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The Effect of *Lupinus Albus* (Termis) and *Hyphaene Thebaica* (Doum) on Some Biochemical Parameters in Streptozotocin-Induced Diabetic Rats

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Abstract

Background: Several plants have been used as dietary adjuvant and in treating the number of diseases because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs. The present study focuses on evaluating the effect of *Lupinus albus* (termis) and *Hyphaene thebaica* (doum) on STZ-diabetic rats at the level of some biochemical parameters **Material and methods:** STZ was injected intraperitoneally at a single dose of 50 mg/kg to induce diabetes. Termis seeds suspension (75 mg/100 g b.wt.) was daily orally administered. Doum palm fruit suspension was daily orally administered (1g/kg b.wt.) and also gliclazide (reference drug) was administered of a dose equivalent to the therapeutic dose of rat. After four weeks of administration, blood samples were collected for biochemical measurement. **Results:** Blood glucose level was significantly increased. Diabetes increased serum total lipids, cholesterol, triglycerides and LDL but serum HDL was decreased. Also activities of serum enzymes ASAT, ALAT, ALP, GGT and LDH were elevated. However, *L. albus* and *H. thebaica* improved the level of mostly all biochemical parameters.

Conclusion: It is concluded that termis and doum may be dietary adjuvant in reducing the harmful of diabetes.

Key words: *Lupinus albus Hyphaene thebaica*, gliclazide, streptozotocin, diabetic rat.

Introduction

Diabetes was first described by the Ancient Greek physician Aretaeus of Cappadocia, who first coined this term **(1)** Diabetes mellitus (DM) is a worldwide menace, escalating at a phenomenal proportion, afflicting the global population **(2)**. Diabetes mellitus is chronic metabolic disorders that affect human body in terms of physical, psychological and social health. It is defined as a group of disorders characterized by hyperglycemia **(3)**. It accounts for a high incidence of morbidity leads to various events including micro and macro vascular complications **(4)**. Diabetes mellitus is classified into type I, type II.Besides drugs classically used for the treatment of diabetes (insulin, sulphonylureas, biguanides and thiazolidinediones), several species of plants have been described in the scientific and popular literature as having hypoglycemic activity **(6)**.

Lupinus albus is a member of the Leguminosae family **(7)**. Lupine is the one that has the highest protein content in its composition apart from being a good source of fibres. However, although lupin seed is one of the legumes with the lowest levels of non-nutritional compounds (trypsin inhibitors, phytic acid, saponins and lectins) **(8)**.

Doum palm fruit (*Hyphaene thebaia*) is a desert palm tree with edible oval fruit, originally native to the Nile valley. It is a member of the palm family, Arecaceae **(9)**. Doum palm fruit is also a source of potent antioxidants **(10)**.

One method which is more uniformly effective and widely used in inducing diabetes is the injection of streptozotocin **(11)**. It causes hyperglycemia by its direct cytotoxic action on pancreatic beta cells **(12)**.

Aim of the study:

The present study was done to elucidate the effect of *Hyphaene thebaia* and *Lupinus albus* (as antidiabetic agents) on some biochemical parameters in STZ-diabetic adult male albino rats.

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Material and methods:

Material:

1- The drugs:

Streptozotocin (STZ): powder, supplied by *Sigma*, used for induction of diabetes.

Gliclazide**:** Diamicron® tablets, 80 mg supplied by *Servier*, used as a reference drug.

2- Plant preparation:

The pulp of *Hyphaene thebaica* was dried and ground into powder. Aqueous suspension was made by suspending 10 gm of the powdered pulp in 100 ml of distilled water **(13)**.

The seeds of *Lupinus termis* were washed dried at 37 °C for 24 h, and meld well into fine powders. The herb powder was suspended in double-distilled water (5g/100ml**) (14)**.

3- The animals:

Adult male albino rats (*Rattus norvegicus*), weighing 100g ±20gm were obtained from the Egyptian Institution of Serum and Vaccine (Helwan, Cairo, Egypt). Animals were kept under normal conditions throughout the experiment. All rats had access free to food and water *ad libitum* throughout the experimental period. The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of STZ(50 mg/kg body weight) in cold 0.9% citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemy. Diabetes was confirmed in STZ rats by measuring glucose concentration 48 h after injection of STZ. The rats with blood glucose level>200 mg/dl were considered to be diabetic. The treatment was started on 7th day after STZ injection.

The animals of this study were divided into the following groups:

A- Normal group (control healthy group).

B- STZ-diabetic group: (Group 2) animals were injected with STZ (50 mg/kg b.wt. interperitoneally).

C- Diabetic rats treated with Lupinus albus: animals were administered plant suspensions, daily at a dose of 75 mg/100 g b.wt. by intragastric tube for 4 weeks.

D- Diabetic rats treated with *Hyphaene thebaica*: animals were administered plant suspension, daily at a dose of (1g/kg b.wt.) by intragastric tube for 4 weeks.

E- Diabetic rats treated with gliclazide: animals were administered drug suspended in distilled water, daily at a dose equivalent to therapeutic dose of rat.

The treatment continued for 4 weeks.

Methods:

1. Biochemical assay

Blood samples were collected from orbital plexus by capillary tubes to collect serum for determination of biochemical parameters, glucose, ASAT, ALAT, ALP, GGT, LDH, cholesterol, triglycerides, LDL and HDL (kits were supplied by RANDOX Laboratories, Antrim, UK and Stainbio Laboratory, Taxas, USA).

2. Statistical analysis:

The statistical analysis of data was carried out by using one way analysis of variance (ANOVA) followed by Duncan's test **(15).** The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 15.

Results:

The effect of the oral administration of aqueous suspension of *Hyphene thebaica* (fruit), on serum glucose, ASAT, ALAT, ALP, GGT, LDH, cholesterol, triglycerides, LDL and HDL are presented in Table (1). The experimentally induced diabetes significantly increased the level of serum glucose. However, treatment of the STZ-diabetic rats with the aqueous suspension of *H. thebaica* reduced their serum glucose levels significantly(P<0.05) when compared with the diabetic group. In STZ-diabetic rats the activities of serum ALAT, ALP, GGT and LDH were significantly (P< 0.05) increased relative to their normal levels by 131.76%,241.11%,292.71%and141.77%respectively.However treatment with doum reduced ALAT, ALP, GGT and LDH by 34.25%, 19.99%, 51.72% and 45.68%, respectively. The experimentally induced diabetic rats also showed increased level of cholesterol, triglyceride, LDL by 56.27%, 45.33%, 89.41% and decreased level of HDL by -38.39% of control value. *Hyphene thebaica* treatment decreased elevated level of cholesterol, triglyceride, LDL by 18.68%, 9.35%, 27.90% and increased the diminished level of HDL by 59.98%, as compared with diabetic group.

The effect of the oral administration of aqueous suspension of Lupinus termis (seeds), on serum glucose, ASAT, ALAT, ALP, GGT, LDH, cholesterol, triglycerides, LDL and HDL are presented in Table 1. The experimentally induced diabetes significantly increased the level of serum glucose.

However, treatment of the STZ-diabetic rats with the aqueous suspension of *L. termis* reduced their serum glucose levels significantly(P<0.05) when compared with the diabetic group. In STZ-diabetic rats the activities of serum ALAT, ALP, GGT and LDH were significantly (P< 0.05) increased relative to their normal levels by 131.76%, 241.11%, 292.71% and 141.77% respectively. However treatment with termis reduced them by 32.64%, 58.53%, 59.67% and 43.05%. The experimentally induced diabetic rats also showed increased level of cholesterol, triglyceride, LDL by 56.27%, 45.33%, 89.41% and decreased level of HDL by -38.39% of control value. *Lupinus albus* treatment decreased elevated level of cholesterol, triglyceride, LDL by 16.81%, 17.16%, 26.69% and increased the diminished level of HDL by 31.27%, as compared with diabetic group.

Table 1: The effect of the oral administration of aqueous suspension of termis (seeds), doum (fruit) and gliclazide on serum glucose, ASAT, ALAT, ALP, GGT, LDH, cholesterol, triglycerides, LDL and HDL of diabetic rats.

	Control	Diabetic	Termis-treated	Doum-treated	Gliclazide-treated
Glucose (mg/dl)	101.91±0.48	438.37±79.95[a]	212.45±0.26[*b]	230.83±.0.67[ab]	289.42±1.00[ab]
Cholesterol (mg/dl)	92.23±6.19	144.13±6.25*	119.9±4.63	117.21±3.42	138.41±2.73*
Triglycerides (mg/dl)	58.24±2.32	84.64±5.75	70.12±4.32	76.73±5.13	83.74±4.22
LDL	35.31±1.24	66.88±1.24*	49.03±2.12*	48.22±1.58	53.17±1.74*
HDL	57.15±3.12	35.21±1.11*	46.22±3.21*	56.33±3.02	47.13±2.27*
ALAT(U/L)	72.11 ±2.13	167.12±6.12*	112.58±5.18*	109.88±4.97*	119.42±7.7*
ASAT(U/L)	83.61 ±5.94	87.16±2.12	102.24±1.8*	103.12±.3.8*	109.71±4.7*
ALP(U/L)	31.11 ±1.09	106.12±1.41*	44.01±1.06	84.91±1.91*	86.42±2.16*
GGT(U/L)	22.22± 3.41	87.26±2.52*	35.19± 1.38*	42.13±1.27*	67.32±2.42*
LDH(U/L)	69.12± 1.12	167.11± 4.33*	95.17±4.32*	90.77±5.62	124.29±6.92*

***: Significant change at p< 0.05 with respect to control group.**

Discussion:

The experimentally induced diabetes significantly increased the level of plasma glucose. However, treatment of the STZ-diabetic rats with the aqueous suspension of *L. termis* and *H. thebaica* reduced their serum glucose levels significantly when compared with the diabetic group. It may be due to presence of saponins in the constituents of lupin which have hypoglycemic activity (**16).** Doum is from medicinal plants that contain flavonoids. It was found that the administration of flavonoids extracts to diabetic rats significantly increased adiponectin levels that stimulate the hypoglycemic action of insulin without altering the concentration of insulin in blood **(17)**. Furthermore, the hypoglycemic effect of these herbs may be due to the increased level of serum insulin **(18)** by increasing the pancreatic secretion of insulin from cells of islets of Langerhans or its release from bound insulin **(19 ,20),** and also may be due to the enhancement of peripheral metabolism of glucose **(21)**.

In STZ-diabetic rats the activities of serum ALAT, ASAT, ALP, GGT and LDH were significantly (P< 0.05) increased relative to their normal levels. Elevated liver enzymes could be expression of excess deposition of fat in liver **(22)** and they may reflect inflammation which impairs insulin signaling both in liver and systemically **(23).**

The increment in transaminases ASAT and ALAT may be explained by oxidant stress from reactive lipid peroxidation, peroxisomal beta-oxidation and recruited inflammatory cells **(24)**. Elevation in ALAT, gluconeogenic enzyme, whose gene transcription is suppressed by insulin, could indicate impairment in insulin signaling **(25).**

Cellular GGT has a central role in glutathione homeostasis by initiating breakdown of extracellular glutathione, a critical antioxidant defense for the cell **(26).** Increase in GGT activity may be response to oxidative stress, making transport of glutathione into cells (**27)**. The treatment of the diabetic rats with Lupinus albus suspension caused reduction in the activity of these enzymes **(14).** On the other hand, treatment of the diabetic rats with *Hyphaene thebaica* showed reduction in ALAT, ALP, GGT and LDH as compared with untreated diabetic group which revealed that administration of doum can improve liver functions and this result is in a harmony with **Al-Masri (2012) and AL-amer et al. (2012) (28,29)**.

In this study, STZ-diabetic rats showed a marked increase in the level of cholesterol, triglycerides, LDL and decrease in HDL. The marked hyperlipidemia that characterizes the diabetic state may be regarded as a consequence of the unlimited action of lipolytic hormones on the fat depot **(30).** High concentrations of plasma lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits hormone sensitive lipase. On the other hand, glucagon and catacholamines and other hormones enhance lipolysis **(20)**.The ability of L. termis to diminish cholesterol and triglycerides levels can be attributed to the mechanism reported by other authors studying lupin proteins and their effects on metabolism. **Sirtori *et al.*** reported that lupin proteins are capable of stimulating the activity of LDL receptors, increasing the capture of LDL from the plasma to the cells **(31)**. On the other hand, the inhibition of HMG-CoA reductase, a key enzyme in the synthesis of cholesterol, regulated by the action of SREBP-2, could also reduce the concentration of LDL cholesterol in plasma **(32). Bettzieche *et al.*** described distinctive effects for different species of lupin proteins in the lipid metabolism **(33)**. The cultivar Vitabor of lupin (Lupinus angustifolius L.) administered to rats, reduced the triglycerides and total cholesterol through the reduction of the expression of genes SREBP-1c and HMG-CoA reductase. The aquous suspension of *H. thebaica* improved serum lipids profile of diabetic rats in which total lipids, cholesterol, triglycerides and LDL levels were reduced but HDL was increased. These results are in agreement with many previous studies **(17, 29, 34) .** Also our biochemical findings are in agreement with previous investigation of **Hetta and Yassin** who reported that doum plant exhibited a highly significant decrease in serum cholesterol and Non-HDL cholesterol **(35)**. **Kamis *et al*** reported that the daily oral administration of the suspension of *Hyphaene thebaica* (L) mart for three weeks revealed a significant decreased in the levels of triglycerides, cholesterol and total lipids compared to the control **(13)**. As mentioned before, *Hyphaene thebaica* is from medicinal plants that contain flavonoids. It was found that the administration of flavonoids extracts into diabetic rats significantly increased adiponectin levels. Some studies have revealed that adiponectin plays important roles in the regulation of lipid metabolism **(17)**.

References:

1) **Ratheau L, Jeandidier N, Moreau F, Sigrist S and Pinget M. (2011):** How technology has changed diabetes management and what it has failed to achieve. J Diabetes & Metabolism, 37(4): S57–S64.
2) **Gurav AN (2012):** Periodontal therapy – An adjuvant for glycemic control. J Diabetes & Metabolic Syndrome: Clinical Research & Reviews,6: 218–223.
3) **Warjeet SL**. **(2011):** Traditional medicinal plants of Manipur as anti-diabetics. J Med Plants Res., 5(5): 677-687.
4) **Srinivasan AR, Niranjan G , Velu VK, Parmar P and Anish A (2012):** Status of serum magnesium in type 2 diabetes mellitus with particular reference to serum

triacylglycerol levels. Diabetes 188 & Metabolic Syndrome: Clinical Research & Reviews, 6:187–189.

5) **American Diabetes Association (2005):** Diagnosis and Classification of Diabetes Mellitus. Diabetes Care, 28: S37-S42.
6) **Colca JR (2006):** Insulin sensitizers may prevent metabolic inflammation. J Biochem. Pharmacol., 72: 125–131.
7) **Litkey J and Dailey MW (2006):** Anticholinergic toxicity associated with the ingestion of lupini beans. J ajem; 215-216.
8) **Martinez-Villaluenga C, Frias J and Vidal-Valverde C (2006):** Functional lupin seeds (Lupinus albus and Lupinus luteus) after extraction of a-galactosides. J Food Chemistry, 98: 291–299.
9) **Aremu1 AK and Fadele OK (2011)**: Some Physical Properties of Doum Palm Fruit (*Hyphaene thebaica Mart.*). J New York Science, 4(3): 63-69.
10) **Hsu B, Coupar IM and Ng K(2006):** Antioxidant activity of hot water extract from the fruit of the Doum palm, Hyphaene thebaica. J Food Chemistry, 98:317 – 328.
11) **Abu Abeeleh M, Ismail ZB, Alzaben KR, Abu-Halaweh SA, Al-Essa MK, Abuabeeleh J, Alsmady MM. (2009):** Induction of Diabetes Mellitus in Rats Using Intraperitoneal Streptozotocin: A Comparison between 2 Strains of Rats. J European Journal of Scientific Research, 32(3):398-402.
12) **Takeshita F, Kodama M, Yamamoto H, Ikarashi Y, Ueda S, Teratani T, Yamamoto Y, Tamatani T, Kanegasaki S, Ochiya T and Quinn G (2006):** Streptozotocin-induced partial beta cell depletion in nude mice without hyperglycaemia induces pancreatic morphogenesis in transplanted embryonic stem cells. J Diabetologia, 49:2948-2958.
13) **Kamis AB, Modu S, Zanna H and Oniyangi TA (2003):** Preliminary biochemical and haematological effects of aqueous suspension of pulp of Hyphaene thebaica (I) mart in rats. J Biokemistri,13(1):1-7.
14) **Mansour H A, Newairy AA, Yousef MI and Sheweita SA (2002):** Biochemical study on the effects of some Egyptian herbs in alloxan-induced diabetic rats. Toxicology, 170: 221–228.
15) **Duncan, D.B. (1955):** Multiple *F-test*. Biometrics; 11:1-42.
16) **Nakashima N, Kimura L and Kimura M (1993):** Isolation of pseudoprototimosaponin AIII from rhizomes of Anemarrhena asphodeloides and its hypoglycemic activity in streptozotocin—induced diabetic mice. J. Nat. Prod.,56(3): 345.
17) **Salah SH, Abdou HS, Abd El Azeem AS and Abdel-Rahim EA (2011):** The antioxidative effects of some medicinal plants as hypoglycemic agents on chromosomal aberration and abnormal nucleic acids metabolism produced by diabetes stress in male adult albino rats. Journal of Diabetes Mellitus, 1(1): 6-14.
18) **Eskander EF and Won -Jun H (1995):** Hypoglycemic and hyperinslinemic effects of some Egyptian herbs used for the treatment of diabetes mellitus (type II) in rats. Egypt. J. Pharm. Sci., 36 (1–6):331–342.
19) **Stanely P, Prince M and Menon VP (2000):** Hypoglycaemic and other related actions of *Tinospora cordifolia* roots in alloxan-induced diabetic rats Journal of Ethnopharmacology, 70 : 9–15.
20) **Dhandapani S, Subramanian VR, Rajagopal SK and Namasivayam N (2002):** Hypoglycemic effect of *Cuminum Cyminum L.* on alloxan-induced diabetic rats.Pharmacological Research,46(3):251-255.
21) **Skim F, Lazrek HB, Kaaya A, El-Amri H and Jana M (1999):** Pharmacological studies of two antidiabetic plants: *Globularia alypum* and *Zygophyllum gaetulum*. Therapie., 54(6):711-715.
22) **Malnick SD, Beergabel M and Knobler H (2003):** Non-alcoholic fatty liver: a common manifestation of a metabolic disorder. QJM., 96:699–709.
23) **Hotamisligil GS (2003):** Inflammatory pathways and insulin action. Int J Obes Relat Metab Disord, 27(3):S53–S55.
24) **Harris EH (2005):** Elevated Liver Function Tests in Type 2 Diabetes. M CLINICAL DIABETES, 23(3):115-119.
25) **O'Brien RM and Granner DK (1991):** Regulation of gene expression by insulin. *Biochem J.*, 278:609–619.
26) **Whitfield JB (2001):** Gamma-glutamyl transferase. Crit Rev Clin Lab Sci., 38:263–355.
27) **Nakanishi, N.; Suzuki, K. and Tatara, K. (2004):** Serumγ Glutamyltransferase and Risk of Metabolic Syndrome and Type 2 Diabetes in Middle-Aged Japanese Men. Diabetes Care, 27:1427–1432.
28) **Al-Masri SA (2012):** Effect of Doum And Methionine Combination on Hepatotoxicity in Rats. Australian Journal of Basic and Applied Sciences, 6(6): 392-397.

29) **AL-amer HA, Nabila and Rashwan M (2012):** Effects of dietary supplementation with doum and selenium on liver injury in experimental rats Journal of Applied Sciences Research, 8(4): 2018-2023.
30) **Murray RK, Granner DK, Mayes PA and Rodwell VW (2000):** eds. *Harper's biochemistry*. 25th edn. Stanford, CT: Appleton and Lange pp: 610–7.
31) **Sirtori CR, Lovati MR, Manzoni C Castiglioni S, Duanti M, Magni C, et al. (2004):** Proteins of white lupin seed, a naturally isoflavone-poor legume, reduce cholesterolemia in rats and increase LDL-receptor activity in hepG2 cells. Journal of Nutrition, 134: 18–23.
32) **Goméz-Pérez FJ, Bustamante F, Vergara A, Villaseñor J, Wong B and Rull JA (1992):** A controlled trial of provastatin vs. Probucol in the treatment of primary hypercholesterolaemia. Revista de Investigación Clínica, 44: 53–61.
33) **Bettzieche A, Brandsch C, Schmidt M, Weibe K, Eder K and Stangl GI (2008):** Differing effect of protein isolates from different cultivars of blue lupin on plasma lipoproteins of hypercholesterolaemic rats. Bioscience, Biotechnology and Biochemistry, 72(12): 3114–3121.
34) **Ismail SH, Ahmed SA, Fayed SA and Van-Breemen RB (2007):** Adiponectin, insulin and nitric oxide levels in hyperlipidemia and hypercholesterolemia in addi-tion of streprozotocin-induced diabetes mellitus and normal rats intraperitonially injected with anthocyanin and epicatechin extracts. New Egyptian Journal of Medicine, **36**: 135-143.
35) **Hetta, M.H. and Yassin, N.Z. (2006):** Comparative studies on hypocholesterolemic effect of different fractions of *Thebaica* (doum) in experimental animals. Pharmazie, 61:230-232.