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Hypoglycemic Effect of the Aqueous Extracts of *Lupinus albus*, *Medicago sativa* (Seeds) and Their Mixture on Diabetic Rats

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ABSTRACT:

Objectives: The number of people suffering diabetes mellitus is increasing worldwide at an alarming rate. A huge number of populations in the world are entirely dependent on traditional medications. This practice may be due to their safety, effectiveness, and availability as well as their fewer side effects when compared to the synthetic hypoglycemic agents. The present study was carried out to investigate and compare the activity of *Lupinus albus* (seeds), *Medicago sativa* (seeds) and the mixture of both plants seeds on some biochemical, hematological and histological parameters in alloxan-induced diabetic rats.

Material and method: Twenty-five male adult albino rats were divided into two groups: *group 1*: control group (five animals) and *group 2*: alloxan induced diabetic rats. Diabetic rats were further divided into four subgroups, five animals each. *Subgroup1*: diabetic untreated rats; *subgroup 2*: diabetic rates treated with aqueous extract of *Lupinus albus* seeds; *subgroup 3*: diabetic rats treated with aqueous extract of *Medicago sativa* seeds; and finally *subgroup4*: diabetic rats treated with aqueous extract of the mixture of *Lupinus albus* and *Medicago sativa* seeds. After thirty days of treatment all rats were sacrificed, blood sample were collected to estimate some biochemical and hematological parameters. Liver samples were collected to determine their glycogen content and pancreatic samples were obtained and processed for microscopic and quantitative evaluation.

Results: In diabetic group, there was reduction in body weight's, hyperglycemia, hypoinsulinemia, significant increase in some parameters of liver and kidney functions as well as significant changes in lipids profile and proteins level with significant decreased liver glycogen content. All treated groups restored most of the mentioned parameters to their normal values. Moreover, these treatments recorded partial improvement in the histopathological changes produced by alloxan.

Conclusion: The aqueous extract of *Lupinus albus* or *Medicago sativa* (seeds) or by their mixture has hypoglycemic and hypolipidemic effects by increasing insulin level and decreasing insulin resistance. In addition, they ameliorate most complications of diabetes.

Key words: Alloxan; Hypoglycemia; Diabetes; *Lupinus albus*; *Medicago sativa*

Introduction:

Diabetes mellitus is a chronic metabolic disease with life-threatening complications. The International Diabetes Federation (IDF) estimates that 285 million people (6.4% of the world population) suffered diabetes in 2010 and this prevalence will increase to 439 million people, 7.7% of the world population by 2030[1]. Despite the great efforts invested in diabetes research, its prevalence continues to grow, and the current medications cannot cover all of the symptoms and complications of the disease [2]. Indeed, alternatives are clearly needed because of inability of the current medications to control most of the pathological effects of diabetes and the high cost and poor availability of current medications for many populations, particularly in developing countries [3]. Most people in Africa rely on herbal concoctions for their primarily health care, but so far, scientific studies supporting the use of plants in traditional medicine remain poor [4].

Lupine is a medicinal food plant with potential value in the management of diabetes. The seeds of *Lupinus termis* are used in the Middle East and Africa as food and in folk medicine. In traditional medicine, the seeds are

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reputed to be effective for diabetes [5]. Many herbal remedies have so far been employed for the treatment and management of various ailments since the beginning of human civilization. *Medicago sativa* (Linn.) family Fabaceae has long been used as traditional herbal medicine in China, Iraq, Turkey, India, and America for the treatment of a variety of ailments [6].

The present study was undertaken to evaluate the antihyperglycemic effects of *Lupinus albus* and *Medicago sativa* (seeds) commonly used in Egyptian folk medicine, in diabetic treatment. In addition, to study the effect of using their mixture as a type of combination therapy because of the complexity of the phytochemicals and bioactivities due to plants interaction.

Material and methods:

Twenty five adult male albino rats of local strain with body weight ranging between (120-140 gm) were divided into two groups asfollows**:** ***group I***= control non diabetic rats, five rats; ***Group II*** = alloxan-induced diabetic which were further divided into four subgroups, five rats each; ***subgroup1***: diabetic untreated, ***subgroup2***: diabetic treated with aqueous extract of *Lupinus albus* seeds (7mg/ 100gm b.wt. daily) by gastric intubation, ***subgroup3***: diabetic treated orally with aqueous extract of *Medicago sativa seeds* (7mg/ 100gm b.wt. daily) and finally ***subgroup4***: diabetic treated orally with aqueous extract of the mixture of *Lupinus albus* and *Medicago sativa* seeds (14mg/ 100gm b.wt. daily).

Induction of diabetes mellitus was done by giving subcuatanous freshly prepared alloxan solution 120 mg/ kg dissolved in 0.5 ml acetate buffer (pH 5.5) to overnight fasting animals. After 48 hours of alloxan injection, rats were fasted overnight (8-10hrs) and administered glucose (3g/kg b.wt.) orally. After 2 hours of glucose administration, blood glucose level was determined by glocometer. The rats with blood glucose levels above 200mg/dl were used for the study [7].

Aqueous extract of *Lupinus albus* seeds: finely grounded *Lupinus albus* seeds then boiling 5g of the seeds powder in 200ml water then filtered. The extract was given to animals (7mg/100g b.wt) orally by oral tube once daily for one month. Aqueous extract of *Medicago sativa* seeds: finely grounded *Medicago sativa* seeds boiling 5g of the seeds powder in 200ml water then filtered. The extract was given to animals (7mg/100g b.wt) orally by oral tube once daily for one month. Aqueous extract of mixture of (*Lupinus albus* and *Medicago sativa* seeds): 5g finely grounded seeds of each of them were boiled in 200ml water then filtered. The extract was given to animals (14mg/100g b.wt) orally by oral tube once daily for one month.

Body weight was determined at the beginning and at the end of the experiment. Blood samples were collected from the orbital plexus using heparinized capillary tubes [8]. Part of the blood was collected on EDTA for hematological studies and the other part collected to separate the serum by centrifugation for 10 min. at 5000 rpm, and the supernatant serum was immediately separated for biochemical analysis. Livers were taken for glycogen determination by using acid-hydrolysis method [9**]**. Pancreases were also taken and processed for the stain with Heamatoxylin and Eosin (HX & E) and modified aldhyde fuchsin [10] for histological study.

Statistical analyses*:*

Data were analyzed using Student (t) test. Data at ($P \leq 0.05$) were considered significant.

Results:

- **Body weight change:**

Treatment with alloxan only led to significant decrease in body weight ($P \leq 0.01$), while the diabetic rats treated with water extract of *Lupinus albus* or *Medicago sativa* (seeds) or by their mixture manifested significant increase in body weight ($P \leq 0.01$).

- **Parameters associated with type II diabetes mellitus:**

The present study investigated some parameters associated with type II diabetes mellitus in order to evaluate the effect of the treatment with water extract of *Lupinus albus* or *Medicago sativa* seed or by the aqueous extract of the mixture as hypoglycemic agent. In diabetic rats liver glycogen and serum insulin recorded significant decrease ($P \leq 0.01$), blood glucose level and HOMA-IR recorded significant increase ($P \leq 0.01$) as compared with control rats. Nevertheless, all treated rats

showed no significant change in serum glucose level, HOMA-IR and liver glycogen when compared with control group except the mixture treated group, which recorded significant increase (P≤0.01) in liver glycogen content. Otherwise, serum insulin level showed significant increase (P≤0.01) in all groups when compared with control group except *Medicago sativa* treated group recorded no significant change in serum insulin level when compared with control group, as shown in table (2).

- **Lipids profile:**

Dislipidemia associated with hypoinsulinemia has been clearly shown in diabetic untreated rats. All serum lipids profile increased significantly (P≤0.01) except HDL-C and HDL-C/LDL-C that recorded significant decrease (P≤0.01), when compared with the control. *Lupinus albus* and *Medicago sativa* treated groups' recorded significant increase (P≤0.01) in total lipids and total cholesterol and no significant change in triglyceride and LDL-C when compared with control group. In addition, HDL-C/LDL-C and VLDL-C showed significant increase P≤ 0.05 and no significant change in *Medicago sativa* treated group. Nevertheless, HDL-C/LDL-C and VLDL-C recorded no significant change and significant increase (P≤ 0.05) in *Lupinus albus* treated group. Additionally, HDL-C recorded significant increase (P≤0.05) in *Lupinus albus* and (P≤0.01) in *Medicago sativa*, when compared with control rats. At the same time, all of these parameters recorded significant decrease when compare with diabetic group except HDL-C and HDL-C/LDL-C, which recorded significant increase (P≤0.01). Otherwise, the mixture treated rats reported significant decrease (P≤0.01) in triglyceride and VLDL-C and recorded no significant change in total lipids, total cholesterol and LDL-C while, significant increase P≤0.01 and P≤0.05 in (HDL-C and HDL-C/LDL-C respectively) when compared with control rats.

- **Proteins level:**

Diabetic untreated rat's recorded significant decrease (P≤0.01) in serum proteins level when compared to the control group except serum globulin level, which, recorded significant, increase (P≤0.05). *Lupinus albus*, *Medicago sativa* and the mixture treated groups showed no significant change in all these parameters when compared to the control rats except serum albumin level in *Medicago sativa* treated rats, which recorded significant decrease (P≤0.05).

- **Some parameters of liver function:**

The toxic effect of alloxan on rats' liver tissues led to significant increase (P≤0.01) in ALAT and ASAT activities when compared to the control group. The treatment with *Lupinus albus*, *Medicago sativa,* and the mixture treated rats showed slight improvement in ASAT when compared with diabetic rats but still significant increase (P≤0.05) in both parameters when compared to control animals.

- **Some parameters of kidney functions:**

Hyperglycemia caused nephropathy that was detected by increasing of serum creatinine and urea. In diabetic untreated rats both creatinine and urea levels were significantly increased (P≤0.01)when compared with control rats as illustrated in table (6). Nevertheless, *Lupinus albus*, *Medicago sativa,* and their mixture treated groups recorded no significant change in serum creatinine, but still recorded significant increased (P≤0.05) serum urea when compared with control rats.

- **Some hematological parameters:**

WBCs and lymphocytes which play a pivotal role in initiating immunity were significantly decreased (P≤0.05 and P≤0.01 respectively) in diabetic rats when compared with control group. These abnormalities in the defense mechanisms returned to normal by treatment with both plants and their mixture. At the same time, other hematological parameters, (RBCs count and Hct %) recorded no significant change in diabetic and all treated groups.

- **Some Histological changes:**

The main three types of pancreatic islet cells (alpha, beta, and delta cells) can be identified by the modified aldehyde fuchsin stain. Where, β-cells are occupying the central portion of the islet and contained numerous granules, α-cells occupied the periphery of the islet (granular and polygonal with central spherical nuclei) and δ-cells were usually adjacent to alpha cells (somewhat larger) as observed clearly in plate (2) specially, in the control group's photograph.

Microscopic examination of pancreatic tissues of the diabetic rats displays islets shrinkage, cytoplasmic vacuolation in β-cells with pyknotic nuclei. Otherwise, *Lupinus albus*

and *Medicago sativa* treated rats showed nearly normal architecture of the pancreatic islets. Where, most of β-cells' cytoplasm became granulated with less vacuoles. While, the mixture treated group showed great improvement of the β-cells' shape and nuclei, some of them were still vacuolated.

The numbers of α, β and δ cells of pancreatic islets recorded significant decrease ($p \leq 0.01$) in diabetic untreated rats when compared with control group. While, *Lupinus albus*, *Medicago sativa* and the mixture treated groups demonstrated no significant changes in the number of α-cells and δ-cells, but showed significant increase ($p \leq 0.01$) in the number of β-cells, except *Medicago sativa* treated rats, which recorded significant decrease ($p \leq 0.05$) in the number of β-cells as compared to control rats.

DISCUSSION:

Scientific investigation of traditional herbal remedies for diabetes may provide valuable treatment for the development of alternative drugs and therapeutic strategies [3]. Plants are rich sources of antidiabetic, antihyperlipidemic and antioxidant agents such as flavonoids, gallotannins, amino acids, and other related polyphenols [11-13]. *Medicago sativa* (lucerne) is used as a traditional plant treatment of diabetes [14]. *Lupinus* species and their derivates are good candidates to be used as hypoglycemic agents [15].

In the present results, hyperglycemia and reduction in body weight were recorded in diabetic untreated rats that could be due to sever hypoinsulinemia and increasing insulin resistance. Where, decrease insulin level and insulin sensitivity lead to alteration in carbohydrates metabolism causing hyperglycemia and decreasing liver glycogen content. In addition, stimulating glycogenolysis and gluconeogenesis resulted from intracellular strafing sensation and inhibition of its effect on peripheral utilization of glucose. Moreover, it trigger the release of triglycerides from adipose tissue (lipolysis) and catabolism of amino acids in muscle tissue (proteolysis) or may be a result to fluid loss as reported by **Morley *et al.*** [16]. The present results are in agreement with **Defronzo and Goodman** [17] who reported that decreasing insulin level and sensitivity causing increase in hepatic glucose production, a decrease in peripheral glucose uptake, and significant decrease in the conversion of glucose to glycogen in the liver, which increase glucose level in blood and decrease its intercellular level.

This hypoinsulinemia could be due to the reduction in β-cells mass or β-cells' cytoplasmic vacuolation as shown in table (8) and plate (2). Also, it could be due to its direct effect on β-cells membrane permeability by causing failure of ionic pumps and increasing cell size which inhibits intracellular energy generation by inhibiting enzymes of tricarboxylic acid cycle and Ca^{+2} dependants dehydrogenises in their mitochondrion, causing ATP deficiency, cessation of insulin production and cell necrosis [18-19]. In addition, β-cells' cytoplasmic vacuolation could be due to the diabetogenic effect of alloxan where, it induced highly reactive oxygen radicals which have cytotoxic effect on β-cells as explained by **Fischer** and **Homburger** [20]. The present result is in agreement with the finding of **Kessler *et al.*** [21] who reported that vacuolation of the islet is more prominent in lesions associated with functional islet abnormality and development of hyperglycemia.

On the other hand, HOMA-IR recorded significant increase ($p \leq 0.01$) in diabetic untreated rats that may be due to increase insulin resistance and severe insulin deficiency. This is in agreement with **Reaven *et al.*** [22] who reported that insulin resistance can occur as a secondary manifestation of insulin deficiency. In addition, it may be due to poor intracellular Mg concentrations, as found in type II diabetes mellitus and in hypertensive, patients may result in a defective tyrosine-kinase activity at the insulin receptor level and exaggerated intracellular calcium concentration. Both events are responsible for impairment in insulin action, and a worsening of insulin resistance in non insulin-dependent diabetic and hypertensive patients [23]. In addition, elevated levels of free fatty acids and triglycerides in the blood stream and tissues have been found in many studies to contribute to diminished insulin sensitivity [24-27].

Otherwise, the improvement of serum glucose level and percentage of body weight gain were recorded in rats treated with extract of *Lupinus albus* or *Medicago sativa* (seeds) or

by the mixture is possibly due to regenerate of β-cells, elevate insulin level and improve its sensitivity, which lowers the concentration of glucose in blood. In addition, insulin inhibits hepatic glucose production and stimulates both glucose uptake and metabolism by muscle and adipose tissues [28]. Increased liver glycogen content was associated with lowering blood glucose level. In addition, amelioration of serum insulin level in all treated groups may be due to increase β-cells' mass number and amelioration of their cytoplasmic vacuolation as recoded in this study.

The amelioration effect of *Lupinus albus* treatment in all previous parameters are in agreement with **Farghaly** and **Hassan** [5] who reported that the treatment with *Lupinus albus* improved insulin resistance in dysglycemic subjects, so ameliorates hyperglycemia. Where, row *Lupinus* beans contain several alkaloids, micro- and macronutrients that could modulate; a. the secretagogues activity of alkaloids, b. the potential effect on insulin receptor, and c. insulin half-life [29]. In addition, the improvement in HOMA-IR observed in subjects with dysglycemia that consumed *Lupinus* could also be the result of decrease blood glucose and increase insulin levels [30]. *Lupinus* includes lupanine and sparteine, which are the main quinolizidine alkaloids (QAs) present in many *Lupinu*s species from the old and new world [31]. Sparteine has been shown to induce hypoglycemic when administered to type II diabetic subjects [32]. **Garcia Lopez *et al.*** [33] reported that quinolizinic alkaloids from *Lupinus* species induced insulin release from cultured pancreatic islets from normal rats. Lupanine, the most abundant alkaloids in *Lupinus*, only induced insulin release by the islets that were cultured in high glucose concentrations [33].

Otherwise, the improvement of these parameters by *Medicago sativa* treated rats are in agreement with **Gray** and **Flatt** [14] who reported that *Medicago sativa* have antihyperglycemic, insulin releasing and insulin-like activity as traditional antidiabetic plant. Where, *Medicago sativa* (seeds) contain a number of hypoglycemic principles as trigonelline, which is the N-methyl derivative and main human metabolite of the vitamin nicotinic acid has hypoglycemic effect when administered orally to diabetic patients. It acts by slowing the metabolism of nicotinic acid, which increase glucose uptake from the blood and its subsequent oxidation, if administered orally. Where, it is converted in the body into nicotinamide, which is an inhibitor of the enzyme poly (ADP-ribose) synthesize, responsible for the depletion of NAD from pancreatic β-cells, and is also a potent hydroxyl-radical scavenger, by which mechanisms nicotinamide can prevent the β-cell toxicity of streptozocin and alloxan [34].

In addition, *Medicago sativa* (seeds) contain Mn^{+2}-ions where, the protein tyrosine kinase associated with insulin receptor has been shown to be Mn^{+2}-dependent [35]. Activation of a Mn^{+2}-dependent-protein tyrosine kinase in the transmembrane β-subunit ensues, resulting in phosphorylation of the receptor and other proteins with phosphate groups from ATP [35]. Activation of a phosphatidylinositol-specific phospholipase C leads to hydrolysis of a membrane glycan phosphoinositide. This produces a cyclic inositol phosphate-glucosamine second-messenger that activates phosphodiesterase, decreasing intracellular cyclic adenosine monophosphate (cAMP) and produces diacylglycerol, which activates protein kinase C [36]. Protein kinase C regulates a number of enzymes and the insulin receptor through phosphorylation [37].

The great effect of mixture of both extracts may be attributed to the presence of synergistic effects leading to hypoglycemic effect. This can be due to one or more of the components, which interacting with each other, or one or more ingredients in one of the used plant, which have potentiating effect on the ingredient of the other, plant thus increasing the hypoglycemic effect. More studies should be done to elucidate the active ingredient(s), responsible for this synergistic actions and the native of the patentees' effect if any.

While the significant increase in body weight resulted by treatment with the extract of *Lupinus albus* or *Medicago sativa* (seeds) or by the mixture may be due to inhibition of both lipolysis and proteolysis process as a result of increasing insulin level and decrease insulin resistance. Where, insulin increases glucose up take, inhibits glycogenolysis and gluconeo-

genesis, and stimulates the entry of amino acids into cells and protein synthesis. **Makimattila *et al.*** [38] reviewed that improved glycemia may promote weight gain by reducing the basal metabolic rate.

According to the increased number of the β-cells in all rats treated with plants extracts or their mixture and increased insulin level, it is suggested that these plants could induce betatrophin secretion from the liver and adipose tissues. Where, betatrophin causes proliferation of mice insulin-secreting pancreatic beta cells. These new β-cells only produce insulin when called for by the body, offering the potential for the natural regulation of insulin and a great reduction in the complication associated with diabetes [39].

Alterations of the metabolism of lipids recorded in diabetic untreated rats may be attributed to insulin deficiency and resistance resulted from islets dysfunctions and less sensitive receptor. Hypertriglyceridemia and hypercholesterolemia, which is associated with increasing production of VLDL and decreasing HDL level, was recorded. These findings are in agreement with **Goodman** and **Gilman's** [28] who explained the same result by the inhibition of the transcription of lipoprotein lipase in the capillary endothelium as a result to deficiency of insulin which lead to elevate the plasma chylomicron and VLDL levels, resulting in hypertriacylglycerolemia and associated with low HDL levels. The metabolic abnormalities of type II diabetes are a result of insulin resistance, which led to dislipidemia in the liver where, fatty acids are converted to triacylglycerol that are packaged and secreted in VLDL [40].

Otherwise, the treatment by the extract of *Lupinus albus* or *Medicago sativa* (seeds) or by the mixture was ameliorating this dislipidemia produced by diabetes. Their effect may be attributed mainly to the relative improvement of insulin level and decreased insulin resistance as discussed above where, insulin decreases the level of circulatory free fatty acids by inhibiting the activity of hormone-sensitive lipase that degrades triacylglycerol in adipose tissue. Insulin also increases the lipoprotein lipase activity of adipose tissue, which leads to reduce triglyceride, cholesterol, VLDL-cholesterol, and LDL-cholesterol levels back to normal values [28]. Saponins and other compounds in *Medicago sativa* (alfalfa) act synergistically to bind the bile acids required for cholesterol absorption from the gut or affect cholesterol metabolism indirectly, by interfering with the enterohepatic circulation of bile acids [41]. While, **Newairy *et al.*** [42] reported that treatment of diabetic rats with *Lupinus* normalized triglycerides, cholesterol, LDL, and VLDL levels that support the present results. Where, alfalfa decreased the intestinal absorption of endogenous cholesterol and increased the bile acid excretion. These effects were attributed to the saponin content of the seed [43].

On the other hand, total protein, albumin, and A/G ratio showed significant decrease in diabetic untreated group, which might be attributed to enhanced rate of gluconeogenesis which secondary effect insulin deficiency and increase insulin resistance as discussed above. This process associated with increased production and excretion of urea as recorded in tables (4&6). This explanation is in agreement with **Shafrir** [18] who attributed the decrease of total proteins and albumin in alloxan diabetic rats to enhanced proteolysis in tissues, which lead to reduce production of growth factors and increase growth factor binding protein by a rapid mechanism and slow, long-losing activations of a myofbrillar protease.

The improvement of protein profile in all treated groups are in a harmony with increased serum insulin level and tissues sensitivity where, insulin plays an important role in protein metabolism which stimulates amino acids uptake and protein synthesis and inhibits protein degradation in muscle and other tissues[28].

The elevation of serum ASAT and ALAT activities, which were recorded in the present study, may be attributed to the excessive release of such enzymes from the damaged liver cells into the blood circulation. Where, there is an inverse relationship between the liver activity and the level of enzymes in serum [44]. Moreover, it may be consistent with their greater need for gluconeogenesis substrates or may reflect damage of the hepatic cells due to hepatotoxic effect of alloxan [45].

All treated groups manifested improvement of the toxic effect of alloxan on the liver when compared with diabetic group. The improvement may be due to increase insulin level and sensitivity, which led to inhibition of gluconeogenesis and improvement of carbohydrate metabolism. This effect may be due to lupine in *Lupinus albus* [46] or saponin present in *Medicago sativa* extract, which efficiently protected the hepatic function [47].

Serum creatinine often rises in type II diabetes due to renal arterial disease and/or cardiac failure rather than to diabetic nephropathy [48-49]. Where, hyperglycemia causes kidney damage through glycosylation, activation of protein kinase C, release of several cytokines, and increase activity of the polyol pathway [50]. In addition, this may be resulted from failure of the body to excrete the metabolic products of proteins [49]. Where, proteins metabolic rate increased in diabetes because of gluconeogenesis increasing rate. Moreover, this result can be caused by the hyperglycemia, hypertension, or hyperlipidemia that occurs with diabetes [50].

The present study recorded an improvement in serum creatinine in all extracts treated groups when compared with diabetic animals, which may be due to improvement of the kidney functions and decrease the excessive loss of albumin in urine in diabetic rats. Therefore, based on these findings, antioxidants have a protective effect against the diabetic nephropathy [50]. Where, *Medicago sativa* contain vitamin C which one of the antioxidants [51]. In addition, the extract of *M. sativa* contains the highest amount of polyphenol compounds and exhibits the greatest antioxidant activity through the scavenging of free radicals, which participate in various pathophysiologies of diseases including ageing [52]. While, *Lupinus* has antioxidant defense mechanism by increasing the reduced glutathione content as well as glutathione reductase, glucose 6-phosphate dehydrogenase, superoxide dismutase and catalase enzymes activity [53]. Moreover, *Lupinus* is a potent free radical scavenger, hypolipidemic, and hypoglycemic agent by the effect of lupaine and sparteine. Where, sparteine has ability to bind or chelate the divalent cation, which lead to decrease oxidative stress and oxidation damage in tissue [54-55]. While, the mixture effects may be attributed to synergistic actions of both plants.

On the other hand, serum urea level still showed highly significant increase in rats treated with *Lupinus albus* or *Medicago sativa* (seeds) or by their mixture when compared with normal rats. This may be a secondary effect of hepatotoxicity produced by alloxan and no treatments can ameliorate that.

Conclusion:

Treatment with any one of the three plant extracts can ameliorate most β-cells dysfunction and increase the insulin's receptors sensitivity, which is associated with improvement of most diabetic complications. Further studies should be done using different doses &periods.

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Table (1): Body weight changes in control, diabetic, *Lupinus albus*, *Medicago sativa* and the mixture treated rats.

	Control	Diabetic	The mixture	*Lupinus albus*	*Medicago sativa*
%Body weight change	21.89±0.93	13.26±1.03	44.63 ± 1.22	42.35±1.16	44.09±2.34
a		**	**	**	**
b			**	**	**

Data expressed as Mean ± standard error,
*=P≤0.05 **= P≤0.01, n.s. = non significant,

(a) = compared to control, (b) = compared to diabetic.

Table (2): Parameters associated with DM in normal, diabetic, *Lupinus albus, Medicago sativa,* and the mixture treated male albino rats.

	Control	Diabetic	The mixture	*Lupinus albus*	*Medicago sativa*
Glucose (mg/dl)	**121.8±1.11**	**286.4±3.36**	**125±1.58**	**123.8±1.24**	**123.6±1.57**
%a		**+135.14**%**	**+2.63$^{n.s}$ %**	**+1.64$^{n.s}$ %**	**+1.48$^{n.s}$ %**
%b			**-56.35**%**	**-56.77**%**	**-56.84**%**
Insulin (µu/l)	**4.06±0.04**	**3.01±0.09**	**6.09±0.08**	**4.99±0.1**	**3.57±0.17**
%a		**-25.86** %**	**+50** %**	**+22.91** %**	**-12.07$^{n.s}$ %**
%b			**+102.33** %**	**+22.91** %**	**+18.6** %**
Glycogen content (mg/dl)	**18.18±0.56**	**2.45±0.16**	**33.96±1.01**	**22.39±2.42**	**20.98±2.9**
% a		**-77.27 **%**	**+86.79**%**	**+23.16$^{n.s}$%**	**+15.4$^{n.s}$%**
%b			**+1286.12**%**	**+1262.86**%**	**+756.33**%**
HOMA-IR	**1.22±0.07**	**2.11±0.08**	**1.48±0.16**	**1.35±0.09**	**1.03±0.1**
%a		**+72.95** %**	**+21.31$^{n.s}$%**	**+18.85$^{n.s}$ %**	**-15.57$^{n.s}$ %**
%b			**-29.85** %**	**-31.28**%**	**-51.18** %**

Data expressed as: Mean ± standard error, % = percentage of change,
*=P≤0.05, **= P≤0.01, n.s. = non significant,
(+) = Increased from control, (-) = Decreased from control
(a) = compared to control, (b) = compared to diabetic.

Table (3): Lipids profile in normal, diabetic, *Lupinus albus, Medicago sativa* and the mixture treated male albino rats

	Control	Diabetic	The mixture	*Lupinus albus*	*Medicago sativa*
T. Lipid	**303.8±2.08**	**436.2±1.36**	**302.8±3.43**	**330.6±2.16**	**338.8±2.6**
%a		**+43.58**%**	**-0.33$^{n.s}$%**	**+8.82**%**	**+11.52**%**
%b			**-30.58**%**	**-24.21**%**	**-22.33**%**
T. cholesterol (mg/dl)	**109.4±1.96**	**199±1.87**	**105.4±1.33**	**121±2.61**	**126.2±2.48**
%a		**+81.9**%**	**-3.66$^{n.s}$ %**	**+10.6**%**	**+15.36**%**
%b			**-47.04**%**	**-39.2**%**	**-36.58**%**
Triglycerides (mg/dl)	**85±1.37**	**92.2±1.02**	**72±1.92**	**87.76±1.34**	**87.56±1.36**
%a		**+8.47**%**	**-15.29** %**	**+3.25$^{n.s}$ %**	**+3.01$^{n.s}$%**
%b			**-21.91 **%**	**-4.82*%**	**-5.03*%**
HDL-C (mg/dl)	**25.2±0.73**	**19.2±0.97**	**29±0.72**	**28.4±0.81**	**32±0.95**
%a		**-23.8** %**	**+15.08** %**	**+12.7*%**	**+26.98** %**
%b			**+51.04** %**	**+47.92**%**	**+66.67** %**
LDL-C (mg/dl)	**67.2±2.34**	**117.36**±1.2**	**63.6±1.72**	**73.76±2.94**	**74.56±2.85**
%a		**+74.64 %**	**-5.36$^{n.s}$ %**	**+9.62$^{n.s}$ %**	**+10.95$^{n.s}$ %**
%b			**-45.81**%**	**-37.15**%**	**-36.47** %**
(HDL-C)/ (LDL-C)	**0.38± 0.02**	**0.25± 0.01**	**0.46± 0.02**	**0.39± 0.03**	**0.43± 0.01**
%a		**-34.21**%**	**+21.05*%**	**+2.63$^{n.s}$ %**	**+13.16*%**
%b			**+84**%**	**+56**%**	**+72**%**
VLDL-C (mg/dl)	**17±0.28**	**64.4**±1.96**	**14.4±0.38**	**19.4±0.93**	**18.44±0.79**
%a		**+278.82%**	**-15.29** %**	**+14.11*%**	**+8.47$^{n.s}$%**
%b			**-78.04**%**	**-69.87**%**	**-71.37** %**

Data expressed as:
Mean ± standard error, % = percentage of change,
*=P≤0.05, **= P≤0.01, n.s. = non significant,
(+) = Increased from control, (-) = Decreased from control
(a) = compared to control, (b) = compared to diabetic.

Table (4): Proteins level in normal, diabetic, *Lupinus albus*, *Medicago sativa* and the mixture treated male albino rats.

	Control	Diabetic	The mixture	*Lupinus albus*	*Medicago sativa*
Total protein (g/dl)	**6.34±0.11**	**5.88±0.09**	**6.2±0.07**	**6.24±0.1**	**6.06±0.16**
%a		**-7.26** %**	**-2.21$^{n.s}$ %**	**-1.58$^{n.s}$ %**	**-4.42$^{n.s}$%**
%b			**+5.44*%**	**+6.12*%**	**+3.06$^{n.s}$%**
Albumin (g/dl)	**3.54±0.13**	**2.84±0.07**	**3.28±0.08**	**3.3±0.07**	**3.14±0.08**
%a		**-19.77** %**	**-7.34$^{n.s}$ %**	**-6.78$^{n.s}$ %**	**-11.3*%**
%b			**+15.49** %**	**+16.2** %**	**+10.56*%**
Globulin (g/dl)	**2.8±0.19**	**3.3±0.06**	**2.9±0.12**	**2.98±0.1**	**2.64±0.1**
%a		**+17.86* %**	**+3.57$^{n.s}$ %**	**+6.43$^{n.s}$ %**	**-5.72$^{n.s}$ %**
%b			**-12.12* %**	**-9.7* %**	**-20** %**
A/G ratio	**1.29±0.06**	**0.86±0.03**	**1.14±0.07**	**1.11±0.04**	**1.19± 0.05**
%a		**-33.33** %**	**-11.63$^{n.s}$ %**	**-13.95$^{n.s}$ %**	**-7.75$^{n.s}$%**
%b			**+32.55* %**	**+29.07* %**	**+38.37**%**

Data expressed as: Mean ± standard error, % = percentage of change,
*=P≤0.05, **= P≤0.01, n.s. = non significant,
(+) = Increased from control, (-) = Decreased from control
(a) = compared to control, (b) = compared to diabetic.

Table (5): Parameters of liver function in control, diabetic, *Lupinus albus*, *Medicago sativa*, and the mixture treated male albino rats.

	Control	Diabetic	The mixture	*Lupinus albus*	*Medicago sativa*
ALAT (U/ml)	**8±0.32**	**10.2±0.37**	**9.4±0.4**	**9.4±0.24**	**9.7±0.51**
%a		**+27.5** %**	**+17.5*%**	**+17.5*%**	**+21.25*%**
%b			**-7.84$^{n.s}$ %**	**-7.84$^{n.s}$ %**	**-4.9$^{n.s}$ %**
ASAT (U/ml)	**7.4±0.51**	**11.2±0.37**	**9.8±0.37**	**9.6±0.51**	**9.8±0.58**
%a		**+ 51.35**%**	**+32.43* %**	**+29.73*%**	**+32.43*%**
%b			**-12.5* %**	**-14.28*%**	**-12.5$^{n.s}$%**

Data expressed as: Mean ± standard error, % = percentage of change,
*=P≤0.05, **= P≤0.01, n.s. = non significant,
(+) = Increased from control, (-) = Decreased from control.
(a) = compared to control, (b) = compared to diabetic.

Table (6): Parameters of kidney function in control, diabetic, *Lupinus albus*, *Medicago sativa* and the mixture treated male albino rats.

	Control	Diabetic	The mixture	*Lupinus albus*	*Medicago sativa*
Creatinine (mg/dl)	**0.62±0.04**	**0.82±0.04**	**0.66±0.02**	**0.68±0.04**	**0.72±0.04**
%a		**+32.25** %**	**+6.45$^{n.s}$ %**	**+9.68$^{n.s}$ %**	**+16.13$^{n.s}$%**
%b			**-19.51** %**	**-17.07* %**	**-12.2$^{n.s}$%**
Urea (mg/dl)	**18.4±0.5**	**28.4±0.51**	**21.4±1.33**	**23.2±1.59**	**23.8±1.56**
%a		**+ 54.35** %**	**+16.3*%**	**+26.09*%**	**+29.34* %**
%b			**-24.65**%**	**-18.31*%**	**-16.2$^{n.s}$ %**

Data expressed as:
Mean ± standard error, % = percentage of change,
*=P≤0.05, **= P≤0.01, n.s = non significant,
(+) = Increased from control, (-) = Decreased from control.
(a) = compared to control, (b) = compared to diabetic.

Table (7): Some hematological parameters in control, diabetic, *Lupinus albus, Medicago sativa,* and the mixture treated male albino rats.

	Control	Diabetic	The mixture	*Lupinus albus*	*Medicago sativa*
RBCs count ($X10^6$/µl)	**8.94±0.29**	**8.35 ±0.26**	**8.58±0.18**	**9.45±0.52**	**8.07±0.26**
%a		**-6.59$^{n.s}$ %**	**-4.03$^{n.s}$ %**	**+5.7$^{n.s}$ %**	**-9.7$^{n.s}$%**
%b			**+2.75$^{n.s}$ %**	**+13.17$^{n.s}$ %**	**-3.35$^{n.s}$ %**
Hct%	**42.92±1.52**	**40.2 ±1.71**	**42.57±0.43**	**45.28±1.69**	**39.46±0.78**
%a		**- 6.34$^{n.s}$ %**	**-0.81$^{n.s}$ %**	**+5.5$^{n.s}$ %**	**-8.06$^{n.s}$ %**
%b			**-3.69$^{n.s}$ %**	**+2.44$^{n.s}$ %**	**-10.72** %**
WBCs ($X10^3$/µl)	**9±0.35**	**7.78±0.16**	**8.12±0.18**	**8.24 ±0.29**	**8.34±0.33**
%a		**-13.56 *%**	**-9.78$^{n.s}$ %**	**-8.44$^{n.s}$%**	**-7.33$^{n.s}$%**
%b			**+4.37$^{n.s}$ %**	**+5.91$^{n.s}$%**	**+7.2$^{n.s}$ %**
Lymph ($X10^2$/µl)	**90.02±1.42**	**82.58±1.62**	**90±1.33**	**90.2±1.34**	**88.58±1.66**
%a		**-8.26** %**	**-0.02$^{n.s}$ %**	**+0.19$^{n.s}$ %**	**-1.29$^{n.s}$ %**
%b			**+8.99** %**	**+9.23** %**	**+7.27* %**

Data expressed as:

Mean ± standard error, % = percentage of change,
*=P≤0.05, **= P≤0.01, n.s. = non significant,
(+) = Increased from control, (-) = Decreased from control.
(a) = compared to control, (b) = compared to diabetic.
Lymph= Lymphocyte

Table (8): Some histological changes in control, diabetic, *Lupinus albus, Medicago sativa* and the mixture treated male albino rats.

	Control	Diabetic	The mixture	*Lupinus albus*	*Medicago sativa*
A-cell count	**3.67±0.33**	**1.67±0.33**	**3.33±0.33**	**3.67±0.33**	**3.67±0.33**
%a		**-54.5** %**	**+10.21$^{n.s}$%**	**0$^{n.s}$ %**	**0$^{n.s}$ %**
%b			**+99.4**%**	**+119.76**%**	**+119.76**%**
B-cell count	**91.67±0.88**	**57.33±0.88**	**132±1.53**	**102.67±1.45**	**87.33±3.18**
%a		**-37.46** %**	**+44** %**	**+12** %**	**-4.73*%**
%b			**+130.25** %**	**+79.09** %**	**+52.32** %**
D-cell count	**4.67±0.33**	**2.33±0.33**	**3.33±0.33**	**5.33±0.67**	**3.67±0.67**
%a		**-50.1** %**	**-28.69$^{n.s}$%**	**+14.13$^{n.s}$%**	**-21.41$^{n.s}$%**
%b			**+42.92$^{n.s}$ %**	+128.76**%	**+47.51$^{n.s}$ %**

Data expressed as:

Mean ± standard error, % = percentage of change,
*=P≤0.05, **= P≤0.01, n.s. = non significant,
(+) = Increased from control, (-) = Decreased from control.
(a) = compared to control, (b) = compared to diabetic.

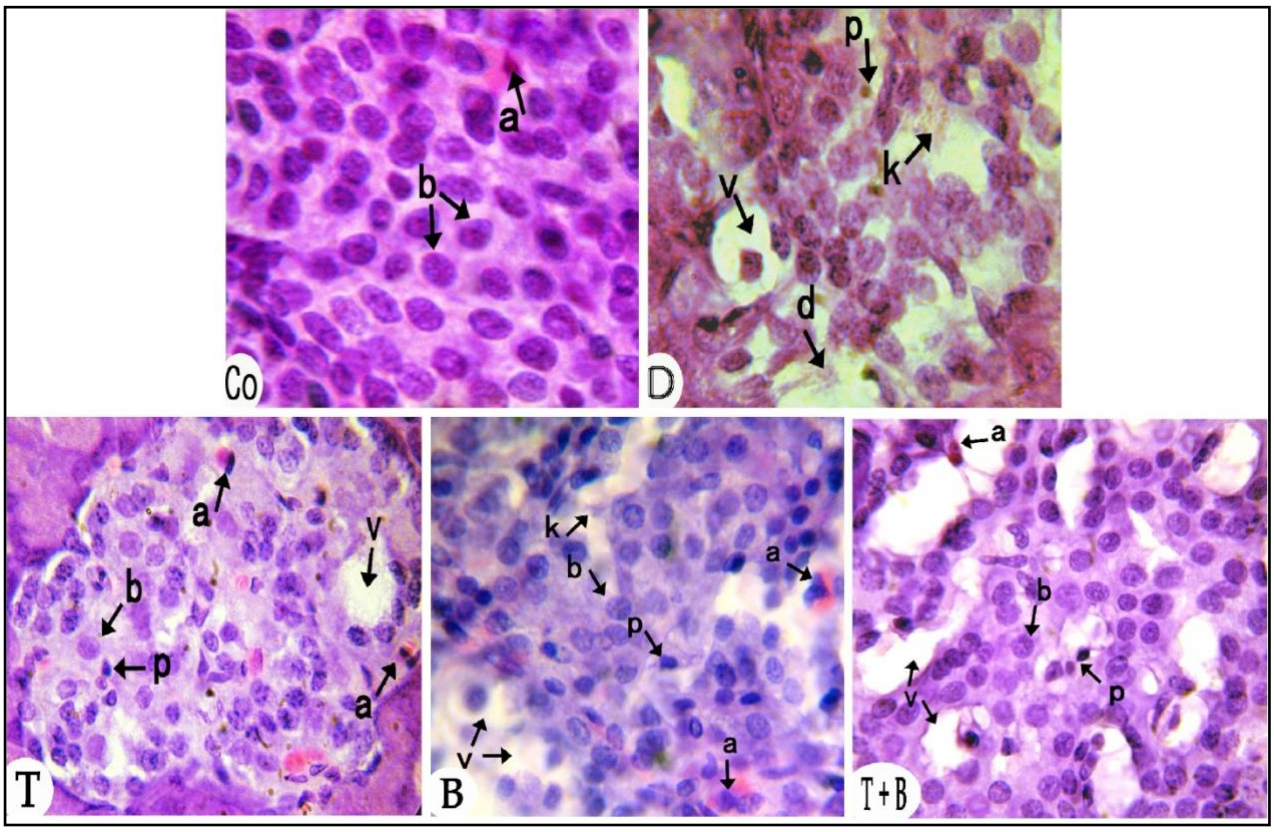

Plate (1): Photomicrograph of pancreatic islets of Control (Co.), Diabetic (D), *Lupinus albus* (T) and *Medicago sativa* (B) and the mixture (T+B) treated rats stained by heamatoxylin and eosin stain (X 1000).

Where,

a; normal alpha cell	b; normal beta cell
P; pyknotic beta cell	v; vacuolated beta cell
k; beta cell karyorrhexis	d; degenerated beta cell

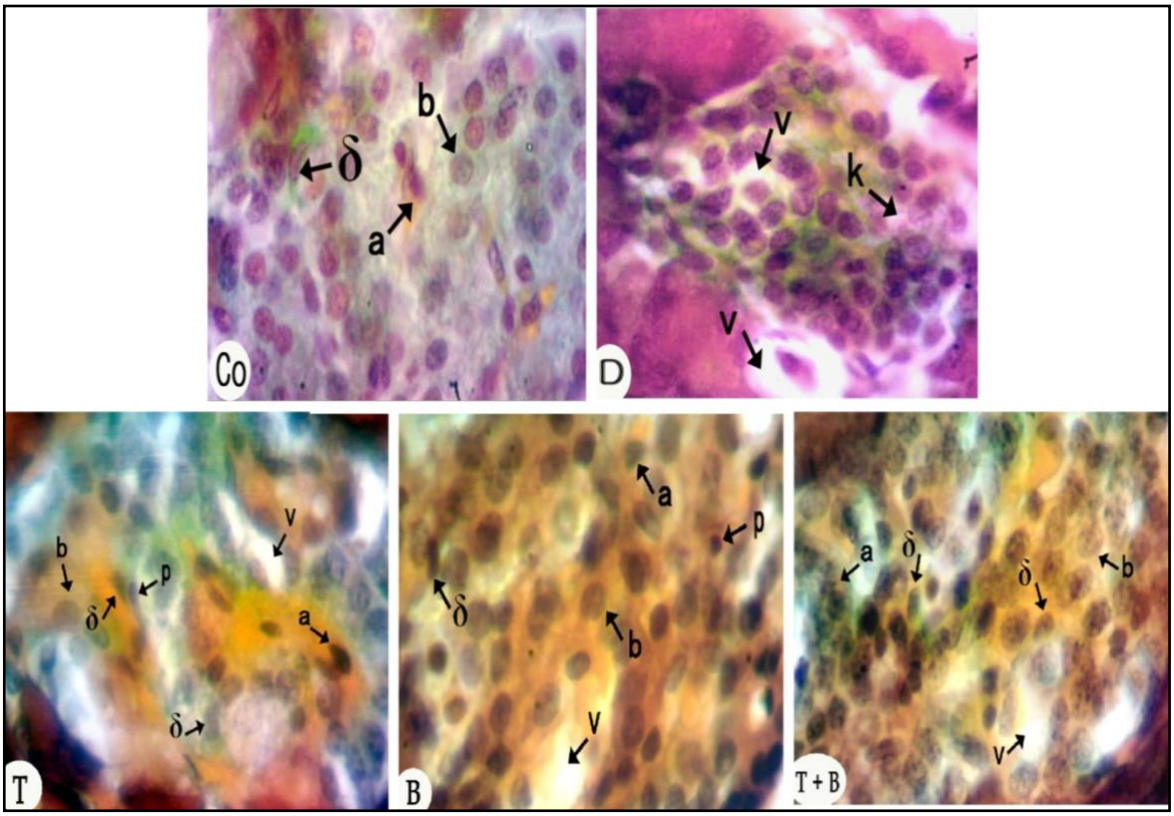

Plate (2): Photomicrograph of pancreatic islets of Control (Co.), Diabetic (D), *Lupinus albus* (T), *Medicago sativa* (B) and the mixture (T+B) treated rats stained by Modified aldhyde Fuchsin stain (X 1000).

Where,

a; normal alpha cell
b; normal beta cell
k; beta cell karyorrhexis
v; vacuolated beta cell
δ; normal delta cell
P; pyknotic beta cell