

## Clinico-Pathological Studies on the Effect of Lupinus Albus (Terminis) on Alloxan-Induced Diabetic in Albino Rats

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**Abstract:** This study was carried out to determine the therapeutic effect of *Lupinus albus terminis* on pancreatic B-cells injury induced by alloxan in diabetic rats. Sixty adult albino rats were divided equally into three groups. Group (1) was orally received distilled water for 5 day followed with *Lupinus albus terminis* (750 mg/kg of b.wt.) orally for 35 days. Group (2) was injected intravenously with one dose of alloxan (60 mg/kg b.wt.). Group (3) was injected intravenously with one dose of alloxan (60 mg/kg b.wt) plus *Lupinus albus terminis* (750 mg/kg of b. w) given orally daily at 5 days post alloxan injection. All rats were sacrificed at 40 days post-alloxan injected (dpt). Blood samples were collected for the detection of serum glucose levels and serum insulin level, besides hematological and biochemical parameters. Diabetic rats (gp 2) showed decrease in level of insulin and an increase in blood glucose in blood compared to other groups. In addition to, necrotic and degenerative changes were seen in B-cells of the pancreas in gp 2 (Alloxan-treated rats). However, Group 3 showed significant decrease in serum glucose levels and increased in the lowered serum insulin concentrations with remodeling in B-cells of the pancreas. It could be concluded that *Lupinus albus terminis* has remodeling effect and regeneration for pancreas in rats.

**Key words:** Alloxan • B-Cells • Diabetes Mellitus • *Lupinus albus Terminis*

### INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Previous authors classified diabetes into two common types, insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) and this nomenclature reflected the need for insulin to survive [1].

*Lupinus albus terminis* is an annual plant belonging to the class of Leguminosae and of Rosales order, Dicotyledons group and Spermatophyta phylum, with flower terminating raceme up to 1.5m high [2]. *Lupinus albus terminis* have been cultivated for over 2000 years, originating around the Mediterranean and along the Nile valley where they were used for human consumption [3].

*Lupinus albus terminis* play an important role in human nutrition since they are rich sources of protein, calories, certain minerals and vitamins [4]. *Lupinus albus terminis* are able to triggering the beta cells to increase insulin production which promotes glucose uptake and utilization [5].

The aim of our study was to investigate the effect of *Lupinus albus terminis* (L. Terminis) on blood glucose and insulin concentrations and histopathology of pancreatic B-cells in alloxan induced diabetic rats.

### MATERIALS AND METHODS

**Lupinus Albus Terminis:** Mature dried seeds of white Lupin were obtained from market then grinded with a grinder into a powder dissolved in distilled water and used (750 mg/kg b. w.).

### Chemicals:

- Alloxan monohydrate (2, 4, 5, 6-pyrimidinetetrone) is an oxygenated pyrimidine derivative Catalog No 2244-11-3 and Insulin ELISA Kits Catalog No SE120086. It was obtained from Sigma Company for Pharmaceutical Drugs, USA.
- Kits of glucose Spectrum Catalog No.250 001, Alanine aminotransferase Liquizyme. Catalog No 292 000, Aspartate aminotransferase Liquizyme Catalog No291 000, Urea/BUN-Liquizyme Catalog No 318 001, Creatinine Catalog No235 001 produced by Spectrum Diagnostic Laboratories, Cairo, Egypt.

**Animals:** Sixty adult male albino rats (Six weeks old in age) weighing  $150 \pm 20$  gm were purchased from Laboratory Animal House, Qena, Egypt and maintained, in a specific pathogen-free environment. The study protocol was approved by the Animal Ethics Committee at South Valley University, Qena, Egypt. All animals were allowed to acclimatize in plastic cages (7 animals/ cage) inside a well-ventilated room for 1 week prior to the experiment. The animals were maintained under standard conditions (Temperature of  $23 \pm 3^\circ\text{C}$ , relative humidity of 60-70% and a 12-hour light/dark cycle), fed a diet of standard commercial pellets and given water ad libitum.

**Induction of Diabetes:** After overnight fasting (Deprived of food for 16 h but allowed free access to water), diabetes was induced in the rats by a single intra-venous injection of freshly prepare 2% alloxan monohydrate solution in a dose of 60 mg/kg body [6]. Control rats were injected with citrate buffer alone. Two days after injection the rats with moderate diabetes having hyperglycemia (Blood glucose level higher than 200 mg/dl) were considered diabetic and used for the experiment.

### Experimental Design

#### Sixty Rats Were Divided into 3 Group (N= 20):

**Group (1):** The rats were received distilled water orally for 5 days then treated orally with 750 mg/kg b. w. of Lupinus albus termis seeds powder dissolved in distilled water for 35 days and used as a control.

**Group (2):** The rats were injected with a single intra-venous 2% alloxan monohydrate solution in a dose of 60 mg/kg body. This group served as diabetic group.

**Group (3):** The rats were injected with a single intra-venous 2% alloxan monohydrate solution in a dose of 60 mg/kg body then treated orally with 750 mg/kg b. w. of Lupinus albus termis seeds powder dissolved in distilled water for 35 days.

Blood samples for hematological and biochemical examinations were collected under general anesthesia by using diethyl ether. All rats were sacrificed after 40 dpi. Specimens from pancreas were collected for histopathological examination.

**Hematological Examination:** The blood was used for the examination of complete blood picture (white blood cells count (WBCs), red blood cells count (RBCs), total hemoglobin, hematocrit assays, differential leucocytic count, MCV, MCH and MCHC).

**Biochemical Analysis:** Serum was used for assessment of blood glucose level, serum insulin level, liver function test and kidney function test.

**Histopathological Analysis:** Specimens from pancreas were collected, then fixed in 10% neutral buffered formalin, dehydrated in gradual alcohol series, cleared with xylene and embedded in paraffin wax. Sections about  $5\mu\text{m}$  thickness were prepared and stained with Harries hematoxylin and eosin (H & E.,) for histopathological examinations [7].

**Statistical Analysis:** Statistical analysis was done using one-way analysis of variance (ANOVA). It was done to compare between control and other treated groups, followed by post-hoc analysis (Dunnett's test) using SPSS (Statistical Package for Social Sciences) version 17 according to Borenstein *et al.* [8]. The data were presented in form of Mean  $\pm$  Standard Deviation. The difference was considered statistically significant when  $P < (0.05)$ .

### RESULTS

**Hematological Findings:** Table 1 shows significant decreases in RBCs, hemoglobin and PCV values in gp 2 and significant increases in WBCs count in gp 2 when compared with group 1. However, in gp 3 there is significantly decrease of WBCs until reach to normal group when compared to gp 2, meanwhile non significantly in other parameters (RBCs hemoglobin, PCV, MCH and MCHC) in gp 3 when compared to gp 2 and significantly decrease comparison with gp 1.

Table 1: The Mean  $\pm$  Standard Deviation of the Blood parameters of the albino rats Group (1) (Control), Group (2) (Alloxan monohydrate), Group (3) (Alloxan plus Termis)

Groups	RBCs ( $\times 10^6$ )	WBCs ( $\times 10^3$ )	Hb. (gm/dl)	PCV %	MCV (fl)	MCH (pg)	MCHC %
Group (1)	4.6 $\pm$ 0.1	7.3 $\pm$ 1.1	14.0 $\pm$ 0.5	42.2 $\pm$ 1.9	90.3 $\pm$ 2.5	31.0 $\pm$ 0.7	33.3 $\pm$ 0.15
Group (2)	3.4 $\pm$ 0.2 <sup>a</sup>	12.6 $\pm$ 3.5 <sup>a</sup>	10.9 $\pm$ 0.7 <sup>a</sup>	32.7 $\pm$ 2.3 <sup>a</sup>	95.0 $\pm$ 1.0	31.5 $\pm$ 0.5	33.2 $\pm$ 0.3
Group (3)	3.8 $\pm$ 0.8	6.7 $\pm$ 2.1	11.8 $\pm$ 1.1	35.4 $\pm$ 3.3	95.7 $\pm$ 12.1	32.0 $\pm$ 4.0	33.5 $\pm$ 0.5

a  $\rightarrow$  The mean difference is significant in comparison with group 1 at 0.05.

Table 2: The Mean  $\pm$  Standard Deviation of the differential leucocytic counts of the albino rats of group (1) (Control), group (2) (Alloxan monohydrate) and group (3) (Alloxan plus terms)

Parameters	Differential Leucocytic Count				
	Neutrophil	Lymphocytes	Monocytes	Eosinophil	Basophils
Group (1)	28.7 $\pm$ 4.1	74.3 $\pm$ 12.0	2.7 $\pm$ 2.1	0.3 $\pm$ 0.5	0 $\pm$ 0.0
Group (2)	60.7 $\pm$ 5.0 <sup>a</sup>	34.0 $\pm$ 8.0 <sup>a</sup>	5.3 $\pm$ 3.1	0 $\pm$ 0.0	0 $\pm$ 0.0
Group (3)	45.0 $\pm$ 5.0 <sup>ab</sup>	51.7 $\pm$ 3.2	3.3 $\pm$ 2.3	0 $\pm$ 0.0	0 $\pm$ 0.0

a  $\rightarrow$  The mean difference is significant in comparison with group 1 at 0.05.

b  $\rightarrow$  The mean difference is significant in comparison with diabetic group (G. 2) at 0.05.

Table 3: The Mean and Standard Deviation of blood glucose level and serum insulin level of group (1) (Control), group (2) (Alloxan monohydrate) and group (3) (Alloxan plus terms)

	Blood glucose level (mg/dl)	Serum insulin level (IU/l)
Group (1)	86.0 $\pm$ 5.2	17.0 $\pm$ 1.0
Group (2)	585.0 $\pm$ 4.3 <sup>a</sup>	10.3 $\pm$ 1.5 <sup>a</sup>
Group (3)	211.6 $\pm$ 10.4 <sup>a</sup>	12.5 $\pm$ 2.5

a  $\rightarrow$  The mean difference is significant in comparison with groups 1 at 0.05.

Table 4: The Mean and Standard Deviation of liver function tests of group (1) (Control), group (2) (Alloxan monohydrate) and group (3) (Alloxan plus terms)

	Liver Function Tests	
	ALT (IU/l)	AST (IU/l)
Group (1)	48.3 $\pm$ 5.1	26.3 $\pm$ 2.5
Group (2)	55.3 $\pm$ 3.5 <sup>a</sup>	31.7 $\pm$ 2.5
Group (3)	47.7 $\pm$ 3.5	28.2 $\pm$ 1.8

a  $\rightarrow$  The mean difference is significant in comparison with groups 1 at 0.05.

Table 5: The Mean and Standard Deviation of kidneys function tests of group (1) (Control), group (2) (Alloxan monohydrate) and group (3) (Alloxan plus terms)

	Kidney Function Tests	
	Urea (mg/dl)	Creatinine (mg/dl)
Group (1)	82.0 $\pm$ 2.0	1.6 $\pm$ 0.1
Group (2)	100.6 $\pm$ 2.1 <sup>a</sup>	2.9 $\pm$ 0.2 <sup>a</sup>
Group (3)	95.0 $\pm$ 2.6 <sup>a</sup>	2.5 $\pm$ 0.4 <sup>a</sup>

a  $\rightarrow$  The mean difference is significant in comparison with groups 1 at 0.05.

Table 2 shows significant increases in neutrophil percentage in group 2, 3 when compared with control group 1. While group 3 showed significant decrease when compared with diabetic group (gp 2). On the contrast, lymphocytes % recorded significant decrease in group 2 when compared with (gps 1, 3). Monocytes, eosinophils and basophils percentage showed non-significant changes in groups 2 in when compared with control group 1.

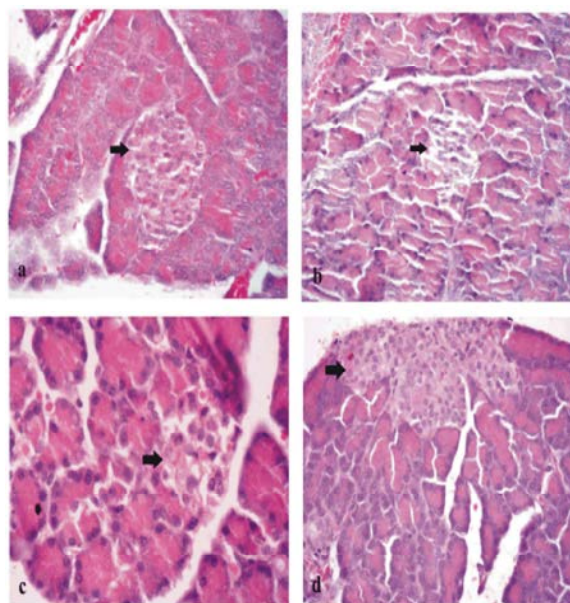


Fig. 1 (a-d): The pancreas in gp 1 (control terms), normal architecture with normal Langerhans cells (a), gp 2, necrosis with disappearance in B-cells of Langerhans (b), in addition to, small scattered of island cells with vacuolar degeneration in acini epithelial cells (c), gp 3, regenerative change in the acinar epithelial cells with hypercellularity in beta cells. (d). (H&E., x 150, 400)

Table 3 showed that group 3 had significant decreases in blood glucose level and slightly significant increases in serum insulin level in comparison with group (2) and non-significantly change when compared with the group 1.

Table 4 shows non significant changes in AST values when compared with the control group1, while serum ALT displayed significant increase in its level in group 2 when compared with group 1. While, group 3 showed no significant comparison with control group1.

Table 5 shows significant increase of urea and creatinine among groups 2, 3 when compared with group 1.

**Histopathology:** The rats of group 1 which were orally treated with *Lupinus albus termis* showed apparently normal acini and Langerhans (Fig. 1a). Pancreas of group 2 which was injected intravenous with one dose of alloxan (60 mg/kg b.wt.) revealed necrosis with disappearance in B-cell of Langerhans (Fig. 1b) and small scattered island cells with vacuolar degeneration in acini epithelial cells in group2 (Fig. 1c). Moreover, group 3 which was received a single intra-venous 2% alloxan monohydrate solution in a dose of 60 mg/kg b.wt. plus 750 mg/k g b.w. of *Lupinus albustermis* exhibited regenerative change with hypercellularity in epithelial cells of acini in addition to increased number of Beta and alpha cells in island of Langerhans (Fig. 1d).

## DISCUSSION

Diabetes mellitus is a systemic metabolic disease characterized by hyperglycemia, hyper lipidemia, hyper aminoacidemia and hypo insulinemia; it leads to decrease in both insulin secretion and insulin action [9]. It is frequently associated with the development of micro and macro vascular diseases which include neuropathy, nephropathy, cardiovascular and cerebrovascular diseases [10].

In group (2), anemia with significant decreases in RBCs, hemoglobin, & PCV values and significant increases in WBCs was observed. The anemic condition that occurred is due to the increased non-enzymatic glycosylation of red blood cell membrane proteins [11]. Oxidation of these proteins and hyperglycaemia in DM cause an increase in the production of lipid peroxides, a marker of oxidative stress in diabetes which consequently have toxic effects on cells through degradation to highly toxic hydroxyl radicals that lead to haemolysis of red blood cell [12]. An increase in the total WBC count of gp2 was recorded. The leucocytes are the mobile units of the

body's defensive mechanism. The cause of enhancement of WBC content may affect the defensive mechanism against the pathophysiological conditions in the body including autolysis caused by some hydrolytic enzymes released by plasma under stress [13].

In our work there were significant increase in blood glucose level and significant decreases in serum insulin level of group (2). The increase in blood glucose level and the decreases in insulin values as a result of the specific necrosis of the pancreatic beta cells [14]. The resulting insulinopenia causes a state of experimental diabetes mellitus called 'Alloxan diabetes' [15]. Alloxan selectively destroys the insulin-producing beta-cells found in the pancreas, hence it is used to induce diabetes in laboratory animals. The toxic action of alloxan on pancreatic beta cells involves oxidation of essential sulphhydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis [16].

In the present study, significant increases were observed in the activity level of serum ALT, urea and creatine in group(2). Therefore, the increment of the activities of AST and ALT in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream [17], which gives an indication on the hepatotoxic effect of alloxan. While the increased concentrations of urea and creatinine due to excessive lipolysis in severe diabetes mellitus leading to ketosis and later on to acidosis. Kidney maintains optimum chemical composition of body fluid by acidification of urine and removal of metabolic wastes such as urea, uric acid and creatinine. During renal diseases the concentration of these metabolites increases in blood [18]. Histopathological examination that revealed vacuolation in epithelial lining of intra lobular duct and disappearance of B-cells of Langerhans with necrosis in acinar epithelial cells induced by alloxan in group ((2). Rapid uptake by insulin-secreting cells has been proposed to be one of the important features determining alloxan diabetogenicity. Further, in long standing diabetes mellitus interstitial fibrosis of the exocrine tissue has been reported [19].

In group (3) administration of *Lupinus albus termis* resulted in significant decreases in blood glucose level and slightly significant increases in serum insulin level due to *Lupinus albus termis* able to triggering the beta cells to increase insulin production which promotes glucose uptake and utilization. Another hypoglycemic mechanism attributed such results to the higher protein and fiber content of *L. termis* stimulating higher insulin response, in addition to several photochemical found in *L. termis* [5]. Histopathological examination revealed

that regenerative change in the acinar epithelial cells with hyper cellularity in beta cells. due to the activities of L-Termis that acted in a way in preventing the death of beta cells and /or helped in the recovery of partially destroyed beta cells [20].

## CONCLUSIONS

It could be concluded that *Lupinus albus* can be used as antidiabetic agent due to its remodeling effect for reparation of pancreas in rats.

## REFERENCES

- World Health Organization (WHO), 1999. Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications: Report of WHO Consultation. WHO/ NCD/NCS/99.2.Geneva.
- Gill, N.T. and K.C. Vear, 1980. Agricultural botany. 3<sup>rd</sup> ed. London: Gerald Duckworth and Co. Ltd., pp: 1321.
- Clapham, A.R., T.G. Tutin and D.M. Moore, 1987. Flora of british Isles. 3<sup>rd</sup> ed. Cambridge University Press, pp: 840-841.
- El-Maki, H.B., S.M. Abdel Rahaman, W.H. Idris, A.B. Hassan, E.E. Babiker and A.H. El Tinay, 2007. Content of antinutritional factors and HCl-extractability of minerals from white bean (*Phaseolus vulgaris*) cultivars: Influence of soaking and/or cooking. Food Chem., 100: 362-368.
- Hall, R.S., S.J. Thomas and S.K. Jahnson, 2005. Australian sweet lupin Flour addition reduces the glycemic index of a white bread breakfast without affecting palatability in the healthy human volunteers, Asia, Pacific, J. Clin Nutr., 14: 91-97.
- Lenzen, S., 2008. The Mechanisms of Alloxan and Streptozotocin-induced Diabetes. Diabetologia, 51(2): 216-226. doi:10.1007/s00125-007-0886-7. PMID 18087688.
- Drury, R.A.B. and E.A. Wallington, 1980. Carleton's Histological Technique, 5<sup>th</sup> ed. Oxford University Press, New York.
- Borenstein, M., H. Rothstein and J. Cohen, 1997. Sample power statistics 1.0.SPSS, Inc., Chicago.
- Wadkar, K.A., C.S. Magdum, S.S. Patil and N.S. Naikwade, 2008. Antidiabetic potential and Indian medicinal plants. J. Herbal Med. Toxicol., 2: 45-50.
- Ruffa, M.J., G. Ferraro, M.L. Wagner, M.L. Calcagno, R.H. Campos and L. Cavallaro, 2002. Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. J. Ethnopharmacol., 79: 335-339.
- Oyedemi, S.O. and D.A. Akinpelu, 2011. Antidiabetic and haematological effect of aqueous extract of stem bark of *Azizelia africana* on streptozotocin induced diabetic wistar rats. Asian Pac J. Trop Biomed., 1(5): 353-358.
- Halliwell, B. and J.M. Gutteridge, 1990. Role of free radicals and catalytic metal ions in human disease: An overview. Meth. Enzymol., 186: 1-85.
- Fortes, Z.B., S.P. Farsky, M.A. Oliveira and J. Garcia-Leme, 1991. Direct vital microscopic study of defective leucocyte-endothelial interaction in diabetes mellitus. Diabet. J., 40: 1267-1273.
- Peschke, E., H. Ebel, H.J. Brömme and D. Peschke, 2000. Classical' and 'New' diabetogens-comparison of their effects on isolated rat pancreatic islets in vitro. Cell Mol Life Sci., 57: 158-164.
- McCance, D.R., R.L. Hanson, M.A. Charles, L.T.H. Jacobsson, D.J. Pettitt and P.H. Bennett, 1994. Comparison of tests for glycated hemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. Brit Med. J., 308: 1323-1328.
- Dhanesha, N., A. Joharapurkar, G. Shah, V. Dhote, S. Kshirsagar, R. Bahekar and M. Jain, 2012. Exendin-4 activates glucokinase. J. Diabet., doi: 10.1111/j.1753-0407.2012.00193.x.
- Navarro, C.M., P.M. Montilla, A. Martin, J. Jimenez and P.M. Utrilla, 1993. Free radicals scavenger and antihepatotoxic activity of *Rosmarinus*. Plant Med., 59: 312-314.
- Virdi, J., S. Sivakami, S. Shahani, A.C. Suthar, M.M. Banavalikar and M.K. Biyani, 2003. Antihyperglycemic effects of three extracts from *Momordica charantia*. J Ethnopharmacol., 88: 107-111.
- Rahier, J., J. Wallon and S. Loozen, 1983. The pancreatic polypeptide cells in the human pancreas: the effects of age and diabetes. J. Clin. Endocrinol. Metab., 56: 441-444.
- Abd Elaziz, E.A., 2011. Pathological and biochemical studies on the effect of *Trigonella foenum-Graecum* and *Lupinus termis* in alloxan induced Diabetic rats, World Appl. Sci. J., 12: 1839-1850.