

Assessing of Antidiabetic and Ameliorative Effect of Lupin Seed Aqueous Extract on Hyperglycemia, Hyperlipidemia and Effect on pdx1, Nkx6.1, Insulin-1, GLUT-2 and Glucokinase Genes Expression in Streptozotocin-induced Diabetic Rats

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Abstract Diabetes mellitus (DM) belong to the major health problems causing mortality and morbidity in the world. Our study aimed to investigate effects of *Lupinus Albus* (LA) seed extract on streptozotocin (STZ)-induced diabetic rats. Thirty mature *Sprague Dawley* male rats were used in this study. Three experimental groups were used as follows: GI- Control rats (Normal), GII- Diabetic rats and GIII- Diabetic rats treated with LA aqueous extract (7mg/100g B.wt) by oral gavage. Insulin, Fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c) and serum lipid profile assays of all rats were carried out. Gene expressions of Pancreatic and duodenal homeobox 1 (Pdx-1), homeobox protein (Nkx6.1), insulin-1, liver glucose transporter-2 (GLUT-2) and glucokinase were performed by real-time PCR. Results obtained for diabetic rats orally gavage with LA extract revealed an improvement in insulin with reduction in FBG, HbA1c% and significant improvement in serum lipid profile. Additionally, LA aqueous extract induced a mild increase in expression of biomarkers genes for pancreatic β cells (β -cells) function, insulin secretion, liver GLUT-2 and glucokinase when compared to STZ-diabetic rats. This research highlights LA seed extract as a nutraceutical for improving insulin secretion in diabetic rats.

Keywords: lupin, diabetes mellitus, hyperglycemia, hyperlipidemia, insulin secretion

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1. Introduction

DM one of the metabolic diseases, consequential to a defect in insulin secretion from β -cells or defect in sensitivity of target tissues to insulin and resulted in hyperglycemia associated with alteration in metabolism of carbohydrate, fat and protein [1]. DM was considered serious health problems resulted in mortality and morbidity in the world [2]. Type-1 Diabetes mellitus (T1DM) happens when beta cells (β -cells) of pancreas recognized by the auto-reactive T cells [3], destroys these cells and subsequent loss of insulin secretion.

The physiological effects of insulin are started with binding of insulin to its membrane receptor that stimulates activity of the intrinsic protein tyrosine kinase and

subsequent phosphorylation of a cascade of interacting proteins, including those of the insulin receptor substrate (IRS) [4]. Pdx-1 sustains the adult beta cells phenotypes and essential for their survival [5]. It is vital for the expression, processing and secretion of insulin as well as glucose homeostatic regulation [6]. Nkx6.1 is important for the final differentiation of β -cells as well as being essential and adequate for specifying the identity of β -cells and production of insulin [7].

STZ is synthesized by *streptomyces achromogenes* and it is used as an antibiotic that produces β -cells pancreatic islets damage and is widely used to induce an experimental model of T1DM [8]. Also, it was accepted by Food and Drug Administration (FDA) in the metastatic cancer of pancreatic islets cells via impeding glucose oxidation and stimulation of insulin secretion from β -cells through DNA fragmentation followed by a continuous

decrease in cellular nicotinamide adenine dinucleotide (NAD⁺) levels that induces cell death [9,10]. STZ induced beta cell damage as well as liver and kidney toxicity [11]. STZ internalized through GLUT-2, which is located on pancreatic beta cells, liver, kidney and small intestine acting as a transmembrane carrier protein and the main glucose transporter and sensor in rodent islets [12]. GLUT-2 gene expression levels in pancreas are defined as a marker for the degree of susceptibility to STZ in different animal models [13]. Single nucleotide polymorphisms (SNPs) in GLUT-2 gene were found to predict conversion to diabetes from impaired glucose tolerance [13].

Micro and macro vascular complications of DM can be reduced through the effective treatments of diabetes-induced hyperglycemia [14]. Nowadays, approach for treatment of diabetes induced-hyperglycemia was directed to use the most potent, effective and safe natural medicinal plants instead of the anti-diabetic drugs to avoid their side effects on diabetic patients [15,16]. *Lupinus Albus (LA)* is an annual plant, one type of legumes, used for human nutrition as a protein source and exemplifies as an anti-hyperglycemic plant [17,18]. It possesses hypoglycemic effects via augmented secretion of insulin from β -cells [19]. Aqueous extract of *LA* seeds encourages the release of insulin from cultured β -cells [20]. Furthermore, *LA* is rich in *lupine* proteins that belong to 11S and 7S globulins; one type of this globulin is conglutin- γ which represented about 5% of the total globulins, show insulin-simulated effects in cultured cells and has hypoglycemic effect that was demonstrated in different experimental animals [21,22].

The anti-hyperglycemic *LA* mechanism of action was not clearly studied; therefore, our study has been carried out to investigate the potential anti-diabetic effects of *LA* seed extract on hyperglycemia and hyperlipidemia in diabetic rats and to evaluate its effect on insulin action based on a molecular level, focusing on its effect on pancreatic insulin-1, Pdx-1 and Nkx6.1 and liver glucokinase and GLUT-2 gene expression.

2. Materials and Methods

2.1. Chemicals and Reagents

Streptozotocin powder (STZ 2-deoxy-2(methyl nitrosamine) carbonyl) amino)-D-glucopyranose), Commercial rodent insulin Elisa kit (Crystal chem), HbA1c commercial Kit (BioSystem, Spain), TC, TAG and HDL-C commercial kit (Spectrum diagnostic kit) were purchased from (Sigma Aldrich Chemical Co (St. Louis, MO, USA). Trizol, SensiFastTM cDNA synthesis kit Bioline, catalogNo. Bio-65053), PCR Master Mix (2x SensiFastTM SYBR, Bioline, catalog No. Bio-98002).

2.2. Animals and Housing Conditions

Thirty mature *Sprague Dawley* male rats, average weight 180 \pm 5 g, obtained from the Helwan animal house- Egypt, kept in separate cages at physiology department- Faculty of Veterinary Medicine-Mansoura University-Egypt. All rats are sustained in standard

laboratory conditions (12 h light: 12 h dark cycles) and provided with standard basal diet required for rats [23] and water ad-libitum for 60 days. All the animal procedures were performed in accordance with the Ethics Committee of the National Research Centre- Egypt with registration number (09/189).

2.3. Plant Material and Preparation of *Lupinus albus (LA)* Aqueous Extract

Lupine seeds, available commercially were purchased from the herbal store-Mansoura, Egypt. *LA* seeds were crushed finely and boiled in distilled water (5g of the seeds powder in 200 ml D.W), the suspension was sieved to remove the deposit. The extract solution was stored in the refrigerator to avoid possible deterioration of active ingredients. *LA* extract was administered to rats with a dose (7mg/100g B.wt) orally by gavage daily for 60 days [24].

2.4. Induction of Experimental Diabetes in Rats by STZ

Twenty male rats were injected intraperitoneal (IP) with STZ (50 mg/kg B.wt) that was dissolved previously in cold 0.1M citrate buffer (PH is 4.5) after overnight fasting [25]. Following STZ injection, 5% glucose water was given to all animals for one day to avoid hypoglycemic mortality due to leakage of insulin from damaged β cells [26]. After three days from STZ injection of STZ, the rats were fasted for 12 hr, anaesthetized with ether, a small drop of blood, obtained by pricking the skin with a lancet, is placed on a disposable test strip of the glucometer (One touch technology) that the meter reads and calculate the blood glucose level in units of mg/dl. Diabetic animals exhibited hyperglycemia (blood glucose higher than 250 mg/dl) and symptoms of polyuria and polydipsia were involved in this experiment [27]. Treatment with *LA* extract was ongoing 3 days after diabetes induction and continued for a period of 60 days.

2.5. Experimental Protocol

Experimental rats were categorized into three groups (Ten rats in each, divided into two cages):

GI-Control normal rats

GII-Diabetic non-treated rats

GroupIII-Diabetic rats, treated with *LA* aqueous extract (7mg/100g B.wt) orally by gavage daily for 60 constitutive days [24].

2.6. Blood Sampling

At the end of the experimental period, rats were fasted overnight, anaesthetized by diethyl ether. Blood samples were collected from retro-orbital plexus, immediately divided into two sterile dry capped tubes; one contained Na₂-EDTA as an anticoagulant for HbA1c analysis. The other tube was used for serum sample separation by centrifugation of blood sample at 3000 g for 15 minutes. Non-hemolyzed serum samples were stored in a deep freezer for further biochemical analysis.

2.7. Tissue Sampling

After the experimental period, the rat's abdomen incised then pancreas and liver were dissected out from each animal, immediately frozen in liquid nitrogen for total RNA extraction and subsequent molecular investigation of genes expression.

2.8. Estimation of Biochemical Parameters

2.8.1. Quantitative Measurement of Insulin

Insulin was assayed by competitive Enzyme-Linked Immune Sorbent Assay (ELISA) method, using a commercial rodent insulin Elisa kit (Crystal chem) [28].

2.8.2. Estimation of Glycosylated Hemoglobin (HbA1c)

Glycosylated hemoglobin (HbA1c) was estimated by a commercial Kit (BioSystem, Spain) [29].

2.8.3. Estimation of Lipid Profile

Serum TC, TAG and HDL-C levels were measured by a commercial kit (Spectrum Diagnostic kit) [30,31,32].

For the determination of LDL-C and VLDL-C, Fried Wald formulae were used as: LDL-C = TC - (VLDL-C+HDL-C) and VLDL-C= TG/5 [32].

2.9. Total RNA Extraction and Reverse Transcription

Total RNA was extracted from pancreatic and liver tissues using Trizol reagent according to the manufacturer's instructions (Direct-zol™ RNA MiniPrep, catalog No. R2050). The amount of extracted RNA was quantified and qualified using NanoDrop® ND-1000 Spectrophotometer. The cDNA of each sample was synthesized following the manufacture protocol (SensiFast™ cDNA synthesis kit, Bioline, catalog No. Bio- 65053). The reaction mixture was carried out in a total volume 20 µL consisted of total RNA up to 1µg, 4 µL 5x Trans Amp buffer, 1 µL reverse transcriptase and DNase free-water up to 20 µL. The final reaction mixture was placed in a thermal cycler and the following program was carried out; primer annealing at 25°C for 10 min, reverse transcription at 42°C for 15 min followed by inactivation at 85°C for 5 min. The samples were held at 4°C.

2.10. Quantitative Real Time PCR

Absolute quantification of mRNA levels of pancreatic Pdx-1, Nkx6.1, insulin-1 and liver GLUT-2, glucokinase was performed by real-time PCR via SYBR Green PCR Master Mix (2x SensiFast™ SYBR, Bioline, catalog No. Bio-98002). Table 1 shows primer sequences and the size of each amplified PCR product. Primers of insulin-1, GLUT-2, glucokinase, Pdx-1 and Nkx6.1 were previously described [33,34]. The housekeeping gene β-actin was used as a constitutive control for normalization [35]. The reaction mixture was carried out in a total volume 20 µL consisted of 10 µL 2x SensiFast SYBR, 3 µL cDNA, 5.4µl H₂O (distilled water), 0.8 µl of each primer. The PCR cycling conditions were as follows: 95°C for 2 min followed by 40 cycles of 94°C for 15s, annealing

temperatures 58°C for 30 s, and 72°C for 20 s. At the end of the amplification phase, a melting curve analysis was performed to confirm the specificity of the PCR product.

Table 1. Oligonucleotide primers sequence and the polymerase chain reaction (PCR) product size of the studied genes

Gene	Oligonucleotide sequence	Size (bp)	Reference
Insulin-1	f 5'-ATGGCCCTGTGGATGCGCTT-3' r 5'-TAGTTGCAGTAGTTCTCCAGCT-3'	331	[33]
GLUT-2	f 5'-TTAGCAACTGGGTCTGCAAT-3' r 5'-TCTCTGAAGACGCCAGGAAT-3'	243	[33]
Glucokinase	f 5'-CACCCAACCTGCGAAATCACC-3' r 5'-CATTGTGGGGTGTGGAGTC-3'	162	[33]
Pdx-1	f 5'-GGGACCGCTCAAGTTTGTAA-3' r 5'-GGCTTAACCTAAACGCCACA-3'	247	[34]
Nkx6.1	f 5'-GGGCTTGTGTAAATCGTCGT-3' R5'-ACTTGCCAGGACCAGAGAGA-3'	209	[34]
β-actin	f5'- TCGTGCCTGACATTAAGAG-3' r5'- ATTGCCGATAGTGATGACCT-3'	260	[35]

2.11. Statistical Analysis

Results obtained were expressed as mean ± standard error (SE). All data obtained were statistically analyzed by software SPSS program package version 17 [36]. One-way analysis of variance (ANOVA) followed by Dennett's test was used to compare among three groups. P- Value was considered significant at the level of (P <0.05). Calculations of real time PCR results were performed by determining the values of Δcycle threshold (ΔCt) using β-actin) as a constitutive control for normalization. Then 2-ΔΔCt for each treatment was calculated and statistical analysis for data was performed as previously described [37].

3. Results

3.1. Influence of LA Seed Extract on Serum Insulin of Diabetic Rats

Quantitative measurement of serum insulin by ELISA technique was assayed in all experimental groups to investigate the effect of LA aqueous extract for 60 days in improvement of pancreatic B-cells function and secretion of insulin. Diabetic rats noticed a significant reduction in insulin concentration when compared to control non-diabetic rats. While treatment of diabetic rats with LA aqueous extract induced an increase in serum insulin when compared to the diabetic group Figure 1 (a).

3.2. Influence of LA Seed Extract on FBG and HbA1c of Diabetic Rats

Fasting blood glucose measurement and glycosylated hemoglobin analysis were performed to evaluate the effectiveness of oral gavage of LA aqueous extract for 60 days on diabetes-induced hyperglycemia in diabetic rats. Figure 1. (b & c) revealed that diabetic rats displayed a significant rise in FBG and HbA1c% as compared to control normal rats. Oral treatments of diabetic rats with LA aqueous extract significantly reduced (P<0.05) FBG and HbA1c% as compared to the diabetic group.

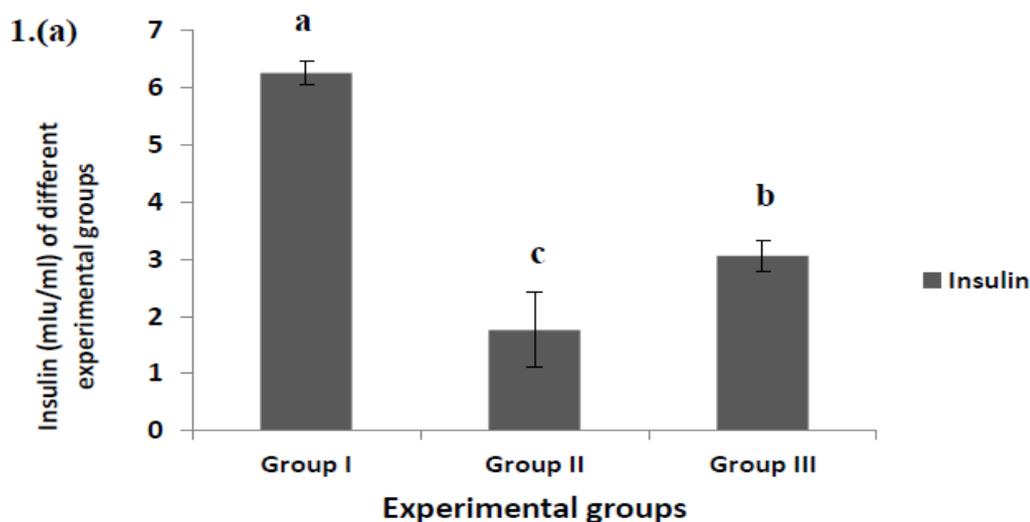


Figure 1. (a). Effect of LA aqueous extract (7mg/100g B.wt) on insulin in STZ-diabetic rats. Number of rats in each group (n=10). Values are mean \pm SE, SE: Standard error. Small alphabetic letters show significance when ($P < 0.05$)

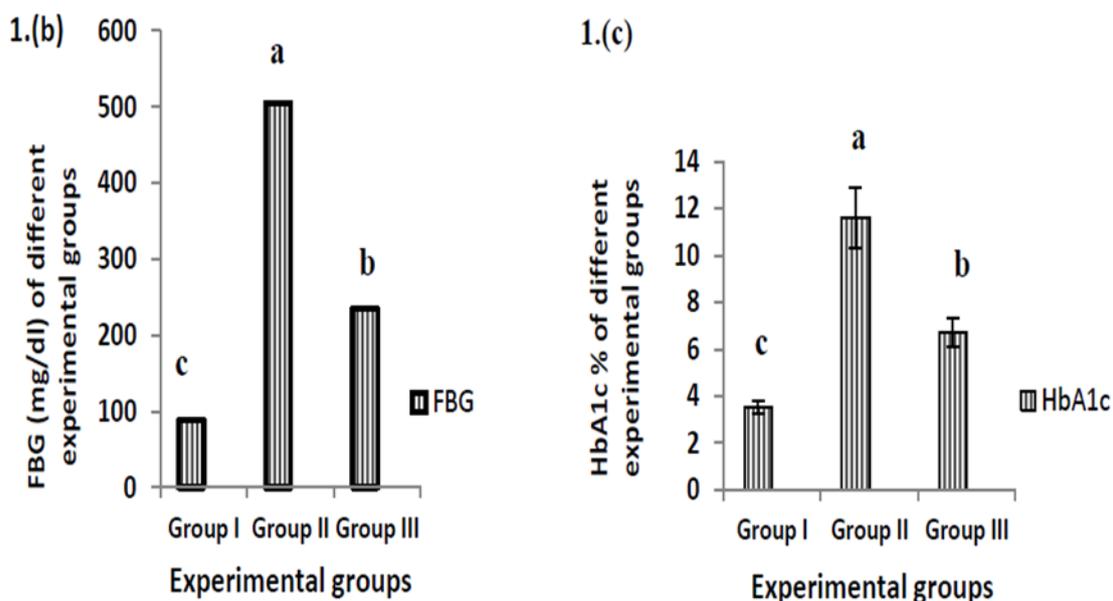


Figure 1. (b): Effect of LA aqueous extract (7mg/100g B.wt) on FBG in STZ-diabetic rats. **(c):** Effect of LA aqueous extract (7mg/100g B.wt) on HbA1c in STZ-diabetic rats. Number of rats in each group (n=10). Values are mean \pm SE, SE: Standard error. Small alphabetic letters show significance when ($P < 0.05$)

3.3. Influence of LA Seed Extract on Serum Lipid Profile of Diabetic Rats

Serum TC, TAG and HDL-C were analyzed using colorimetric methods, in addition to calculation of LDL-C and VLDL-C levels in order to investigate the effect of oral gavage of LA aqueous extract for 60 days on diabetes-induced hyperlipidemia in STZ-diabetic rats. Results showed in Figure 2 (a & b) revealed that TC, TAG, LDL-C & VLDL-C were increased in diabetic rats; while HDL-cholesterol was decreased when compared to normal rats. Following gavage of diabetic rats with LA aqueous extract for constitutive 60 days, they showed significant ($P < 0.05$) reduction in TC, TAG, LDL-C and VLDL-C, while HDL-C showed a significant rise as compared to diabetic group.

3.4. Influence of LA Seed Extract on Pancreatic Expression of Pdx-1, Nkx6.1, Insulin-1, Liver GLUT-2 and Glucokinase of Diabetic Rats

Real-time PCR analysis was performed to determine mRNA levels of pancreatic Pdx-1, Nkx6.1, insulin-1, liver GLUT-2 and glucokinase in normal, diabetic and diabetic-treated rats with LA seed extract for 60 days. Dosing of STZ-diabetic rats by LA could affect the expression of biomarkers genes for β cell function and insulin effectiveness. Results summarized in Figure 3. (a,b,c,d,e) showed that STZ significantly down regulate the expression of pancreatic and liver genes in diabetic rats in comparison to normal and diabetic-treated rats. Gavage of LA seed extract to STZ-diabetic rats induced slight increase in mRNA of the after mentions genes than in diabetic rats.

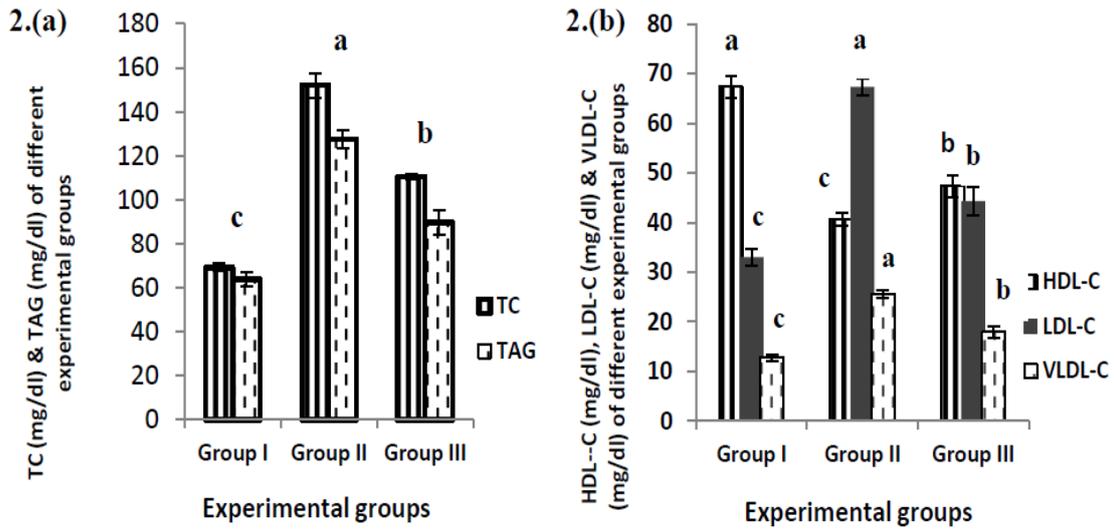


Figure 2. (a): Effects of LA aqueous extract (7mg/100g B.wt) on TC and TAG in STZ-diabetic rats; (b): Effects of LA aqueous extract (7mg/100g B.wt) on HDL-C, LDL-C and VLDL-C in STZ-diabetic rats. Number of rats in each group (n=10). Values are mean ± SE, SE: Standard error. Small alphabetic letters show significance when (P <0.05)

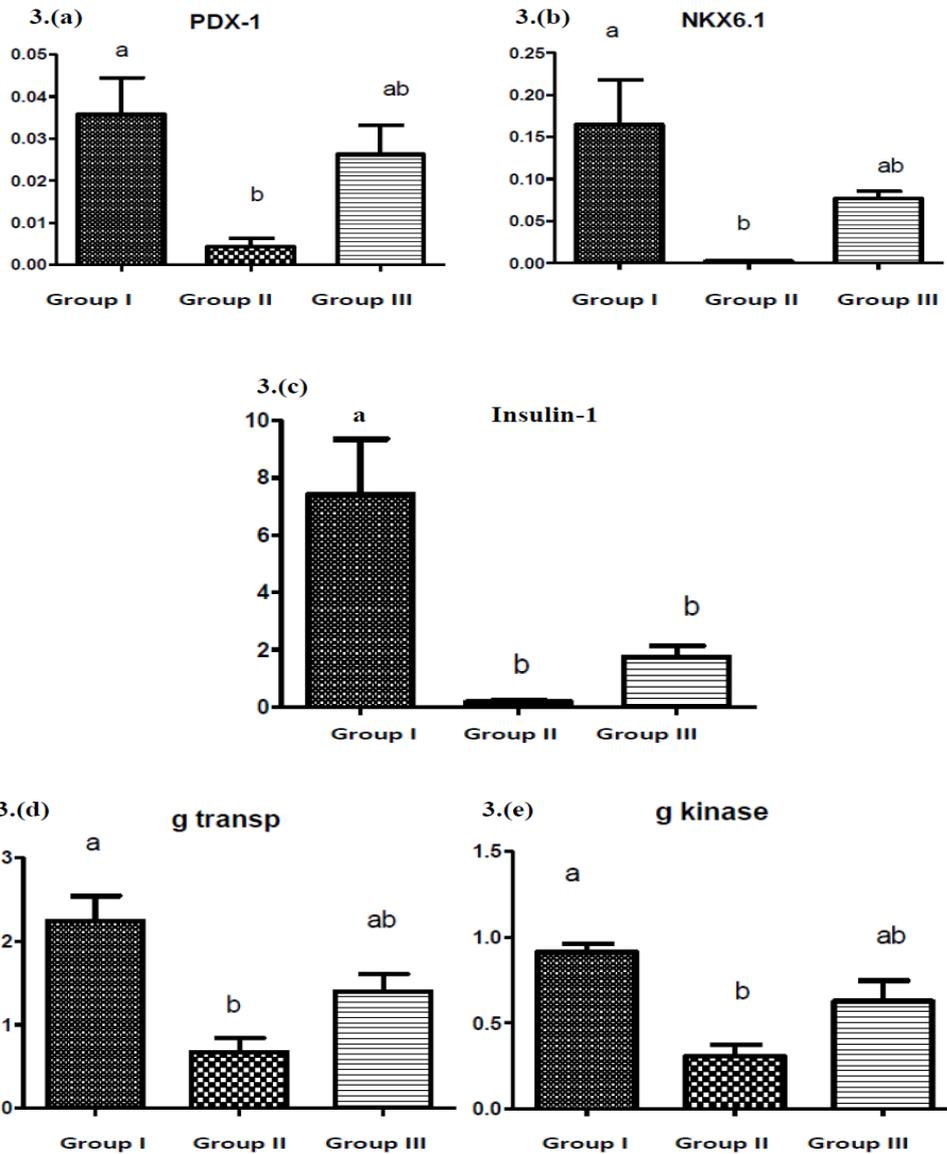


Figure 3. (a,b,c,d,e): m.RNA levels of pancreatic Pdx-1, Nkx6.1, insulin-1, liver, glucose transporter-2 and glucokinase in the control, STZ-diabetic and diabetic rats treated with LA aqueous extract (7mg/100g B.wt) for 60 days. Number of rats in each group (n=10). Values are mean± SE, SE: Standard error. Small alphabetic letters show significance when (P <0.05)

4. Discussion

DM is a chronic disease characterized by hyperglycemia and hyperlipidemia [38]. DM type 1 is an auto-immune disease, characterized by long term damage of B-cells and absolute deficiency in insulin secretion [39]. Liver has a vital role in both glucose and lipid homeostasis because it is an insulin-dependent tissue, severely affected by DM [40]. Oral hypoglycemic agents showed high drug tolerance [41]. Some patients have insulin resistance and do not respond well to the conventional drugs but show a good response to natural products [42]. Different medicinal plants have been known for their anti-diabetic effects by a diversity of mechanisms by way of enhancing pancreatic β -cells regeneration, enhancing glucose dependent-insulin secretion in diabetic rats and improving insulin sensitivity [43]. A number of anti-diabetic herbs have been used for a long period for treatment of hyperglycemia and hyperlipidemia in different diabetic animal models [44,45,46,47].

In this context, the diabetic rats exhibited hyperglycemia which indicated by increased blood glucose and glycosylated hemoglobin percent with reduction in serum insulin concentration when compared to control group. Similar results reported a significant reduction in insulin sensitivity in STZ-treated rodents and explained this by the hyperglycemic state which modulates insulin signaling at the insulin receptor (IR) level in liver and skeletal muscle [48]. Also, STZ leads to beta cell damage, impedes glucose oxidation and glucose stimulated insulin secretion in pancreatic β -cells through DNA methylation and fragmentation corresponding to its cytotoxic effect followed by a continuous drop in cellular NAD⁺ levels that induces cell death as well as kidney and liver toxicity [10,12,49].

STZ also increased glucose binding to hemoglobin and producing glycosylated hemoglobin which increased up to 16% in DM, this metabolite is a good indicator of diabetes control [50,51]. The obtained results revealed that diabetic rats treated with LA extract showed a significant reduction in fasting blood glucose and glycosylated hemoglobin levels, while there was a significant rise in serum insulin concentration when compared to diabetic non-treated rats. This result in accordance with that reported by [51] who showed that repeated *Lupinus albus* dry extract in type 2 diabetic adult patients significantly diminished fasting and postprandial plasma glucose, fasting as well as HbA1c. It was shown that LA supplementation in normal and alloxan-diabetic animals increased serum insulin as well as in diabetic and healthy Humans [52,53]. LA extract contains bioactive compounds like polyphenol, Saponins and lupanine alkaloids which induce anti-hyperglycemic effects via stimulation of insulin action on peripheral tissues, suppression of carbohydrate digestion and its absorption [54,55]. The main quinolizidine alkaloids (QAs) present in many *Lupinus* species Lupanine and Sparteine [56]. Quinolizinic alkaloids from *Lupinus* species induced insulin release from cultured pancreatic islets of normal rats, and established that Lupanine, the most abundant alkaloids in *Lupinus* that potentiates glucose-stimulated insulin release via increasing insulin gene expression [57].

There are major signaling pathways in the pancreatic β -cells through which, *Lupinus* alkaloids induce a direct

stimulatory effect on insulin secretion [58]. The major intracellular signals in insulin secretion are Ca²⁺, ATP, cAMP and phospholipid-derived signals such as inositol 1, 4, 5-triphosphate (IP3) and diacylglycerol (DAG) [59]. The principal mechanism of insulin secretion is glucose-stimulated insulin secretion (GSIS). An increase in mitochondrial ATP concentration (or ATP/ADP ratio) due to increased glucose metabolism closes the ATP-sensitive K⁺ (K_{ATP}) channels, depolarizing the β -cell membrane and opening the voltage-dependent Ca²⁺ channels (VDCCs), which allows Ca²⁺ influx. The resultant rise in [Ca²⁺] in the β -cell triggers exocytosis of insulin granules. Thus, K_{ATP} channels couple metabolic changes to membrane potential [60]. Treatment with *Lupinus* γ -conglutinin had enhanced the state of insulin resistance as discovered by a reduction of HOMAS in treated diabetic animals [61]. Also, γ -Conglutinin declines plasma glucose level in mice and healthy Humans [62]. It was approved that *Lupinus* γ -conglutinin activates the IRS-1/PI-3-kinase pathway, which is vital in glucose homeostasis and protein synthesis and the translocation of GLUT-4 to the cell surface [63].

DM is a metabolic disease, altered lipid metabolism, leads to abnormal lipid profile in diabetic patients [64]. Insulin regulates different steps in lipid metabolism, promotes lipid synthesis and inhibits lipid degradation in adipose and liver tissues [65]. In diabetic rats, serum lipid profile was elevated 2-fold more when compared to control rats [66]. Our results obtained revealed that, diabetic rats showed hyperlipidemia with significant elevation of TC, TAG, LDL-C, VLDL-C and AI, as well as significantly decreased HDL-C. Lipid abnormalities usually associated with DM, leads to hypercholesterolemia and hypertriglyceridemia [67]. Defective insulin secretion in DM leads to defective lipoprotein lipase activation, thus inducing hypertriglyceridemia [68].

Treatment of diabetic rats with LA seed extract for successive 60 days induced a significant decline in TC, TAG, LDL-C and AI. Meanwhile, it exerted an elevation in HDL-C as compared with diabetic non-treated rats. This improvement in lipid profile of diabetic rats indicated that LA extract induced hypolipidemic and anti-atherosclerotic effects. Anti-hyperlipidemic effect of LA extract was previously reported in type-2 diabetic patients, diabetic rats and rabbits [69,70]. The potential hypolipidemic effect of LA extract in diabetic-treated rats may be attributed to its soluble dietary fibers, which retard the absorption of fats [71]. Additionally, the two major elements albumin and globulin of LA proteins which represent a ratio of 1:9, induced an increment in low-density lipoprotein cholesterol receptors activity, enhanced LDL-uptake from plasma and their destruction [72]. Also, LA proteins inhibit 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) the enzyme responsible for cholesterol synthesis [73]. Lupin proteins had vital role in reduction of the expression of genes SREBP-1c and HMG-CoA reductase [74]. Moreover, *Lupine* seed encloses high quantities of proteins and non-starch polysaccharides that able to reduce intestinal absorption of lipids through its binding to bile acids and improve loss of these acids in the feces [75]. Upon *Lupine* therapy, a better glycemic control improved serum lipid profile in diabetic rats [76].

Concerning the gene expression pattern, diabetic rats treated with LA modulate the expression of some selected biomarkers genes for beta cell activity and insulin effectiveness. Results revealed that, LA supplementation exert a non-significant effect on the genes expression of glucokinase, glucose transporter, PDX-1 and NKX6.1; although it is mildly increased than that expressed in diabetic group but did not reach to control levels. In addition, STZ significantly downregulate the expression of insulin genes in diabetic group as previously reported in diabetic rats [77]. The potential anti-diabetic effects of LA seed extract on diabetic rat might be achieved by increased insulin production or increased tissues insulin sensitivity that could lead to tissue glucose storage and utilization. Moreover, results demonstrated that, LA could significantly increase insulin reduce and reduce fasting blood glucose levels as compared to diabetic group. Similarly, insulin gene expression significantly increased in LA treated group than diabetic one. The possible anti-hyperglycemic effect of LA seed extract might be manifested by increasing insulin synthesis, stimulating its release from the beta cells and increasing cell receptors sensitivity to insulin. This appears through the induction of expression of the insulin gene in pancreatic cells [78]. Additionally, STZ induces a selective destruction of pancreatic beta cells, but leaving many of the surviving beta cells, which can be regenerated [79]. LA seed extract enhanced beta cells regeneration, improved insulin gene expression secretion and so increased insulin in the blood. Insulin receptors are expressed in different insulin sensitive tissues which can progress glucose utilization [80]. Human studies stressing the potential of lupin raw material for improvement of glycemic control [81].

5. Conclusion

In summary the present study concluded that diabetes induced hyperglycemia, hyperlipidemia and decreased tissues insulin sensitivity. LA aqueous extract increased insulin with significant reduction in FBG, HbA1c% and significantly improved serum lipid profile in addition to a mild increase in expression of biomarkers genes for pancreatic β cells (β -cells) function, insulin gene, liver glucokinase and GLUT-2 in diabetic rats. Thus, results obtained from our study, suggested that LA a nutraceutical could be recommended for its hypoglycemic, hypolipidemic effects and its potential effect in improving insulin secretion from beta cells.

Ethics Approval and Consent to Participate

All the animal procedures were performed in accordance with the Ethics Committee of the National Research Centre- Egypt with registration number (09/189). Authors are equally contributed in this work.

Competing Interests

The authors declare that they have no competing interests.

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List of abbreviations

LA: *Lupinus Albus*; FBG: Fasting blood glucose; HbA1C: Glycosylated hemoglobin; TC: Total cholesterol; TAG: Triacylglycerol; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; VLDL-C: Very low density lipoprotein cholesterol; Ins-1: insulin-1; Pdx-1: Pancreatic and duodenal homeobox-1; Nkx6.1: homeobox protein; GLUT-2: glucose transporter-2.

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