Conglutin Gamma, a lupin Seed protein Ameliorates Lipid profile in Diabetics

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ABSTRACT

Background: Lupine seeds (Lupinus albus) are good sources of protein which can regulate intestinal absorption of glucose and attenuate risk of hyperlipidemia and T2DM diseases. Consumption of lupines induces hypoglycemic effects in diabetic patients, which decreases hyperglycemic features. Gamma Conglutin (Cγ) lupines induce cellular glucose uptake, and improve glucose metabolism and insulin sensitivity. γ-conglutin is significantly decreases the levels of fasting glucose, improves glucose tolerance and insulin resistance (IR), and increases cellular glucose uptake in diabetic rats. Aim: In this study we aimed to evaluate and compare between the preclinical therapeutic effects of the γ-conglutin whole white lupine seeds powder (CγWWLSP) (Lupines albus L./Lupinus terms L./sweet lupines) and the γ-conglutin standard lupine proteins isolate (CγSLPI/reference commercial extract) on the high fat high cholesterol diet feeding plus streptozotocin injection (HFHCD+STZ)-induced T2DM male albino rats model.

- We targeted to determine hypoglycemic, hyperinsulinemia, powerful β-cells homeostasis, peripheral insulin sensitivity, cellular glucose uptake and hypolipidemic properties of CγWWLSP and CγSLPI on the HFHCD+STZ-induced T2DM male albino rats model.
- The hepatic and pancreatic histopathological examinations were performed to evaluate preclinical therapeutic potentials of CγWWLSP and CγSLPI on the liver and pancreas tissue sections of the HFHCD+STZ-induced T2DM male albino rats model.

Methods: the experiment, the rats were divided into two main groups including SNC group (standard negative control /12 rats) and HFHCD group (High fate High cholesterol Diet/72 rats) which later divided into 6 groups (positive control (diabetes) –
Introduction:
Diabetes Mellitus
Diabetes Mellitus (DM) is the most common endocrine metabolic disorder in the world. Chronic metabolic disorder that represents several metabolic disturbances in :Carbohydrates- Proteins- Fat metabolism, Hyperglycemia, Hyperlipidemia, dyslipidemia, Oxidative stress (free radicals/oxidants) generation and Antioxidants depletion. That results from insulin secretion and/or insulin action abnormalities (1,2). There are three types of diabetes depending upon its basic cause, type 1, type 2, and type 3 diabetes. Type 1 diabetes (T1DM) is also called as an insulin-dependent diabetes mellitus (IDDM) and (juvenile or childhood diabetes). It is characterized by insufficient or no pancreatic β-cells insulin production. It is common in children and is divided into two types including Type 1A and Type 1B diabetes (4). Hyperglycemia increases reactive oxygen species (ROS) production and decreases enzymatic and non-enzymatic antioxidants signaling (activities of ROS scavenging enzymes) as well as induces glycation and peroxidation of proteins (arterial walls damage) (1,2).

Hyperglycemia and lipid profile
DM accelerates harmful risk to develop atherosclerosis disorder and cardiovascular disease complications. Which increases levels of blood total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) and decreases level of blood high-density lipoprotein cholesterol (HDL-C). Insulin is considered as a hepatic very low density lipoprotein cholesterol (VLDL-C) secretion suppressor. In DM, insulin deficiency leads to hyperglycemia, dyslipidemia, hypertriglyceridemia, and hypercholesterolemia (6). Lupines belong to a group of medicinal plants that contain several bioactive components in their leaves and seeds. The sweet lupine seeds contain a great number and safe non-toxic substance as in: L. albus (white lupine), L. angustifolius (blue narrow-leaved lupine), L. luteus (yellow lupine) and L. mutabilis seeds (Pearl lupine). The white lupine, Lupinus albus (sweet) has large seed size and small amounts from toxic pyrolizidine alkaloids and have hypoglycemic agents (7) Lupine seeds (Lupinus albus) are good sources of vitamins including vitamin B group (niacin - thiamin - riboflavin). Lupine seeds are a good source of iron and zinc. Lupine seeds (Lupinus albus) are good sources of protein which can regulate intestinal absorption of glucose and attenuate risk of hyperlipidemia, hypertension, T2DM, and cardiovascular diseases. (12,13) Previous studies reported that lupine seeds of L. albus species have significant amounts of different flavonoids including aglycones, kaempferol, kaempferol has anti-diabetic, antioxidant, anti-inflammatory, antihyperlipidemic, and pancreatic β-cell protective properties(5). Lupines also contain phenolic compounds including catechin and rutin. Rutin has anti-obesity, anti-diabetic, antioxidant, and anti-inflammatory properties (12). Lupine seed proteins act about 38% of total weight of lupine content. Lupine seeds proteins have two types of proteins including albumin and globulin in a 1:5 fractions ratio. Albumins are functional proteins, are represented as metabolic (storage function) and defense enzymes. Globulins are introduced as salt-soluble storage proteins. which have four main fractions including α-, β-, γ-, and δ-
Lupine seed proteins are greatly introduced health benefits and potential therapeutic properties including hypolipidemic, hypoglycemic, hypotensive, anti-carcinogenic, and anti-obesity (8). Gamma conglutin (Cγ) protein storage as glycoprotein, presents as a globulin fraction of white L. albus L. seeds and represents about 5% of the total white lupine seeds proteins (9). (Cγ) protein consist of a basic 7S protein that includes two heterogeneous disulfide-linked subunits (15). All of these proteins have been characterized at the molecular level, although Only conglutin γ has a known amino acid sequence (9). Cγ can resist intestinal proteolytic digestive enzymes at pH > 4, interact with insulin and potentiate its effect, Gamma Conglutin (Cγ) lupines induce cellular glucose uptake, and improve glucose metabolism and insulin sensitivity (10,11). Numerous studies demonstrated therapeutic properties and health benefits of Gamma Conglutin (Cγ) lupines toward patients with T2DM as lupine-enriched foods that improved glucose metabolism and insulin sensitivity as hypoglycemic agents (12,13). γ-conglutin is significantly decreases the levels of fasting glucose, improves glucose tolerance and insulin resistance (IR), and increases cellular glucose uptake in diabetic rats. Previous work demonstrated that γ-conglutin has insulin-mimetic properties in murine myoblasts (12). γ-Conglutin is synthesized as a single polypeptide chain fused to an N-terminal signal peptide (8). Gliclazide is an oral sulfonylurea anti-hyperglycemic agent used to treat non-insulin dependent diabetes mellitus (NIDDM). Gliclazide improves pancreatic β-cells homeostasis, increases pancreatic insulin secretion and its cellular sensitivity, reduces blood glucose levels circulation, and improve peripheral and systemic IR. which is available in market in the form of different brand like Diamicron MR (15).

Material and Methods:

Chemicals:
1- Experimental Induction of Diabetes (84 Male albino rats module)
2 – Whole Dried Sweet lupine Powder
3- Standard lupine protein isolate
4- Diamicron drug (glicazide)
5- Chemicals and suppliers
6- Instruments and software

Animals:
Eighty-four adult Wistar Albino male rats (130-150 g) were purchased from the experimental animal house, Faculty of Pharmacy, Suez Canal University (Ismailia), Ismailia, Egypt.

Experimental design:
1- Experimental Induction of Diabetes
1- All animals were acclimatized for one week before starting of the experimental study.

At the beginning of the experiment, the rats were divided into two main groups including:
A – SNC group (standard negative control)/ 12 rats
B - HFHCD group (High fate High cholesterol Diet)/ 72 rats.

3- HFHCD group after 4 weeks form the beginning of the Experiment were intraperitoneally (IP) injected with a single dose of freshly prepared 40 mg/kg STZ that was dissolved in fresh cold 0.1M sodium citrate buffer (pH 4.5) to induced T2DM rat model/positive control/ 72 rats.

4- After one Week from STZ administration we collected Blood samples.
5- After 4 weeks from STZ administration we collect blood samples.
6- Four weeks after STZ administration (eight weeks from the beginning of the experiment), HFHCD+STZ-induced T2DM rats (n = 72) were randomly divided into six subgroups (12 rat in each group) as the following:
6 – 1 - HFHCD+STZ-induced T2DM group (Positive group).

6-2-300WWLSP group received orally administrated with 300 (5 mg Cγ) mg WWLSP/kg of rats/day for four week (Co-administration).

6-3-600WWLSP group received orally administrated with 600 (10 mg Cγ) mg WWLSP/kg of rats/day for four weeks (Co-administration).

6-4-100SLPI group orally administrated with 100 mg SLPI (5 mg Cγ) /kg of rats/day for four weeks (Co-administration).

6-5-200SLPI group orally administrated with 200 (10 mg Cγ) mg SLPI/kg of rats/day for four weeks (Co-administration).

6-6-Gliclazide group orally administrated with 5 mg DIAMICRON MR/kg of rats/day for four weeks (Co-administration).

Blood, Liver, and Pancreas Samples Collection: After twelve weeks from the beginning of the experiment study Blood samples, Liver and Pancreas Tissue Specimens was collected.

3- Serum Biochemical Parameters. Determination of the Effects of 300/600WWLSP, 100/200SLPI, and Gliclazide on:

1-The Level of Blood Fasting Glucose.
2-The Levels of Blood Insulin, HOMA-IR, and HOMA-β Cells Function Indexes
3-The Activity of Blood Alanine Transaminase (ALT) Enzyme
4-The Levels of Blood Total Cholesterol (TC), Triglycerides (TG), and Very Low-Density Lipoprotein Cholesterol (VLDL-C) as a Serum Lipid Profile.

4. Histopathological Examinations

Liver and Pancreas Tissue Specimens.

5. Statistical Analysis.

Evaluation of the Effect of HFHCD Feeding plus STZ Injection on the Level of Blood Glucose after One Week from STZ Administration (after Five Weeks from the Experimental Duration)

In details, our experimental HFHCD+STZ-induced T2DM rat model was previously described in section Materials & Methods Experimental Induction of Diabetes). As shown in Figure 1, after five weeks from the experimental study, the HFHCD+STZ-induced hyperglycemia rats introduced a significant (p < 0.05) increase in the levels of serum fasting glucose compared to the SNC group rats (12.37 ± 0.68 vs. 4.32 ± 0.34 mmol/L).

Evaluation of the Effects of 300/600WWLSP, 100/200SLPI, and Gliclazide on the Levels and Activities of Different Serum Biochemical Parameters of the HFHCD+STZ-Induced T2DM Rat Model before and after Drugs Administration/ after Eight and Twelve Weeks from the Experimental Duration/ Intra-group and Inter-group Differences

1- Serum fasting glucose (mmol/L)

Figure 2 A show that the Pre and post-treatment values of serum
fasting glucose (mmol/L) showed a significant ($p < 0.05$) increase in the SNC and HFHCD+STZ-induced T2DM groups and significant ($p < 0.05$) decrease in the 300 (5 mg $C\gamma$)/600 (10 mg $C\gamma$) WWLSP, 100 (5 mg $C\gamma$)/200 (10 mg $C\gamma$) SLPI, and gliclazide (5 mg) groups compared to their pre-treatment values (intra-group differences).

**Figure 2 B** show post-treatment values that inter-group differences, the HFHCD+STZ-induced T2DM rat model introduced a significant ($p < 0.05$) elevation in the levels of serum fasting glucose compared to the SNC group. Furthermore, 300/600WWLSP, 100/200SLPI, and gliclazide groups demonstrated a significant ($p < 0.05$) attenuation in the levels of serum fasting glucose compared to the HFHCD+STZ-induced T2DM rat model.

At the end of the experimental study, after 12 week, 600WWLSP (10 mg $C\gamma$) rats showed a significant ($p < 0.05$) reduction in the levels of serum fasting glucose compared to 300WWLSP and 100/200SLPI rats and a non-significant ($p > 0.05$) decrease compared to the gliclazide treatment (post-treatment).

**2- Serum Fasting insulin (μIU/mL ) and Serum HOMA-IR index**

**Figures 3 A and 5 A** describe the Pre and post-treatment values of serum fasting insulin (μIU/mL) and HOMA-β cells function index demonstrated a significant ($p < 0.05$) reduction in the HFHCD+STZ-induced T2DM rat group and elevation in the SNC, 300/600WWLSP, 100/200SLPI, and gliclazide groups compared with their pre-treatment values (intra-group differences).

**Figures 3 B and 5 B** report that: post-treatment values in inter-group differences, the HFHCD+STZ-induced T2DM rat model introduced a significant ($p < 0.05$) depletion in the levels of serum fasting insulin and HOMA-β cells function index compared to the SNC group. The 300/600WWLSP, 100/200SLPI, and gliclazide groups demonstrated a significant ($p < 0.05$) improvement in the levels of serum fasting insulin and HOMA-β cells function index compared to the HFHCD+STZ-induced T2DM rat model.

At the end of the experimental study, after 12 week, 600WWLSP (10 mg $C\gamma$) rats showed a significant ($p < 0.05$) restoration in the levels of serum fasting insulin and HOMA-β cells function index compared to the 300WWLSP, 100/200SLPI, and gliclazide administration (post-treatment).

**3- HOMA- IR index**

**Figure 4 A** demonstrated that the Pre and post-treatment values of serum HOMA-IR index introduced a significant ($p < 0.05$) elevation in the SNC group and alleviation in the HFHCD+STZ-induced T2DM, 300/600WWLSP, 100/200SLPI, and gliclazide rat groups compared with their pre-treatment values (intra-group differences).

**Figure 4 B** demonstrated inter-group differences, the HFHCD+STZ-induced T2DM rats demonstrated a significant ($p < 0.05$) increase in the levels of serum
HOMA-IR index compared to the SNC rats. The 300/600WWLSP, 200SLPI, and gliclazide rats showed a significant (p < 0.05) reduction in the levels of serum HOMA-IR index compared to the HFHCD+STZ-induced T2DM rats.

At the end of the experimental study, after 12 week, 600WWLSP (10 mg Cγ) rats showed greatly attenuation in the levels of serum HOMA-IR index but non-significant (p > 0.05) compared with the 300WWLSP, 200SLPI, and gliclazide rats (post-treatment).

4- Serum ALT activity (IU/L)

Figure 6 A explain that, the Pre and post-treatment values of serum ALT activity (IU/L) demonstrated a significant (*p < 0.05) increase in the HFHCD+STZ-induced T2DM rat group and decease in the 300/600WWLSP and gliclazide rat groups as well as a non-significant (*p > 0.05) elevation in the SNC rat group and attenuation in the 100/200SLPI rat groups compared with their pre-treatment values (intra-group differences).

Figure 6 B report that, post-treatment values inter-group differences, the HFHCD+STZ-induced T2DM rat model showed a significant (p < 0.05) increase in the activities of serum ALT enzyme compared to the SNC group. The 300/600WWLSP, 100/200SLPI, and gliclazide-treated groups introduced a significant (p < 0.05) reduction in the activities of serum ALT enzyme compared to the HFHCD+STZ-induced T2DM rats.

At the end of the experimental study, after 12 week, the 600WWLSP (10 mg Cγ) oral administration significantly (p < 0.05) attenuated the activities of serum ALT enzyme compared to the 300WWLSP and 100/200SLPI-treated groups as well as non-significantly (p > 0.05) compared with the gliclazide rats (post-treatment).

5- Serum Total Cholesterol(TC), Serum Total Triglyceride (TG) mg/dl and Serum VLDL-C

Figures 7 - 9A determined that, the pre and post-treatment values of serum TC, TG, and VLDL-C (mg/dL) introduced a significant (*p < 0.05) elevation in the HFHCD+STZ-induced T2DM group and reduction in the 300/600WWLSP, 200SLPI, and gliclazide rat groups compared with their pre-treatment values (intra-group differences).

Figures 7 – 9 B introduced post-treatment values in inter-group differences, the HFHCD feeding plus STZ injection introduced a significant (p < 0.05) increase in the levels of serum TC, TG, and VLDL-C compared to the SNC group. The 300/600WWLSP, 100/200SLPI, and gliclazide oral administration showed a significant (p < 0.05) reduction in the levels of serum TC, TG, and VLDL-C compared to the HFHCD+STZ-induced T2DM rats.

At the end of the experimental study, after 12 week, the 600WWLSP (10 mg Cγ) rats showed a significant (p < 0.05) amelioration in the hyper- and dyslipidemic features compared with the 300WWLSP, 100/200SLPI, and gliclazide-treated rats (post-treatment).
Bivariate Correlation Standardized Coefficient Relationships between Several Serum Biochemical Variable Parameters (post-treatment values) within the Different Experimental Rat Groups (Simple Linear Regression Analysis/Linear Curve Fit Estimation Model)

Figures 10 show strength of the bivariate correlation standardized coefficient relationships between each two different serum biochemical variable parameters (glucose and insulin; glucose and HOMA-IR index; glucose and HOMA-β-cells function index; insulin and ALT; insulin and TC; insulin and TG; insulin and VLDL-C within the different experimental rat groups. As shown in Figures 10 the presence of strong negative bivariate inversely relationships between each two different serum biochemical variable parameters (glucose and insulin; glucose and HOMA-β-cells function index; insulin and ALT; insulin and TC; insulin and TG; insulin and VLDL-C within the different experimental rat groups were observed. Under pathological conditions, impairment of HOMA-β-cells function and low levels of pancreatic β-cells insulin content were accompanied by a greatly increase in the levels of serum fasting glucose, ALT, TC, TG, and VLDL-C within the different experimental rat groups. After treatment, upregulate the function of pancreatic β-cells and improvement their insulin secretion were also related to retardation the levels of serum fasting glucose, ALT, TC, TG, and VLDL-C within the different experimental rat groups.

Figures 10 demonstrate strength of the bivariate correlation standardized coefficient relationships between each two different serum biochemical variable parameters glucose and HOMA-IR index within the different experimental rat groups. Figures 10 also strongly explain positive bivariate directly relationships (glucose and HOMA-IR index between each two different serum biochemical variable parameters within the different experimental rat groups. Under pathological conditions, hyperglycemia was accompanied by a highly increase in the values of serum HOMA-IR index, in the different experimental rat groups. After treatment, improvement of peripheral insulin sensitivity (attenuation HOMA-IR index values) was also related to reduction the levels of serum fasting glucose, within the different experimental rat groups.

Histological changes
Liver and pancreatic specimens from all experimental rat groups were fixed for 24 h at room temperature in 10% neutral buffered formalin for the histopathological analysis. After fixation, these tissues were gradually dehydrated by using different concentrations of ethanol and then embedded in paraffin. The solid paraffin tissue sections were cut with a thickness of 5-6 µm, which stained with haematoxylin and eosin (H&E) stain for the histopathological investigations. These tissue sections were also examined and photomicrographed under a light Olympus BX53 optical microscopy.
Figure 11: show that Histopathological Evaluations of Pancreatic Tissue Specimens show that: The SNC rats showed normal endocrine islets of Langerhans’ cells (ICs). T2DM specimens showed vacuolar degeneration, necrosis, and loss of zymogen granules of ACS. T2DM3 specimens showed atrophic ICs with few necrotic cells, eosinophilic necrotic material, and coagulated necrotic ACs. also showed a marked degeneration, necrosis, nuclear pyknosis, and loss of ICs. 300WWLSP specimens showed good restoration of ICs and ACs with a mild vacuolation and necrosis of some scattered ICs and proliferated pancreatic duct epithelium. 600WWLSP specimens showed a mild degeneration and necrotic changes of ICs with few kayomegally (arrow) and Ess. 100SLPI specimens showed a good restoration of ICs, which included scattered degenerated cells with vacuolar degeneration. 200SLPI specimens showed a scattering degeneration and necrotic changes of both ACs and ICs with coagulation necrosis (dashed arrow) of ACs. gliclazide specimens showed a vacuolar degeneration and necrosis of some ICs Histopathological Evaluations of liver Tissue Specimens show that: The SNC rats showed normal central vein (CV), portal areas, and hepatic cells (HCs). T2DM specimens showed a marked expansion of the portal area with proliferation of the bile duct epithelium. Also showed per ductal fibrosis and inflammatory cells infiltration (dashed arrow), and scattering necrotic hepatocytes. 300WWLSP specimens showed good restoration of the hepatic parenchymal cells with a mild infiltration of the inflammatory cells in the portal areas. 600WWLSP specimens showed normal hepatic cells with only few scattered degenerated ones. 100SLPI specimens showed mild pericholangitis with periducteolar inflammatory cells infiltration, edema, and fibrosis. 200SLPI specimens showed vacuolar degeneration and focal necrosis of the hepatic artery wall. gliclazide specimens showed normal appearance of HCs, CV, and portal areas with few infiltrated inflammatory cells. Figure 12: explains microscopic examinations of the liver tissue specimens of the different experimental rat groups. The SNC rats showed normal central vein (CV), portal areas, and hepatic cells (HCs). The liver T2DM specimens showed a marked expansion of the portal area with proliferation of the bile duct epithelium including newly formed bile ductules, inflammatory cells infiltration, fibrous proliferation (short arrow), and dilated portal vessels (PV) with hepatocellular degeneration and necrosis. Also demonstrated picture of cholangitis and per cholangitis, proliferation of the bile duct epithelium with periductal fibrosis and inflammatory cells infiltration (dashed arrow), and scattering necrotic hepatocytes. The liver 300WWLSP tissue specimens introduced a good restoration of the hepatic parenchymal cells with a mild infiltration of the
inflammatory cells in the portal areas. The liver 600WWLSP tissue specimens showed normal hepatic cells with only few scattered degenerated ones. The liver 100SLPI tissue specimens explained a picture of mild pericholangitis with periducteolar inflammatory cells infiltration, edema, and fibrosis. The liver 200SLPI tissue specimens showed a vacuolar degeneration and focal necrosis of the hepatic artery wall, marked portal tract fibrous proliferation (dashed arrow), and infiltration of the inflammatory cells. The liver gliclazide tissue specimens introduced a normal appearance of HCs, CV, and portal areas with few infiltrated inflammatory cells (Figure 12, X400 H&E staining).

Discussion
Diabetes is a harmful metabolic disorder that currently effects on >220 million people worldwide, and expects to effect on 400 million by 2030 Coronary artery disease, atherosclerosis, hypertension, hyperlipidemia, nephropathy, retinopathy, and neuropathy are considered as the most serious IR-induced diabetic complications. In diabetes, the elevated levels of glucose and cholesterol highly increase production of the inflammatory cytokines that develop cellular injury and progress chronic diabetic complications by generating reactive oxygen species (ROS) and attenuating the antioxidant signaling via glycation of the antioxidant enzymes (19).

In the Fabaceae (Leguminosae) family and subfamily Papilionoidea, Lupinus L. is a large genus that includes different herbaceous, soft-woody shrubs, and small tree species. Herbaceous lupines are 200–500 species mainly used as grain crops. The species of lupine are grouped as Old World and New World species. The Old World species are 13 lupine species distributed in the Mediterranean and African regions, which are introduced as annual, herbaceous, and large seeds like L. albus L. seeds as a white lupine (8). Lupine seeds improve soil quality, which have high contents of protein (vegetable proteins, up to 40%), dietary fibers, and antioxidants, very low value of starch, and gluten-free (18).

L. albus seeds were used as a food by the ancient Romans, Egyptians, and Greeks (14). Lupine seed proteins, vegetable proteins, are considered as an important source for essential amino acids like arginine, leucine, and lysine, which contain low levels of sulfur-containing amino acids such as methionine (7). Lupine is considered as an excellent alternative legume to soybean or any other legumes, which has high proteins content, low starch (glycemic carbohydrates) and lipid contents, and high dietary fibers (non-starchy polysaccharides). White lupine (L. albus) seeds have 38.2 g vegetable proteins, 9.5 g fats, 3.1 g ash, and 14.0 g fibers/100 g dried seeds (14). The lupine proteins are greatly introduced health benefits and potential therapeutic properties including hypolipidemic, hypoglycemic, hypotensive, anticarcinogenic, and anti-obesity. Storage globulins are considered as the bulk protein content of the legume seeds, which contain high levels of essential amino acids. White lupine proteins contain high levels of histidine, isoleucine, leucine, lysine, cystine, tyrosine, phenylalanine, threonine, and valine as essential amino acids (8).
L. albus seeds contain carbohydrates as oligosaccharides and non-starchy polysaccharides. Oligosaccharides as sucrose and nondigestible galactosides (raffinose, stachyose, and verbascose) and non-starchy polysaccharides are introduced as important sugars that maintain homeostasis of the intestinal microbial flora fermentation. The vitamins profile of lupine seeds is similar to other legumes. Lupine seeds are a good source of vitamins as vitamin B groups including niacin, thiamin/thiamine (VitB1), and riboflavin (VitB2). Furthermore, calcium is low in all lupine species. Manganese, a trace element, is usually found in lupine seeds in high amounts, especially in the L. albus seeds. Moreover, lupine seeds are considered as a good source of iron and zinc. Flavonoids, a class of phenolic compounds, have antioxidant properties such as anticancer, anti-inflammatory, and antiviral activities (14). Furthermore, flavonoids, isoflavonoids (lupinoisolone A, lupinoisolone C, lupinisol A, and lupinoisolavone G), and fatty acids are greatly responsible for the antioxidant activity of L. albus seeds. Moreover, primary metabolites such as alpha-tocopherol and amino acids (asparagine, thiamine, and proline) present in high amounts in L. albus seeds, which greatly have free radical scavenging and antioxidant properties. In addition, some flavonoids are considered as potent α-glucosidase inhibitors such as kaempferol (17).

Sweet lupine seeds flour has several bioactive components instead of proteins including phytochemicals (phenolic compounds, alkaloids, tannins, saponin, and flavonoids), fibers, and essential FAs that have powerful antioxidant, anti-inflammatory, and antidiabetic properties. Foods can be supplemented with certain proteins (vegetable proteins). Lupine proteins have beneficial health properties in T2DM. These vegetable proteins are a mixture of albumin and globulin proteins that can improve insulin releasing and glycemic response in T2DM and healthy human participants. Sweet lupine flour proteins also include low content of sulfur-containing amino acids such as cysteine and methionine (20).

As described by previous studies, our SND included 3 Kcal energy density/g diet, 22% proteins, 12% fats (4% total fats by body weight, 0.6% SFAs, 0.7% MUFAs, 2.1% PUFAs, and 0% cholesterol content), and 66% carbohydrates content. Furthermore, our HFHCD also represented 6.5 Kcal energy density/g diet, 6% proteins, 86% fats (61.9% total fats by body weight, 36.4% SFAs, 20.4% MUFAs, 2.1% PUFAs, and 1.5% cholesterol content), and 8% carbohydrates content (21).

**At the beginning of the experiment, the rats were divided into two main groups as the following:**

1. **SNC group** (negative control group/12 rat) received ad libitum pure drinking water and standard normal diet (SND) (14) during all the experimental period (12 week) as well as were intraperitoneally (IP) injected with a single dose of cold 0.1M citrate buffer (pH 4.5) alone after one month from the beginning of the experiment (16).

2. **HFHCD+STZ-induced T2DM rat model** (positive control group/72 rat) was fed ad libitum with a HFHCD during all the experimental period (12 week) (21) as well as intraperitoneally (IP) injected with a single dose of freshly
prepared 40 mg/kg STZ that was dissolved in cold 0.1M citrate buffer (pH 4.5) after four weeks from the beginning of the experiment (16). Following STZ injection, 5% glucose solution was administrated to all animals for one day to prevent hypoglycemic mortality due to leakage of insulin from the damaged pancreatic β-cells (21).

One week after STZ administration, the overnight tail vein fasted blood glucose levels of all the animals were determined. Diabetic animals represented hyperglycemia, and the blood glucose level was demonstrated as a 222.88±11.77 mg/dL (12.37±0.68 mmol/L) compared to the value of SNC group 77.88±6.11 mg/dL (4.32±0.34 mmol/L), which matched glycemic results from another previous literatures (glycemic > 200 mg/dL) (2).

Four weeks after STZ administration (eight weeks from the beginning of the experiment), HFHCD+STZ-induced T2DM rats (n = 72) were randomly divided into six subgroups (12 rats in each group) as the following:

2.1. HFHCD+STZ-induced T2DM group received HFHCD feeding for another four weeks.
2.2. 300WWLSP group received HFHCD feeding plus orally administrated with 300 (5 mg Cγ) mg WWLSP/kg of rats/day for four weeks (Co-administration).
2.3. 600WWLSP group received HFHCD feeding plus orally administrated with 600 (10 mg Cγ) mg WWLSP/kg of rats/day for four weeks (Co-administration).
2.4. 100SLPI group received HFHCD feeding plus orally administrated with 100 (5 mg Cγ) mg SLPI/kg of rats/day for four weeks (Co-administration).
2.5. 200SLPI group received HFHCD feeding plus orally administrated with 200 (10 mg Cγ) mg SLPI/kg of rats/day for four weeks (Co-administration).
2.6. Gliclazide group received HFHCD feeding plus orally administrated with 5 mg DIAMICRON MR/kg of rats/day for four weeks (Co-administration) (22).

For each experimental group (seven groups), blood samples were taken to measure the levels of different serum biochemical parameters before (baseline/pre-/after eight weeks from the beginning of the experiment study) and after (post-/after twelve week from the beginning of the experiment study) treatment that represented conditions of each group before and after drug administration.

**Conclusion:**

In conclusion, the HFHCD feeding for continuous 12 week plus IP injection with a single dosage of 40 mg/kg STZ were developed a HFHCD+STZ-induced T2DM rat model.

In this study, the HFHCD+STZ-induced T2DM rat model was characterized by degeneration, infiltration, and necrotic features as well as impairment of pancreatic β-cells function, reduction the levels of pancreatic β-cells insulin secretion and its serum content, increasing hyperglycemia, peripheral IR, and hyperlipidemia, compared to the SNC group.

Our study demonstrated that the 600WWLSP oral administration greatly restored pancreatic β-cells homeostasis and its insulin content, decreased hyperglycemia, increased peripheral insulin sensitivity, induced cellular glucose uptake, metabolism, and its utilization, inhibited hepatic glycogenolysis, lipolysis, and
gluconeogenesis, reduced hyperlipidemia, improved cellular lipids metabolism and homeostasis compared to the 300WWLSP, 100/200SLPI, and gliclazide treatment.

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Figure 1 - Evaluation of the Effect of HFHCD Feeding plus STZ Injection on the Level of Blood Glucose after One Week from STZ Administration (after Five Weeks from the Experimental Duration)

Figure 2 - A: show that the Pre and post-treatment values of serum fasting glucose (mmol/L).

Figure 2- B: Post-treatment: inter-group show that 300/600WWLSP, 100/200SLPI, and gliclazide groups
**Figure 3 - A:** show that the pre and post-treatment values of serum insulin (μIU/mL).

**Figure 3 - B:** show that Post-treatment inter-group differences, 300/600WWLSP, 100/200SLPI, and gliclazide groups.

**Figure 4 - A:** show that the pre and post-treatment values of serum HOMA-IR index.
**Figure 4 - B**: Post-treatment show that inter-group differences. 300/600WWLSP, 100/200SLPI, and gliclazide groups.

**Figure 5 - A**: show that the pre and post-treatment values of **HOMA-β cells function index**

**Figure 5 - B**: Post-treatment show that inter-group differences: 300/600WWLSP, 100/200SLPI, and gliclazide groups.
Figure 6 - A show that the pre and post-treatment values of serum ALT activity (IU/L).

Figure 6- B: Post-treatment show that inter-group differences, the 300/600WWLSP, 100/200SLPI, and gliclazide groups

Figure 7- A show that the pre and post-treatment values of serum TG mg/dl.
Figure 7 - B: Post-treatment show that inter-group differences, the 300/600WWLSP, 100/200SLPI, and gliclazide groups.

Figure 8 - A: show that the pre and post-treatment values of serum VLDL-C mg/dl

Figure 8 - B: Post-treatment show that inter-group differences, the 300/600WWLSP, 100/200SLPI, and gliclazide groups.
Figure 9 - A: show that the pre and post-treatment values of serum TC mg/dl

Figure 9 - B: Post-treatment show that inter-group differences, the 300/600WWLSP, 100/200SLPI, and gliclazide groups.
Figure 10. Simple linear regression analysis. Bivariate correlation standardized coefficient relationships (linear curve fit estimation model) (A-F). The bivariate correlation standardized coefficients including the relationships between each two different serum biochemical variable parameters such as serum fasting glucose and insulin (A), serum fasting glucose and HOMA-IR index (B), serum fasting glucose and HOMA-β-cells function index (C), serum fasting insulin and ALT (D), serum fasting insulin and TC (E), and serum fasting insulin and TG (F) within the different experimental rat groups.
Figure 11: Histopathological evaluations of pancreatic tissue specimens of the different experimental rat groups. The SNC group showed a normal endocrine islets of Langerhans’ cells (ICs) and exocrine acinar cells (ACs). (H&E, X400). The pancreatic T2DM tissue specimens showed a vacuolar degeneration (arrow) and necrosis (dashed arrow) of the ACs. (H&E, X400). The pancreatic 300WWLSP tissue specimens showed a mild vacuolation (arrow), necrosis (dashed arrow) of some scattered islets cells, and proliferated pancreatic duct (short arrow) epithelium. (H&E, X400). The pancreatic 600WWLSP tissue specimens showed mild necrobiotic changes of the Islets cells and few kayomegally (arrow) and ESs (dashed arrow). (H&E, X400). The pancreatic 100SLPI tissue specimens showed a good restoration of the ICs that included scattered degenerated cells with vacuolar degeneration (arrow) and necrosis (dashed arrow), few nuclear pyknosis (short arrow), and large number of proliferated cells at the periphery (thick arrow). (H&E, X400). The pancreatic 200SLPI tissue specimens showed a mild to moderate vacuolar degeneration (arrow), few necrotic ICs, and coagulant necrosis (dashed arrow) of the ACs. (H&E, X400). The pancreatic gliclazide tissue specimens showed a vacuolar degeneration (arrow) and necrosis of some ICs. The ACs also showed a mild degeneration and necrotic changes (dashed arrow) after gliclazide treatment. (H&E, X400).
Figure 12: Histopathological evaluations of liver tissue specimens of the different experimental rat groups. The SNC rats showed normal central vein (CV) and hepatic cells (HCs). (H&E, X400). The liver T2DM tissue specimens showed a marked expansion of the portal area that included proliferation of the bile duct epithelium with newly formed bile ductules (arrow), inflammatory cells infiltration (dashed arrow), fibrous proliferation (short arrow), and dilated portal vessels (PV). (H&E, X400). The liver T2DM tissue specimens showed proliferation of the bile duct epithelium that included newly formed bile ductules (arrow) with periductal fibrosis and inflammatory cells infiltration (dashed arrow), hepatocellular degeneration, and necrosis (short arrow). (H&E, X400). The liver 300WWLSP tissue specimens showed a good restoration of the hepatic parenchymal cells with mild inflammatory cells infiltration (arrow) in the portal areas. (H&E, X400). The liver 600WWLSP tissue specimens showed a normal HCs with only few scattered degenerated ones (arrow). (H&E, X400). The liver 100SLPI tissue specimens showed a picture of mild pericholangitis (arrow), periducteolar inflammatory cells infiltration, edema, and fibrosis. (H&E, X400). The liver 200SLPI tissue specimens showed a vacuolar degeneration (short arrow) and focal necrosis of the hepatic artery wall, marked portal tract fibrous proliferation (dashed arrow), and inflammatory cells infiltration (arrow). (H&E, X400). The liver gliclazide tissue specimens showed a normal appearance of the HCs, CV, and portal areas (arrow) with few inflammatory cells infiltration. (H&E, X400).