The protective role of alcoholic extract of (*Anethum graveolens*) seeds on renal function in alloxan induced diabetic rabbits

Weam A. Shakir and Luma W. Khalil
Department of Physiology and Pharmacology, College of Veterinary Medicine, Baghdad University, Iraq.
E-mail: waleedluma@yahoo.com
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Summary

This study was designed to investigate the effect of dill (*Anethum graveolens)* seeds in mitigating the complications of induced diabetes mellitus such as nephropathy of adult female rabbits. Thirty adult female rabbits were randomly assigned to 5 equal groups and treated daily for 45 days as follows: Control group (C) drenched tap water, control group intubated orally with 700 mg/kg B.W ethanolic extract of dill (T1), diabetic group drenched tap water (T2), diabetic group intubated orally700 mg/kg B.W of dill (T3) and diabetic group treated with insulin injection 3 I.U S/C (T4). Blood samples were collected by heart puncture at 0 and 45 days of experiment to assess renal efficiency: Serum creatinine, serum urea, and serum glutathione (GSH). The results indicated that diabetes mellitus without treatment group (T2) caused renal damage manifested by significant ($P<0.05$) increase in creatinine, urea and significant ($P<0.05$) decrease in serum glutathione (GSH) concentration. Oral intubation of alcoholic extract of dill seeds in diabetic treated group (T3) improved renal function through significant ($P<0.05$) decrease in serum urea, creatinine, and significant ($P<0.05$) increase in serum glutathione concentration (GSH). It was concluded that alcoholic extract of dill seeds was effective in reducing the complications of diabetes mellitus such as nephropathy.

Keywords: Diabetic nephropathy, Dill seed extract, Diabetes mellitus.

Introduction

Herbal medicines have been the main source of primary health care in many nations. About 80% of the world's populations are still dependent on traditional medicines. In fact, medicinal plants which form the backbone of traditional medicine, have in the last few decades been the subject for very intense pharmacological studies, this has been brought about by the acknowledgement of the value of medicinal plant as potential sources of lead compounds in the drug development (1). Spices, widely recognized as food additives have been used traditionally to prevent and treat various disease of various and spices, *Anethum graveolens* linn. (Umbelliferae), is popular aromatic herb. As a folk remedy, *Anethum graveolens* is used for some gastrointestinal ailments such as flatulence, indigestion, stomach ache and colic (2). The fruit has an antispasmodic effect on the smooth muscles of the gastrointestinal tract (3). With regard to central nervous system, *Anethum graveolens* has been used to alleviate tiredness from disturbed rights and strengthen brain: The aerial parts of the plant are often cooked with fish to add flavor to it and to stimulate the brain (4). *Anethum graveolens* contains essential oil, moisture, proteins, carbohydrates, fiber, ash, furanocoumarin, polyphenols and minerals, previous studies revealed that *Anethum graveolens* have some pharmacological effects such as antimicrobial, anti- inflammatory, analgesic, gastric mucosal protective, increased progesterone concentration, anti-diabetic, and antihyperlipidaemic effects (5). Diabetic nephropathy is one of the most common causes of this problem (6). Moreover, it is the commonest cause of and stage renal failure in many countries and is associated not only with a high morbidity rate but also an increase in morality (7). Therefore, this study was designed to investigate the possible protective effect of dill seeds extract against the deleterious effects of diabetes mellitus as anti- oxidant agent on renal function of adult female rabbits.

Materials and Methods

A 100 gm of ground dill seeds put in conical flask, 1000 ml of 70 % ethanol was added. The mixture was subjected to a continuous mixing for 24 hours by using

magnetic stirrer. The whole mixture was filtered first by medical gauze and followed by filtration on Whatman filter paper No.1, the filtrate was concentrated in incubator at 40°C for 72 hours. The resulting crude extract was kept at -20 °C until used.

Thirty adult female local rabbits weighed between 1500-2000g and aged 6 months were bought from local market. They were managed and housed in cages in condition room 22-25°C in the animal house of the College of Veterinary Medicine/ University of Baghdad. Rabbits were randomly divided into five equal groups. They were treated daily for 45 days as follows: Control group was adiminstered tap water serving as (negative control), group T1 treated with alcoholic extract of dill *anethum graveolens* seeds at 700mg/kg BW orally. Group T2: Animals in this group were treated with single dose of alloxan monohydrate at 150mg/kg B.W. I.P serving as (positive control). Group T3: diabetic animals were treated with alcoholic extract of dill *anethum graveolens* seeds at 700mg/kg BW orally and group T4: Diabetic animals were treated with insulin at (3 I.U) S/C (8). Blood samples were collected at 0, 45 days of experiment via cardiac puncture technique by using vacuum test tubes. Serum was separated from blood by centrifugation at 2500 rpm for 15 min. and kept at -20 C̊ until use. Serum samples were used for measuring creatinine as described by (9), urea according to (10) and reduced glutathione (GSH) as described by (11). Results are expressed as mean+SE. Statistical analysis of data were performed using two-way analysis of variance (ANOVA II). Group differences were determined using least significant difference (LSD) test at $P<0.05$ (12).

Results and Discussion

Table (1) Shows a significant ($P<0.05$) increase in the mean value of creatinine concentration in untreated diabetic rabbits (T2 group) as compared with controls (C), diabetic rabbits treated with dill extract (T3) and diabetic rabbits treated with insulin (T4), after 45 days. Likewise, creatinine concentration ($P<0.05$) decreased significantly in diabetic treated with dill extract (T3) after 45 days as compared with diabetic untreated rabbits (T2) and diabetic treated with insulin group (T4). Nevertheless, insulin treatment causes a significant ($P<0.05$) decrease in creatinine concentration in (T4 group) as compared with untreated diabetic group (T2). Finally, creatinine concentration in control treated with dill extract (T1) group shows a significant ($P<0.05$) decrease as compared with other groups after 45 days.

Kidney maintains optimal chemical composition of body fluids by acidification of urine and removal of metabolite wasts such as creatinine (13). In renal disease, the concentration of metabolites such as creatinine increases in blood. Therefore, elevated creatinine is the significant renal marker and related to renal dysfunction in diabetics. Serum creatinine levels in alloxan diabetic rabbits may be due to hyperglycemia and metabolic disturbances. Moreover, protein glycation in diabetes may lead to muscle wasting and increase degradation of protein and release non protein nitrogenous compound such as creatinine (14). Also creatinine concentration increased in condition associated with extensive muscle breakdown as in starvation and poorly controlled diabetes (15). The current study demonstrated that continuous oral administration of the alcoholic extract of dill seeds for 45 days improved serum creatinine level in diabetic rabbits as compared with untreated diabetic rabbits. This indicated that dill seed extract could be help in improving secondary complications like kidney damage in diabetes mellitus.

One of the major classes of natural antioxidants found in the studied plant that could remove free radicals, is polyphenols. These compounds are able to neutralize free radicals and scavenging singlet and triplet oxygen and breakdown peroxide (16). Furthermore, these compounds protect cells constituents against oxidative stress produced from hyperglycemia by acting directly on reactive oxygen species by stimulating endogenous antioxidant defense systems. Consequently, they may decrease the risk of various metabolic disorders, which are closely associated with oxidative stress damage (17). The excellent antioxidant activity of dill extract is attributed not only to the presence of a high content of polyphenols but also to the

volatile constituents that are present abundantly in dill (18). Therefore, dill possessed antioxidant capacity that improve the normal renal function in paracetamol-intoxicated rats. At the same time decreased muscle breakdown and restored the elevated blood creatinine to normal range (19).

Table, 1: Effect of alcoholic extract of dill (*Anethum graveolens*) on serum creatinine concentration (mg/dl) in control and diabetic female rabbits. Mean ± S.E. (n = 6).

Group / Day	C	T1	T2	T3	T4
Zero time	1.08+0.007 A a	1.07+0.006 A a	1.07+0.006 A b	1.09+0.008 A b	1.08+0.017 A b
45 days	1.07+0.006 C a	1.06+0.006 C a	1.73+0.016 A a	1.37+0.325 B a	1.39+0.325 B a

C: control group, T1: Animals received of alcoholic extract of dill seed orally for 45 days, T2: Animals received single dose of alloxan monohydrate I.P, T3: Diabetic animals received alcoholic extract of seed orally for 45 days, T4: Diabetic animals received (3 I.U) of insulin S/C for 45 days. Small letters denote significant differences within group (P<0.05). Capital letters denote significant differences between groups (P<0.05).

Table (2) after 45 days induced diabetes by alloxan causes significant (P<0.05) increase in urea concentration of diabetic group (T2) as compared with other groups. Meanwhile, treated diabetic rabbits (T3) with dill seed extract lead to significant (P<0.05) decrease in urea as compared with other groups. In addition, urea concentration decreased (P<0.05) significantly in diabetic rabbits treated with insulin group (T4) after 45 days as compared with diabetic group (T2). While urea concentration decreased (P<0.05) significantly after treatment with dill seed extract given to control rabbits (T1) as compared with (T2), (T3) and (T4) groups. The data of the present study revealed a significant (P<0.05) elevation in blood urea in alloxan induced diabetic rabbits. A similar effect was recorded previously (20). However, the highly significant (P<0.05) increase in serum urea concentrations of diabetic rabbits might be due to depletion of serum protein, increase in the rate of circulating amino acids and deamination that consequently leads to the formation of large amount of ammonia which is eventually converted to urea. Moreover, the breakdown of amino acids during gluconeogenesis in the liver results in increased production of urea fostering negative nitrogen balance (21).

Table, 2: Effect of alcoholic extract of dill (*Anethum graveolens*) on serum urea concentration (mg/dl) in control and diabetic female rabbits. Mean ± S.E. (n = 6).

Group / Day	C	T1	T2	T3	T4
Zero time	31.59+0.34 B a	31.48+0.33 B a	32.65+0.26 AB b	31.63+0.30 B b	32.71± 0.35 A b
45 days	31.42+0.33 D a	30.85+0.40 D a	69.32+0.48 A a	36.22+0.33 C a	40.11+0.32 B a

C: control group, T1: Animals received alcoholic extract of dill seed orally for 45 days, T2: Animals received single dose of alloxan monohydrate I.P, T3: Diabetic animals received alcoholic extract of dill seed orally for 45 days, T4: Diabetic animals received (3 I.U) of insulin S/C for 45 days. Small letters denote significant differences within group (P<0.05). Capital letters denote significant differences between groups (P<0.05).

Hyperglycemia has been recently implicated in the initiation and development of various types of diabetic complications. Nephropathy is one of these serious microvascular complications that has been observed in diabetic individuals (22). In addition blood urea concentration increased among uncontrolled diabetic individuals and this increase could be a result of impaired renal function due to an increased blood glucose level (23). The excellent performance of dill seeds extract in reversing the negative effects of alloxan induced diabetes in rabbits may be due to the presence of some antioxidants, vitamins and minerals, as well as

polyphenols, flavonoids and alkaloids in dill seeds extract (24). Hence, the decrease in blood urea in dill treated diabetic rabbits may be due to the antioxidant and antidiabetic action resulting in alleviation of altered metabolic status in animals. Recent studies show that the presence of antioxidants such as flavonoids, polyphenols and vitamins in strawberry may be responsible for the restoring urea, creatinine, albumin and uric acid in alloxan diabetic animals (25). Therefore the presence of these constituents in dill may exert the same effect. The experimental results indicated that dill seed extract exhibited a potent blood GSH increasing property in diabetic rabbits. Dill seed extract reverted the GSH level to normal in diabetic rabbits. Table (3) shows that GSH concentration decreased ($P<0.05$) significantly in diabetic group (T2) as compared with other groups at 45 days. While dill seeds extract causes significant ($P<0.05$) increase in control treated with dill extract group (T1) and diabetic treated with dill extract group (T3), within the same period. During diabetic state, increased generation of reactive oxygen species (ROS) occur and causes an imbalance between the oxidant and antioxidant status. Sustained hyperglycemia has been identified as a mediator of increased reactive oxygen species (ROS) generation in diabetes. This might be the result of suppression of β-cell proliferation and inhibition of insulin gene transcription that caused impairment of insulin release (26). However, evidence suggests that oxidative stress and free radicals play an important role in the pathogenesis of diabetes mellitus and diabetic complications (27). Reduced glutathione an important part of none enzymatic antioxidant system is a major non protein thiol in living organism, which plays a central role in coordinating the body antioxidant defense processes. It helps maintain cellular oxidation reduction balance and protect cells against free radical species and detoxifying foreign compounds (28). Perturbation of GSH status of a biological system can lead to serious consequences (29). Accordingly high glucose has been postulated to generate ROS in numerous cell types. Antioxidants exert their effects by donating at least one hydrogen atom to a free radical resulting in the termination of radical chain reactions, or preventing the addition of free radical chain reaction (30). Interestingly, the available polyphenols, flavonoids and carvone in dill seed extract prevent oxidative stress and activates antioxidant enzymes thus helping in prevention of disease (31).

Table, 3: Effect of alcoholic extract of dill (*Anethum. graveolens*) on serum glutathione concentration ((µmol/l) in control and diabetic female rabbits. Mean ± SE, n = 6.

Group / Day	C	T1	T2	T3	T4
Zero time	55.33±1.60	56.00±3.03 b	54.50±1.94 a	54.16±1.30	55.66± 1.80
45 days	55.50±2.96 B	69.66±1.40 A a	43.66±1.33 C b	52.83±1.70 B	51.33±1.53 B

C: Control group, T1: Animals received alcoholic extract of dill seed orally for 45 days, T2: Animals receivedsingle dose of alloxan monohydrate I.P, T3: Diabetic animals received alcoholic extract of dill seed orally for 45 days, T4: Diabetic animals received (3 I.U) of insulin S/C for 45 days. Small letters denote significant differences within group ($P<0.05$). Capital letters denote significant differences between groups ($P<0.05$).

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الدور الوقائي للمستخلص الكحولي لبذور الشبت في وظائف الكلى في الأرانب المصابة بداء السكر التجريبي المستحدث بالالوكسان

وئام عدنان شاكر و لمى وليد خليل

فرع الفسلجة والادوية، كلية الطب البيطري، جامعة بغداد، العراق.

E-mail: waleedluma@yahoo.com

الخلاصه

أجريت هذه الدراسة لمعرفة تأثير نبات الشبت في التخفيف من الإصابه بداء السكر التجريبي المستحدث بالالوكسان والتي من ضمنها الاعتلال الكلوي في إناث الأرانب البالغة. استعملت 30 انثى بالغة من الأرانب المحلية، قُسمت الى 5 مجاميع متساوية عشوائياً كالآتي: مجموعه سليمة للسيطرة (C)، مجموعة سليمة ومعالجة بالشبت (T1)، مجموعة مصابة بداء السكر المستحدث بالالوكسان (T2)، مجموعة مصابة بداء السكر ومعالجة بالشبت (T3)، مجموعة مصابة بداء السكر ومعالجة بالانسولين (T4). جرعت حيوانات المجموعتان (T1) و (T3) ، (700 ملغم / كغم) بالمستخلص الكحولي لبذور الشبت يومياً، في حين حقنت المجموعة المصابة بالسكري والمعالجة بالانسولين (T4) بـ 3 وحدات من الانسولين تحت الجلد يومياً طيلة مدّة التجربة. وفي مدّة التجربة البالغة 45 يوماً جمعت عينات الدم في المدد صفر، 45 يوم من التجربه، وسحب الدم بطريقه الوخز القلبي لغرض دراسة معايير الكفاءة الكلوية: قياس تركيز الكرياتنين واليوريا والكلوتاثايون في مصل الدم. أظهرت نتائج التجربة أنّ الإصابة بداء السكر وبدون العلاج في المجموعة (T2)، أدت إلى حصول تلف كلوي وخلل وظيفي تمثل بحدوث انخفاض معنوي ($P<0.05$) في تركيز الكلوتاثيون وارتفاع معنوي ($P<0.05$) في مستوى الكرياتنين واليوريا. كما أوضحت النتائج أنّ التجريع الفموي بالمستخلص الكحولي لبذور الشبت في المجموعه (T3) ادى إلى حصول تحسن في وظيفه الكليتين تمثل بانخفاض معنوي ($P<0.05$) في اليوريا والكرياتنين كذلك حصول ارتفاع معنوي ($P<0.05$) في تركيز الكلوتاثيون. نستنتج من هذه الدراسة أن المستخلص الكحولي لبذور الشبت له تاثير فعال في الحد من مضاعفات الإصابة بداء السكر كالاعتلال الكلوي.

الكلمات المفتاحية: اعتلال الكلى السكري، مستخلص بذور الشبت، داء السكري.