Study of Hypoglycemic Activity of Cuscuta chinensis Lam. on Type 1 Diabetes Mellitus in White Male Rats

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Abstract. This study was conducted to evaluate the Hypoglycemic activity of Methanolic-Watery Extract of Cuscuta chinensis Lam. plant by screening about the presence of some antidiabetic and antioxidant phytochemical compound by HPLC technique in addition to estimate the effect of this extract on fasting blood glucose (FBG) of type 1 diabetic rats. Four runs of Preparative reversed-phase HPLC was performed using column C18-ODS (25cm X 4.6 mm X 5 μm) as a stationary phase, while the mobile phase determined based on the type of detected phytochemical, which were (Berberine, Kampferol, quercetin and β-carotene). And the experiment demand deviding 54 Rattus rattus male rats weighting (180-200 g) into 3 groups: A normal control daily administrated with Dwater, B Diabetic control daily administrated with Dwater and C diabetic group daily administrated with 400 mg/Kg body weight of C. chinensis 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 showed the presence of (Berberine, Kampferol, quercetin and β-carotene) in the following respectively in concentration (246, 13.3, 320 and 17977) ppm, in addition to that the results showed significance decrease in FBG of group C as the period of extract administration increase in compare with group B, which had FBG (125, 90 and 59) mg/dl after (30, 60, 90) days of extract administration respectively. So we concluded that administrated diabetic rats with 400 mg/Kg body weight of C. chinensis Lam. extract for two month retained the FBG to the normal level.

Key words: Antidiabetic activity, Type 1 Diabetes Mellitus, HPLC technique and cuscuta chinensis Lam.

1. Introduction

Cuscuta chinensis Lam. or Dodder plant, is an annual voluble parasitic plant of the family Convolvulaceae, its leafless plant and its unable for metabolism, so it uptake nutrient that ready to absorption from its host plant by wrapping his parasitic stem around host plant and sucking nutrients by haustorians (Mavlonov et al., 2008).

Cuscuta chinensis Lam. has found its use as a traditional medicine in China, Korea, Pakistan, Vietnam, India and Thailand. It was commonly used as an anti-aging agent, anti-Inflammatory agent and pain reliever (He et al., 2010). Moreover, it has been listed in the Pharmacopoeia of the People's Republic of China since 1995 as treatment to improve Sexual function, prevent and treat cardiovascular diseases, treatise-Proses and prevent for the treatment of premature ejaculation and spleen and kidney.
deficiency. In addition, many classical medicine books in China have recorded that this plant treat various diseases such as Impotence, infertility, wet dreams, urinary retention, urinary incontinence, lower abdominal and back pain (Li et al., 2013; Zhang, 2013). Furthermore, C. chinensis was applied as a treatment for chronic ulcers, wounds and inflammations (Qureshi et al., 2010). And treatment for, inflamed eyes, Jaundice and for removing hair dandruff (Shubhangi and Patil, 2012).

Diabetes mellitus (DM) is a group of metabolic syndrome in which there are high blood sugar levels over a prolonged period due to either the pancreas not produced enough insulin (Diabetes mellitus type1) or the cells of the body not responding properly to the insulin by insulin receptors on the cell membrane (Diabetes mellitus type 2), which also called insulin resistance (Sesti et al., 2001; Serrano et al., 2005). Diabetes mellitus was one of the most common non-communicable disease with macro and micro vascular complications that result in significant morbidity and mortality in the world (Nawale et al., 2006) and Around 300 million people of the world suffer from this disease (Vats et al., 2003).

The high blood glucose with time lead to neurological, cardiovascular, retinal and renal complications (Moore et al., 2009). And the artificial drugs cannot be cured from it and not restore normal glucose homeostasis and moreover have side-effects (Subodh, 2004). On the other hand, traditional medicinal practitioners of various countries claim to cure diabetes or at least alleviate the major symptoms and progression of this disease through administration of medicinal plants (Wanzala et al., 2005). Limited pharmacognostic studies had been done to discover therapeutically effect of Cuscuta chinensis on many healthy problems and chronic disease (Anjum & Khan, 2003). So this study aimed to Characterize and identify phytochemical compounds of C. chinensis Lam. by HPLC technique, and Demonstrate from hypoglycemic activity of Watery – methanol of this plant by testing the change in FBG diabetic rats.

2. Materials and methods

Plant Collection, identification and drying

The hallow plant of Cuscuta chinensis 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.

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. 1g 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 °C. the dried concentrated material was milled by using electric mill and the final powder was sterilized by UV equipment for 20 min (Ekpenyong et al., 2012).

Biochemical and physiological studies

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- Mustanseriyyah 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 conditions.
light/dark cycle. Animals were divided into 3 main 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 Cuscuta chinesis Lam. methanol extract), each main group consisted of (18) rats and further divided into (3) sub-group 1, 2 and 3. According to the period of administration 30, 60 and 90 day respectively.

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 (Ganesh et al., 2010).

Detection effective administrated dose of plant crud

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.

Blood samples and FBG test:

The blood samples of 6 rats from each group were withdrawn by puncturing the retro-orbital plexus under ether anesthesia and then Fasting blood glucose (FBG) were estimated by using ACCU-CHEK Active System (Germany). Were one drop of rat blood was putted in specific strep and then automatic result was taken.

Statistical analysis:

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

3. Results and discussion

3.1. HPLC Analysis

3.1.1. Quantitative and qualitative Identification of Berberine in C. chinesis extract by HPLC.

HPLC was performed in a stationary phase and a step gradient polarity system of mobile phase which appropriate to Identify Berberine.

Berberine compound in the extract of C. chinesis sample was identified under the chromatographic conditions Figure(1), appearance of retention time 7.313 min and area 5182.938 in C. chinesis extract compared with berberine standard retention time and area which was respectively 7.253 and 42173.110, revealed the presence of berberine in concentration 246 ppm. Other studies confirm the presence of berberine in C. chinesis Lam. plant like Sineeporn et al in (2014) by screening about many phytochemical compound in C. chinesis Lam. plant and they got that C. chinesis contain many important phytochemical compound one of them was the berberine specially in seeds and whole plant and previous study submitted by Kwon et al (2000) justified antioxidant activity of C. chinesis Lam. plant to their ability to accumulate high amount of berberine.

3.1.2. Quantitative and qualitative Identification of Kaempferol in C. chinesis extract by HPLC.

To identify the presence and concentration of Kaempferol, appropriate chromatographic conditions from stationary phase and a step gradient polarity system of mobile phase was performed. as shown in Figure (2), where the retention time of C. chinesis extract was 5.907 min and area was
299.965 in compared with Kaempferol standard retention time and area which was respectively 5.927 and 44672.236. This result indicate the presence of Kaempferol in concentration 13.3 ppm. kaempferol is a flavonol that is relatively abundant in C. chinensis Lam and its able to diminish the increased serum glucose level and increase glucose uptake in the rat soleus muscle as efficiently as insulin (DuPont et al., 2004). Jun-Zeng et al in (2014) studies the anti-diabetic activity of traditional medicinal plants by integrating ethnobotanical, phytochemical, and pharmacological approaches and they listed C. chinensis Lam as an antidiabetic medicinal plant due to has many type of Flavonoids, especially rutin, quercetin, isorhamnetin and kampferol which regard as biologically active constituents in C. chinensis Lam. and they exhibited various pharmacological activities such as antidiabetic , antioxidant and anti-inflammatory agent.

![Berberine Sample

| Reten Time (Min) | Area (mAU.s) | Height (mAU) | Area (%) | Height (%) | WOS (Min) |
|-----------------|--------------|--------------|----------|------------|-----------|
| 1               | 7.253        | 42173.110    | 130.524  | 100        | 0.5       |
| Total           | 42173.110    | 130.524      | 100      | 100        | 0.5       |

| Reten Time (Min) | Area (mAU.s) | Height (mAU) | Area (%) | Height (%) | WOS (Min) |
|-----------------|--------------|--------------|----------|------------|-----------|
| 1               | 1.867        | 136.296      | 19.611   | 1.1        | 3.8       |
| 2               | 2.593        | 873.406      | 53.274   | 7.1        | 10.4      |
| 3               | 5.283        | 1114.406     | 59.932   | 9.1        | 11.6      |
| 4               | 6.513        | 1363.906     | 51.372   | 11.2       | 10.0      |
| 5               | 7.313        | 5182.938     | 180.920  | 42.4       | 35.1      |
| 6               | 8.133        | 3548.258     | 149.045  | 29.0       | 29.0      |
| 7               | 9.453        | 10.570       | 0.566    | 0.1        | 0.1       |
| 8               | 14.983       | 0.010        | 0.0010   | 0.0        | 0.0       |

| Reten Time (Min) | Area (mAU.s) | Height (mAU) | Area (%) | Height (%) | WOS (Min) |
|-----------------|--------------|--------------|----------|------------|-----------|
| 1               | 12299.971    | 514.722      | 100      | 100        | 0.5       |

**Figure (1)** HPLC detection of Berberine in Cuscuta chinensis watery- methanol extract
3.1.3. Quantitative and qualitative Identification of Quercetin in C. chinesis extract by HPLC.

the presence and concentration of Quercetin was identified by performing HPLC analysis in a proper condition of stationary phase and a step gradient polarity system of mobile phase as shown in figure (3), where the retention time of C. chinesis extract was 4.337 min and area was 2520.812 in compared with Quercetin standard retention time and area which was respectively 4.124 min and 6253.816. This result revealed the presence of Quercetin in concentration 320 ppm.

Quercetin is a polyphenolic chemical structure that stops oxidation by acting as a scavenger of free radicals that are responsible for oxidative chain reactions, so it regard as a strong antioxidant compound (Prabhu et al., 2017) the presence of Quercetin in C. chinesis was confirm by many researchers like (Ye et al., 2002 and Sineeporn et al., 2014) and they regarded it as one of a causative effect of anti oxidant and antidiabetic activity of C. chinesis plant.

3.1.4. Quantitative and qualitative Identification of Quercetin in C. chinesis extract by HPLC.

the presence and concentration of Quercetin was identified by performing HPLC analysis in a proper condition of stationary phase and a step gradient polarity system of mobile phase as shown in
figure (3), where the retention time of *C. chinesis* extract was 4.337 min and area was 2520.812 in compared with Quercetin standard retention time and area which was respectively 4.124 min and 6253.816. This result revealed the presence of Quercetin in concentration **320 ppm**.

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3.1.5. Quantitative and qualitative Identification of β- Carotene in *C. chinesis* extract by HPLC.

To identified the presence and concentration of β- Carotene, appropriate chromatographic conditions from stationary phase and a step gradient polarity system of mobile phase was performed. as shown in Figure (4), where the retention time of *C. chinesis* extract was 5.907 min and area was 299.965 in compared with β- Carotene standard retention time and area which was respectively 5.927 and 44672.236. This result indicate the presence of β- Carotene in concentration **17977 ppm (1.7%)**. Many nutrition scientist regard the yellow/orange color of fruits, vegetables and herbs indicate to presence of high amount of beta-carotene, so the yellow/orange color of *C. chinesis* plant was regarded as a confirmed indication about the presence of high amount of beta-carotene and this suggest was Supported by the study of (Schierle *et al.*, 2004) who showed that *C. chinesis* plant was a good source of pro-vitamin A (β – carotene).

| Reten Time (Min) | Area (mAU.s) | Height (mAU) | Area (%) | Height (%) | WOS (Min) |
|------------------|--------------|--------------|----------|------------|-----------|
| 1                | 4.124        | 6253.816     | 639.724  | 100        | 0.16      |
| Total            | 6253.816     | 639.724      | 100      | 100        |           |
| Quercetin Con. = 320 ppm | 3.673        | 228.253      | 37.793   | 2.6        | 0.10      |
| 2                | 4.010        | 743.251      | 96.024   | 8.3        | 0.13      |
| 3                | 4.337        | 2520.812     | 278.793  | 28.2       | 0.15      |
| 4                | 5.907        | 299.965      | 38.020   | 3.4        | 0.13      |
| 5                | 6.820        | 1621.247     | 207.176  | 18.1       | 0.13      |
| 6                | 7.053        | 676.793      | 83.298   | 7.4        | 0.13      |
| 7                | 9.257        | 1447.176     | 192.362  | 17.1       | 0.12      |
| 8                | 9.887        | 1408.980     | 195.379  | 17.4       | 0.12      |
| Total            | 8946.478     | 1125.845     | 100      | 100        |           |

**Figure (3) HPLC detection of Quercetin in Cuscuta chinesis watery- methanol extract**
**β-carotene Sample**

| Reten Time (Min) | Area (mV.s)   | Height (mV) | Area (%) | Height (%) | WOS (Min) |
|------------------|---------------|-------------|----------|------------|-----------|
| 1                | 2.748         | 16708.867   | 575.932  | 57.4       | 0.21      |
| 2                | 10.500        | 10574.906   | 426.599  | 42.6       | 0.36      |
| Total            | 27283.773     | 1002.530    | 100      | 100        | 0.5       |

**Cuscuta chines extract**

| Reten Time (Min) | Area (mAU.s) | Height (mAU) | Area (%) | Height (%) | WOS (Min) |
|------------------|--------------|--------------|----------|------------|-----------|
| 1                | 0.050        | 3.278        | 0.0      | 0.0        | 0.09      |
| 2                | 4.653        | 131.181      | 0.1      | 0.2       | 0.26      |
| 3                | 4.902        | 4604.339     | 4.4      | 10.6       | 0.26      |
| 4                | 5.813        | 1279.055     | 1.2      | 2.8        | 0.27      |
| 5                | 8.960        | 2732.737     | 2.6      | 4.5        | 0.36      |
| 6                | 10.593       | 95012.960    | 90.8     | 80.9       | 0.72      |
| 7                | 14.800       | 853.084      | 0.8      | 1.0        | 0.53      |
| Total            | 104616.597   | 2388.796     | 100      | 100        |           |

β-Carotene Con. = 17977 ppm

**Figure (4)** HPLC detection of β-carotene in Cuscuta chinesis watery-methanol extract

3.2. **Fasting blood glucose (FBG) analysis**

The results showed in table (1) and figure (5). Mean of fasting blood glucose in all experimental groups, where explained that the alloxan injection caused significant increase (p<0.05) in mean of FBG levels (283) mg/dl as compared to normal control group (111) mg/dl and the FBG level in control diabetic group was increased as the period of experiment increased (283, 372, 473) in periods (30, 60 and 90) days 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 (p>0.05) 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 maybe regard to its hypoglycemic phytochemical compounds like alkaloid (berberine), flavonoids (kaempferol and 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 (Ming and Li, 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 (1): Mean of 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 difference among groups and Small letters for comparison among different periods.
- Normal control group and DM control group orally administered with D.W for different periods.
- DM + Treatment group orally administered with C. chinesis (400 mg/kg body weight) for different periods.

Figure (5) Mean fasting blood sugar of rats groups during different periods of experiment

4. Conclusions

In conclusion, The C. chinesis plant was effective in reduced blood glucose of type 1 diabetes mellitus rats when it was administrated orally for 60 day in dose 400 mg/kg body weight via its ability to return FBG to normal level, by its containing many hypoglycemic and antioxidant compounds like berberine, kaempferol, quercetin and beta-carotene.
5. References

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