The antihyperglycemic effect of LUPINES (termis) in alloxan-induced diabetic rats

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ABSTRACT

The goal of the current investigation was to determine whether LUPINES (termis) has an antihyperglycemic effect on diabetic rats produced by alloxan. El Osman Farm in Cairo, Egypt provided a total of 40 male albino rats. Animals were kept in stainless steel cages with unlimited access to food and water. Each animal was sound and clinically disease-free. Four equal groups of 10 rats each were formed by randomly dividing the rats into groups, and each group received one of the following diets: group one: normal control feed normal food, group two: normal rat feed normal diet plus 5% LUPINES, group three: diabetic rat feed normal diet, Group 4: Normal diet with 5% Lupine-fed diabetic rats. Each group had blood samples taken after 30 and 60 days since the experiment's start. Blood samples were centrifuged at 3000 rpm for 15 minutes to separate the serum, which was then stored in the freezer for later analysis. Additionally, blood samples were collected at the conclusion of the experiment to be used in the determination of the hematological parameters. The current study showed that LUPINES (termis) medicinal herbs reduced the risks associated with diabetes. Therefore, it is important to pay attention to the sources of LUPINES (termis) in foods. Additionally, eating diets high in LUPINES (termis) medicinal herbs may help reduce the risks associated with diabetes.

Keywords: alloxan; hypoglycemic; lupines.

INTRODUCTION

Alternative approaches to the current pharmacotherapy of diabetes mellitus are urgently needed due to the etiopathogenesis of the disease, harmful side effects of synthetic drugs, the inability of existing modern therapies to control all the pathological aspects of the diabetic disorder, the exorbitant cost of modern drugs, as well as the poor accessibility of the advanced therapies for many rural populations in developing countries (Tanaka et al., 1992). In order to achieve a breakthrough in the treatment of diabetes, the use of medicinal plants in primary healthcare for diabetic mellitus will be increased (Udupa, 1985). Recent evidence indicates that natural medicines are generally safe, free of major adverse effects, and relatively non-toxic (Momoin, 1987). The green elements on our planet are plants, which transform carbon dioxide, water, and nitrogen into carbohydrates and amino acids, respectively. In addition to providing food, plants are regarded as nature's "green pharmacy," providing medicines to uphold and restore human health. When people first started using preventative methods to ease their pains, sufferings, and other illnesses, the medical arts were born (Badr et al., 2012).

The goal of the current investigation was to determine the positive effects of LUPINES (termis) on a few serum markers in rats with diabetes induced by alloxan.

MATERIALS AND METHODS

The Animal House Laboratory of the Animal Production Department, Faculty of Agriculture, Al- Azhar University, served as the site of this investigation.

Animals: El Osman Farm in Cairo, Egypt provided a total of 40 male albino rats. Animals were kept in stainless steel cages with unlimited access to food and water. Each animal was sound and clinically disease-free.

Experimental design:

Medicine plant: The bulbs of, LUPINES (termis) (obtained from the local market in Cairo) is grounded to crush garlic and used as feed additives.

Animal: Rats were randomly divided into 4 equal groups, each group contained 10 rats and fed on one of the following diets:

Group 1: Normal Control feed normal diet,

Group 2: Normal rat feed normal diet+ 5% LUPINES

Group 3: diabetic rat feed normal diet

Group 4: diabetic rat feed normal diet+ 5% LUPINES.

Induction of diabetes mellitus in rates:

Diabetes mellitus was induced in selected experimental rats by intraperitoneal injection of Alloxan solution (0.1 ml/100 gm. body weight). Alloxan solution consists of 0.12 gm. (120 mg/kg) alloxan hydrasin per 1 ml 1 buffer solution. Alloxan buffer is prepared by the addition of 7.5 ml of 5.7% glacial acetic acid to 29.5 ml of 8.2% sodium acetate solution. Injected animals were fasting for 18 and 2 hr. before and after injection, respectively according to (Malaisse, 1982 and Mohammed, 2005). One week later, injected animals with alloxan were considered diabetic through the appearance of certain symptoms (such as increased urine volume and body weight loss). Some rates were resistant to Alloxan so were excluded.

Serum Collection:

Blood tests were gotten from rates by pulling back blood from the orbital venous plexuses employing a capillary tube. Tests were collected in two events at 4th week and 8th week from the begin of the explore at the proteins but at all hormones tests were collected after conclusion of 8th week as it were. The collected blood tests collected and centrifuged at 3000 rpm for 20 min to get serum. Serum was exchanged to Ependorff tube and put away at 20 Co until ensuing examinations (Glucose, Add up to Protein, Egg whites, ALT, AST, Creatinine and Urea). NB: Moment examination for glucose after each blood draw was done.

parameters:

Glucose: The glucose concentration determined by glucose oxidase method (Trinder, 1969) using commercial kit (bio Meieux, Lyon, France).

Total Protein : Serum total protein assay: Serum total protein was determined using colorimetric method according to Burtis (1999)

Albumin: Serum albumin assay: Serum albumin was measured using kits depending on the method according to Gindler and Westgard (1973)

Alanine Transaminase (ALT): Serum alanine transaminase (ALT) assay: Serum ALT was determined by using a colorimetric method according to Mathieu et al. (1982a).

Aspartate Transaminase (AST): Serum aspartate transaminase (AST) assay: Serum AST was determined using colorimetric method according to Mathieu et al. (1982b).

Creatinine : Creatinine was determined by a kinetic method according to Henr., (1974) using Diamond-Egypt kits.

Urea:Serum urea assay: Serum urea was measured by colorimetric method based on the method of Tabacco et al. (1979).

Body Wight: The animals were weighed at the beginning of the experiment to standardize the weights the initial Wight were 100±10 g. - the weight was performed twice in the fourth and eighth week.

Statistical analysis:

Statistical analyses were carried out using SPSS program. One-way analysis of variance (Procedure ANOVA of SPSS version 20) followed by Duncan's multiple range test (Duncan, 1955) to test the effect of Nigella sativa and Allium sativum after 4 and 8 weeks from the experiment within.

RESULTS:

Glucose

Tabel (1) shows that after 4 weeks from the start of the experiment treatment of healthy rats with medicinal plant (Lupines) did not show any significant effect on serum glucose as compared with the control group .

The results in table (1) also showed that after 4 or 8 weeks from the start of the experiment diabetes significantly increased serum glucose level as compared with the control rats. While treatment of diabetic rats with medicinal plant (Lupines) from 4 or 8 weeks significantly decreased serum glucose levels as compared with diabetic rat, but did not reach to the normal range in the control group .

These results agree with Saha (2012) who found that blood glucose was significantly increased in diabetic group compared with non-diabetic group. Sultan (2008) reported that blood sugar levels of both diabetic groups were significantly higher than in controls. Blood glucose levels are mainly regulated by insulin and glucagon hormones which are released from Langerhans islets of pancreas. Thus, insulin regulates blood glucose levels during the anabolic phase by transformation of blood glucose into glycogen in muscles and liver, glucagon's regulates the blood glucose levels during the reverse catabolic phase.

The results also revealed that medicinal plant (Lupines) can decrease the elevated of serum glucose due to diabetes. This result indicated that medicinal plant have ant

diabetic properties. These results are in accordance with those found by Ayed et. al (2011) showed that the blood glucose of both control and experimental groups showed a highly significant ($p < 0.001$) increase in the levels of blood glucose, registering increases of 246.2% after 7 weeks compared to the controls.

Total Protein & Albumin

Tabel (2&3) shows that after 4&8 weeks from the start of the experiment treatment of healthy rats with medicinal plant (Lupines) did not show any significant effect on serum Total Protein & Albumin as compared with the control group.

The results in table (2&3) also showed that after 4 or 8 weeks from the start of the experiment diabetes significantly decreased serum Total Protein & Albumin as compared with the control rats. While treatment of diabetic rats with medicinal plant (Lupines) from 4 or 8 weeks significantly increased serum Total Protein & Albumin as compared with diabetic rat , and reach to the normal range in the control group.

These results agree with those found by. Hady Maha M. (2013) It was observed that diabetic group demonstrated significant ($P<0.05$) increment in the urea, creatinine levels and AST and ALT activities, on the contrary, the levels of total protein and Albumin showed significant ($P<0.05$) decrement, . All treatments were relatively able to regain the normal values of the control healthy rats, however the treatment in the degree of improvement.

ALT, AST

Table (4&5) Table (4&5) shows that after 4 or 8 weeks from the start of the experiment treatment of healthy rats with the medicinal plant(Lupines) did not show any significant effect on serum ALT & AST as compared with the control group .

The results in table (4&5) also showed that after 4 or 8 weeks during summer season diabetes significantly increased serum ALT &AST activity as compared with the control rats.

Treatment of diabetic rats with medicinal plant(Lupines) for 4&8 weeks significantly decreased serum ALT activity as compared to diabetic rats to reach to or near the normal level in the control group.

These results are in agreement with those found by Abdel Ghfar (2016) and Radhia (2012) who found that Alanine amino transferees (ALT), significantly increase in diabetic groups compared with control group.

El-Demerdash (2005) observed that the activities of plasma AST&ALT, were significantly ($p < 0.05$) increased by 49& 60, %, respectively in Alloxan-diabetic rats as compared to the control group . They also indicated that diabetes might induce hepatic dysfunction.

Celik et al (2002) observed that ALT was slightly higher in the diabetic group than in the control group.

These results agree with those found by Faoziyat et al. (2014) showed that serum ALT and AST activities significantly increased in rats treated with highly active antiretroviral therapy when compared with the rats in normal control group. They also showed that administration of rats with Nigella sativa seed extract (100, 200, 400 and 800 mg/kg) plus highly active antiretroviral therapy significantly decreased serum ALT and AST concentrations when compared with rats treated with highly active antiretroviral therapy.

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These results are in agreement with those found by El-Demerdash et al. (2005) showed that when rats injected subcutaneously with a single dose of alloxan (120 mg/kg BW) plasma AST and ALT significantly increased as compared with the control group. While treatment of diabetic rats with garlic juice (1 ml/100g BW/day) significantly decreased activities of AST and ALT in the liver tissue when compared with the control group.

The result also revealed that treatment of diabetic rats with medicinal plant (Lupines) significantly decreased the activities of ALT & AST that well increased after induction of diabetes.

Creatinine

Table (6) shows that after 4 or 8 weeks from the start of the experiment treatment of healthy rats with the medicinal plant (Lupines) did not show any significant effect on serum

Creatinin as compared with the control group

The results in table (6) also showed that after 4 or 8 weeks from the start of the experiment serum Creatinin in diabetic rats were significantly increased as compared with the control group. Treatment of diabetic rats with medicinal plant (Lupines) significantly decreased serum Creatinine as compared with diabetic rats to reach to or near the normal rang in the control r

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The above results indicated that diabetes significantly increased serum Creatinin these results may be due to rise of Clothes results are in according with Ayed et. al. (2011) showed that a significant ($p < 0.001$) increase in blood uric acid, urea and creatinine by 23.7%, 155.4% and 29.7% respectively, compared to the control. Eidi et. al. (2006) showed that serum Creatinine increased in diabetic rats, when compared with normal rats.

Urea

Table (7) shows that after 4 or 8 weeks from the start of the experiment treatment of healthy rats with the medicinal plant(Lupines) did not show any significant effect on serum Urea as compared with the control group

The results in table (6) also showed that after 4 or 8 weeks from the start of the experiment serum Urea in diabetic rats were significantly increased as compared with the control group. Treatment of diabetic rats with medicinal plant (Lupines) significantly decreased serum Creatinine as compared with diabetic rats to reach to or near the normal rang in the control r

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Body Wait

Table (8) shows that after 4 or 8 weeks from the start for the experiment on healthy rats, LUPINES did not show any significant effect on body weight as compared with the control group.

The results also show that body weight significantly decreased in diabetic rats compared with control group. Treatment of diabetic rats with medicinal plant (LUPINES) did not show any significant effect on body weight as compared with diabetic rats group.

These results are in agreement with Abdel Ghfar (2016) and Johan el at, (1990) who observed that after 12 weeks of diabetes, the adult diabetic body weight rats had lost considerable weight; their final weight was only 60% of that of control rats. Bernard, et.al. (1970) observed that animals respond to severe alloxan-induced diabetes. Approximately half of all the examined animals either became "obese" or strikingly emaciated and moribund.

DISCUSSION

Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. Besides the enzymatic antioxidants. such as GST and CAT, reduced GSH is also an important antioxidant.

Administration of seeds LUPINES (termis) , caused an increase in the level of the antioxidant enzymes of the normal and diabetic rats. These findings suggested that LUPINES (termis), seeds might be involved in the restoration of the antioxidant defense system by regulating the activities of antioxidant enzymes (GST and CAT) and non-enzymatic one (GSH) in STZ-induced diabetic rats. Reduced GSH could protect the cells from the toxic effects of ROS and GST and CAT play a prominent role in scavenging free radical .

CONCLUSION

The present study demonstrated that the seeds LUPINES (termis) only significant hypoglycemic effect to STZ-diabetic rats. seed powder to diabetic animals has been shown to lower blood glucose levels and partially, restore the activities of key enzymes of carbohydrate and lipid metabolism close to normal In our experiment we suggest that in diabetic animals increased a lot of diseases. the present results suggest the use of medicinal plant (LUPINES) 5 % in improving the condition of health in normal and diabetic animals.

It could be concluded that the addition of 5% of Lupines in rats diet cause a several effect on some blood components under this study. Feeding of diet contain Lupines decrease glucose levels in some experimental groups. Thus, we can add Lupines to rat's diet by 5% from the diet to increase the performance in these animals.

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Table : Composition of the control diet:

Ingredient	Quantity (%)
Yellow corn	56.65
Soybean meal (44%)	14.60
Wheat bran	20.30
Lime stone	7.40
Caco3	0.40
Mineral and vitamin premix	0.30
Salt	0.25
Fish male	0.05
Methionine	0.05

Table 1: Mean ± S.E for Protective effect of Lupines against diabetic on serum Glucose (mg/dl)

Groub	30days		60days	
	Mean±SE	D T.	Mean±SE	D T.
Group 1: Normal Control feed normal diet,	84.25±2.462	C	105.00±5.182	C
Group 2: Normal Control feed normal diet+ 5% LUPINES	80.25±1.123	C	100.00±5.182	C
Group 3: Diabetic rat feed normal diet	357.50±22.592	A	352.80±16.429	A
Group 4: Diabetic rat feed diet+ 5% LUPINES	139.80±10.220	B	153.05±3.064	B

Table 2: Mean ± S.E for Protective effect of Lupines against diabetic on serum total protein concentrations (g/dl)

Groub	30days		60days	
	Mean±SE	D T.	Mean±SE	D T.
Group 1: Normal Control feed normal diet,	7.100±.0.057	A	6.900±0.032	A
Group 2: Normal Control feed normal diet+ 5% LUPINES	7.900±.157	A	7.110±0.022	A
Group 3: Diabetic rat feed normal diet	5.650±0.160	B	5.486±0.239	B
Group 4: Diabetic rat feed diet+ 5% LUPINES	7.400±0.264	A	6.310±0.164	A

Table 3: Mean ± S.E for Protective effect of Lupines against diabetic on serum Albumin concentrations (g/dl)

Groub	30days		60days	
	Mean±SE	D T.	Mean±SE	D T.
Group 1: Normal Control feed normal diet,	4.900±0.057	A	4.693±0.054	A
Group 2: Normal Control feed normal diet+ 5% LUPINES	5.200±0.037	A	5.553±0.054	A
Group 3: Diabetic rat feed normal diet	3.133±0.033	B	3.796±0.071	B
Group 4: Diabetic rat feed diet+ 5% LUPINES	4.430±0.176	A	4.503±0.214	A

Table 4: Mean ± S.E for Protective effect of Lupines against diabetic on serum ALT concentrations (U/L)

Groub	30days		60days	
	Mean±SE	D T.	Mean±SE	D T.
Group 1: Normal Control feed normal diet,	45.333±2.027	C	61.000±0.577	C
Group 2: Normal Control feed normal diet+ 5% LUPINES	44.245±1.044	C	60.090±0.987	C
Group 3: Diabetic rat feed normal diet	55.333±1.201	A	74.333±1.763	A
Group 4: Diabetic rat feed diet+ 5% LUPINES	40.666±0.881	D	66.333±0.333	B

Table 5: Mean ± S.E for Protective effect of Lupines against diabetic on serum AST concentrations (U/L)

Groub	30days		60days	
	Mean±SE	D T.	Mean±SE	D T.
Group 1: Normal Control feed normal diet,	354.666±1.855	B	215.333±1.452	B
Group 2: Normal Control feed normal diet+ 5% LUPINES	312.321±1.342	B	211.44±1.432	B
Group 3: Diabetic rat feed normal diet	377.000±3.464	A	234.666±2.027	A
Group 4: Diabetic rat feed diet+ 5% LUPINES	334.666±2.603	C	200.000±1.732	C

Table 6: Mean ± S.E for Protective effect of Lupines against diabetic on serum Creatinine concentrations (U/L)

Groub	30days		60days	
	Mean±SE	D T	Mean±SE	D T
Group 1: Normal Control feed normal diet,	0.730±0.005	B	0.803±0.043	B
Group 2: Normal Control feed normal diet+ 5% LUPINES	0.690±0.002	B	0.793±0.011	B
Group 3: Diabetic rat feed normal diet	0.936±0.020	A	0.976±0.008	A
Group 4: Diabetic rat feed diet+ 5% LUPINES	0.756±0.016	B	0.733±0.176	C

Table 7: Mean ± S.E for Protective effect of Lupines against diabetic on serum Urea concentrations (mg/dl)

Groub	30days		60days	
	Mean±SE	D T	Mean±SE	D T
Group 1: Normal Control feed normal diet,	27.666±2.603	B	73.333±1.452	B
Group 2: Normal Control feed normal diet+ 5% LUPINES	26.444±2.435	B	72.143±1.424	B
Group 3: Diabetic rat feed normal diet	35.666±2.027	A	83.833±2.602	A
Group 4: Diabetic rat feed diet+ 5% LUPINES	28.000±6.350	B	72.400±0.461	B

Table 8: Mean ± S.E for Changes of BW (gr.) as affected by feeding the healthy and diabetics rats on Lupines during

Groub	30days		60days	
	Mean±SE	D T	Mean±SE	D T
Group 1: Normal Control feed normal diet,	171.766±4.227	A	241.775±5.100	A
Group 2: Normal Control feed normal diet+ 5% LUPINES	170.766±4.227	A	242.775±5.100	A
Group 3: Diabetic rat feed normal diet	105.345±1.443	B	121.234±5.0234	B
Group 4: Diabetic rat feed diet+ 5% LUPINES	115.768±8.423	B	119.244±6.567	B

التأثيرات الوقائية للترمس علي الفئران المصابة بالسكري

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الملخص العربى

تم الحصول على 40 من ذكور الجرذان البيضاء من مزرعة عثمان ، القاهرة ، مصر. تم إيواء الحيوانات في أقفاص من الفولاذ المقاوم للصدأ وتم تزويدها بالطعام والماء لمدة اسبوع للتالقلم. كانت جميع الحيوانات صحية وخالية من الأمراض إكلينيكيًا . تم تقسيم الجرذان عشوائياً إلى 4 مجموعات متساوية ، احتوت كل مجموعة على 10 فئران وتغذت على إحدى الحميات التالية: المجموعة 1: المجموعة الضابطة وتم تغذيتها علي عليقة طبيعية، ، المجموعة 2: المجموعة مجموعة طبيعية وتم تغذيتها علي عليقة طبيعية مضاف اليها 5% من مطحون نبات الترمس المجموعة 3: المجموعة المصابة بالسكري وتم تغذيتها علي عليقة طبيعية، المجموعة 4: المجموعة المصابة بالسكري وتم تغذيتها علي عليقة طبيعية مضاف اليها 5% من مطحون نبات الترمس. تم جمع عينات الدم من كل مجموعة بعد 30 و 60 يومًا من بداية التجربة. تم طرد عينات الدم عند 3000 دورة في الدقيقة لمدة 15 دقيقة. وتم فصل المصل وحفظها مجمدين حتى التحليلات اللاحقة. وفي نهاية التجربة تم جمع عينات الدم أيضًا في أنابيب EDTA لتحديد المعلمات الدموية. أخيرًا ، أشارت النتائج إلى أن السكري له آثار سلبية على صحة الإنسان والحيوان. أظهرت الدراسة الحالية أن اضافة (الترمس) الي العيقة قلل من مخاطر السكري علي الحيونات حيث عمل تحسين وظائف الكبد والكلي. وبالتالي ، فان استخدام الترمس كجزاء من الوجبات او العلائق المقدمة يعمل علي تحسين العديد من وظائف الجسم المختلفة بلاضافة الي انة يقي من الاثار السلبية للسكري

الكلمات الاسترشادية: السكري، الوقائي, الترمس.