

Biopharmacological studies of the aqueous extract of *Lepidium sativum* seeds in alloxan - induced diabetes in rats

Shahbaa M. Al-khazraji

Medical Technical Institute / Mansour Foundation of Technical Education

Summary

Garden cress (*Lepidium sativum*) seeds are considered extensively in the folk medicine as an antidiabetic agent in many countries. The current investigations focuses attention on the glucose and lipid lowering effect of the aqueous extract of *L. sativum* seeds on experimentally induced diabetes in rats. The biochemical parameters studied were plasma glucose, insulin, total cholesterol, triglycerides, phospholipids, hemoglobin, and glycosylated hemoglobin.

In addition body weight and renal glucose reabsorption were notified. Aqueous extract of *L. sativum* were orally administered daily for 30 days in a dose of 20 mg / kg body weight to alloxan – diabetic rats , and a significant reduction in the parameters measured was investigated compared to diabetic rats. Meanwhile, Glibinclamide was used as standard reference drug. In conclusion , *L. sativum* seeds possess a hypoglycaemic with concurrent hypolipidemic effect in diabetic states , and may further suggests that *L. Sativum* may be useful in the therapy and managements of diabetic hyperlipidemia through reducing lipids levels .

Keywords:- Alloxan , diabetes mellitus , hyperlipidemia , *Lepidium sativum*

دراسة بايوفارماكولوجية حول بذور نبات الرشاد في الجرذان المصابة بمرض داء السكري

شهباء مسلم الخزرجي

قسم الصيدلة / المعهد الطبي التقني / منصور / هيئة التعليم التقني / العراق

الخلاصة

تعتبر بذور نبات الرشاد من البذور المستخدمة بشكل ملموس وعلى نطاق واسع في الطب الشعبي لعلاج مرضى داء السكري في بلدان مختلفة من العالم , تركز الدراسة الحالية على تأثير المستخلص المائي لبذور نبات الرشاد على خفض تركيز الجلوكوز والدهون بانواعها في دم الجرذان المخلقة بداء السكري , كذلك دراسة تأثير المستخلص المائي للبذور على تركيز هرمون الانسولين , الهيموغلوبين , والهيموغلوبين الغليكوزيدي بالاضافة الى قياس وزن الجسم للحيوانات المختبرية المخلقة بداء السكري قبل وبعد التجربة وكذلك حساب تركيز السكر بالبول .
تم اعطاء المستخلص المائي لبذور النبات عن طريق الفم وبجرعة 20 ملغم/كغم وزن جسم لمدة 30 يوم للجرذان المصابة بداء السكري مختبريا , وتم الحصول على نتائج ايجابية في التأثير على مستويات السكر . الدهون بانواعها وكل القياسات البايوكيميائية التي تم قياسها بالدراسة مقارنة للجرذان المصابة بداء السكري والتي لم يتم اعطائها المستخلص المائي لبذور الرشاد وتم استخدام Glibinclamide كمادة قياسية في التجربة .
اثبتت النتائج ان المستخلص المائي لبذور نبات الرشاد لها تأثير ايجابي في تخفيض نسبة السكر والدهون بانواعها وبشكل ملحوظ وبالتالي يمكن السيطرة على مضاعفات مرض داء السكري.

Introduction

Diabetes mellitus is a syndrome resulting from variable interactions of hereditary and environmental factors, and characterized by depleted insulin secretion, hyperglycaemia, and altered metabolism of lipids, carbohydrates, and proteins, and damage of Beta – cells of pancreas, with increased risk of complication of vascular disease (1). A number of pharmacological and chemical agents act as diabetogenic and produce variety of diabetic complication. Alloxan induction of diabetes in experimental models widely used to study glycaemic and lipidemic changes in plasma. Many species of plants and herbs are known to act as anti – diabetic agents, but only few of them have been investigated (2) . *Lepidium sativum* (Garden cress, Fam :Cruciferae) is annual erect herbaceous plant , growing up to 30

cm . It is wellknown culinary herb and the leaves are widely used as a garnish and are consumed raw insalads. The plant is known to posses varied medical properties, leaves of this plant are diuretic and gently stimulant. The seeds are aperient, diuretic, tonic, demulcent, aphrodisiac, carminative, galactogogue and emmenagogue (3) . The seeds are rubefacient and are applied as poultice for hurts and sprains (4). The plant also shows teratogenic effect and anti – ovulatory properties. The root is used in the treatment of secondary syphilis and tenesmus (5).

L. sativum seeds had a marked influence on fracture healing in rabbits, clearly supporting their effects on human beings, as a well – known natural element to promote fracture healing in traditional medicine (6). A preliminary pharmacological study on seeds of *L. sativum* has suggested the presence of cardioactive substance and is shown to have probable action through adrenergic mechanism (7). The aqueous extract of *L. sativum* seeds has been reported to have anti-hypertensive and diuretic effect when studied in normotensive and spontaneously hypertensive rats (8, 9). The effectiveness of this plant in treatment of bronchial asthma, hiccups, coughs with expectoration (4, 5). The aqueous extract of *L. sativum* has been reported to exhibit a potent hypoglycaemic activity in normal and streptozotocin induced diabetic rats (10). Aqueous extract of *L. sativum* caused a potent inhibition of renal glucose reabsorption which in turn reduce blood sugar (9,11). However, no scientific studies are so far carried out to investigate the efficacy of *L. sativum* in the management of hyperlipidemia, and other biochemical changes associated with diabetic state. The present study was undertaken to investigate the effect of aqueous extract of *L. sativum* on serum glucose , insulin , lipid profile, haemoglobin , glycosylated haemoglobin , body weight and glucose reabsorption in alloxan induced diabetes in rats .

Materials and Methods

Dried seeds of *L. sativum* obtained from commercial suppliers were identified and authenticated by Iraqi National Herbarium in Abu-Ghraib. Dried seeds were powdered and used for the study. Powder of *L. sativum* seeds (100g) was taken, and 200ml of distilled water was added and boiled and later it was filtered off by using filter. The final concentration of the extract was 150 mg / ml. the filtrate obtained were served as crude extract, and administered orally to the experimental animals at the concentration of 20 mg / kg body weight / day for 30 days (10, 11).

All the experiments were carried out with Wister male rats (180- 200 g) obtained from the animal house of College of Pharmacy in University of Baghdad , Iraq . The animals were housed in polypropylene cages, and provided with water and standard pellets diet *ad libitum*.

Diabetes was induced in rats by intraperitoneal injection of 100 mg / kg body weight of alloxan monohydrate (5 % w/v), freshly dissolved in physiological saline immediately before use (12) The diabetic state was confirmed 48 h after alloxan injection by body weight loss , glucosuria (13), and hyperglycaemia , and the animals which presented blood glucose level above 200 mg / 100 ml , as well as with the clinical signs of polydipsia, polyuria, and polyphagia were selected for the experiments (14) . Animals were divided into four groups of 6 of each:

Group I: normal rats received only physiological saline .

Group II: control diabetic rats received physiological saline .

Group III: diabetic rats received aqueous extract of *L. sativum* seeds (20 mg / kg body weight) / Orally / daily for 30 days (10, 11).

Group IV: diabetic rats received glibenclamide 600 microgram / kg body weight / orally / daily for 30 days (10). At the end of the 30 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in two different tubes (i.e.) one with anticoagulant (potassium oxalate and sodium fluoride) for plasma , and another without anticoagulant for serum which is separated by centrifugation . Fasting blood glucose level was estimated by O- toluidine method by Sasaki,(15). Plasma insulin level was assayed by

Enzymetic Linked Immunosorbant Assay (ELISA) kit using human insulin as standard. Haemoglobin was estimated by method of Drabkin and Austin (16), and glycosylated haemoglobin by method of Sudhakar and Pattabiraman(17).

Total cholesterol and triglyceride were estimated by method of Zlatkis(18) and Foster and Dunn (19) respectively. Phospholipids were analyzed by method of Zilversmit(20). The results were expressed as Mean \pm SEM statistically significance of the differences in parameters before and after treatment was calculated using Students - paired t - test.

Results

Table 1 – shows the levels of blood glucose, plasma insulin, total haemoglobin , and glycosylated haemoglobin. There was significant elevation in blood glucose and glycosylated haemoglobin levels, while the plasma insulin and total haemoglobin levels decreased significantly in alloxan diabetic rats when compared with normal rats . Administration of *L. sativum* seeds and glibiclamide tends to bring the parameters significantly towards the normal .In diabetic rats , there is significant changes in body weight , and the urine sugar , since urine containing sugar were noticed (+++) , but treatment with 20 mg /kg body weight of aqueous extract of *L. sativum* seeds caused decrease in urine sugar (+) , these effects were compared to glibinclamide , see Table - 2. Table -3 shows the level of cholesterol , triglycerides , and phospholipids in the plasma of control and experimental rats , Diabetic rats showed significantly increase levels of cholesterol, triglycerides, and phospholipids when compared with normal rats . In rats treated with aqueous extract of *L. sativum* seeds and glibinclamide, there was a significant decrease in the levels of cholesterol, triglycerides, and phospholipids when compared with diabetic control rats.

Table-1: Changes in blood glucose, plasma insulin, haemoglobin and glycosylated Haemoglobin levels of control and experimental animals.

Groups	Blood glucose level mg/dl	Plasma insulin μ u/ml	Haemoglobin mg / dl	Glycosylated Haemoglobin mg/g haemoglobin
Group I	96.5 \pm 7.04	15.83 \pm 1.02	12.65 \pm 0.7	0.24 \pm 0.01
Group II	236 \pm 13.8 +++	4.85 \pm 0.96 +++	5.49 \pm 0.42 +++	0.81 \pm 0.07 +++
Group III	158 \pm 14.9 ***	13.05 \pm 0.48 ***	9.54 \pm 0.93 ***	0.49 \pm 0.03 ***
Group IV	124 \pm 10.13 ***	12.53 \pm 0.65 ***	10.31 \pm 1.03 ***	0.42 \pm 0.04 ***

+++ represents $P < 0.0005$ compared to control

*** represents $P < 0.0005$ compared to control diabetics

Table-2: Changes in the body weight, and glucose renal reabsorption in control and Experimental animals.

Groups	Body weight g		Urine sugar
	initial	Final	
Group I	198 \pm 0.94	206 \pm 8.9	Nil
Group II	202 \pm 14.2	150 \pm 14.6 +++	+++
Group III	193 \pm 14.8	196 \pm 15.3 **	+
Group IV	194 \pm 10.9	205 \pm 13.14 ***	trace

+++ represents $P < 0.0005$ compared to control

*** represents $P < 0.0005$ compared to control diabetics

** represents $P < 0.005$ compared to control diabetics

Table-3: Change in serum cholesterol, triglyceride, phospholipids in control and experimental animals

Groups	Cholesterol mg / 100 ml	Triglycerides mg / 100 ml	Phospholipids mg / 100 ml
Group I	73.1 +- 1.49	44.13 +- 3.76	80.15 +- 1.37
Group II	99.33 +- 4.03+++	62.43 +- 1.5 +++	97.95 +-2.28 +++
Group III	89.82 +- 2.18 **	54.53 +- 3.4 **	88.5 +- 2.86 **
Group IV	90.13 +- 1.36 **	59.16 +- 1.69 *	89.91 +- 2.32 **

+++ represents P<0.0005 compared to control

***representsP<0.0005 compared to control diabetic

**representsP<0.005 compared to control diabetic

*representsP<0.01 compared to control diabetic

Discussion

Diabetes mellitus is one of the most common metabolic disease associated with hyperlipidemia and co-morbidities such as obesity, hypertension. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes (21).Alloxan, a Beta cytotoxin induces alloxan diabetes in a wide variety of animal species by damaging the insulin secreting pancreatic B cells resulting in a decrease in endogenous insulin release which paves the ways for the decreased utilization of glucose but the tissues (22) . In my study, aqueous extract of *L. sativum* seeds decreases blood glucose level in alloxan – diabetic rats. The possible mechanism of the extract could be correlated with the reminiscent effect of hypoglycaemic sulphonylureas that promotes insulin secretion by closure of K⁺ ATPase channels, membrane depolarization and stimulation of Cal²⁺ influx , an initial key step in insulin secretion. The resultof this study was contraversary to the results obtained by Eddouks (10), in which the hypoglycaemic effect seems to be independant on insulin secretion. Moreover, total haemoglobin decreament during diabetes was notified, and this may be due to the formation of glycosylated haemoglobin. Increase in the levels of haemoglobins in animals given aqueous extract of *L. sativum* may be due to the decreased level of blood glucose and glycosylated haemoglobin. *L. sativum* administration to alloxan – induced diabetic animals reversed the weight loss , this ability of *L. sativum* seeds to recover the body weight loss seems to be due to its hypoglycaemic effect Excess of fatty acids in serum produced by alloxan – induced diabetes promote conversion of excess fatty acids into phospholipids and cholesterol in the liver . These two substances along with excess triglycerides formed at the same time in theliver may be discharged into the blood in the forms of lipoproteins (23). The abnormal high concentration of serum lipids in the diabetic subject is due, mainly to the increase in the metabolism of free fatty acids from the peripheral fatty depots, since insulin inhibits the hormone sensitive lipase. Hyper cholesteremia and hyper triglyceridemia have been reported to occurs in alloxan diabetic rats (24), and a significant increases observed in this experiment was in accordance of these studies. The marked hyperlipdemia that characterize the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on fat depots (25). Activation of enzymes suggests that enhanced lipid metabolism during diabetes is shifted towards carbohydrates metabolism, and it enhances the utilization of glucose at peripheral sites. One of the possible actions of *L. sativum* seeds may be due to its inhibition of endogenous synthesis of lipids. Metabolic aberration in alloxan induced diabetes in rats suggests a high turnover of triglycerides and phospholipids. *L. sativum* was antagonizing the metabolic aberration, and thereby restore the normal metabolism by tilting the balance from high lipids to high carbohydrate metabolism.

On the basis of the above results, it could be concluded that *L. sativum* seeds exert a significant hypoglycaemic and hypolipidemic effects, since both diabetes and hyperlipidemia are considered to be major risk factors for the premature atherosclerosis and essentially all cholesterol in atherosclerotic plaques is derived from that of circulating cholesterol the actual mechanism for the hypoglycaemic and hypolipidemic effects of *L. Sativum* is not clear, and further biochemical and pharmacological investigations needed to isolate and identify the active ingredient(s) in the composite extract.

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