

A Comparative Study of the Therapeutic Effect of Lupine Extract with Metformin on the Liver Function in Induced Type II Diabetic Male Rats

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Abstract

Diabetes mellitus (DM) is classified as a metabolic disorder characterized by high blood sugar levels and impaired glucose tolerance. Type II diabetes represents the predominant variant, constituting almost 90% of all instances. It's primarily associated with insulin resistance and insufficient insulin secretion, along with other complications such as hypertension and dyslipidemia. The aim of this experiment was to evaluate the effects of lupine extract on metabolic parameters in a rat model of T2DM and compare its effects with metformin. For the experiment, type 2 diabetes was induced in male rats (depend on a high-fat diet) by one-time intraperitoneal injection of Streptozotocin (STZ) at a dose of 40 mg/kg. To the necessary need for new treatment alternatives, the affected rats were divided into two treatment protocols: one with lupine extract (100) mg/kg at every day in experiment (30 days) and the other with metformin treatment (200) mg/kg at every day in experiment (30 days). The analyses included measuring random body weight, blood glucose levels, serum insulin, and liver enzyme profile in the effected rats. Since the primary goals of diabetes treatment are to improve glycemic control, reduce metabolic complications, and preserve organ function., lupine extract has shown promising efficacy comparable to metformin in controlling the disease. It significantly contributed to regulating glucose concentration in blood, improving glucose tolerance and increasing cellular sensitivity of insulin in the treated rats, in addition to reducing body fat accumulation .

Key words: Diabetes, lupine, Metformin, Anti-diabetic therapy, Glucose

خلاصة:

يُصنف داء السكري كاضطراب أيضي يتميز بارتفاع مستويات السكر في الدم وضعف تحمل الجلوكوز. ويُعد داء السكري من النوع الثاني هو النوع السائد، إذ يُشكل ما يقرب من 90% من الحالات. ويرتبط هذا النوع بشكل أساسي بمقاومة الأنسولين وعدم كفاية إفرازه، بالإضافة إلى مضاعفات أخرى مثل ارتفاع ضغط الدم واضطراب شحوم الدم. هدفت هذه التجربة إلى تقييم تأثير مستخلص الترمس على المؤشرات الأيضية في نموذج فأر مصاب بداء السكري من النوع الثاني، ومقارنة تأثيره مع تأثير الميتفورمين. ولإجراء التجربة، تم تحفيز الإصابة بداء السكري من النوع الثاني في ذكور الفئران (عن طريق نظام غذائي غني بالدهون) عن طريق حقنة واحدة داخل الصفاق من الستريبتوزوتوسين (STZ) بجرعة 40 ملغم/كغم. ونظرًا للحاجة الملحة إلى بدائل علاجية جديدة، قُسمت الفئران المصابة إلى مجموعتين علاجيتين: الأولى باستخدام مستخلص الترمس (100) ملغم/كغم يوميًا لمدة 30 يومًا، والثانية باستخدام الميتفورمين (200) ملغم/كغم يوميًا لمدة 30 يومًا. شملت التحليلات قياس وزن الجسم العشوائي، ومستويات سكر الدم، ومستوى الأنسولين في المصل، ومستوى إنزيمات الكبد لدى الفئران المصابة. ولأن الأهداف الرئيسية لعلاج داء السكري هي تحسين التحكم في نسبة السكر في الدم، والحد من المضاعفات الأيضية، والحفاظ على وظائف الأعضاء، فقد أظهر مستخلص الترمس فعالية واحدة تضاهي فعالية الميتفورمين في السيطرة على المرض. وقد ساهم بشكل ملحوظ في تنظيم تركيز الجلوكوز في الدم، وتحسين تحمل الجلوكوز، وزيادة حساسية الخلايا للأنسولين لدى الفئران المعالجة، بالإضافة إلى تقليل تراكم الدهون في الجسم.

الكلمات المفتاحية: مرض السكري، التورم، الميتفورمين، العلاج المضاد لمرض السكري، الجلوكوز

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia and associated with increased morbidity, mortality, and reduced quality of life. Hyperglycemia is a hallmark of diabetes [1]. Arising from a deficiency in the release or function of insulin, or the both [2]. There are two broad categories of DM: insulin-dependent diabetes mellitus (Diabetes type I) and insulin-independent diabetes mellitus (Diabetes type II) [3]. Numerous comorbidities, such as cognitive decline,

functional impairment, emotional disorders, obstructive sleep apnea, and liver disease, are significantly correlated with diabetes mellitus [4]. Streptozotocin is the most widely used diabetogenic agent for producing diabetes in animal models [5]. It has the capacity to specifically kill necrotic pancreatic beta cells in animal experiments [6], through DNA alkylation, and prevents their development [7]. Numerous medications are employed to manage diabetes, including metformin, one of the primary medications that decreases the generation of glucose in the liver and increases

the sensitivity of bodily tissues to insulin [8]. but frequently results in gastrointestinal (GI) adverse effects, which may lower therapy efficacy and adherence [9]. Consequently, there is a necessity to identify a treatment derived from natural remedies with less adverse effects. lupine extract showed encouraging antibacterial, antihypertension, antidiabetic, anticancer, antioxidant, and anti-inflammatory properties [10].

Materials and methods

Animal Preparation and Habitation

This study utilized 60 mature male Wistar rats weighing between 140 and 160 grams. Animals were housed in the animal facility of the College of Science at the University of Al-Qadisiyah, maintained on a 12-hour light/12-hour dark cycle, with the temperature regulated at $(24 \pm 2^\circ\text{C})$. The animal was administered a standard meal and distilled water during the trial. Following a week of acclimatization to the academic environment, the examination commenced. [11].

Preparation method of lupine extract

Lupine seeds were extracted using a Soxhlet apparatus. Lupine seed powder was prepared using approximately 50 g of the fine powder, which was weighed and placed into the paper thimble of the Soxhlet apparatus. Next, some cotton wool was placed on the upper part of the extraction flask to prevent the sample from flowing onto other apparatus parts. A 500 mL round-bottom flask with a condenser was attached to the Soxhlet extractor and filled with distilled water, and then the distillation process was begun. The extraction assembly was then heated on a heating mantle at 100°C to initiate the process. After the extraction procedure was completed within the allotted time, the extract solution was allowed to cool to room temperature. The resulting liquid extract was transferred into

clean Pyrex containers, then placed in a drying oven at 40°C and evaporated for 1-3 days to evaporate the solvent. The final extracted lupine material was weighed to calculate the extraction yield [12].

The design of experiment

Sixty male westar rats were divided into two groups as follows: (the time of experiment was 30 days)

- 1) The control negative group (15 rats) was administered distilled water daily by oral gavage.
- 2) Induced diabetes groups (45 rats, 15 rats in every group).
 - a) Diabetes group: the control positive group (STZ): rats received no treatment.
 - b) Metformin group: received orally by gavage metformin (200) mg/kg/day [13] for 30 days.
 - c) Lupine extract-treated group: received daily by oral gavage (100 mg/kg body weight) [14].

Streptozotocin Induced diabetes

The rats subjected to a 12-hour fast in the three induction groups received a single intraperitoneal injection of STZ in sterile saline. STZ, formulated by dissolving in 0.01 M citrate buffer at pH 4.5, was delivered intraperitoneally in a single dosage of 40 mg/kg body weight. [15].

Body weight

All animals were weighed and measured for blood sugar levels during the acclimatization period, and then they were weighed at the end the experiment in the 30 day.

Sample prepare method

At the end of the research period, blood samples were drawn for laboratory tests and analyses to assessment some parameters

(blood glucose, insulin and liver enzymes profile).

Results

1. Body weight

The result is illustrated in the table (1) showed no significant differences between all groups male rats in beginning of experimental. While, the results recorded a significant ($P<0.05$) increase of body weight gain in control group compare with all other groups,

whereas the result showed the significant ($P>0.05$) decrease in diabetes group compare with all other groups in started and ended of the experiment. Conversely, the lupine group recorded significant ($P<0.05$) increase compare with metformin group male rats at both experimental periods.

Data were expressed as Mean $\pm$ SD based on 15 samples. Distinct lowercase letters indicate a significant difference ($P<0.05$) among groups.

Table (1): the effect of (metformin and lupine) treatment on Body Weight in streptozotocin induced diabetes rats at (start and final) experiment.

Group	Wt_Start (g) (Mean $\pm$ SE)	Wt_Final (Mean $\pm$ SE) (g)
Control	151.5 $\pm$ 2.43 a	202.33 $\pm$ 2.12 a
Diabetes	153.7 $\pm$ 1.49 a	127.50 $\pm$ 1.48 d
Metformin	153.7 $\pm$ 0.95 a	164.17 $\pm$ 1.82 c
Lupine	153.6 $\pm$ 1.07 a	176.00 $\pm$ 2.27 b
LSD	22.53	5.74

The significant differences between groups in each period is represented by small letters.

2. Blood glucose

The result illustrated in table (2) recorded no significant ($P<0.05$) differences between all groups male rats in started of experiment. Whereas the results showed that a significant ($P<0.05$) increase of glucose blood concentration in diabetes group compare with all other groups, as well as the result recorded a significant decrease in control group

compare with all other groups in the ended of the experiment. On the other hand, the lupine group recorded significant increase compare with metformin group male rats at end of experimental periods.

Data were expressed as Mean $\pm$ SD based on 15 samples. Distinct lowercase letters indicate a significant difference ($P<0.05$) among groups.

Table (2): the effect of (metformin and lupine) treatment on blood glucose level in streptozotocin induced diabetes rats at (before and after) experiment.

Group	Glucose(mg/dL) (Before) Mean $\pm$ SE	Glucose(mg/dL) (After) Mean $\pm$ SE
Control	91.5 $\pm$ 1.87 a	97.83 $\pm$ 2.15 d
Diabetes	92.1 $\pm$ 2.60 a	396.67 $\pm$ 9.50 a
Metformin	90.25 $\pm$ 1.97 a	238.83 $\pm$ 10.97 c
Lupine	93.21 $\pm$ 0.956 a	263.50 $\pm$ 8.38 b
LSD	22.12	18.92

The significant differences between groups in each period is represented by small letters.

3. insulin

As illustrated in table (3), a relative insulin blood glucose control group revealed significant ($P \leq 0.05$) elevation, and the diabetes group significant ($P > 0.05$) decrease, after 30-day period, in comparison with all other groups, whereas Lupine group was

significantly ($P < 0.05$) higher than metformin and diabetes groups.

Data were expressed as Mean $\pm$ SD based on 15 samples. Distinct lowercase letters indicate a significant difference ($P < 0.05$) among groups.

Table (2): the effect of (metformin and lupine) treatment on blood insulin lveles in streptozotocin induced diabetes rats at (before and after) experiment.

Group	Mean $\pm$ SE (ng/mL)
Control	0.655 $\pm$ 0.027 a
Diabetes	0.182 $\pm$ 0.009 d
Metformin	0.378 $\pm$ 0.012 c
Lupine	0.385 $\pm$ 0.009 b
LSD	0.028

The significant differences between groups in each period is represented by small letters.

liver enzyme profiles (ALT, AST and ALP)

At the end of the experiment, serum ALT, AST and ALP concentration of diabetes-treated male rat's results in table (4) recorded a significant ($P<0.05$) increase. While, control

male rats revealed significant ($P>0.05$) decrease in comparison with control female rats. Whereas, metformin group significant ($P<0.05$) increase with lupine group.

Data were expressed as Mean $\pm$ SD based on 15 samples. Distinct lowercase letters indicate a significant difference ($P<0.05$) among groups.

Table (2): the effect of (metformin and lupine) treatment on liver enzymes in streptozotocin induced diabetes rats at (before and after) experiment.

Groups	ALT (U/L) (Mean $\pm$ SE)	AST (U/L) (Mean $\pm$ SE)	ALP (U/L) (Mean $\pm$ SE)
Control	30.18 $\pm$ 1.13 d	35.27 $\pm$ 1.40 d	120.31 $\pm$ 2.95 d
Diabetes	84.84 $\pm$ 2.12 a	90.54 $\pm$ 2.90 a	279.36 $\pm$ 6.87 a
Metformin	50.56 $\pm$ 1.70 b	56.03 $\pm$ 2.14 b	172.53 $\pm$ 4.44 b
Lupine	46.71 $\pm$ 2.30 c	52.14 $\pm$ 1.78 c	160.02 $\pm$ 4.33 c
LSD	3.51	3.27	11.33

The significant differences between groups in each period is represented by small letters.

Discussion:

Diabetes mellitus is one of the most prevalent chronic metabolic disorders worldwide and represents a major public health challenge due to its progressive nature and the severity of its complications. The disease is characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Among the animal models available for diabetes research, streptozotocin (STZ)-induced diabetes in rats is one of the most widely accepted and reproducible, as it selectively destroys pancreatic beta (β)-cells through reactive oxygen species-mediated DNA damage and poly-ADP-ribose polymerase (PARP) over activation [17], [18]. The present study evaluated the effects of *Lupinus albus* (white lupine) seed extract as a

potential natural therapeutic agent compared with the standard antidiabetic drug metformin in STZ-induced diabetic male rats, examining a comprehensive range of physiological, biochemical, and histopathological parameters. The following sections provide a detailed discussion of each finding in the context of current scientific literature.

The significant increase in body weight gain in the control group in the present study confirms that the rats live with normal growth by stabilization of metabolic mechanisms and hormones, which is causing normal protein synthesis and adipose tissue aggregation. In contrast, the significant decrease in body weight gain in the diabetes group male rats, might be attributed to insulin-dependent glucose reduction which is resulted from destruction of pancreatic beta cells by

the effect of streptozotocin induced diabetes, therefore, the body suffer from decrease energy production by glycolysis inside the cells, and consequently, increase skeletal muscles catabolism and breakdown of protein and adipose tissues [17]. On the other hand, the results of this study showed a significant weight gain recovery in the lupine-treated group compared with the metformin and diabetes groups. Although metformin is considered an amazing drug for stabilizing blood sugar levels, it often reduces appetite slightly, which reduces its ability to restore lost body weight in diabetics [19], [20], and [21].

The weight recovery amelioration of the lupine group might be attributed to the unique component (conglutin-gamma protein), which performs insulin-like action through the increase in skeletal muscle uptake of glucose and finally prevents muscle protein degradation and increases muscle protein synthesis [22]. Moreover, researchers have suggested that the high nutritional content of lupine, including fiber and amino acids, improves feeding efficiency, which increases the animal's ability to repair tissues and gain weight.

The STZ-mediated diabetes caused an increase in serum glucose concentrations in male rats when STZ was injected intraperitoneal at a dose of 40 mg/kg. The results exhibited an increase in blood glucose levels in a time-dependent manner. This result agreed with other researchers [23], [24]. In STZ-diabetes rats, the significant increase of serum glucose and significant decrease of serum insulin concentrations could be due to specific pancreatic beta cells of Langerhans islets being damaged by affinity of glucose plasma membrane protein transporter 2 which is expressed in huge amount on Langerhans islets lead to DNA alkylation and pancreatic cell necrosis, finally, insulin synthesis and

secretion is suppressed [25]. Skeletal muscles and adipose tissues prevent the consumption of glucose because insulin is decreased and, at the same time, rapid production of glucose by glycogen metabolism and gluconeogenesis in the liver, therefore increasing blood glucose levels [26]. where the pancreas is the major organ for insulin production and secretion. The pathological changes of the pancreas could be a confirmation of the glucose and insulin levels reported in this study.

On the other hand, chronic hyperglycemia is caused by diabetes in experimental animal models, showing increased oxidative stress, which causes increased production of free radicals and decrease the activation of the mechanism of antioxidant defense [27], [28] reported that cellular dysfunction is caused by increased free radical availability with decreased effect of the antioxidant defense. Also, the lipid peroxidation, DNA destruction, and sulfhydryl oxidation resulting from increased generation of superoxide radicals might be attributed to a decrease in the scavenger of free radicals because of the cytotoxic effect of Streptozotocin on pancreatic beta cells [29].

Conversely, the result in this study showed ameliorative effect of (lupine, metformin) treated diabetes male rats' groups which significantly corrected insulin level and successfully decreased blood glucose level, this result for lupine is in agreement with [30], [31] and for metformin agrees with [32]. This phenomenological divergence indicates that although both treatments drove a comparable numerical recovery in pancreatic islets insulinogenic secretory capacity, their primary downstream, insulin-independent target pathways varied in potency.

Metformin is antidiabetic drug reducing blood glucose level and prevent gluconeogenesis. The result of current study

showed amelioration effect of metformin in decrease blood glucose level over lupine groups may be thought to its heavy extra pancreatic pathway, which was explained by reporter [26]. Liver gluconeogenesis is suppressed by triggering (AMPK) and accelerated glucose elimination by location change of (GLUT4) in to skeletal muscles through metformin effect, regardless of high insulin.

On other side, the significant inhibition of blood glucose levels in the lupine group male rats compare with STZ group illustrated in this study might be attributed to treatment role of the lupine against diabetes through certain methods.

According to the results, the hepatic enzymes (ALP, ALT, AST) reported a significant increase in diabetic groups compared with other groups, indicating to hepatic necrosis which may be attributed to hyperglycemia and increased free radical because oxidative stress. Whereas the results of both therapy groups reported a significant decrease, the lupine male rats group reported a greater recovery than the metformin male rats group. Actually, it is well recognized that the drain of liver enzymes in the diabetic group is a results of lipid peroxidation triggered of liver cell membranes and poor liver antioxidant defense [33]. The high protection effect of lupine in the liver is may be due to lupine is flavonoid composition of lupine, mainly genistein, daidzein, and luteolin that exhibit strong anti-inflammatory and antioxidant activities. These substances have been demonstrated to inhibit the activation of nuclear factor kappa B (NF- κ B), decreasing the synthesis of pro-inflammatory cytokines that aggravate the liver cells' toxicity [34]. As well as, lupine protein also has regulatory effects on lipid metabolism in the liver by induction of genes involved in fatty acid

oxidation and suppression of lipogenic pathways, resulting in reduced fat accumulation and cellular stress in the liver [35]. The integrated pathways lead to the remarkable hepatoprotection seen in the lupine male rats group.

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