

# Effect of Lupinus albus conglutin gamma protein on experimentally induced diabetes in rats.

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## Research Article

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# Abstract

Early insulin resistance and a progressive loss of pancreatic  $\beta$  cell function combine to cause type 2 diabetes (T2D), which leads to insufficient insulin production followed by hyperglycemia. Purified from *Lupinus albus* seed, conglutin gamma (Cy) is a protein that lowers blood sugar. The primary function of adipocytokines, hormones released by adipose tissue, is to alert important organs to maintain metabolic balance. This study aimed to identify and compare the role of Cy and glimepiride in controlling hyperglycemia, insulin secretion, insulin resistance and hyperlipidemia in high-fat diet and low-dose streptozotocin (STZ) induced diabetes in experimental rats. Male Sprague Dawley rat groups were divided into seven groups; normal, Cy control, T2D control, and four T2D groups which received Cy (30, 60, and 120 mg/Kg) and Glimepiride (0.1 mg/kg) treatments. Administration of Cy successfully eliminated hyperglycemia and increased insulin secretion and sensitivity. Additionally, when compared to (STZ+HFD) control rats, treatment with Cy improved the expression of leptin, adiponectin, and their blood concentrations, as well as the activity of the enzyme chitotriosidase. It also significantly decreased the expression of apelin, NAMPT and RBP4. The present data suggests that Cy has an effective role in controlling hyperglycemia induced by diabetes through amelioration of leptin, adiponectin, lipid profile and metabolic syndrome.

## INTRODUCTION

Early insulin resistance and increasing loss of pancreatic  $\beta$  cell function are both factors in type 2 diabetes, which leads to insufficient insulin production and resultant hyperglycemia.[1]

Anti-diabetic drugs control blood sugar levels by (a) boosting peripheral insulin sensitivity, (b) reducing liver glucose synthesis, (c) raising insulin secretion from beta cells, or (d) altering the enzymatic activity of enzymes involved in glucose metabolism. [[2], [3], [4]]

The ability of several *Lupinus* genus proteins to reduce blood pressure, glycemia, and cholesterolemia, among other consequences, has recently given these proteins increased prominence. [[5], [6], [7]]. The proteins in the seeds of the extensively cultivated, domesticated species *Lupinus albus*- that have hypoglycemic effects both in vivo and in vitro- have attracted the attention of scientists. The globulin fraction of *L. albus* seeds contains a protein called gamma conglutin (Cy), which was found to have these effects. [[7], [8], [9]]. The homo-tetramer Cy is a component of the 7S glycoprotein, which accounts for 4-5% of all plant protein. Each Cy monomer is made up of two subunits of 17 and 29 kDa that are connected by disulfide links and has a relative mass of roughly 50 kDa. [10]. At pH greater than 4, Cy is resistant to proteolytic enzymes. [11]. A high-fat diet/streptozotocin treatment (HFD/STZ) rat model of diabetes is an illustration of an experimentally created animal model of the disease. In this paradigm, a meal heavy in fat and, occasionally, sugar is combined with therapy with the  $\beta$ -cell toxin STZ to induce hyperinsulinemia, insulin resistance, and/or glucose intolerance[10], [11]. This causes a significant decrease in the mass of functioning  $\beta$  cells. These two stresses work together to simulate the pathogenesis of type 2 diabetes, albeit on a shorter time than that experienced by humans.

Adipokine profiles are altered in patients with type 2 diabetes and obesity, which increases metabolic risk and affects insulin sensitivity. [[12], [6]].

The chitinolytic enzyme chitotriosidase (CHIT1) was discovered to have significantly increased activity in type 2 diabetes patients and to be favourably correlated with blood pressure and glycemic control parameters (levels of glucose and glycated haemoglobin). [13] Although Cy has been assessed in several metabolic scenarios, no research has investigated how it may affect the expression of adipocytokines or the levels of the chitotriosidase enzyme in the blood in a chronic experimental T2D model.

The current study aimed to determine and compare the function of Cy and glimepiride in regulating hyperglycemia, insulin secretion, insulin resistance, and hyperlipidemia in high-fat diet and low-dose streptozotocin (STZ) induced type 2 diabetes (T2D) experimental rats. This was done by influencing the expression of various adipocytokines and serum chitotriosidase enzyme activity.

## **MATERIALS AND METHODS**

### **Isolation of Cy and Characterization by PAGE**

Dehulled lupin seeds were made into flour, then the fat was removed using hexane. With some adjustments, the proteins were isolated as previously described by Martínez et-al [14]. In two processes, the defatted flour was added to double-distilled water (ddH<sub>2</sub>O) and stirred for two hours at 4 °C to remove the albumin fraction. After filtering the solution, the albumin fraction in the supernatant was discarded. Step 1 was repeated after the pellet was resuspended in ddH<sub>2</sub>O in step 2. The whole globulin fraction was then resuspended in 10% NaCl (pH 7) and stirred for 12 hours at 4 °C. The filtrate was then recovered after the solution had been filtered at 4 °C. The filtrate's globulins were precipitated out with ammonium sulphate until they reached 85% saturation. The pellet was filtered, then dissolved in phosphate buffer (0.1 M, pH 6.8), and dialyzed for 18 hours against 0.2 M acetate buffer (pH 4.8). After filtering the solution, the alpha conglutin (C $\alpha$ ) was found in the pellet, while the beta and gamma conglutins (C $\beta$  and C $\gamma$ , respectively) were found in the supernatant. The supernatant was dialyzed against distilled water at 4 °C for 48 hours to separate C $\beta$  and C $\gamma$ . Following filtering, the C $\gamma$ -containing supernatant was lyophilized (Laboratory freeze dryer Alpha 1-2 LSCbasic) for 48 hours at -55 °C, 0.021 mbar. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) at 12 percent under reducing and non-reducing conditions was used with a small Protean®Tetra cell (BioRad, Milan, Italy) apparatus to confirm the presence of C $\gamma$  in the isolate fraction (2 mg per sample) [15]. Gels were stained with Coomassie brilliant blue G-250 following electrophoresis (BioRad, Milan, Italy). By contrasting native and denatured C $\gamma$  with a protein ladder (BenchMark™ Prestained protein ladder, Invitrogen), the relative molecular weight of each was measured.

### **Animals**

The study was carried out in National Nutrition Institute (NNI) Cairo, Egypt on one hundred and five adult male Sprague Dawley (SD) albino rats weighing (160 $\pm$  10g), bought from EGY VAC- The Egyptian Company for production of vaccines and drugs (Vaccsera), in Helwan- October 2020, kept individually in

cages. The rats were divided into 7 groups each consisted of 15 rats. Feed and water were provided ad libitum. Rats were maintained on normal light-dark schedule and temperature  $25\pm 3$  °C throughout the experiment and left 1 week for acclimatization. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the Faculty of Pharmacy, Suez Canal University (Ismailia, Egypt).

### **Experimental induction of Type2 diabetes by high fat diet and low-dose STZ**

The rats were divided into two dietary plans: the normal pellet diet (NPD), which contains 12 % of calories as fat (30 rats), and the high fat diet (HFD), which contains 58 % fat, 25 % protein, and 17 % carbohydrate (75 rats) [16]. The typical diet was made in accordance with Reeves et- al [17]. The makeup of it was as follows (g/100g): Casein 14 (Difco, Becton Dickinson, France), Cellulose 5 (Oxford Lab, Mumbai, India), corn Oil 10 (Egyptian market), vitamin and mineral mix 5 (ADWIC Co., Cairo, Egypt), Corn starch 66 (Egyptian market), DL-methionine, 0.3 (Sigma–Aldrich, MO, USA; ADWIC Co., Cairo, Egypt), cholin chloride 0.2 (Oxford Lab, Mumbai, India). Srinivasan et-al [18] described the following components (g/kg) as the composition and preparation of HFD: powdered NPD, 365 (prepared); DL-methionine, 3.0 (Sigma-Aldrich, MO, USA; ADWIC Co., Cairo, Egypt); lard, 310 (Egyptian market); casein, 250 (Difco, Becton Dickinson, France); cholesterol, 10 (Oxford Lab, Mumbai, India); vitamin and mineral mix, 60 (ADWIC Co., Cairo, Egypt); yeast powder, 1.0 (Egyptian market); sodium chloride (Egyptian market). After two weeks of dietary manipulation, 75 rats on HFD were given an intraperitoneal (i.p.) injection of STZ 35 mg/kg that had just been freshly dissolved in di-sodium citrate (pH 4.5) buffer [19]. The glucose level in blood samples taken from the tail tip ten days after STZ injection was assessed using the glucometer method (Perfecta-Granzia- Italy). After that, the study only included rats with fasting blood glucose levels greater than 250 mg/dl [20]

### **Experimental design**

Diabetic rats received Glimepiride or the different concentrations of the extract under study orally and once daily dissolved in the carrier solution for 4 weeks starting from day 11 after induction of diabetes. Rats were randomly allocated into seven groups of fifteen animals each.

Groups of male Spargue Dawely rats were assigned to normal group, Cy control group, T2D control group and four T2D groups treated with Cy (30, 60, and 120 mg/Kg) and Glimepiride (0.1 mg/kg) once daily oral gavage for 4 weeks.

Group I: non-diabetic rats, fed NPD, received only single i.p. injection of citrate buffer (1 ml/kg) and served as normal control group.

Group 2: non-diabetic rats, fed NPD, received only single i.p. injection of citrate buffer (1 ml/kg) and daily oral dose of Cy (120 mg/Kg) served as positive control for "non- diabetic Cy group.

Group 3: Type 2 Diabetes; diabetic rats received only vehicle and served as Type 2 Diabetes control group.

Group 4: Type 2 Diabetes rats received "Cy at doses of 30mg/kg [9]

Group 5 Type 2 Diabetes rats received "Cy at doses of 60mg/kg.

Group 6 Type 2 Diabetes rats received "Cy at doses of 120mg/kg [21]

Group 7 Type 2 Diabetes rats received " Glimipiride at doses of 0.1 mg/kg [22]

### **Blood sampling and biochemical analysis**

Rats were starved overnight and given 50 mg/kg of thiopental sodium to put them to sleep [23]. The hepatic portal vein was used to collect blood, which was transferred into centrifuge tubes containing anti-coagulants. Whole blood was used to measure blood glucose levels using the haemoglobin and Perfecta-Granzia, Italy, glucometer technique. The remaining blood was centrifuged at 2000 rpm for 15 min after 30 min of collection and stored at -80 °C until assayed. Triglycerides (TGs), total cholesterol (TC), alanine Transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), urea, creatinine, and uric acid (UA), were measured by automatic biochemistry analyzer BT 1500 Biotechnica Instrument. According to the manufacturer's instructions, HDL-C was measured colorimetrically using assay kits from (Stanbio, Texas, USA).

### **Enzyme-linked immunosorbent assay**

Serum insulin- Catalog No: E-EL-R3034, C – peptide- Catalog No: E-EL-R3004, Visfatin- Catalog No: E-EL-R1067, Adiponectin- Catalog No: E-EL-R3012 and Leptin-Catalog No: E-EL-R0582, were determined using the rat ultrasensitive ELISA kit (elabscience, USA) by ELISA reader. CHIT1(chitotriosidase enzyme activity) was determined using the rat CHIT1 ultrasensitive ELISA kit (elabscience, Catalog No: E13980m, USA) by ELISA reader.

### **Calculation of insulin resistance.**

Insulin resistance was determined using the homeostasis model assessment index for insulin resistance (HOMA-IR) using the following formula:  $\text{HOMA-IR index} = \frac{\text{fasting glucose (mg/dl)} \times \text{fasting insulin (mU/ml)}}{405}$  [24]. To assess insulin sensitivity, the revised quantitative insulin sensitivity check index (R-QUICKI)  $\frac{1}{1/\log \text{fasting insulin (mU/ml)} + \log \text{fasting glucose (mg/dl)}}$  was used [25].

### **Expression of NAMPT, Leptin, Adiponectin, RBP4 and Apelin genes in adipose tissue:**

A mortar and pestle were used to smash 30 mg of frozen adipose tissue into liquid nitrogen. A rotor-stator homogenizer was used to blend the lysate. We pipette-mixed 450 µL of 96–100% ethanol into the mixture, centrifuged the mixture for 5 minutes at > 12000 x g, and then transferred the supernatant into a fresh microcentrifuge tube devoid of RNase. The GeneJET RNA Purification Column received 700µL of Wash Buffer 1, and we centrifuged it at 12000 x g for 1 minute. The purification column was then infused with 250 µL of wash buffer 2 before being centrifuged at 10,000 x g for two minutes. We put the lysate into the

column after centrifuging and discarded the flow-through solution. Denaturing agarose gel electrophoresis followed by ethidium bromide staining was used to evaluate the integrity of the RNA before cDNA synthesis. Total eukaryotic RNA, which includes both 18S and 28S rRNA, was intact. We prepared a reaction master mix by adding the components according to the manufacturer's instructions. (except template DNA) for each 20 µl reaction to a tube at room temperature. The reaction mix was mixed gently and transferred into the PCR machine 42 °C for 60 min and 70 °C for 5 min. We programmed the thermal cycler according to the recommendations below.

| Step                 | Temperature, °C | Time   | Number of cycles |
|----------------------|-----------------|--------|------------------|
| Initial denaturation | 95              | 10 min | 1                |
| Denaturation         | 95              | 15 s   | 40               |
| Annealing/Extension  | 60              | 60 s   |                  |

The primers used to amplify the genes are shown in Table (1).

**Table 1: Primer's sequence used for qRT-PCR.**

| Name        | Forward sequence              | Reverse sequence            |
|-------------|-------------------------------|-----------------------------|
| Leptin      | CCT GTG GCT TTG GTC CTA TCT G | AGG CAA GCT GGT GAG GAT CTG |
| (Nampt)     | AGCGGCAGAGCACAGTACCATA        | CCACAGACACAGGCACTGATGA      |
| RBP4        | GACAAGGCTCGTTTCTCTGG          | AAAGGAGGCTACACCCCACT        |
| Apelin      | GGCTAGAAGAAGGCAACATGC         | CCGCTGTCTGCGAAATTTC         |
| Adiponectin | AATCCTGCCCAGTCATGAAG          | CATCTCCTGGGTCACCCTTA        |
| Actein      | GTAAAGACCTCTATGCCAACA         | GGACTCATCGTACTCCTGCT        |

## Statistical Analysis

All data were expressed as mean± SD and analyzed using the Statistical Package of Social Sciences (SPSS) program version 17, (Chicago, IL, USA). For all parameters, comparisons among groups were carried out using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test [26]. All p-values reported are two-tailed and P< 0.05 was considered significant.

# RESULTS

## Characterization of Cy by SDS-PAGE

Protein isolated from -conglutin was examined using SDS-PAGE. Proteins were isolated in non-reducing (Fig. 1 lane 2) and reducing (lanes 3) conditions. Coomassie brilliant blue was used to stain the gels. The SDS-PAGE profiles of the Cy used in the Cy treatment groups are displayed in Figure 1. When there are no reducing conditions, only one band of roughly 50 kDa is visible for Cy. (lane 2). As anticipated, under reducing circumstances, two prominent bands of approximately 17 and 29 kDa formed; these bands were the two Cy sub-units (Fig. 1 lane 3) [27]

## Effect of *Conglutin Gamma* on serum lipid profile

The serum concentrations of cholesterol and TG in diabetic rats of the positive control group significantly increased ( $p < 0.05$ , Table 1). On the other hand, when compared to the values of normal control rat, HDL-C was significantly decreased. When compared to diabetic control rats, oral treatment of Cy significantly reduced the blood levels of TG and TC while significantly increasing the level of HDL-C. When compared to diabetic control rats, Conglutin Gamma and Glimipiride treatment effectively reduced the abnormalities in the lipid profile ( $P < 0.05$ , Table 1). Notably, when compared to diabetic controls, the medium dose of conglutin gamma (60 mg/kg) did not significantly improve HDL-C levels.

Table 1. Effect of Conglutin Gamma on serum lipid profile in diabetic rats

| Groups                                       | Parameters | Total Cholesterol<br>mg/dl | Triglyceride<br>mg/dl | HDL-C<br>mg/dl   |
|----------------------------------------------|------------|----------------------------|-----------------------|------------------|
| Normal                                       |            | 72.973±8.9                 | 56.373±14.7           | 69.67±6.7        |
| positive control for "non-diabetic CG group" |            | 60.033±6.19                | 49.900±12.26          | 72.13±7.43       |
| Type 2 Diabetes                              |            | 256.267±24.4<br>ac         | 359.273±191.3<br>ac   | 35.47±3.9<br>Ac  |
| Type 2 Diabetes + "Cy 30mg/kg"               |            | 111.087±39.4<br>abc        | 77.773±7.24<br>b      | 62.87±5.6<br>Ab  |
| Type 2 Diabetes + "Cy 60mg/kg"               |            | 137.540±33.8<br>abc        | 75.227±8.9b           | 52.87±7.4<br>Abc |
| Type 2 Diabetes + "Cy 120mg/kg"              |            | 76.627±9.5<br>b            | 63.380±11.9<br>b      | 71.60±6.9<br>B   |
| Type 2 Diabetes + Glimipiride                |            | 78.180±9.7<br>b            | 98.260±35.1<br>b      | 61.93±8.4<br>Abc |

TG, triglycerides; TC, total cholesterol; HDL-C high density lipoprotein cholesterol; Rats were treated for 4 weeks. Results were expressed as mean±SD and analyzed using one-way ANOVA followed by Bonferroni's post hoc test (n=15)

\*a is significant vs. positive control for "non diabetic CG group"/ b is significant vs positive control/ c is significant vs. negative non diabetic

**Effect of *Conglutin gamma* on fasting blood glucose, serum insulin, HOMA-IR index and serum C – peptide in diabetic rats.**

Injection of STZ after two weeks of HFD significantly ( $p < 0.05$ ) raised blood glucose levels in contrast to the normal control group, as indicated in Table 2. When compared to normal controls, diabetic rats' serum insulin significantly dropped, and the computed HOMA-IR significantly rose ( $P < 0.05$ , Table 2). In comparison to the diabetic control group, oral administration of conglutin gamma and glimipiride produced a significant decrease in serum glucose, as well as insulin resistance and a significant increase in serum insulin. ( $P < 0.05$ ; Table 2). Notably, when compared to diabetic controls, the lowest and middle doses of conglutin gamma (30 mg/kg and 60 mg/kg) did not significantly alter HOMA-IR. Regarding insulin resistance during the 4-week treatment, there was no significant difference between the two doses. Compared to diabetic control group, serum C-peptide levels significantly increased after oral administration of conglutin gamma and glimipiride. Serum C-peptide levels was significantly reduced in diabetic rats of positive control group.

Table 2. **Effect of *Conglutin gamma* on fasting blood glucose, serum insulin, calculated HOMA-IR index, and serum C– peptide in diabetic rats.**

| Parameters<br>Groups                             | fasting blood<br>sugar mg/dl | C – peptide<br>pg/ml | Insulin<br>pg/ml | Homa IR         |
|--------------------------------------------------|------------------------------|----------------------|------------------|-----------------|
| Normal                                           | 103.20±4.7                   | 1053.1±149.3         | 251.77±17.3      | 1.83±0.10       |
| positive control for "non-<br>diabetic CG group" | 106.13±4.22                  | 1107±146.4           | 359.273±191.3    | 1.88±0.14       |
| Type 2 Diabetes                                  | 365.67±46.5<br>ac            | 296.1±100.9<br>ac    | 95.5±44.0<br>ac  | 2.48±1.19<br>Ac |
| Type 2 Diabetes + "Cy<br>30mg/kg"                | 161.60± 19.4 abc             | 716.8±87.4<br>abc    | 243.8±16.9<br>b  | 2.79±0.4<br>Ac  |
| Type 2 Diabetes + "Cy<br>60mg/kg"                | 168.60± 13.76<br>abc         | 825.3±15.9<br>abc    | 259.4±19.4<br>b  | 3.1±3.8<br>Abc  |
| Type 2 Diabetes + "Cy<br>120mg/kg"               | 110.47±3.06<br>b             | 1104.1±130.6<br>b    | 237.5±30.5<br>b  | 1.85±0.23<br>B  |
| Type 2 Diabetes + Glimipiride                    | 112.87±4.12<br>b             | 890.9±132.9<br>abc   | 235.2±35.3<br>b  | 1.88±0.29<br>B  |

HMOA-IR index, homeostasis model assessment index for insulin resistance index; Rats were treated for 4 weeks. Results were expressed as mean±SD and analyzed using one-way ANOVA followed by Bonferroni's

post hoc test (n=15).

\*a is significant vs. positive control for "non diabetic CG group"/ b is significant vs positive control/ c is significant vs. negative non diabetic

### **Effect of *Conglutin gamma* on liver enzyme activity, kidney function tests and LDH in diabetic rats.**

Serum levels of AST, urea, and creatinine were not significantly different between normal control rats and those fed the HFD for two weeks before receiving a low dosage of STZ. Conglutin gamma oral treatment resulted in a non-significant change in serum levels of these parameters.

The serum levels of ALT, ALP, LDH, GGT, and U.A. in diabetic rats, however, increased significantly.

In comparison to the diabetic group, blood levels of ALT, ALP, LDH, GGT, and U.A. exhibited a significant decline after oral administration of Conglutin gamma at the end of the study period. The serum levels of ALP and AST following oral administration of Conglutin gamma (30 mg/kg and 60 mg/kg, respectively) and glimepiride (0.1 mg/kg ) did not differ significantly from those of diabetic positive control rats (Table 3).

**Table 3. Effect of *Conglutin gamma* on liver enzymes activities, kidney function tests and LDH in diabetic rats.**

| Parameters                                   | ALT<br>(GPT)<br>u/l | AST<br>(GOT)<br>u/l | ALP<br>I U/L        | LDH<br>u/l         | GGT<br>I U/L   | Urea<br>mg/dl     | Creat mg/dl     | U.A<br>mg/dl    |
|----------------------------------------------|---------------------|---------------------|---------------------|--------------------|----------------|-------------------|-----------------|-----------------|
| Groups                                       |                     |                     |                     |                    |                |                   |                 |                 |
| Normal                                       | 48.9±3.7            | 115.09±11.82        | 412.7±66.6          | 421±62.3           | 1.08±0.49      | 49.2±16.1         | 0.53±0.07       | 1.75±0.63       |
| Positive control for "non-diabetic CG group" | 56.1±7.6            | 353.2±39.56         | 500.5±155.9         | 306.3±67.22        | 0.98±0.47      | 34.8±3.9          | 0.51±0.56       | 3.06±0.78       |
| Type 2 diabetes                              | 148.4±17.9<br>ac    | 252.83±28.98<br>ac  | 2099.2±205.3<br>ac  | 1988.5±278.6<br>ac | 5.08±1.8<br>ac | 59.3±12.3         | 0.56±0.06       | 4.8±0.6<br>Ac   |
| Type 2 diabetes + "Cγ 100mg/kg"              | 57.4±5.1<br>b       | 174.2±26.73<br>abc  | 1645.8±180.5<br>abc | 410.1±21.8<br>b    | 1.31±0.77<br>b | 69.4±14.3<br>ac   | 0.45±0.07<br>bc | 2.3±0.47<br>Ab  |
| Type 2 diabetes + "Cγ 200mg/kg"              | 90.5±14.9<br>abc    | 153.55±15.45<br>ab  | 1770.7±181.6<br>abc | 376.6±21.8<br>b    | 2.16±0.7<br>ac | 104.4±22.5<br>abc | 0.57±0.06       | 2.5±0.34<br>Bc  |
| Type 2 diabetes + "Cγ 400mg/kg"              | 61.9±4.9<br>bc      | 262.15±72.14<br>ac  | 631.7±266.9<br>bc   | 392.4±42.2<br>b    | 1.54±0.56<br>b | 41.8±13.4<br>b    | 0.6±0.06<br>a   | 2.1±0.75<br>Ab  |
| Type 2 diabetes + mipiride                   | 62.7±10.4<br>bc     | 232.87±27.13<br>ac  | 113.0±58.2<br>abc   | 974.3±83.2<br>abc  | 1.40±0.53<br>b | 63.5±8.7<br>a     | 0.58±0.83       | 2.16±0.42<br>Ab |

\*a is significant vs. positive control for "non diabetic CG group"/ b is significant vs positive control/ c is significant vs. negative non diabetic

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.; LDH: Lactate dehydrogenase; GGT, Gamma-glutamyltransferase; U.A.: uric acid. Rats were treated for 4 weeks. Results were expressed as mean±SD and analyzed using one-way ANOVA followed by Bonferroni's post hoc test. ( n=15).

### Effect of *Conglutin gamma* on serum Visfatin, Adiponectin and Leptin of diabetic rats

Induction of T2DM increased blood levels of leptin and visfatin significantly (p< 0.05) compared to the normal control group, however diabetic rats' serum levels of adiponectin significantly (p< 0.05) dropped when compared to controls.

In comparison to the diabetic control group, oral administration of conglutin gamma and glimipiride resulted in a significant decrease in blood leptin, visfatin, and a rise in serum adiponectin. ( $p < 0.05$ ; Table 4). Notably, when compared to diabetic controls, the highest dose of conglutin gamma (120 mg/kg) showed the greatest drop in serum leptin and visfatin levels. When compared to diabetic controls, the highest dose of conglutin gamma (120 mg/kg) caused and the greatest increase in blood adiponectin levels ( $p < 0.05$ , Table 4).

**Table 4: Effect of *Conglutin gamma* on serum Visfatin, Adiponectin and Leptin of diabetic rats**

| Parameters                                          | Visfatin<br>ng/ml | Adiponectin<br>ng/ml | Leptin<br>ng/ml  |
|-----------------------------------------------------|-------------------|----------------------|------------------|
| <b>Groups</b>                                       |                   |                      |                  |
| <b>Normal</b>                                       | 1.86±0.2          | 10.0±2.5             | 7.8±2.6          |
| <b>positive control for "non-diabetic CG group"</b> | 1.973±46          | 11.96±2.7            | 8.38±1.7         |
| <b>Type 2 Diabetes</b>                              | 3.66±0.34<br>Ac   | 2.06±0.5<br>ac       | 49.5±3.6<br>Ac   |
| <b>Type 2 Diabetes + "Cγ 30mg/kg"</b>               | 2.82±0.44<br>Abc  | 6.02±1.3<br>abc      | 18.77±3.9<br>Abc |
| <b>Type 2 Diabetes + "Cγ 60mg/kg"</b>               | 2.63±0.35<br>abc  | 7.2±1.3<br>abc       | 19.0±3.4<br>Abc  |
| <b>Type 2 Diabetes + "Cγ 120mg/kg"</b>              | 2.44±0.4<br>abc   | 10.4±1.8<br>b        | 12.5±3.6<br>Bc   |
| <b>Type 2 Diabetes + Glimipiride</b>                | 2.58±0.5<br>abc   | 8.89±1.7<br>ab       | 14.74±6.1<br>Abc |

\*a is significant vs. positive control for "non-diabetic CG group"/ b is significant vs positive control/ c is significant vs. negative non-diabetic

#### **Effect of *Conglutin gamma* on serum Chitotriosidase enzyme activity of diabetic rats**

Injection of STZ after two weeks of HFD elevated blood chitotriosidase enzyme levels significantly compared to the normal group,  $p < 0.05$  as demonstrated in Table (5). Conglutin gamma and glimipiride were administered orally, and the results demonstrated a significant decrease in serum chitotriosidase enzyme levels compared to the diabetic control group. ( $p < 0.05$ ). Notably, compared to diabetic controls, the highest dose of conglutin gamma (120 mg/kg) showed the largest decrease in serum chitotriosidase

enzyme levels, and the three doses of conglutin gamma caused a greater decrease than the glimepiride group ( $p < 0.05$ , Table 5).

**Table 5: Effect of *Conglutin gamma* on serum Chitotriosidase enzyme activity of diabetic rats**

| Group                                        | Parameter CHIT1 (chitotriosidase enzyme activity)<br>pg/ml |
|----------------------------------------------|------------------------------------------------------------|
| Normal                                       | 475.0±201.5                                                |
| positive control for "non-diabetic CG group" | 567.75±146.6                                               |
| Type 2 Diabetes                              | 1594.1±418.8<br>ac                                         |
| Type 2 Diabetes + "Cγ 30mg/kg"               | 679.94±128.3<br>B                                          |
| Type 2 Diabetes + "Cγ 60mg/kg"               | 671.69±192.1<br>B                                          |
| Type 2 Diabetes + "Cγ 120mg/kg"              | 655.82±151.7<br>Bc                                         |
| Type 2 Diabetes + Glimipiride                | 834.7±132.6<br>Abc                                         |

\*a is significant vs. positive control for "non-diabetic CG group"/ b is significant vs positive control/ c is significant vs. negative non-diabetic

### Effect of *Conglutin gamma* on the expression of NAMPT, Leptin, Adiponectin, RBP4 and Apelin genes in adipose tissue of diabetic rats

The values of the fold change of the relevant adipokine genes in the test condition compared to the control condition are shown in Table (6) and have all been normalised to the housekeeping gene.

The effects of oral administration of conglutin gamma and glimepiride on expression of adipokines was measured in adipose tissue following 4 weeks treatments. There was a reduction in the expression of adiponectin and an increase in the expression of leptin, NAMPT, RBP4 and apelin genes in the adipose tissue for rats of positive diabetic control in comparison with normal control. Oral administration of conglutin gamma and glimepiride for 4 weeks resulted in an up- regulation of adiponectin expression in adipose tissue of rats of treated groups compared to positive diabetic control, while the expression of leptin, NAMPT, RBP4 and apelin genes in the adipose tissue for rats of treatment groups after oral

administration of conglutin gamma and glimepiride was down-regulated compared to positive diabetic control.

**Table 6: Effect of *Conglutin gamma* on the expression of NAMPT, Leptin, Adiponectin, RBP4 and Apelin genes in adipose tissue of diabetic rats**

| Groups                                      | Parameters | NAMPT expression | Leptin expression | Adiponectin expression | RBP4 expression | Apelin expression |
|---------------------------------------------|------------|------------------|-------------------|------------------------|-----------------|-------------------|
| Normal                                      |            | 1.09±0.05        | 1±0.02            | 1±0.01                 | 1±0.021         | 1±0.11            |
| positive control for "nondiabetic CG group" |            | 1.07±0.01        | 1.5±0.05          | 0.73±0.02              | 1.6±0.25        | 1.17±0.9          |
| Type 2 Diabetes                             |            | 2.2±1.04         | 5.23±1.1          | 0.27±0.13              | 4.48±0.3        | 6.59±0.14         |
|                                             |            | ac               | ac                | ac                     | ac              | Ac                |
| Type 2 Diabetes + "Cy 30mg/kg"              |            | 1.87±0.9<br>a    | 4.3±0.9<br>abc    | 0.29±0.002<br>abc      | 2.21±0.01<br>ab | 3.34±1.04<br>Bc   |
| Type 2 Diabetes + "Cy 60mg/kg"              |            | 1.5± 0.01<br>abc | 3.8±0.5<br>abc    | 0.31±0.01<br>abc       | 2.1±0.017<br>ab | 3.1±0.20<br>Bc    |
| Type 2 Diabetes + "Cy 120mg/kg"             |            | 1.29±1.03<br>ab  | 2.52±0.06<br>ab   | 0.53±0.2<br>bc         | 1.88±0.04<br>b  | 1.6±0.03<br>B     |
| Type 2 Diabetes + Glimipiride               |            | 1.1±0.6<br>abc   | 2.75±0.1<br>b     | 0.57<br>c              | 1.81±0.25       | 1.51±0.014        |

\*a is significant vs. positive control for "non-diabetic CG group" / b is significant vs positive control/ c is significant vs. negative non-diabetic

NAMPT, Nicotinamide Phosphoribosyltransferase; RBP4, Retinol Binding Protein 4

## DISCUSSION

The goal of the current study was to assess and compare the potential effects of Conglutin gamma (Cy) and glimepiride as hypoglycemic medications, as well as their effects on the expression of different adipocytokines, serum chitotriosidase enzyme activity, and serum adipocytokines in type 2 diabetic rats fed a high-fat diet and treated with low doses of STZ.

First off, when compared to normal control rats, the current data revealed no statistically significant variations in the serum levels of AST, urea, and creatinine. As it is crucial to confirm the lack of kidney and liver toxicity after delivering any treatment, oral administration of Conglutin gamma demonstrated a substantial decrease in blood levels of ALT, ALP, LDH, GGT, and U.A. as compared with the diabetic group at the end of the study period. In this instance, the group receiving Cy from *L. albus* did not experience any appreciable alterations in their serum urea and creatinine levels.

Comparing this group to normal levels revealed no change in the serum ALT and AST levels. As a result, it appears that there is no liver or kidney damage caused by the oral administration of Cy from *L. albus* at the dose and duration examined in this investigation. As a result, it appears that there is no liver or kidney damage caused by the oral administration of Cy from *L. albus* at the dose and duration examined in this investigation. This is in line with earlier findings for the species *L. albus* Cy [28]

By combining HFD with a low dose of STZ, as described in the literature, our data showed that blood glucose levels and insulin resistance increased significantly [29]. The glucose-fatty acid cycle is the primary mechanism that can explain insulin resistance. In brief, consuming excessive amounts of fat can result in high levels of triglycerides, which can enhance the bioavailability and oxidation of fatty acids, which blunts the insulin-mediated decrease in hepatic glucose output and decreases glucose uptake or utilisation in skeletal muscle, resulting in insulin resistance [30].

Along with abnormalities in glucose metabolism, the diabetic rats in the current study also exhibited abnormalities in lipid metabolism, as shown by significantly elevated TG and TC levels coupled with decreased HDL-C, like type 2 diabetic rats, which may be a risk factor in several cardiovascular complications. Increased dietary cholesterol absorption following the use of HFD in a diabetic situation may be the cause of hypercholesterolemia. [31], [32].

Conglutin Gamma (Cy) has an impact on the serum lipid profile in diabetic rats when compared to normal control rats. These findings support the notion that Cy has the capacity to improve the dyslipidemic profile linked to diabetes. [33]

According to Trujillo & Scherer, insulin resistance is directly correlated with hyperlipidemia because high blood lipid concentrations release humoral factors like resistin and adiponectin that affect insulin sensitivity and result in insulin resistance [34]. Therefore, the hypolipidemic effect of Conglutin Gamma (Cy) in the current study may help reduce insulin resistance.

Comparing the oral administration of Conglutin Gamma to the diabetic control group, produced a significant rise in serum insulin levels was observed. These results are consistent with earlier research by Vargas et-al, which showed that rats given -conglutin gamma had higher insulin levels and lower blood glucose levels than the control group [35]. Additionally, Glimipiride significantly boosted insulin secretion in diabetic rats, which is consistent with a study by [36]. It has previously been demonstrated that several intestinal and exogenous enzymes could not break down the Cy protein. [11]. Its oral administration is facilitated by this characteristic. Additionally, Capraro and colleagues [37] revealed that Cy could cross the Caco-2 cells' basolateral membranes, an in vitro model that resembled the human intestinal epithelium, in an undamaged state. We proposed that, following absorption, Cy may induce pancreatic insulin secretion similarly to glucagon-like peptide-1 (GLP-1), which binds to its pancreatic receptor and stimulates insulin exocytosis [38].

Our research also offers novel understandings of how Conglutin Gamma affects serum adipocytokines. We found that Conglutin Gamma treatment was linked to elevated serum levels of adiponectin and

decreased serum levels of leptin and visfatin. This is consistent with recent research that discovered an association between adiponectin and insulin sensitivity [39], as well as the functions of visfatin and leptin in insulin resistance and the diabetic metabolic syndrome. [40]

One of the unique characteristics of this study is how it investigates the effects of Conglutin Gamma and Glimipiride on blood indicators linked to chitotriosidase enzyme activity. The substantial increases in this parameter that have been seen are important proof of the favourable effects of these therapies on various aspects of metabolic dysfunction.

The thorough examination of adipokine gene expression in response to therapy is one of this study's additional novel features. The potential of Conglutin Gamma and Glimipiride in modulating the expression of important adipokines involved in metabolic regulation is highlighted by the observed upregulation of adiponectin gene expression and downregulation of leptin, NAMPT, RBP4, and apelin gene expression in the adipose tissue of treated diabetic rats.

The use of animal models and the lack of long-term follow-up are two drawbacks of our work. The underlying processes by which Conglutin Gamma impacts adipocytokines and glucose metabolism were also not examined. Future research could look at the cellular and molecular mechanisms underlying these effects.

## CONCLUSION

Cy administration effectively abrogated hyperglycemia and enhanced insulin secretion and sensitivity. Moreover, administration Cy ameliorated Chitotriosidase enzyme activity, the expression of leptin, adiponectin, and their serum concentration, NAMPT and RBP4 expression compared to (STZ+HFD) control rats and effectively downregulated apelin expression. The present data suggests that Cy has an ameliorative effect on liver damage induced by diabetes.

## Declarations

### Ethical Approval

All experimental protocols were approved by the Institutional Animal Care and Use Committee at the Faculty of Pharmacy, Suez Canal University (Ismailia, Egypt ).

### Authors' contributions

Marwa bhieldeen & Afaf Tawfik: prepared the protein extract.

Marwa bhieldeen: Marwa bhieldeen: Did the biological experiments and collected blood samples and adipose tissue samples.

Marwa bhieldeen: did the ELISA and PCR assays for the samples.

Marwa bhieldeem & Fatema El-shaarawy: Analysed the results and prepared data.

Marwa bhieldeem: prepared the manuscript.

Fatema El-shaarawy: helped in editing the manuscript.

All authors reviewed the manuscript.

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## Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Declaration of interest

According to the authors, there are no conflicts of interest that might be thought to compromise the objectivity of the research presented.

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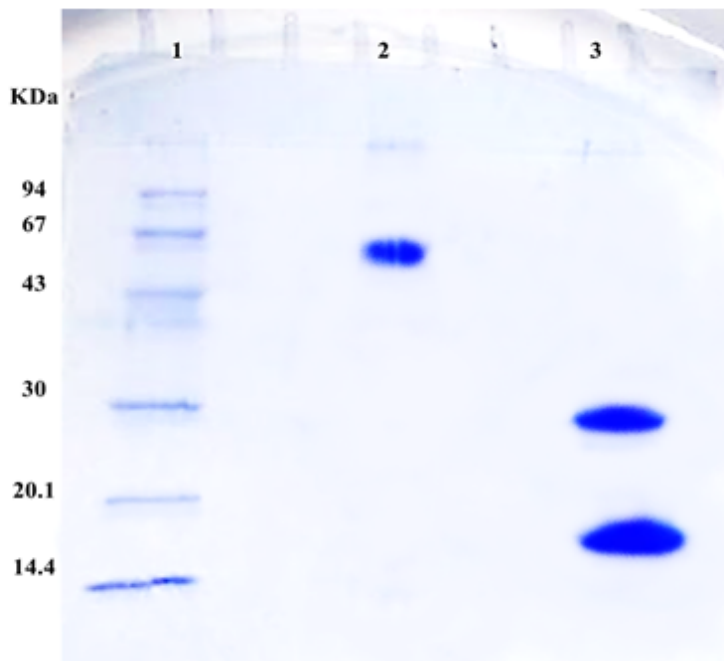
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## Figures



**Figure 1**

characterization of purified lupin protein,  $\gamma$ -conglutin: