



Potential Effect of Lupine Seeds (Sweet and Bitter) in Alloxan-Induced Diabetic Rats

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ABSTRACT:

Lupinus, a genus of plants belonging to the legume family Fabaceae, is widely recognized as Lupines. Both sweet and bitter lupine seeds are considered promising candidates for diabetic treatment. This study aimed to evaluate the effect of sweet and bitter lupine seeds on diabetic rats induced with alloxan. A total of 48 male albino rats were divided into eight groups. After a 7-day acclimatization period on a basal diet, rats in the second group were given an injection of 150mg/kg alloxan injection to induce diabetes. Group 1 was the negative control, while groups 3 and 4 were fed sweet lupine powder (2.5% to 5% of their diet weight), and groups 5 and 6 received bitter lupine powder in the same proportion. Groups 7 and 8 were given a mixture of sweet and bitter lupine powder. The results indicated that the rats receiving lupine powders significantly improved in various biochemical parameters compared to the positive control group. These impacted blood glucose, liver enzymes, lipid profiles, and kidney function ($P \leq 0.05$). Also, the treated groups had significantly elevated insulin secretion, and HDL-c levels were significantly higher in the treated groups. In conclusion, these findings suggest that sweet and bitter lupine seeds may positively influence the biochemical condition of diabetic rats, indicating their potential as a natural adjunct in diabetes management.

Keywords: *Lupine Seeds, Blood Glucose, Rats*

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1. INTRODUCTION

The inability of the pancreas to generate an adequate amount of insulin or the body's inability to effectively utilize the insulin it produces is the cause of diabetes mellitus, a chronic, noninfectious disease (1&2). It affects how the body converts food into energy. Hence the high level of sugar in the

blood leads to the risk of damage to micro blood vessels (retinopathy, nephropathy and polyneuropathy) (3&4). It is a disease of the endocrine system, a group of metabolic diseases It is characterized by changes in lipid, protein, and carbohydrate metabolism (5&6). Since the early Roman era, lupine has been utilized as both a human sustenance and animal fodder. Lupines are cultivated on a

global scale, with more than 300 species in existence. Lupine is capable of withstanding drought, frost, and substandard soil conditions. [7&8].

People have been consuming lupine seeds for thousands of years, despite the fact that their use in contemporary food production remains restricted [9] Several fundamental compounds, such as proteins, reducing sugars, glycosides, and cardiac glycosides, Within the phytochemical analysis of the methanolic seed extract of lupin, these compounds were identified. No carboxylic acid, lipids, or fatty acids were identified, however. rendering it a suitable food for the management of obesity and diabetes [10]. As a result, we were compelled to study the effects of lupine seed on several biochemical markers and enzyme activity in rats with diabetes.

2. MATERIAL & METHODS

2.1 Materials

2.1.1 Lupine

The main materials used were (sweet lupine and bitter lupine) as powder were obtained from local market. These lupine products will be sun dried and milled. At this study, two concentrations of sweet lupine and bitter lupinas powder 2.5% and 5% during the experimental period will use.

2.1.2 Chemicals

Chemical Company, USA provided pure white crystalline cholesterol powder and saline solutions. Casein, cellulose, choline chloride powder, and DL methionine powder, were obtained from Morgan Co. Cairo, Egypt.

2.1.3 Animals:

normal male albino rats (48 animals) of the Sprague Dawley strain were purchased from Helwan Farm , The Ministry of Health's Vaccine and Immunity Organization , Cairo , Egypt. Each rat weighed 140 ± 10 g .

2.1.4 Chemical kits

Chemical kits for the determination of TC, TG, HDL-c, ALT, AST, ALP, urea, uric acid, creatinine.

2.1.5 Alloxan

Alloxan obtained from Al-Gomhoria. for the trading of chemicals, drugs, and medical instruments in Cairo, Egypt.

2.2 Methods

2.2.1 Induction of diabetes

According to the methodology detailed by Desai and Bhide, rats with a blood glucose level higher than 200 mg/dl were considered diabetic (11).

2.2.2 Experimental design

The Scientific Research Ethics Committee of The Institutional Animal Care and Use Committee (IACUC) Menoufia University accepted the research protocol No. (#20-SREC-11-2018) of the Science Research Ethics Committee of Faculty of Home Economics.

For the purpose of the study, forty-eight adult male Sprague Dawley rats 28 day old weighed 140 ± 10 g were used. Rats were fed a basal diet (casein diet) for seven days. as per the American Institute of Nutrition (AIN). Then were divided into eight distinct groups each of 6 rats as follows.

Group I: Normal rats fed on basal diet (negative group)

Group II: The rats that were injected were administered 150mg of alloxan per kilogram of body weight in order to establish a positive control group.

Group III: In the diabetic population, 2.5% of the diet's weight is comprised of delectable lupine powder.

Group IV: Five percent of the weight of a diabetic group's diet consists of sweet lupine powder.

Group V: Acid lupine powder is being administered to a group of diabetics who have contracted the infection. About two and a half percent of the weight of the diet.

Group VI: Acid lupine powder is being administered to a group of diabetics who have contracted the infection. 5% of the diet of weight.

Group VII: A combination of astringent and saccharine consumables is administered to a diabetic group. Lupin powder constitutes 2.5% of the diet's weight.

Group VIII: A combination of astringent and saccharine consumables is administered to a diabetic group. Lupin powder accounts for five percent of the diet's weight.

Rats' general behavior and body weight were recorded weekly and their food consumption and body weight were monitored throughout experimental. Blood samples will be collected and each rat will be individually weighed after the 28-day investigation concludes. Ultimately, the rats were eradicated.

2.2.3 Blood sampling

The hepatic portal vein was used to collect blood samples at the end of each session, following a 12-hour fast. After spinning the blood samples at 3000 rpm for 10 minutes, the serum was extracted. Subsequently, until it was analyzed in accordance with Schermer's method, The serum was separated from the blood samples by centrifuging them at 3000 rpm for 10 minutes, The blood samples were placed in centrifuge glass tubes that were desiccated and immaculate, and they were permitted to coagulate for a period of 10 minutes (12).

2.2.4 Biochemical analysis

The calorimetric method was employed to enzymatically determine the serum glucose. The method of measuring serum insulin (4-12) was followed. The following parameters were assessed: HDL-c, LDL-c, VLDL-c, and triglycerides (T.G) overall cholesterol (13).

Using the identification approach, the serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined correspondingly (14). The enzymatic

technique was employed to determine serum creatinine and serum urea to the following specifications. Conversely, the method of calorimetry was employed to quantify serum uric acid. (15-16)

2.2.5 Statistical analysis

The data were shown as the mean $\pm$ standard deviation using the student t-test program. Using an ANOVA with a single experimental variable (17).

3. RESULTS AND DISCUSSION

Data in table (1). (1) Demonstrated the impact of sweet and astringent lupine seeds on the random blood sugar levels of rats. A mean of 106.25 ± 6.994 ($P > 0.05$) provided evidence that the negative control group and any of the experimental groups did not exhibit any significant differences. Nevertheless, the positive control group's mean was 262.50 ± 36.629 , which was statistically significantly higher than all of the categories that were examined ($P \leq 0.05$). In any of the categories that were thoroughly examined, no significant changes were identified ($P > 0.05$). These findings are consistent with (18)

Lupine was asserted to affect the transfer of glucose from the intestines to the liver and into the general circulation. This hypothesis is supported by the observation that animals treated with lupine demonstrate a diminished insulin response in response to a glucose challenge. This outcome is consistent with a decrease in the stimulation of the insulin response by glucose, rather than an increase in insulin levels, which could potentially result in a precipitous decline in glucose levels.

(19-20-21) They disclosed that conglutins, which are present in Lupine seeds, are a significant protein component of the seed. Despite the absence of apparent structural similarity, The Conglutin-g has been demonstrated to bind endogenous insulin and, more importantly, to exhibit insulin-mimetic properties in murine myoblasts. (22-

23) They discovered that lupine seeds are a product that is high in fiber and protein (24). It is involved in the mitigation of the risk of diabetes and insulin resistance, because soluble dietary fiber may affect insulin resistance by delaying stomach emptying after eating and reducing glucose levels.

Table (1) Effect of sweet and bitter lupine seeds on RBS of rats

Groups	RBS
Negative control (G1)	106.25a±6.994
Positive control (G2)	262.50b±36.629
Sweet Lupine 2.5% (G3)	139.00a±35.935
Sweet Lupine 5% (G4)	127.50a±21.252
Bitter Lupine 2.5% (G5)	125.25a±40.966
Bitter Lupine 5% (G6)	128.25a±25.448
Sweet & Bitter Lupine 2.5% (G7)	113.25a±26.700
Sweet & Bitter Lupine 5% (G8)	160.75a±34.942
LSD	101.75

Means under the same column bearing different superscript letters are different significantly ($p \leq 0.05$)

Each value is presented as Mean $\pm$ standard deviation ($n=4$)

Data in table (2) demonstrated the impact of sweet and bitter lupine seeds on the lipid profile of rats, The following are included: triglycerides, cholesterol, very low-density lipoprotein, and low-density lipoprotein. Regarding VLDL, the negative control group demonstrated an average of 18.60 ± 5.279 , the G7 group had a significantly lower value ($P < 0.05$). The mean of the positive control group was 15.70 ± 2.873 , and there was no significant difference in the findings compared to any of the categories that were considered ($P > 0.05$). $P < 0.05$ indicates that the G7 group had a significantly lower mean compared to the G3 and G4 groups. The G5, G6, and G8 groups did not show any significant changes ($P > 0.05$).

For LDL, the mean of the positive control group was 15.70 ± 2.873 . The results were not significantly different from those of all other categories that were tested ($P > 0.05$). However, the mean of the G7 group was considerably lower than that of the G3 and G4 groups ($P \leq 0.05$). The mean of the positive control group was 28.05 ± 7.902 , which was not significantly different from that of all tested groups ($P > 0.05$). Nevertheless, the G4 group

exhibited a significant difference from the G3, G7, and G8 groups ($P > 0.05$). The G5 and G6 groups did not exhibit any statistically significant differences ($P > 0.05$).

For HDL, observed that negative control group was 35.75 ± 2.500 , Analysis of all categories yielded no significant differences ($P > 0.05$). Additionally, the mean of the positive control group was 38.004.243, which was not significantly different from that of all other analyzed groups ($P > 0.05$). In any of the categories that were examined, no significant changes were observed ($P > 0.05$).

For TRI, the negative control group exhibited a mean of 93.00 ± 26.394 . On the other hand, the mean of the positive control group was 78.50 ± 14.364 , which was markedly greater than that of the G7 group ($P \leq 0.05$). Nevertheless, TRI did not differ significantly from any of the categories that were examined ($P > 0.05$). However, the significance level of the G7 group was markedly lower than that of the G3 and G4 groups ($P \leq 0.05$). The G5, G6, and G8 groups did not exhibit any significant differences ($P > 0.05$).

For CHOL, observed that negative control group was 93.00 ± 8.327 , Additionally, the mean of the positive control group was 81.75 ± 7.411 , which was not significantly different from that of all tested groups ($P > 0.05$), which did not demonstrate a significant correlation with any of the categories that were examined ($P > 0.05$). Nevertheless, the G4 group was significantly more differentiated than the G8 group ($P > 0.05$). Substantial alterations were not observed in the G3, G5, G6, and G7 groups ($P > 0.05$). The results are consistent with [25-26]. they discovered that Lupine protein isolates and fiber may reduce serum lipids and lower total cholesterol concentration. These findings are also consistent with [27]

However, they demonstrated that HDL cholesterol increased, albeit not statistically significant. The accumulation of body fat was diminished by additional lupine constituents, such as protein isolate and whole lupine seed.

Lupine kernel fiber was determined to be as effective as -glycan or guar gum in reducing LDL cholesterol without affecting HDL cholesterol. Due to the high water-soluble fiber content Kernel fiber is believed to be responsible for the reduction in LDL levels through the formation of short-chain fatty acids [28]. Lupine kernel fiber and lupine proteins are the primary components of dehulled lupine. In numerous investigations, it has been observed that pure lupine flour significantly reduces LDL cholesterol levels, potentially due to a reduction in cholesterol absorption in the intestinal tract. Also,

phytosterols contribute to the reduction of LDL cholesterol, in addition to kernel fiber and proteins. A variety of mechanisms are employed by phytosterols to inhibit the absorption of cholesterol through the intestine, as reviewed by [29]. 10% of the population may experience a reduction in their LDL cholesterol by consuming 11.4 g of phytosterols per day. The oil fraction contains phytosterols, which are fat-soluble. Lupine oil contains 2.4% phytosterols, which surpasses the concentration of any other known phytosterol-rich oil.

Table (2) Effect of sweet and bitter lupine seeds on Lipid profile of rats

Groups	VLDL	LDL	HDL	TRI	CHOL
Negative control (G1)	18.60b±5.27	37.65bc±9.36	35.75a±2.50	93.00b±26.39	93.00ab±8.32
Positive control (G2)	15.70ab±2.87	28.05abc±7.90	38.00a±4.24	78.50ab±14.36	81.75ab±7.41
Sweet Lupine 2.5% (G3)	19.30b±2.36	19.45a±9.62	39.75a±2.98	96.50b±11.81	83.50ab±16.42
Sweet Lupine 5% (G4)	19.30b±3.24	41.45c±11.33	40.00a±3.91	96.50b±16.21	100.50b±17.17
Bitter Lupine 2.5% (G5)	16.65ab±2.14	24.60abc±7.70	37.50a±3.10	83.25ab±10.72	78.00ab±7.52
Bitter Lupine 5% (G6)	14.35ab±1.39	26.40abc±3.98	38.25a±3.59	71.75ab±6.99	84.00ab±11.13
Sweet & Bitter Lupine 2.5% (G7)	11.45a±1.60	18.38a±3.918	37.75a±2.50	58.75a±8.01	76.25ab±11.95
Sweet & Bitter Lupine 5% (G8)	12.60ab±2.55	20.90ab±3.333	33.25a±2.63	63.00ab±12.75	66.75a±5.62
LSD	7.15	18.20	6.76	34.25	33.75

Means under the same column bearing different superscript letters are different significantly ($p \leq 0.05$)

Each value is presented as Mean $\pm$ standard deviation ($n=4$)

Data in table (3) and demonstrated the impact of sweet and bitter lupine seeds on the kidney profile of rats, which includes uric acid, creatinine, and urea. For UA, the negative control group's mean was 2.00 ± 0.183 , which was significantly lower than that of the G8 group ($P \leq 0.05$). Noted. Similarly, the positive control group demonstrated a mean of 3.60 ± 0.216 , which was significantly lower than that of the G8 group ($P \leq 0.05$). The Group of Eight (G8), however, demonstrated a mean of 2.43 ± 0.411 , which was significantly greater than that of the G4 and G7 groups ($P \leq 0.05$). Nevertheless, there was no statistically significant difference between the G3, G5, and G6 groups ($P > 0.05$).

For CREAT, the negative control group presented a mean of 0.71 ± 0.057 ; which was significantly greater than that of the G4 and G7 groups ($P \leq 0.05$). Nevertheless, there was

no statistically significant difference between the G3, G5, and G6 groups ($P > 0.05$). Additionally, ($P > 0.05$) indicated that the results were not significantly different from those of all other tested categories. Nevertheless, the mean of the G3 group was significantly higher than that of the G5 and G7 groups ($P \leq 0.05$). However, there were no statistically significant differences between the G4, G6, and G8 groups ($P > 0.05$).

For UREA, noted that the negative control group had a mean of 52.50 ± 9.678 . The findings were not significantly different from all of the categories that were examined ($P > 0.05$). Furthermore, the mean of the positive control group was 51.00 ± 11.690 , which was not markedly different from that of all other tested groups ($P > 0.05$). There were no significant changes observed in any of the categories that were examined ($P > 0.05$). These

results are consistent with [30--31].

Table (3) Effect of sweet and bitter lupine seeds on Kidney profile of rats

Groups	UA	CREAT	UREA
Negative control (G1)	2.00a±0.183	0.71ab±0.057	52.50a±9.678
Positive control (G2)	2.43a±0.411	0.72ab±0.050	51.00a±11.690
Sweet Lupine 2.5% (G3)	2.80ab±0.698	0.91b±0.197	53.50a±4.203
Sweet Lupine 5% (G4)	2.03a±0.299	0.80ab±0.106	54.75a±6.801
Bitter Lupine 2.5% (G5)	2.95ab±0.466	0.67a±0.043	55.25a±3.862
Bitter Lupine 5% (G6)	2.55ab±0.545	0.71ab±0.040	51.50a±5.260
Sweet & Bitter Lupine 2.5% (G7)	2.23a±0.670	0.67a±0.065	43.25a±4.031
Sweet & Bitter Lupine 5% (G8)	3.60b±0.216	0.74ab±0.054	47.25a±5.058
LSD	1.17	0.24	12.01

Means under the same column bearing different superscript letters are different significantly ($p \leq 0.05$)

Each value is presented as Mean $\pm$ standard deviation ($n=4$)

Data in table (4) demonstrated the impact of sweet and astringent lupine seeds on the liver function of rats (glucagon like peptide, alkaline phosphatase, albumin, glutamic pyruvate transaminase, and glutamic oxaloacetic transaminase). For GLP, observed that negative control group was 3.93 ± 0.386 , This did not vary significantly from any of the categories that were examined ($P > 0.05$). Furthermore, In the positive control group, the mean was 4.03 ± 0.465 . In the analysis of the groups, no statistically significant difference was observed ($P > 0.05$). In any of the categories that were investigated, there were no significant changes ($P > 0.05$).

For ALP, the mean of the negative control

group was 305.25 ± 48.134 , There was no significant difference between the results and all of the categories that were analyzed ($P > 0.05$). Furthermore, the mean of the positive control group was 265.75 ± 54.616 , The results were not significantly different from those of all other tested categories ($P > 0.05$). Nevertheless, the G6 group exhibited a significantly lower mean than the G3 and G5 groups ($P \leq 0.05$). There were no statistically significant differences between the G4, G7, and G8 groups ($P > 0.05$).

For ALB, the negative control group was observed to have a mean of 3.86 ± 0.136 , This was not significantly different from all of the categories that were examined ($P > 0.05$).

Table (4) impact of sweet and astringent lupine seeds on the liver function of rats (glucagon like peptide, alkaline phosphatase, albumin, glutamic pyruvate transaminase, and glutamic oxaloacetic transaminase).

Groups	GLP	ALP	ALB	GPT	GOT
Negative control (G1)	3.93a±0.386	305.25ab±48.13	3.86ab±0.13	60.25a±2.986	113.25a±21.172
Positive control (G2)	4.03a±0.465	265.75ab±54.61	3.92ab±0.27	54.00a±13.089	96.75a±17.783
Sweet Lupine 2.5% (G3)	3.93a±0.499	328.00b±63.440	3.83ab±0.66	67.50a±11.818	131.75a±40.631
Sweet Lupine 5% (G4)	3.79a±0.296	285.75ab±85.30	3.68ab±0.19	60.75a±15.650	105.25a±18.822
Bitter Lupine 2.5% (G5)	4.28a±0.386	351.75b±72.261	3.23a±0.332	77.00a±11.460	120.25a±28.123
Bitter Lupine 5% (G6)	3.90a±0.345	197.25a±39.263	3.88ab±0.17	64.25a±11.236	109.25a±18.246
Sweet & Bitter Lupine 2.5% (G7)	3.63a±0.303	235.25ab±7.500	4.14b±0.138	60.50a±13.723	101.25a±16.520
Sweet & Bitter Lupine 5% (G8)	3.75a±0.592	258.00ab±35.54	3.74ab±0.44	66.25a±15.735	86.50a±40.927
LSD	0.66	130.75	0.91	23.01	45.26

Means under the same column bearing different superscript letters are different significantly ($p \leq 0.05$)

Each value is presented as Mean $\pm$ standard deviation ($n=4$)

Data in table (5) illustrated the feed efficiency ratio and body weight gain of rats in response to sweet and astringent lupine seeds.

For BWG, the mean of the negative control group was 7.95 ± 3.527 , which was not significantly different from any of the other

groups that were examined ($P > 0.05$), as was observed. Furthermore, the mean of the positive control group was 9.23 ± 9.619 , and no significant changes were observed in any of the categories that were examined ($P > 0.05$). For FER, the mean in the negative control group was 0.04 ± 0.019 .

which was obviously evident, no significant differences were observed between the positive control group and the other groups, and the examined groups did not exhibit any significant differences ($P > 0.05$). Additionally,

none of the categories that were investigated showed any significant changes ($P > 0.05$). These results are in accordance with the initial hypothesis.

[37-38-39 -40] they disclosed that One of the properties of lupine seed fibers is their ability to bind with water, especially in the upper digestive tract Also, foods that contain lupine seeds may reduce appetite after eating, which may result in a substantial reduction in food consumption and, as a result, the maintenance of body weight (41-42).

Table (5) Effect of sweet and bitter lupine seeds on BWG & FER of rats

Groups	BWG	FER
Negative control (G1)	7.95a $\pm$ 3.527	0.04a $\pm$ 0.019
Positive control (G2)	9.23a $\pm$ 9.619	0.04a $\pm$ 0.038
Sweet Lupine 2.5% (G3)	18.10a $\pm$ 9.632	0.09a $\pm$ 0.039
Sweet Lupine 5% (G4)	18.35a $\pm$ 11.619	0.11a $\pm$ 0.063
Bitter Lupine 2.5% (G5)	23.65a $\pm$ 13.085	0.10a $\pm$ 0.044
Bitter Lupine 5% (G6)	18.40a $\pm$ 6.065	0.08a $\pm$ 0.022
Sweet & Bitter Lupine 2.5% (G7)	19.15a $\pm$ 18.086	0.08a $\pm$ 0.078
Sweet & Bitter Lupine 5% (G8)	9.25a $\pm$ 3.799	0.05a $\pm$ 0.019
LSD	15.71	0.08

Means under the same column bearing the same superscript letters are different significantly ($p > 0.05$)

CONCLUSION:

Blood sugar levels can be enhanced by consuming lupine seeds, including both sweet and astringent varieties. Liver and kidney function, insulin, and lipid profile. A high bioactive chemical content in specific plant components may be responsible for these effects, as it endows them with robust antioxidant properties.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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نوع المقالة: بحوث أصلية
التغذية وعلوم الاطعمة

التأثير المحتمل لبذور الترمس (الحلو والمر) في الفئران المصابة بمرض السكري الناتج عن تأثير الألوكسان

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الملخص العربي:

اللوبين جنس من النباتات ينتمي الى عائلة البقوليات ومعروف على نطاق واسع باسم الترمس. تعتبر بذور الترمس، الحلوة والقابضة، مرشحة واعدة لعلاج مرض السكري. هدفت هذه الدراسة إلى تقييم تأثير بذور الترمس الحلوة والمر على الفئران المصابة بمرض السكري التي تم إحداثه بالألوكسان. تم تقسيم العدد الإجمالي من ذكور الفئران البيضاء البالغ 48 فأراً إلى ثمانية مجموعات. بعد فترة تأقلم لمدة 7 أيام على نظام غذائي أساسي، تم حقن الفئران في المجموعة الثانية حقنة ألوكسان 150 مجم/كجم لحدث الإصابة بمرض السكري. كانت المجموعة 1 هي المجموعة الضابطة السلبية، بينما تم تغذية المجموعتين 3 و4 على مسحوق الترمس الحلو (2.5٪ إلى 5٪ من وزن النظام الغذائي)، وتلقت المجموعتان 5 و6 مسحوق الترمس المر بنفس النسبة. تم إعطاء المجموعتين 7 و8 مزيجاً من مسحوق الترمس الحلو والمر. أشارت النتائج إلى أن الفئران التي تلقت مسحوق الترمس أظهرت تحسناً كبيرة مقارنة بمجموعة المقارنة الإيجابية في معايير كيميائية حيوية مختلفة. وشملت هذه انخفاضاً في نسبة الجلوكوز في الدم، وأنزيمات الكبد، ومستويات الدهون، ووظائف الكلى ($P \leq 0.05$). بالإضافة إلى ذلك، كانت مستويات إفراز الأنسولين وHDL-C أعلى بشكل ملحوظ في المجموعات المعالجة. تشير هذه النتائج إلى أن بذور الترمس الحلوة والمر قد تؤثر بشكل إيجابي على الحالة الكيميائية الحيوية للفئران المصابة بمرض السكري، مما يشير إلى إمكاناتها كمساعد طبي في إدارة مرض السكري.

الكلمات الكاشفة: بذور الترمس، جلوكوز الدم، الفئران

الاستشهاد الي:

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