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Effect of *Lupinus albus* on Glycaemic Control, Plasma Insulin Levels, Lipid Profile and Liver Enzymes in Type 2 Diabetics

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Abstract *Lupinus* species and their derivatives are good candidates to be used as hypoglycaemic agents. The aim of this study was to evaluate the effects of supplementation with *Lupinus albus* dry extract in type 2 diabetic patients. The study included 47 adult patients (21 men and 26 women) with type 2 diabetes. While consuming their usual medication, patients received a daily dose of 400 mg of *Lupinus albus* dry extract during 12 weeks. Fasting and post meal test glucose and insulin were measured at baseline and after 2 and 12 weeks. Plasma lipids, Alanine and Aspartate aminotransferases activities and glycated haemoglobin were assessed at baseline and at the end of the supplementation period**.** Compared to baseline values, fasting and postprandial plasma glucose levels were decreased at 2 and 12 weeks. Blood glucose area under the curve significantly decreased after 2 weeks ($p<0.01$) and 12 weeks ($p<0.05$) of lupine administration. Fasting insulin concentrations (10.3±5.34 *vs.* 11.9±6.58; $p<0.05$) and homeostasis model assessment of insulin resistance (3.50±2.01 *vs.* 4.40±2.80; $p<0.01$) were significantly lower at 12 weeks, but not at 2 weeks. The area under the curve for insulin response did not differ from the baseline. After 12 weeks of *Lupinus albus* administration, glycated haemoglobin (-5.71%), plasma total cholesterol (-8.12%), LDL cholesterol (-5%), triglycerides (-23.2%), and Alanine aminotransferase activity (-21.1%) were significantly decreased compared to baseline. The study showed that administration of *Lupinus albus* results in a hypoglycaemic effect and an improvement of diabetes control, but does not affect insulin secretion. These findings suggest that *Lupinus albus* has insulin mimetic action.

Keywords: *Blood glucose, Glycated haemoglobin, Insulin, Lupinus albus, Type 2 diabetes mellitus*

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1. Introduction

Diabetes is a global epidemic with an estimated worldwide prevalence of 246 million people in 2007 and expected to rise to 300 million by 2025 [1]. In Tunisia, type 2 diabetes prevalence increased from 9.9 % in 1996 to 15.1 % in 2010 [2,3]. Many herbal medicines have been recommended for diabetes mellitus because of their low toxic effect compared to oral hypoglycaemic agents such as sulfonylureas and metformin [4]. The antidiabetic effect of plant extracts could be linked to stimulation β cells-based insulin secretion or insulin action [5]. Among these plants, some lupine species have been used as a natural remedy [6]. Lupine is a leguminous seed used to prepare food supplements or foods [7]. Several previous studies have shown beneficial effects of lupine species on glucose metabolism. Most studies were done in normal and experimentally diabetes-induced animals and cultured cells, mainly testing the effect of lupine derivatives, especially the γ-conglutin protein on insulin secretion [8-13]. Effects of prolonged lupine extract administration in Humans and its impact on insulin sensitivity and overall diabetes control are rarely investigated [8,14]. Notably, the effect of lupine on insulin secretion and peripheral sensitivity remains controversial. The aim of this study was to test the effect of *Lupinus albus* administration on blood glucose and insulin levels, and on diabetes control in type 2 diabetics' mellitus patients. To this end, we monitored plasma glucose and insulin concentration upon a standardized meal test before and after 2 and 12 weeks of *Lupinus albus* dry extract administration. We also tested change in glycated haemoglobin, plasma lipids and

aminotransferases activities. The hypothesis of this study was that lupine will improve the diabetes control in supplemented patients.

2. Material and Methods

2.1. Participants

A total of 47 volunteers type 2 diabetics (21 males and 26 females) aged 35 to 65 years participated in this study. Participants were recruited from the Endocrinology Service in Rabta Hospital (Tunis, Tunisia). Diagnosis of diabetes mellitus was based on the World Health Organization and the American Diabetes Association criteria [15,16]. Exclusion criteria were type 1 diabetes, insulin use, haemoglobin A1c > 9%, known allergy to lupine, nuts or soya, change in regular medications in the previous three months. The duration of illness varied from 3 months to 5 years (24.4±18.2 months). Mean body mass index (BMI) was 30.6±4.71 kg/m^2. Participants were hyperglycaemic with baseline fasting glucose values of 150±44.1 mg/dL. Among the 47 participants, six (12.8%) were on diet alone, 24 (51.1%) were treated with metformin, three (6.4%) were taking glibenclamide and 14 (29.8%) were prescribed both metformin and glibenclamide. The study's protocol was approved by the Rabta Hospital Ethics Committee and a written consent was obtained from all participants.

Two capsules (200 mg per capsule) of *Lupinus albus* dry extracts were administered to the subjects, one capsule 30 minutes before breakfast and one capsule 30 minutes before dinner, totalling 400 mg per day for a period of 12 weeks. *Lupinus albus* dry extract was kindly provided by Vital laboratories (Tunisia). Each participant was instructed to visit the hospital after 2, 4, 8 and 12 weeks from the start of the treatment. At each visit, participants were asked to return their capsule bottles to allow the investigators to check compliance with the supplements. The safety and harmlessness of the supplements were checked by looking for functional signs (nausea, malaise, weakness, breathing difficulty, visual disturbances, ataxia, or coma, etc.) and performing biochemical tests of renal and liver functions. Participants were requested to maintain their usual diet, physical activity and medications for the duration of the study. They were asked to consume the same meal on the evening before each visit in order to avoid a second-meal effect [17]. A capsule of 200 mg of *Lupinus albus* extract was administered per os 30 minutes before the meal test. Anti-hyperglycaemic medications were kept on the morning of each study visit.

2.2. Biochemical Analyses

Participants attended visit around 8 AM, following a 12 hours fast. An intravenous cannula was inserted into the antecubital vein through which 10 ml blood sample was drawn after a rest period of 15 min. Participants then consumed a standardized meal test for 5-10 min (55% carbohydrate, 33% fat, and 12% protein with a calorific value of 500 kcal and 75 g carbohydrate). Further blood samples (2-3 mL) were drawn at 30, 60, 90 and 120 min after the meal consumption. After the final blood sample was collected, the cannula was removed. The meal test was only carried out at baseline and at 2 weeks and 12 weeks from the onset of lupine administration. The samples were centrifuged at 2000 x g for 25 minutes and plasma was frozen at - 40° C until analysis (within 3 months).

Plasma insulin concentrations were measured by chemiluminescence immunoassay using a Liaison analyzer and the respective reagents kit (DiaSorin Inc., Stillwater, MN) with an intra-assay CV below 5%. Blood haemoglobin A1c (Hb A1c) was assessed by a competitive turbidimetric inhibition immunoassay method (Tina-quant HbA1c Gen. 2) using a Cobas 400 plus analyzer (Roche Diagnostics Ltd., Rotkreuz, Switzerland). Plasma glucose, creatinine, uric acid, total cholesterol, HDL cholesterol, triglyceride and Alanine (ALT) and Aspartate (AST) aminotransferases were assessed by colorimetric methods using an Architect C8000 analyzer and the respective reagents kits (Abbott Laboratories, Abbott Park, IL). LDL cholesterol was calculated using the Friedwald formula [18]. Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR) index as follows; HOMA-IR=[fasting insulin (mU/L)*fasting glucose (mmol/L)/22.5] [13].

2.3. Statistical Analysis

Calculations and statistical analysis were performed using the Systat ver. 19 software for Windows from SPSS. The primary endpoints of interest were fasting serum glucose and insulin and the respective response upon meal test. Descriptive statistics and 95% confidence intervals were used to estimate the parameters. Results are reported in terms of means with their standard deviations. Differences between pre (baseline) and post supplementation (after 2 and 12 weeks) values for different parameters were tested using paired-samples t-test. Differences between areas under the curve for glucose and insulin responses to meal test before and after lupine supplementation were compared with the method of DeLong et al. (1988). A p-value <0.05 based on two-sided calculation was considered significant.

3. Results

The tolerability of *Lupinus albus* extract was satisfactory. No side effect or adverse event was recorded and biochemical tests for renal (creatinine) and liver (AST, ALT) functions remained unchanged and stable upon administration and during the time course of the study. Compared to baseline values, BMI, waist circumference, body fat mass and weight excess significantly decreased after 2 and 12 weeks (Table 1). Fasting and 30 min, 60 min, 90 min and 120 min post meal test glucose levels after 2 weeks of lupine administration were significantly decreased compared to baseline values (Table 2). After 12 weeks, only 90 min and 120 min post meal test glucose were significantly lower than the respective baseline values (Figure 1). However, areas under the curve for glucose response to meal test were significantly lower at 2 weeks and 12 weeks compared to baseline values (Table 2 and Table 3). Compared to baseline, fasting insulin concentration was lower after 12 weeks (Table 3). But, postprandial insulin concentrations and area under the curve for insulin response to meal test was not different from the respective baseline values at both 2 and 12 weeks (Table 3). However, HOMA-IR at 2 weeks (3.69±2.00)

and 12 weeks (3.50±2.01) following lupine intervention were significantly lower (P<0.05 and P<0.01, respectively) than baseline values (4.40±2.80). Haemoglobin A1c, total and LDL cholesterol and triglycerides, as well as AST and ALT activities significantly decreased following 12 weeks *Lupinus albus* administration (Figure 2).

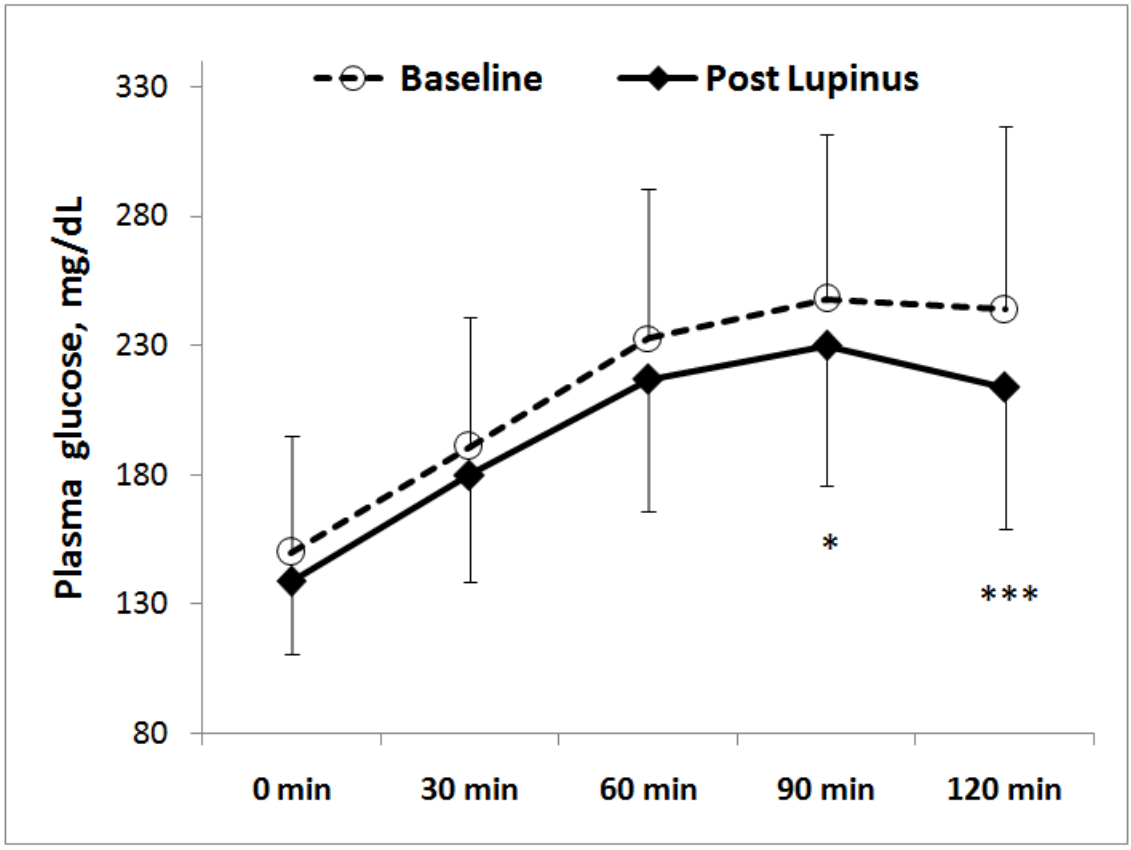

Figure 1. Comparative plasma glucose response to a standardized meal test before and after 12 weeks of *Lupinus albus* dry extract administration in type 2 diabetics (*, P< 0.05; ***, P<0.001)

Table 1. Anthropometric characteristics of participants at baseline and after 2 weeks and 12 weeks from the onset of *Lupinus albus* administration

	Baseline	After 2 weeks	After 12 weeks
Weight (kg)	82.6±14.2	82.2±14.1**	81.6±13.8**
Body mass index (kg/m^2)	30.6±4.71	30.4±4.70**	30.2±4.65**
Waist circumference (cm)	99.8±9.55	99.3±9.42	98±8.99**
Hip circumference (cm)	106±8.97	106±8.83	104±8.38**

Values were expressed as mean ± standard deviation; **, *P<0.01 (*compared to baseline).

Table 2. Plasma glucose concentrations and area under the curve following a meal test at baseline and after 2 weeks from the onset of *Lupinus albus* administration

		Baseline	After 2 weeks	p-value
Glucose (mg/dL)	0 min	150±44.8	137±32.2	0.015
	30 min	191±50.3	176±55.0	0.013
	60 min	233±58.1	219±38.6	0.045
	90 min	248±63.9	229±49.1	0.008
	120 min	244±71.0	215±53.8	0.000
Area under the curve		26130±6631	24071±4354	0.005

Values were expressed as mean ± standard deviation.

Table 3. Plasma glucose and insulin concentrations and area under the curve following a meal test at baseline and after 12 weeks (Post Lupinis) from the onset of *Lupinus albus* administration

	Glucose (mg/dL)			Insulin (mIU/L)		
	Baseline	Post *Lupinus*	(%)	Baseline	Post *Lupinus*	(%)
0 min	150±44	139±27	-7.33	11.9±6.58	10.3±5.34*	-13.8
30 min	191±50	180±40	-5.75	26.3±19.1	23.7±11.0	-9.90
60 min	233±58	217±50	-6.86	37.7±23.2	33.7±15.6	-10.7
90 min	248±63	230±54*	-7.25	39.9±18.7	41.4±21.5	+3.75
120 min	244±71	214±55***	-12.3	42.1±17.7	41.8±21.3	-0.30
AUC	26130±6631	24189±5285*	-7.42	3932±1964	3747±1669	-4.69

Data are expressed as mean ± standard deviation; (%), percent of variation from baseline; AUC, area under the curve; *, P< 0.05; ***, P<0.001.

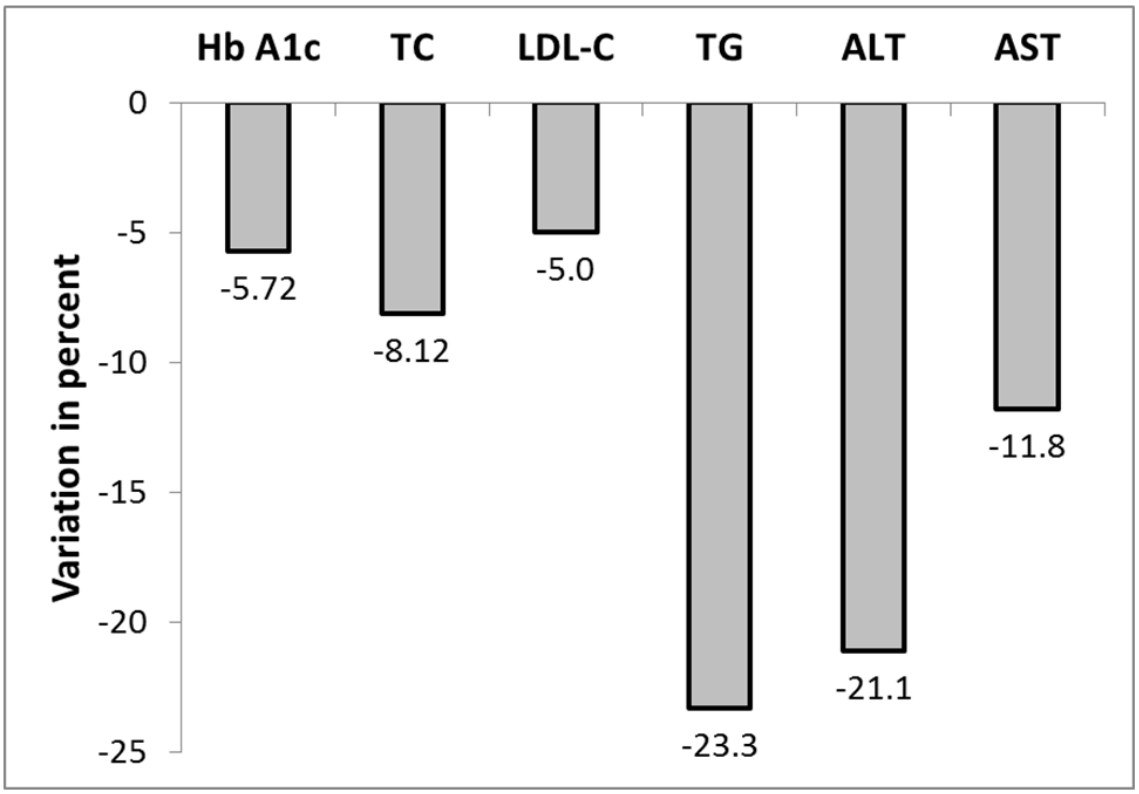

Figure 2. Effect of 12 weeks of *Lupinus albus* dry extract administration on haemoglobin A1c (HbA1c), total cholesterol (TC), LDL cholesterol (LDL-C), triglycerides (TG) and Alanine (ALT) and Aspartate (AST) aminotransferases activities in type 2 diabetics

4. Discussion

Repeated *Lupinus albus* administration significantly decreased fasting and postprandial plasma glucose, fasting insulin level and HOMA-IR, as well as HbA1c, plasma lipids and aminotransferases in type 2 diabetics. Compared to baseline, subjects who received *Lupinus albus* showed significant lower fasting and postprandial serum glucose concentrations after 2 and 12 weeks. The AUC of glucose response was significantly below the baseline, which agrees with the results of Dove et al. [14]. The hypoglycaemic effect of *Lupinus albus* was mainly attributed to a lupine glycoprotein seed named γ-Conglutin. Previous studies showed that γ-Conglutin decreases plasma glucose concentrations upon glucose overload in mice [9,10,11] and healthy Humans [8]. Studies indicated that isolated lupine conglutin-γ has hypoglycaemic effect in glucose loaded non-diabetic experimental animals [8,10,12] and shows insulin-mimetic effects in cultured cells [11,13]. Terruzi et al. [11] have shown that γ-conglutin stimulation of mouse myoblastic cells elicited the activation of intracellular kinases, very similar to the effects provoked by insulin. γ-conglutin activates the IRS-1/PI-3-kinase pathway, which is critical in glucose homeostasis and protein synthesis and the translocation of the glucose transporter GLUT-4 to the surface of the cell [11].

The herein studies showed a significant decrease of fasting insulin and a decreasing trend of postprandial insulin under *Lupinus albus* supplementation. This finding corroborates that of Lovati et al. [9], who observed increased glucose consumption by HepG2 cells and a reduced blood glucose and insulin in hyperglycaemic mice treated with γ-conglutin. In contrast, numerous studies showed increased serum insulin after *Lupinus* supplementation in normal and alloxan-diabetic animals [8,19], as well as in healthy and diabetic Humans [8]. In this study, although insulin concentrations have decreased upon *Lupinus albus* therapy, HOMA-IR has also decreased, it was 20.5% below the baseline values, indicating an improvement in responsiveness to insulin. In a similar vein, Lovati et al. [9] reported that chronic treatment with γ-conglutin had improved the state of insulin resistance as determined by a decrease of HOMAS in treated animals. These findings corroborate the potential insulin mimetic effect of *Lupinus albus*. On the other hand, decreased insulin resistance may explain the observed reduction in insulin levels after lupine supplementation. Indeed, the circulating insulin level depends on both β cell secretion and peripheral tissues' sensitivity to insulin.

In addition to blood glucose and insulin resistance decrease, *Lupinus albus* administration resulted in a significant decrease in HbA1c, which reflects a better diabetes control. Similar finding (reduction in HbA1c) was previously obtained following Fenugreek seed powder supplementation in diabetics [20, 21]. The present study is the first to report the reducing effect of *Lupinus albus* on HbA1c in diabetics.

Lipids disorders are common in diabetics' mellitus and play crucial roles in developing cardiovascular complications in diabetics [22]. *Lupinus albus* administration for 12 weeks resulted in a substantial decrease in serum total and LDL cholesterol and triglyceride levels, but no significant change in HDL cholesterol in these diabetics was observed. A previous study has shown that *Lupinus albus* is effective in reducing atherogenic lipid levels (total and LDL cholesterol and triglyceride) in hypercholesterolemic Human patients [23]. The hypolipemic and cholesterol-

lowering effects of diverse *Lupinus albus* derivatives were also observed in different animal species [24,25,26,27,28]. It was shown that *Lupinus albus* proteins increase LDL receptors' activity and then LDL-uptake and degradation. This mechanism has been suggested to be one of the mechanisms behind the hypocholestrolemic effect of lupine [29]. Moreover, lupine seed contains high amounts of proteins and viscous nonstarch polysaccharides that could bind bile acids and lead to a significant loss of these acids in the faeces, which may reduce intestinal absorption of lipids [30,31]. Finally, improvement in serum lipid profile may be secondary to a better glycaemic control upon lupine therapy.

The study also showed a significant decrease in plasma AST and ALT activities after lupine supplementation. Likewise, Harisa and Alanazi [32] concluded to the beneficial effect of *Lupinus luteus* on plasma AST and ALT activities in individuals with metabolic syndrome. A decrease in the activity of these enzymes was also observed following *Lupinus albus* treatment in diabetic or hypercholesterolemic rats [21,33,34]. Our findings together with the literature data suggest that lupine could reduce liver steatosis associated with diabetes and dyslipemia as well as liver damage associated with alloxan diabetes.

Lupine seeds of wild varieties may contain up to 6 % of quinolizidine alkaloids [35]. However, sweet lupines used in this study contain 0.01% to 0.03% alkaloids, making these products safe for human and animal consumption [35,36].

Some limitations have to be mentioned. The study did not include a control group, which would have permitted attributing the changes observed to lupine supplementation with greater firmness. However, in this pre-post test study, the baseline could be seen as a reference and the observed changes can be mainly attributed to the lupine supplementation. Due to a small sample size, the study may have missed detecting significant changes in some variables, such as insulin. Finally, the study did not control for dietary intake and energy expenditure, which may affect the parameters studied. However, no participant has changed his eating habits or physical activity while participating to the trial. Therefore, it is improbable that these factors had influenced the study results.

5. Conclusions

The study demonstrated glucose and lipid lowering effects and a superior diabetes control upon *Lupinus albus* dry extract administration in type 2 diabetics. These antidiabetic and hyolipemic effects suggest that *Lupinus albus* exerts insulin mimetic activities. With the above reported results, repeated administration of *Lupinus albus* dry extract in diabetic adults could be an effective supportive therapy in managing diabetes and preventing its long-term complications.

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Conflict of Interests

The authors declare that there is no conflict of interests.

Non-standardized Abbreviations

BMI, body mass index; Hb A1c, glycated haemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; AST, Aspartate aminotransferase; ALT Alanine aminotransferase; GLUT-4, glucose transporter type 4, HepG2, liver hepatocellular cells; IRS-1, insulin receptor substrate-1; PI-3-kinase, phosphatidyl inositol 3-kinase.

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