulin Gene and Hepatic Insulin Receptor Gene in Type 1 Diabetic Rats Treated with Cuscuta chinesis Lam.

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Abstract
This research was conducted to study the hypoglycemic activity of C. chinesis Lam. on type 1 diabetic disease and investigate the molecular and histological mechanism of its action. Many parameters were investigated, Fasting blood glucose (FBG), Fasting serum insulin, Hepatic Insulin Gene Expression, pancreas Insulin Gene Expression, Hepatic Insulin Receptor Gene expression and histological sections of pancreas and liver. 54 Rattus rattus male rats weighting (180 - 200 g) were divided into 3 groups: A normal control daily administrated with Dw, B diabetic control daily administrated with Dw and C diabetic group daily administrated with 400 mg/Kg body weight of C. chinesis Lam. methanolic extract, each group consisted of 18 rats and further divided into (3) sub-groups 1, 2, and 3. According to the period of administration 30, 60, and 90 days respectively. The results showing the daily administration of 400 mg/Kg body weight of C. chinesis Lam. methanolic extract for 60 days causing significance decrease in FBG and in the other hand each of fasting serum insulin, hepatic insulin gene expression, pancreas insulin gene expression and hepatic insulin receptor gene expression was increased in group C in compare to B group and return all studied parameters involving pancreas and liver texture to the normal state, which were statically morphologically not appeared any significant difference from A group. This study concluded that the daily administration type 1 diabetic rats with 400 mg/Kg body weight of C. chinesis Lam. extract for 60 days was return fasting serum insulin and FBG to normal value by upregulated the gene expression of hepatic INS Gene, INSR gene, pancreas INS Gene, regenerate pancreatic beta-cell and return the texture of both liver and pancreas to the normal state.

Key words: up-regulation, hepatic INS Gene, INSR gene, pancreas INS Gene, histological sections of pancreas and liver, Cuscuta chinesis Lam., type 1 diabetic rat

الخلاصة

تُنجز هذه الدراسة للكشف عن فعالية مستخلص نبات الحامول الخافض لسكر الدم، دراسة آلية عمله على المستوي العصبي والخليجي للإعفاء، ذات العلاقة مع مرض السكر (البلدي والكبد)، حيث تم قياس العديد من المؤشرات مثل مستوى سكر الدم، مستوى الانسولين في المصل، التعبير الجيني لكل من جين الانسولين في الكبد، جين الانسولين في البنكرياس وجنين مستقبل الانسولين في الكبد بالإضافة إلى إجراء فحصات لمقاطع في نسيج البنكرياس والكبد. لإجراء هذه التجربة، تم تقسيم اربعي وخمسون ذكرًا من الجرذان نوع Rattus rattus والذين تراوحت أوزانهم من 180-200 غم إلى ثلاثة مجموعات رئيسية: مجموعة A (مجموعة السيطرة للجرذان الطبيعي والتي تم تجريعها بالماء المقطر يوميًا، مجموعة ب (مجموعة السيطرة للجرذان المصابة بداء السكري النوع الأول والتي تم تجريعها أيضاً بالماء المقطر يوميًا) أما المجموعة الثالثة فكانت (مجموعة الجرذان المصابة بداء السكري النوع الأول والتي تم تجريعها يوميًا بالمستخلص الميثانولي بمقدار 400 ملغ/ كغم من وزن الجسم). تم تقسيم كل مجموعة رئيسية والتي كانت تتألف من 18 جرذًا إلى ثلاث مجموعات ثانوية وفقًا لنتيجة التجريع: مجموعة رقم (1) ، مجموع
1. Introduction

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes such as transfer RNA (tRNA) or small nuclear RNA (snRNA) genes, the product is a functional RNA (Brueckner et al., 2009).

Gene expression is a tightly regulated process that allows a cell to respond to its changing environment. However, scientists are interested in study gene expression to examine the changes increase or decrease in the expression of gene or several genes through measuring the abundance of gene-specific transcripts quantity with Real-Time PCR. This quantity will monitor the gene response to defined treatment under specific set of circumstances. Gene expression can also be used to look at profiles or patterns of expression of several genes (Joyce, 2002). Many cellular functions are regulated by changes in gene expression. Thus, quantification of transcription levels of genes plays a central role in the understanding of gene function and of abnormal alterations in regulation that may result in a disease state. The innovation of the real-time polymerase chain reaction (PCR) technique played a crucial role in molecular medicine and clinical diagnostics. Examples are the quantitation of relative gene expression, detection the level of disease progress, diseases diagnostics, and measurements of DNA or transgene copy number, and allelic discrimination. Analysis of messenger RNA (mRNA) is widely used to investigate the change in the level of expression of any target gene (Jump up and Varkonyi-Gasic, 2010).

Many researchers like Joyce (2002) and Jump up and Kang (2010) using Real-time PCR analysis and Real-time quantitative reverse transcriptase PCR (RT-PCR) to evaluated the changes in gene expression of genes, hormones and metabolite- or to estimating the activity of some drug-in modulation the metabolic and hormonal milieu in most organs.

Mariani et al. (2003) and Jump et al. in (2014) showed that the folding change is a measure describing how much a quantity changes going from an initial to a final
value. And the folding changes is calculated simply as the ratio of the difference between final value and the initial value over the original value.

In rat there are two preproinsulin genes have been conclusively demonstrated, preproinsulin genes I and II, which are nearly identical (93% homologous in their coding sequences) (Muglia and Lockor, 2004). The two preproinsulin genes of rats are both expressed and are nonallelic. The rat preproinsulin genes I and II are found 100,000 kilobase pairs (kbp) apart on chromosome 1 (Ohlsson and Thor, 2002). The insulin gene is expressed almost in pancreatic β-cells and Glucose in blood is the major stimulant that regulates the insulin gene expression and enables the beta cells to produce insulin and maintain an adequate store of intracellular insulin to sustain the secretory demand. (Hedeskov, 2005).

Low insulin production in diabetes may occur if there is continued high levels of glucose or lipids in blood. This leads to glucotoxicity or lipotoxicity respectively. This leads to worsening of β-cell function in type 2 diabetes, in part via inhibition of insulin gene expression. This glucotoxicity involves decreased binding activities of PDX-1 and MafA and increased activity of C/EBPβ((Karlsson et al., 2007). High levels of glucose also leads to damage due to generation of oxidative stress. Lipotoxicity also leads to de novo ceramide synthesis and involves inhibition of PDX-1 nuclear translocation and MafA gene expression (Urinir et al., 2011).

Saunders and Terblanche (2003) and Jump up and Aggarwal (2012) showed that the INS gene expression was an instructions to determined producing the hormone insulin, which is necessary for the control of glucose levels in the blood. And others like Jun et al in (2012) studying the effects and mechanisms of some phytochemical compound like berberine to treat type 1 DM by up-regulating the gene expression of both INS and INSR genes.

Renewed attention to alternative medicines and natural therapies has raised researcher interest in traditional herbal medicine. Because of their perceived effectiveness, with minimal side effects and relatively low costs, herbal drugs are prescribed widely, even when the contents of their biologically active constituents are unknown (Ekor, 2017). Hence, people are seeking traditional medicines for the management of DM. So this resent study aimed to detect about the hypoglycemic activity of C. chinesis Lam. on type 1 diabetic disease and investigate the molecular and histological mechanism of its action.

2. Material and Method

2.1. Plant Collection, Identification and Drying

The hollow plant of Cuscuta chinesis Lam. was collected at duration mid of November to mid of December 2016 from gardens of Babylon university, then the plant was identified by Dr. Nedaa Adnan (Plant herbarium / department of biology / college of science / university of Babylon). The collected plant was dried in shad at room temperature for 10 days. Dried plant was milled by using electric mill.
2.2. Plant extract preparation

The dried and powdered plant materials were extracted with solvent methanol – water (1:1 V/V) according to Ekpenyong et al. (2012) with some modification. One gram of plant powder : 10 ml of solvent was blended for 30 min at room temperature. The suspension was filtered by guise and the filtrated liquid was concentrated to dryness in oven at 45 ºC. The dried concentrated material was milled by using electric mill and the final powder was sterilized by UV equipment for 20 min.

2.3. Animals

Fifty four healthy White Male adult of Rattus rattus - rats weighing 180-200 gram at the age of 2.0-2.5 months have been used. Animals were obtained from the animal house, Pharmacology college, Al- Mustanseriyah University and were housed in animal place with room temperature being maintained at 25±2 ºC. Animals were fed on a commercial pellet diet and kept under normal light/dark cycle. Animals were divided into 3 groups A (normal control daily administrated with Dw ), B (Diabetic control daily administrated with Dw ) and C ( diabetic group daily administrated with 400 mg/Kg body weight of C. chinesis Lam. methanolic extract ), each group consisted of (18) rats and further divided into (3) sub- groups 1, 2 and 3. According to the period of administration 30, 60 and 90 days respectively.

2.4. Type 1 Diabetes mellitus rats induction

For experimental induction of Type 2 diabetes mellitus in rats alloxan dose of 160 mg/Kg body weight given intrapritonealy in single dose to fasting rats (Chougale et al., 2007).

2.5. Administered dose of crud plant

Effective dose of plant extract was detected by testing the orally administration of three doses(200 , 300 , 400) mg/kg body weight to 18 diabetic rats ( 6 rats for each dose) and after one month the most effective dose with no mortality recorded was chosen , which was 400 mg/kg body weight to perform experiment.

2.6. Blood samples and biochemical tests:

The blood samples of 6 rats from each group were withdrawn by puncturing the retro-orbital plexus under ether anesthesia. The blood samples were allowed to clot for 30-40 min. at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 min. and various biochemical parameters were estimated like Fasting blood glucose (FBG), serum insulin serum, were carried out as the following:

2.7. Fasting blood glucose (FBG)

Fasting blood glucose were measured by using ACCU-CHEK Active System (Germany). Were one drop of rat blood was putted in specific strep and then automatic result was taken.

2.8. Serum insulin measurement

Insulin serum was measured by Rat Insulin Elisa Kit (CALBIOTECH , USA)
2.9. The gene expression and Real-Time PCR test

To study the regulation effect of *Cuscuta chinesis* Lam. on Gene expression for Insulin gene 1 in liver and pancreas and Insulin receptor gene in liver, Real-Time PCR were investigated and analysis according to (Livak & Schmittgen, 2001).

2.9.1. Real-Time PCR Primers

The primers of this genes were designed by using Gene-Bank data base NCBI (https://www.ncbi.nlm.nih.gov/nuccore/XM_003235234.1) In addition to primer 18S was designed by Humanizing Genomics macrogen /south korea in 29 /5 /2017 as shown in table (3-1).

**Table (3-1): Primers used for gene expression according Gene-Bank data base (NCBI).**

| organ       | Primer               | Sequences (5'-3')                  | Amplicon size bp |
|-------------|----------------------|------------------------------------|------------------|
| Liver       | Insulin gene 1       | F 5'- CCA GTT GGT AGA GGG AGC AG - 3' | 179              |
|             |                      | R 5'- CAC CTT TGT GGT CCT CAC CT - 3' |                  |
| Liver       | Insulin receptor gene| F 5'- CGT CAT CAA TGG GCA GTT - 3'  | 83               |
|             |                      | R 5' – GTG ACT TAC AGA TGG TTG GG - 3' |                  |
| Pancreas    | Insulin gene 1       | F 5'- CCA GTT GGT AGA GGG AGC AG - 3' | 179              |
|             |                      | R 5'- CAC CTT TGT GGT CCT CAC CT - 3' |                  |
| House keeping | 18s gene            | F 5' – GTA ACC CGT TGA ACC CCA TT – 3'  | 156              |
|             |                      | R 5' – CCA TCC AAT CGG TAG TAG CG –3' |                  |

2.9.2. Total RNA Extraction

The concentration of the total RNA yield was measured by a Quantus Florometer (Promega, USA).

2.9.3. Quantitative Real Time PCR (qRT–PCR)

The expression levels of Insulin gene 1 in liver and pancreas and Insulin receptor gene in liver, were estimated by One Step qRT-PCR. To confirm the expression of target gene, quantitative real time One step qRT-PCR SYBR Green assay was used by using M-MLV Reverse Transcriptase kit, FicoScript/ Canada and done according to company instructions as showed in the following tables.
Table (3-2) Reaction volume and components of qRT–PCR

| Components       | Concentration | Volume (μl) |
|------------------|---------------|-------------|
| Master Mix       | 2X            | 10 μl       |
| RT Master mix    | -             | 0.4 μl      |
| Forward Primer   | 10μM          | 2 μl        |
| Revers Primer    | 10μM          | 2 μl        |
| RNA              | 1-2ng         | 5 μl        |
| RNase-free water | -             | 0.6 μl      |
| **Total volume per reaction** |               | = 20 μl    |

Table (3-3) qRT–PCR Thermal Cycler Programming

| Steps          | Temperature | Time | Cycle number |
|----------------|-------------|------|--------------|
| cDNA Synthesis | 37 C°       | 15min| 1            |
| Initial Denaturation | 95 C°     | 5 min| 1            |
| Denaturation    | 95 C°       | 30 sec| 40          |
| Annealing       | 60 C°       | 30 sec| 40          |
| Extension       | 72 C°       | 30 sec| 40          |
| Final extention | 65-90 C°   |      | 1            |

2.4.4. Data Analysis of qRT-PCR

The data results of qRT-PCR for target and housekeeping gene were analyzed by the relative quantification gene expression levels (fold change) The ΔΔCT Method Using a Reference Gene that described by (Livak and Schmittgen, 2001). The relative quantification method, quantities obtained from qRT-PCR experiment must be normalized in such a way that the data become biologically meaningful. In this method, one of the experimental samples is the calibrator such as (Control samples) each of the normalized target values (CT values). The ΔCT of control and target gene are calculated as in the following equation:

\[ \Delta \text{CT target gene} = \text{Ct}_{\text{target gene}} - \text{Ct}_{\text{reference gene/target}} \]

\[ \Delta \text{CT control} = \text{Ct}_{\text{control}} - \text{Ct}_{\text{reference gene/control}} \]

After that, ΔΔCT was calculated after the calculation of ΔCT of target gene and control as in the following equation:

\[ \Delta\Delta \text{CT} = \Delta \text{Ct}_{\text{target gene}} - \Delta \text{Ct}_{\text{control}} \]

Then \( 2^{-\Delta\Delta \text{CT}} \) was calculated as fold change in expression.
2.10. Histological study

Histological Processing are performed according to (Bancroft & Stevens, 1982) where the haemotoxylin-eosin staining and Peroidic acid shiff (PAS) staining were used.

2.11. Statical analysis

The data were reported as mean ± standard error. For determining the statistical significance one-way analysis of variance (ANOVA), Duncan test was employed. P-values of less than 0.05 were considered significant (Verma & Ahmed, 2009).

3. Results and disscusion

1. Fasting blood glucose (FBG)

The results showed in table and figure (3-1) the variation of fasting blood glucose in normal control and all experimental groups, where explained that the alloxan injection caused significant increase in mean of FBG levels (283) mg/dl as compared to normal negative control (111) mg/dl and that significant increase of FBG level in control diabetic group as the period increase(283, 372, 473) in periods (30, 60 and 90) day respectively. Alloxan has been used in induction of diabetes in experimental models due to its ability to destruction pancreatic β- cells islets which producing insulin and the persistent and increasing hyperglycemia revealed the ordinary progress of this disease (Rajagopal and Sasikala, 2008). These results agree with (Amer, 2012 and Alshukri, 2016) whose showed that alloxan causing asignificant increase in the FBG of experimental animals compared with control.

When comparing FBG level of treated Diabetic group with control Diabetic group. There was significant decrease in mean of FBG level in treated Diabetic group after treatment with C. chinesis extract and that hypoglycemic effect increased when the period of administration increased as compared to diabetic control group. Administration treated Diabetic group with C. chinesis for 60 day reached the FBG level to (90mg/dl), which was significantly not different than normal group, which were (102) mg/dl. The strong hypoglycemic effect of C. chinesis regard to that was rich in many anti hyperglycemic phytochemical compounds like berberine, kaempferol, quercetin, coumarins, and glycosides (Sineeporn et al., 2014). Berberine inhibited mitochondrial function and activated AMPK to enhances glucose uptake, decrease G6Pase gene expression to inhibit the gluconeogenesis and decrease intestinal glucose absorption by inhibition of α-glucosidase (Jun et al., 2012). In addition to that kaempferol and quercetin also have the ability to decrease fasting blood glucose, serum HbA1c levels and improved insulin resistance (Ramachandran and Baojun, 2015) and the presence of a large amount of β-carotene in C. chinesis may be the other cause of its ability to reduce FBG level, by its ability to regenerate pancreatic β- cells islet (Mustafa et al., 2008). Recent study support our results by justify the use of C. chinesis to treat diabetes, and suggest that administration of it might also serve as an effective way to bring blood sugar in diabetic patients under control (Sineeporn et al., 2014).
Table 3-1: Mean fasting blood sugar (mg/dl) of rats groups during the periods of experiment

| Period | Normal Control | DM Control | DM + Treatment |
|--------|----------------|------------|----------------|
|       |                |            |                |
| 30 day | 111 ±10.99     | 283 ±32.74 | 125 ±5.75      |
|        | C,a            | A, c       | B, a           |
| 60 day | 102 ±10.78     | 372 ±35.47 | 90 ±4.23       |
|        | B, b           | A, b       | B, b           |
| 90 day | 94 ±7.05       | 473 ±12.68 | 59 ±3.06       |
|        | B, c           | A, a       | C, c           |

- Different letters mean there is significant difference at P≤0.05
- Capital letters for defence among groups and Small letters for comparison among different periods

Figure 3-1: Mean fasting blood sugar of rats groups during the periods of experiment

2. Serum insulin and histological section of pancreas

The results in table (3-2 ) showed highly significant decrease in fasting serum insulin level of diabetic control group compared to normal control group and the figures(3-8:3-10) showed sever decrease in number of endocrine β cells (islets of Langerhans) and derangement in exocrine acini of diabetic control group as compared to normal control group figures (3-2:3-4). Alloxan was used in this study to induction type 1 DM and it well known in affected the antioxidant status and selectively induced destruction of pancreatic b-cells through reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide,Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction.The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of B cells which consequently lead to deficiency in insulin secretion (Szkudelski, 2001). The formation of reactive oxygen species (ROS) is involved in the etiology and pathogenesis of diabetes and the development of diabetic complications(Dar et al., 2014) and the Prolonged exposure to hyperglycemia increased oxidative stress and reduces capacities of the endogenous antioxidant defense system via the production of several reducing sugars (through glycolysis and the polyol pathway) (Szkudelski, 2001)These reducing sugars can easily react with
lipids and proteins (nonenzymatic glycation reaction), and increase the production of ROS (Bojuna et al., 2004).

The results table (3-2) revealed high significantly increase in insulin level in treated diabetic group which in the (30, 60, 90) day were (18.01, 15.16 and 10.23) µIU/ml respectively compare to diabetic control group which were (6.28, 2.32 and 2.68) µIU/ml and we saw that the administration period 60 days returned serum insulin of treated diabetic group near to normal level. and figures (3-5 :3-7) showed that the extract administration increase β cells number (islets of Langerhans) with preserved exocrine pancreatic acini. This effect of C. chinesis methanolic extract may be returned to its strong antioxidant activity which confirmed by yen et al. In (2008) whose cleared the potent antioxidant activity of C. chinesis ethanolic extract and its organic fractions for preventing free radical damage to cell membranes through scavenging of free radicals and inhibition of the lipid peroxidation. Other study submitted by Gao et al (2013) show that the water extract of C. chinesis can significantly inhibited the reactive oxygen species (ROS) generation, malondialdehyde (MDA) production, and increased the activity of superoxide dismutase (SOD), GR and GST. All these results revealed the strong antioxidant activity of C. chinesis plant which increase the possibility of it to elevate the oxidative stress and its complication in diabetic rats. The our data here coincided with Sineeporn et al in (2014) that showed quercetin has ability to decrease in serum glucose, increased serum insulin, preservation of pancreatic beta-cells from oxidative damage and regeneration of the pancreatic islets (Prabhu et al., 2017).

Table 3-2: The concentrations of Serum insulin (µIU/ml) of rats groups during the periods of experiment

| Period | Normal Control | DM Control | DM + Treatment |
|--------|----------------|------------|----------------|
| 30 day | 15.41±3.09     | 6.28±1.14  | 18.01±1.97     |
|        | B, a           | C, a       | A, a           |
| 60 day | 14.63±1.26     | 2.32±0.47  | 15.16±1.46     |
|        | A, a           | B, b       | A, b           |
| 90 day | 15.46±1.47     | 2.68±0.82  | 10.23±1.53     |
|        | A, a           | C, b       | B, c           |

- Different letters mean there is significant difference at P≤0.05
- Capital letters for defence among groups and Small letters for comparison among different periods
Figure (3-2) pancreas section of normal group in experiment period (30day) shows normal exocrine pancreatic acini and normal endocrine (islets of Langerhans) H&E. 400X.

Figure (3-3) pancreas section of normal group in experiment period (60day) shows normal endocrine (islets of Langerhans) surrounded by normal exocrine pancreatic acini H&E. 400X.

Figure (3-4) pancreas section of normal group in experiment period (90day) shows normal exocrine pancreatic acini and normal endocrine (islets of Langerhans) H&E. 400X.
Figure (3-5) pancreas section of treated diabetic group after 30 day of extract administration shows normal endocrine (islets of Langerhans) surrounded by normal exocrine pancreatic acini H&E. 400X.

Figure (3-6) pancreas section of treated diabetic group after 60 day of extract administration shows increase in β cells (islets of Langerhans) surrounded by normal exocrine pancreatic acini. H&E. 400X.

Figure (3-7) pancreas section of treated diabetic group after 90 day of extract administration shows normal hyper functioning β cells surrounded by normal exocrine pancreatic acini. H&E. 400X.
Figure (3-8) pancreas section of diabetic group in experiment period (30 day) shows decrease in number of endocrine β cells (islets of Langerhans) with preserved exocrine pancreatic acini. H&E. 400X.

Figure (3-9) pancreas section of diabetic group in experiment period (60 day) shows severe decrease in β cells with derangement in exocrine acini. H&E. 400X.

Figure (3-10) pancreas section of diabetic group in experiment period (90 day) shows severe decrease in β cells and derangement in exocrine acini. H&E. 400X.
3- Insulin gene 1 expression in pancreas

The data which were revealed by table (3-3) and figure (3-11) showed significantly down-regulate gene expression of pancreas Insulin gene 1 in diabetic rats groups in each period of experiment compared to both normal control group and treated diabetic group. In addition to that the data showed significantly up-regulate gene expression of pancreas Insulin gene 1 of treated diabetic rats groups during experiment periods which were (2.104, 1.970 and 2.064) fold in the periods (30, 60 and 90) day respectively compared to gene expression of pancreas Insulin gene 1 of diabetic rats groups during same periods which were (0.938, 0.634 and 0.553) fold.

Insulin was known to modulate the expression of over 100 genes at transcriptional level in mammals (Brien et al., 2001). The transcriptional effects of insulin was wide spread and concern multiple biological phenomena (Brien and Granner, 1996). Deficiency of insulin serum down-regulated gene expression of insulin gene (Brien et al., 2001) and since the decrease in the beta-cells number and insulin secretion from pancreatic beta-cells was common features of type 1 diabetes mellitus (Thomas et al., 2010). So the reason of down-regulating in the expression of insulin gene 1 in the pancreas of diabetic control group compared normal control group was became clarified and as a consequently the ability of C. chinesis Lam. extract to increase the number of beta-cells in pancreatic islets and improving insulin secretion which was showed in previous results may was the reason of its ability to up-regulating insulin gene expression of insulin gene 1 in pancreas of treated diabetic group compared with diabetic control group.

Table (3-3): Folding change in gene expression of pancreas Insulin gene 1 in rats groups during experiment period

| Period | Group            | House Keeping Ct | Insulin gene 1 Ct | DCt  | DDCt | Folding | P   |
|--------|------------------|------------------|-------------------|------|------|---------|-----|
| 30 day | Normal Control   | 22.354           | 17.125            | -5.228 | 0.000 | 1.000   | B  |
|        | DM Control       | 22.354           | 17.218            | -5.136 | 0.091 | 0.938   | C  |
|        | DM + Treatment   | 23.253           | 16.952            | -6.301 | -1.073| 2.104   | A  |
| 60 day | Normal Control   | 21.174           | 16.918            | -4.256 | 0.000 | 1.000   | B  |
|        | DM Control       | 20.636           | 17.036            | -3.599 | 0.656 | 0.634   | C  |
|        | DM + Treatment   | 22.300           | 17.065            | -5.234 | -0.978| 1.970   | A  |
| 90 day | Normal Control   | 20.994           | 17.432            | -3.562 | 0.000 | 1.000   | B  |
|        | DM Control       | 19.874           | 17.164            | -2.709 | 0.852 | 0.553   | C  |
|        | DM + Treatment   | 21.799           | 17.191            | -4.608 | -1.046| 2.064   | A  |

Ex. Period = Experimental period; Ct=Cycling threshold; DCt=Delta cycling threshold; DDCt=Delta Delta cycling threshold; Folding= \(2^{-\Delta\Delta Ct}\)

- Different letters mean there is significant difference at \(P\leq0.05\)
- Capital letters for defrence among groups and Small letters for comparison among different periods
4- The gene expression of Insulin gene 1 and insulin receptor gene in liver

When investigated the results of table (3-4) and figure (3-12), we conclude that administration of C. chinesis extract was more effective in up-regulating gene expression of Insulin gene 1 in liver than in pancreas compared to their diabetic control groups by rising the gene expression of Insulin gene 1 from (1.067, 1.262 and 1.684) fold in diabetic control group to (1.934, 3.394 and 4.447) fold in the periods of administration (30, 60, 90) day respectively. Whereas the data in table (3-4) and figure (3-12) didn’t reveal any statically difference between normal control group and DM control group in each same period. And the data of table (3-5) and figure (3-13) revealed that administration of C. chinesis extract was significantly up-regulating gene expression of Insulin receptor gene in liver of treated diabetic groups by rising the gene expression of Insulin receptor gene from (0.976, 0.760 and 0.607) fold in diabetic control group to (1.934, 3.394 and 4.447) fold in treated diabetic group at the periods of administration (30, 60, 90) day respectively. Whereas the data didn’t reveal any statically difference between normal control group and DM control group in gene expression of Insulin receptor gene at the same periods.

In the liver of type 1 diabetic rats, there is a tiny amount of hepatic insulin gene was constitutively expressed which prevented ketoacidosis and death associated with severe diabetes induced by alloxan (Hengjiang et al., 2002). Many studies were conducted to induction insulin gene expression in liver (hepatic beta-like cells) and were opened the windows for researchers to get pancreatic alternative organ as a therapy for type 1 DM (Thule et al., 2000 and Lee et al., 2000). The presence of berberin in C. chinesis Lam. plant (Sineeporn et al., 2014) justified and support the results in table (3-4 and 3-5) about the effect of this plant to up-regulating both insulin gene (INS) and insulin receptor gene (INSR) in liver, because many researchers like (Zhou et al., 2008; Yan et al., 2008 and Liu et al., 2010) were showed the ability of berberine to modulate genes expression of many metabolic genes in liver which related to blood glucose homeostasis like up-regulated peroxisome proliferator-activated receptor s (PPARs) expression in liver which responsible for
glucolipid metabolism in addition to up-regulated glucose transporter 4 in liver (GLUT4) which lead to increase the uptake of glucose.

Recent study submitted by Jun et al., (2012) showed that berberine was able to lower blood glucose and increase serum insulin in type 1 DM by repairing destructed or exhausted islets and up-regulating the gene expression of both insulin gene(INS) and insulin receptor gene(INSR) in liver and up-regulating both of glucose transporter 4 GLUT4 and GLUT1 in liver, and science the berberin represent as one of main C. chinesis lam. phytochemical compounds (Wen-Huang et al., 2016), So the recent results become supported and more accepted.

**Table(3-4): Folding change in gene expression of Liver Insulin gene 1 in rats groups during experiment period**

| Period | Group             | House Keeping Ct | Insulin gene 1 Ct | DCt     | DDCt    | Folding       | P    |
|--------|-------------------|-------------------|-------------------|---------|---------|---------------|------|
| 30 day | Normal Control    | 9.642             | 17.244            | 7.601   | 0.000   | 1.000         | B, a |
|        | DM Control        | 9.981             | 17.489            | 7.507   | -0.094  | 1.067         | B, b |
|        | DM + Treatment    | 10.994            | 17.644            | 6.649   | -0.952  | 1.934         | A, c |
| 60 day | Normal Control    | 9.332             | 17.148            | 7.816   | 0.000   | 1.000         | B, a |
|        | DM Control        | 9.894             | 17.374            | 7.479   | -0.336  | 1.262         | B, b |
|        | DM + Treatment    | 10.958            | 17.011            | 6.052   | -1.763  | 3.394         | A, b |
| 90 day | Normal Control    | 9.129             | 17.291            | 8.161   | 0.000   | 1.000         | B, a |
|        | DM Control        | 10.234            | 17.644            | 7.409   | -0.752  | 1.684         | B, a |
|        | DM + Treatment    | 11.084            | 17.093            | 6.009   | -2.152  | 4.447         | A, a |

Ex. Period = Experimental period; Ct=Cycling threshold; DCt=Delta cycling threshold; DDCt=Delta Delta cycling threshold; Folding= $2^{-\Delta\Delta Ct}$

- Different letters mean there is significant difference at $P\leq0.05$
- Capital letters for defence among groups and Small letters for comparison among different periods

**Figure(3-12): Folding change in gene expression of Liver Insulin gene 1 in rats groups during experiment period**
### Table (3-5): Folding change in gene expression of liver Insulin receptor gene in rats groups during experiment period

| Period | Group          | House Keeping Ct | Insulin receptor gene Ct | DCt  | DDCt | Folding   | P |
|--------|----------------|------------------|--------------------------|------|------|-----------|---|
| 30 day | Normal Control | 9.642            | 21.871                   | 12.228 | 0.000 | 1.000     | B, a |
|        | DM Control     | 9.981            | 22.245                   | 12.626 | 0.034 | 0.976     | B, a |
|        | DM + Treatment | 10.994           | 21.903                   | 10.909 | -1.319| 2.495     | A, c |
| 60 day | Normal Control | 9.332            | 20.327                   | 10.994 | 0.000 | 1.000     | B, a |
|        | DM Control     | 9.894            | 21.283                   | 11.388 | 0.394 | 0.760     | C, b |
|        | DM + Treatment | 10.958           | 20.064                   | 9.106  | -1.888| 3.702     | A, b |
| 90 day | Normal Control | 9.129            | 22.170                   | 13.040 | 0.000 | 1.000     | B, a |
|        | DM Control     | 10.234           | 23.993                   | 13.759 | 0.718 | 0.607     | C, b |
|        | DM + Treatment | 11.084           | 21.011                   | 9.926  | -3.114| 8.659     | A, a |

Ex. Period = Experimental period; Ct=Cycling threshold; DCt=Delta cycling threshold; DDCt=Delta Delta cycling threshold; Folding= $2^{-\Delta\Delta Ct}$

- Different letters mean there is significant difference at P≤0.05
- Capital letters for defense among groups and Small letters for comparison among different periods

![Figure 3-13](image)

**Figure (3-13):** Folding change in gene expression of liver Insulin receptor gene in rats groups during experiment period

### Conclusions

This study concluded that the daily administration with 400 mg/Kg (60 day) of *C. chinesis* extract for type 1 diabetic rats was return fasting serum insulin and FBG to normal value by upregulated the gene expression of hepatic INS Gene, INSR gene, pancreas INS Gene and regenerate pancreatic beta-cell and return the pancreas texture to the normal state.
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