Original Article

Helianthus Annuus Seed Hydro-Alcoholic Extract Chemical Composition and Effects on Body Weight and Blood Parameters among Iraqi Patients Suffering from Type 2 Diabetes Mellitus

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Abstract

Background & Objectives: The purpose of the present study was to calibrate and analyze the effects of *Helianthus annuus (H. annuus)* seeds extract on body weight and blood parameters of type-2 diabetic-mellitus (T2DM) patients.
Materials & Methods: Three-hundred cases of T2DM patients were involved in this study including 180 males and 120 females and divided into two groups (each with 150 members). The herbal extract of *H. annuus* with a concentration of 500mg/kg was subjected to group1 (case population), but group 2 received normal diet for three months. Gas Chromatography mass spectrometry (GC/MS) was used to analyze *H. annuus* compounds.
Results: Major *H. annuus* fraction included chlorogenic acid (CGA) being 40%. The extract conferred significant decreasing effects on hypertension but not body weight and body mass index (BMI) values. The extract receiving group vs control group regarding the mean blood factors respectively included fast blood glucose (FBS) (39.2% vs 3.22%, p<0.0001), cholesterol (6.9% vs 3.22%, p=0.0237), triglyceride (5.4% vs 2.30, p=0.0113), and increase in high-density lipoprotein (HDL) (22.3% vs 5.26%, p<0.001) and low-density lipoprotein (LDL) (1% vs 2.6%, p=0.0112).
Conclusion: *H. annuus* major compounds included CGA (40%) contributed to the control of the hypertension, lipid profile and the blood factors among T2DM patients compared to those patients who received normal diet. More detailed investigations by participation of higher number of patients are required in this regard.

Keywords: *Helianthus-annuus*, Diabetes Mellitus type 2, blood factors

Introduction

Helianthus-annuus (H. annuus) or sunflower, the courtly-plants have been originated from North-America, but were expanded universally due to yield of the seed containing 30–40% oil, in which ranks as the fourth oil in the world according to Guo etal, 2017 (1).

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H. annuus seeds contain high levels of proteins, vitamins, minerals, as well as anti-oxidants. Particularly, phenolic-compounds account for 1-5% of the total mass of oil extract, and the chlorogenic acid (CGA) fraction exist with high levels (2, 3). The sunflower seeds contain high content of tryptophan-rich proteins crucial for growth mainly for children (4). It is a well-heeled source of niacin, folic acid, B-complex vitamins (B1, B2) and pantothenic acid, , as well as iron, Zinc, Manganese, Copper,

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Selenium and "Calcium" which play a major part in productivity of hormone, enzymes-synthetic, synthesis of RBC, bones-mineralization, metabolic regulation and cardio-skeleton muscles function (5).

According to world health organization (WHO), nearly half of individuals were overweight "BMI>25-30 and BMI≥30". Moreover, fatness is significantly correlated with several diseases "diabetes", hypertension, cardio-vascular diseases, arthritis, atherosclerosis, and cancers (6). As per the WHO in 2014, the rate of cancers, obesity diabetes and heart diseases included 43%, 40%, 25% and 9%, respectively (7).

Type-2 Diabetes mellitus (T2DM) occurred mainly as a metabolic disorder with more common characteristics with hyperglycemia in case of the insulin-resistance conditions. In addition, in type-1 diabetes, the low-level insulin production is appeared as a collapse in 'β-cell of langerhans islets in the pancreatic organ (8). The general symptoms include "excessive drought, frequently urinating, urinating in the night as well as firm starvation". More than 95% of diabetic cases are T2DM, and sometimes have pregnancy diabetes. The fatness ranks as the highest risk factor of T2DM, mostly among persons who already have genetic predisposition to metabolism disorders (9). Around 286 million people in 2010 were diagnosed as diabetes compared to 32 million at 1985. Long-time complications for hyperglycemia involves 'stroking', diabetes-retinopathy "trouble in vision", diabetes-neuropathy, "proceeding the kidney disability" which lead to request of dialysis and cardio-malady (10). Several medicinal plants such as *Asteraceae, Lamiaceae* and *Apiaceae* families have deciphered antidiabetic traits (11). The antidiabetic effect of *H. annuus* hydroalcoholic extract was exhibited in Choudhary study (12). In a study by Adeleke Ojo, the leaf of *H. annuus* exhibited anti-diabetic effect via inhibition of α-amylase and α-glucosidase enzymes (13). The aim of this study was to assess the *H. annuus* seed extract effects on FBS, weight and body composition among patients with T2DM.

Materials & Methods

Study population

In this cross-sectional cohort study, 300 samples were collected from 180 males and 120 female patients with T2DM with ages ranging from 30-50 (mean=34±3.5) and BMI of 31-47kg/m^2. Moreover 300 healthy subjects (163 men and 137 women) were considered as the control group. The BMI of control group was between 35 and 45 kg/m^2. Inclusion criteria of control group included age match with patients group (29-52 years), no pre-diabetic conditions, no use of drugs and no family history of diabetes.

Preparation of herbal hydro-alcoholic extract

The seeds were collected from field in the country and 100 gr of it was powdered for extract preparation. The extract was obtained using 500 mL of 70% methanol plus ddH_2O at 1:1 ratio in a percolator apparatus for 72 hr. Next, the extract was dried and weighed.

Analysis of H. *annuus* compounds

The gas chromatography (GC) and mass spectrometry (MS)/GC methods were used for the identification and analysis of *H. annuus* herbal fractions. GC (Agilent 6890 model) and MS (Agilent 5973 model, 70 EW) devices had 30 m long column, 0.25 mm inside diameter and 0.25 µm layer thickness of HP- 5MS type. The ionization method was EI and the source temperature included 220°C. The spectra were identified using their inhibition index and compared with the indices of previous studies using the mass spectra of standard compounds.

Analysis of *H. annuus* effects

The control group received only diet counseling (normal diet), whereas, the patients group received two capsules of *H. annuus* (sunflower) extract (before breakfast and before lunch) daily for 3 months and diet counseling. Each capsule included 250 mg. Blood samples were collected in vacuum anticoagulant tubes for serum extraction. Thereafter, several parameters including FBS (mg/dL), hemoglobin Hb (%), cholesterol (mg/dL), HDL (mg/dL), LDL (mg/dL),

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triglyceride (TG) (mg/dL), free fatty-acids (nmol/dL) were measured. Additionally, the control group received diet counseling and various tests were conducted as mentioned above.

Data analysis

All data before supplementation for both control groups and patients were gathered by fully assisting for the diet-surveys, vital measures (bio-chemical test the comprising for the dietary historic, diabetic historic, high-chart, BMI/FBS) then after supplementation of data were recorded with different biochemical parameters which were checked at the end of every month then made a statistical analysis using SPSS 20 and Chi-square test.

Results

Patients' demographic data

In this cross-sectional cohort study, 300 samples were collected from 180 males and 120 female patients with T2DM with ages ranging from 30-50 (mean=34±3.5) years old and BMI 31-47kg/m^2. Moreover 300 healthy subjects (163 men and 137 women) were considered as the control group. The BMI of control group was between 35 and 45 kg/m^2. Inclusion criteria of control group included age match with patients group (29-52 years), no pre-diabetic conditions, no use of drugs and no family history of diabetes.

Effect of H. *annuus* extract

Our findings revealed that 40% CGA of *H. annuus* extract significantly reduced the BMI, body weight, and fat accumulation following three months.

Among the patients consuming 500mg/kg/day of the *H. annuus* extract, its effect was observed on blood factors including FBS (196.2mg/dL to 119.5mg/dL), cholesterol (159.2mg/dL to 148mg/dL), TG (129mg/dL into 122mg/dL), HDL (36mg/dL to 44mg/dL) and LDL (96mg/dL to 97mg/dL). In the control group, the counseling diet effect included FBS (99.2mg/dL to 89.5mg/dL), cholesterol (170.5mg/dL to 165mg/dL), TG (130mg/dL to 127mg/dL), HDL (38mg/dL to 40mg/dL) and LDL (114mg/dL to 117.3mg/dL) (Tables 1 and 2).

Table 1. The blood parameters of patients' group receiving *H. annuus* extract

Patients group	FBS		Cholesterol		TG		HDL		LDL	
Before/ After	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.
Mean	196.2	119.5	159.2	148	129	122	36	44	96	97
S.D.	35.46	8.58	26.84	17.10	31.12	18.39	7.30	10.60	16.95	16.91
p- value	0.00*		0.001*		0.001*		0.013*		0.628**	
Sign./ non. sign.	Sign.		Sign.		Sign.		Sign.		Non sign.	

FBS: Fasting blood sugar, TG: triglyceride, HDL: High density lipoprotein, LDL: Lowdensity lipoprotein, B: before, a: after, S.D.: Standard Deviation, *Sign.: significant, **

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Table 2. The blood parameters of control group receiving counseling diet

Patients group	FBS		Cholesterol		TG		HDL		LDL	
Before/ After	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.
Mean	173.2	129.5	170.5	165	130	127	38	40	114	117.3
S.D.	30.91	7.11	20.34	14.21	13.83	10.11	4.71	1.08	14.99	11.23
p- value	0.00*		0.001*		0.002*		0.00*		0.099**	
Sign./ non. sign.	Sign.		Sign.		Sign.		Sign.		Non sign.	

FBS: Fasting blood sugar, TG: triglyceride HDL: High density lipoprotein, LDL: Low density lipoprotein, B: before, a: after, S.D.: standard Deviation, *Sign.: significant, **non sign.: non-significant

Among T2DM patients receiving *H. annuus* extract, p-values of the FBS, cholesterol, TG and HDL changes included 0.00 and 0.001, 0.001 and 0.013, being significant but that of LDL was non-significant (0.628) (Table1).

In addition, among the control group, the p-values of FBS, cholesterol TG and HDL included 0.00, 0.001, 0.002 and 0.00 being significant, but the LDL p value was 0.099 being a non-significant change.

Furthermore, as a comparison of the mean of blood factors changes between groups, the H. annuus extract receiving group vs control group included FBS (39.2% vs 3.22%, $p<0.0001$), cholesterol (6.9% vs 3.22%, $p=0.0237$), TG (5.4% vs 2.30, $p=0.0113$), and increase in HDL (22.3% vs 5.26%, $p<0.001$) and LDL (1% vs 2.6%, $p=0.0112$), respectively.

Discussion

The *H. annuus* (sunflower) extract active components anti-obesity effects have been previously studied and several clinical trials have revealed the anti-diabetic traits (14). It was supposed that the extract regulates the lipids metabolism and energy homeostasis through the AMPK pathway, as well as adipogenesis during interaction with PPAR.

In a comprehensive review study, it was revealed that seeds of flax and sunflower exert pivotal anti-diabetic effects (14). In another study, it was stated that anti-diabetic drugs have side effects and therefore, combination of herbal and synthetic drugs will overcome these problems. These herbal drugs/ nutraceuticals

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included *Panax quinquefolius* L., *Radix astragalus, Gymnema sylvestre, Aloe vera, Bitter melon, Cinnamonum cassia, Fenugreek, Zingiber officinale, Trigonella foenum-graecum, Allium sativum and Curcuma longa* (2-4, 8, 13-17). It was also deciphered that consumption of traditional herbs and modified lifestyle pattern can improve the normoglycemic conditions (17).

According to our findings, the CGA was the major fraction (40%) of *H. annuus* seed extract. The *H. annuus* seeds extract can reduce the blood levels of cholesterol, LDL-cholesterol, as well as TG (18), being consistent to our results. Some animal studies have documented that dose-dependent lipid profile is associated with exhaustion in CGAs (19). Wan et al, 2012 reported the reduction in sera of cholesterol and the LDL following treatment by 10mg/kg of CGA for one month (20). Whereas, (Shimoda et al, 2006) revealed the significant decreasing effect on mouse sera TG by 400 mg/kg of green-coffee-beans extract containing 27% CGA with 108mg/kg CGAs. Also, 8.8mg CGAs per kg, exerted weaker effect on lipid-profile, hence its effect was dose-dependent. On the other hand, time spanning of exposure to the extract affects anti-diabetic effects through influences on blood parameters such as cholesterol, TG, LDL and HDL. It is notable that the non-toxic level of the components on normal cells must be outlined. Thus, regarding atherogenes characteristic on hyper-lipidemia (high level of cholesterol and TG) is an important risk-factor of cardiac disease reported by Castelli (1996) and Noor Al-Huda (2012) (21-23). Major limitations of our study included narrow number of samples and lack of gene expression analysis to disclose *H. annuus* extract mechanisms of action.

Conclusion

H. annuus seeds extract plays a major role in reducing the levels of FBS among T2DM patients, through increasing the HDL, therefore daily consumption of this extract not only confers anti-diabetic effects but also provides proteins, vitamins, minerals and antioxidants. Major *H. annuus* fractions such as CGA contributes to the alteration of blood parameters such as FBS among T2DM patients according to (Krimer et al, 2011).

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Conflict of interest

All the authors have no conflict of interest.

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