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Physiological Effect Of Peri winkle (*C.roseus)* On Diabetic Albino Rat

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ABSTRACT

Back ground: Diabetes mellitus (DM) is a major health problem worldwide in recent time. Asia and Africa are the most viable areas where the disease is feared to raise 2–3 folds. Many herbal products have been recommended for the treatment of DM in ancient literature of Ayurveda in India and other worldwide.

Material and method: Thirty male adult albino rats were used to investigate the effect of *Catharanthus rosea* (*C.roseus*) on diabetic rats. Rats were divided into three equal groups, control, diabetic non treated and diabetic *C.roseus* treated groups. After thirty days of treatment all rats of each group were sacrificed. The body weight of each rat was determined at the beginning and the end of each period. Blood glucose, serum insulin, lipid and protein profiles, liver and kidney functions, blood picture and liver glycogen were determined for each rat at the end of each period. Pancreatic samples were obtained and processed for microscopic and quantitative evaluation after staining the prepared sections with heamatoxylin and eosin as well as special stain for demonstration of the different pancreatic cells in the islets of Langerhans.

Results: The obtained results showed that the diabetic rats were diagnosed by laboratory assessment to body weight loss, hyperglycemia, and hypoinsulinemia, significant increase in liver and kidney functions, lipid and protein profiles and decreased liver glycogen content. While, *C.roseus* treatment led to a significant improvement in these parameters except liver function. Microscopically there was definite vaculation, degeneration, karyolysis and pyknosis of beta pancreatic cells in the diabetic group, while other pancreatic cells were not affected (alpha and delta cells). The use of *C.roseus* treatment of this study greatly improves such cellular changes.

Conclusion: It was recommended that the use of the water extract of *C. roseus* levies as a hypoglycemic agent may offer a new hope to the diabetics in future. It's well recommended to use variable doses and different periods of treatment to evaluate the best dose and period.

Key words: Alloxan- Hypoglycemia- Diabetic- Pancreas.

INTRODUCTION

The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It is predicted that by 2030, India, China and the United States will have the largest number of people with diabetes **(Wild *et al.*, 2004).** Alloxan has been commonly utilized as an animal model of diabetes. Alloxan exerts its diabetogenic actions when administered intravenous, intraperitoneal or subcutaneous. The action of alloxan on the pancreas is preceded by its rapid uptake by the insulin-secreting cells (β-cells) **(Heikkila *et al.*, 1976)**, and also due to

autoimmune destruction of the β-cells of the pancreas **(Atkinson and Maclaren, 1994)**.

Catharanthus rosea **(Raja *et al.*, 2009)**, family Apocynaceae originates from Madagaskar, but now spreads throughout the tropics and subtropics region of the world. The other names of this plant are periwinkle, madagaskar periwinkle, and sadabahar. Plant extract of different parts of *C. rosea* possesses antibacterial **(Careew and Patterson, 1970)**, antifungal **(Jaleel *et al.*, 2007)**, antiviral **(Fornsworth *et al.*, 1968)**, and antioxidant properties **(Zheng and Wang, 2001)**. The alkaloids of *C. rosea* are famous for anticancer activities **(El-Sayed and Cordell, 1981; El-Sayed *et al.*, 1983; Ueda *et al.*, 2002)**. Several animal studies showed that ethanolic **(Chattopadhyay *et al.*, 1991, 1992; Ghosh and Gupta, 1980)** as well as aqueous extract of leaves of *C. rosea* lowered the glucose level of blood exhibiting antidiabetic properties **(Shabeer *et al.*, 2009)**. There were immunoenhancing properties of a hetero polysaccharide isolated from the leaves of *C. rosea* **(Sukesh Patra *et al.*, 2010)**.

Leaves and twigs of *Catharanthus roseus* have been reported to have hypoglycaemic activity in streptozotocin induced diabetic rats **(Singh *et al.*, 2001)**. The methanolic extracts also showed improvement in parameters like body weight and lipid profile as well as regeneration of β - cells of pancreas in diabetic rats. Histopathological studies reinforce the healing of pancreas, by methanolic *Vinca rosea* extracts, as a possible mechanism of their antidiabetic activity **(Ahmed *et al.*, 2010).**

MATERIAL AND METHODS

Material:

A-Animals:

Thirty adult male albino rats of local strain with body weight (b. wt.) ranging between 120-140 gm were divided into three equal groups:

Group I (Control group),

Group II (Diabetic group) rats were given subcutaneous alloxan (120 mg / kg b. wt.) in order to induce diabetes mellitus.

Group III (*C.roseus* treated group) rats were given alloxan to induce diabetes then given aqueous extract of *C.roseus* (L.) 3 ml/ 100 gm b.wt orally.

B-Drugs and chemicals:

Alloxan (powder from B.D.H chemical LTD, England)

Aqueous extract of *C.roseus* was prepared by boiling 3 gm in 200 ml water then filtered.

Methods:

- Induction of diabetes mellitus: By giving subcutaneous freshly prepared alloxan solution 120 mg / kg dissolved in 0.5 ml acetate buffer (pH 5.5) to an overnight fasting of the animals according to **(Helal *et al.*, 2012)**. After 48 hours blood glucose level was determined by glocometer. The rats having blood glucose levels above 200mg/100ml were used for the study.

-Preparation of aqueous extract of *C. roseus* (L.): The extract prepared by boiling 3 gm of *C. roseuse* (L.) in 200 ml of water and filtered, then given to animals (3ml/100g

b.wt as used in the egyption folk medicine) orally by oral tube once daily for one month.

- **Preparation of serum and determination of various parameters**: All animals were weighted at the beginning and end of the experiment, and blood samples collected from the orbital plexus using heparinized capillary tubes. Blood was collected part on EDTA for hematological studies and the other part in clean centrifuge tubes to separate the serum by centrifugation for 10 min. at 5000 rpm, and the supernatant serum was immediately separated for biochemical analysis. Animals were then killed and samples were collected for histological examination. Livers were taken for glycogen determination. Samples from the pancreases were also taken, stained with heamatoxylin and eosin (HX & E) and modified aldhyde fuchsin **(Halami, 1952)** for the histological study.

Student (t) test was used to compare between groups, P≤ 0.05 was considered significant **(Snedecor and Cochron, 1980).**

RESULTS

As shown in tables (1&2), alloxan led to a significant decrease in the body weight, liver glycogen and serum insulin with significant increase in blood glucose level and HOMA-IR (P≤0.01), at the same time, *C.roseus* treatment ameliorated these parameters to the normal values as compared to the control group.

As indicated in table (3), there was a highly significant increase in serum T-Lipid, T-cholesterol, Triglyceride, LDL-C and vLDL-C with a significant decrease (P≤0.01) in HDL-C and HDL-C/LDL-C in diabetic group when compared with the control group. While, in *C.roseus* treated group, no significant changes in these parameters were recorded when compared with the control group.

As shown in table (4), a significant decrease (P≤0.01) in total protein, albumin and A/G ratio, and a significant increase (P≤0.05) in globulin level in the diabetic group were observed when compared to the control group. On the other hand, *C.roseus* treated group nearly ameliorated these parameters, where this group showed a significant decrease (P≤0.05) in serum total protein and albumin levels and showed no significant change in serum globulin level and A/G ratio when compared to the control.

As indicated in tables (5and 6), the current study showed a highly significant increase (P≤0.01) in ALT and AST activities in the diabetic group, while *C. roseus* treated group, showed the same effect on AST activity but serum ALT activity showed a little correction where it recorded a significant increase (P≤0.05) in comparison with the control rats. At the same time, serum urea showed a highly significant increase in both diabetic and *C.roseus* treated groups. But, creatinine levels showed highly significant increase (P≤0.01) in the diabetic group and recorded no significant change in *C.roseus* treated group.

In table (7), the present results showed no significant change in RBCs count Hct and granulocytes in both diabetic and *C.roseus* treated rats, but WBCs and

lymphocytes showed significant decrease (P≤0.5) in diabetic rats, while *C.roseus* treated group showed no significant change when compared with the normal rats.

Histological examination of the pancreatic tissues from the control group stained with Hx & E showed normal pancreatic islets. Modified aldhyde fuchsin stain showed the three main types of cells of the pancreatic islets (alpha, beta and delta cells). B-cells are more abundant, occupying the central portion of the islet and contained numerous granules. Alpha and delta cells occupy the periphery of the islets. Delta cells are usually adjacent to alpha cells and are somewhat larger in size. Alpha cells are granular and polygonal with central spherical nuclei (Plates 1&2). Alloxan administration led to shrinkage of the pancreatic islets. The cytoplasm of the cells was vacuolated with pyknotic nuclei and many necrotic cells were seen and others showed hydropic degeneration (Plates 1&2). Pancreatic tissues from *C.roseus* treated rats showed nearly normal architecture of the pancreatic islets. Most of the cytoplasm became granulated, less vacuoles appeared in β-cells and nuclei became normal (Plates 1&2). As shown in table (8), a significant decrease (p≤0.01) was realized in number of A-cell, B-cell and D-cell of the islets in the diabetic group, while *C.roseus* treated group showed no significant change in both A-cell and D-cell number, but showed significant decrease (p≤0.01) in B-cell number when compared to the control group, but the percentage change became better when compared with the diabetic group.

Table (1)

Percentage of body weight change in the control, diabetic and *C.roseus* treated male albino rats.

	Control	**Diabetic**	***C.roseus***
% Body weight change	**21.89±0.93**	**13.26**±1.03**	**24.97$^{n.s}$+3.27**

Data expressed as:

Mean ± standard error, *=P≤0.05,

**= P≤0.01, n. s. = non significant,

(+) = Increased from control, (-) = Decreased from control.

Table (2) : Serum glucose (mg/dl) and insulin (pg/ml) levels, glycogen content and HOMA test in the liver (mg/dl) in normal, diabetic and *C.roseus* treatment on male albino rats.

	Control	Diabetic	*C.roseus*
Glucose (mg/dl)	121.8±1.11	286.4**±3.36	128.8 n.s±2.71
%		+135.14%	+5.74 %
Insulin (µu/l)	4.06±0.04	3.01**±0.09	4.26 n.s±0.07
%		-25.86 %	+4.93 %
Glycogen content (mg/dl)	18.18±0.56	2.45**±0.16	20.92 n.s±1.56
%		-77.27 %	+17.07 %
HOMA-IR	1.22±0.07	2.11**±0.08	1.35 n.s+0.05
%		+72.95 %	+10.66 %

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.

Table (3) : Serum cholesterol (mg/dl), triglycerides(mg/dl),HDL-cholesterol (mg/dl), LDL-cholesterol(mg/dl) and vLDL-cholesterol (mg/dl)levels in normal, diabetic and C.roseus treatment on male albino rats.

	Control	Diabetic	*C.roseus*
T. Lipid	303.8±2.08	436.2**±1.36	298.4 n.s±2.42
%		+43.58%	-1.78 %
T. cholesterol	109.4±1.96	199**±1.87	105.6 n.s±1.63
%		+81.9%	-3.47 %
Triglycerides	85±1.37	92.2**±1.02	87.6 n.s±1.12
%		+8.47%	+3.06%
(HDL-C)	25.2±0.73	19.2**±0.97	26.4 n.s±0.93
%		-23.8 %	+4.76
(LDL-C)	67.2±2.34	77.76**±0.98	61.68 n.s±1.73
%		+15.72 %	-8.21%
(HDL-C)/ (LDL-C)	0.38± 0.02	0.25**± 0.01	0.43 n.s±0.02
%		-34.21%	+13.16%
(VLDL-C)	17+0.28	28.44**±0.2	17.52 n.s±0.23
%		+67.29%	+3.06%

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pressed as: Mean ± standard error, % = percentage of change, *=P≤0.05, **= P≤0.01, n.s. = non significant, (+) = Increased from control, (-) = Decreased from control.

Table (4): Serum total protein (g/dl), albumin (g/dl) and globulin (g/dl) concentration and A/G ratio in normal, diabetic and C.roseus treated male albino rats.

	Control	Diabetic	*C.roseus*
Total protein	**6.34±0.11**	**5.7^{**}±0.04**	**5.94^{*}±0.1**
%		**-10.09 %**	**-6.31%**
Albumin	**3.54±0.13**	**2.84^{**}±0.07**	**2.92^{*}±0.14**
%		**-19.77 %**	**-17.51 %**
Globulin	**2.8±0.19**	**3.3^{*}±0.06**	**$2.86^{n.s}$±0.17**
%		**+17.86 %**	**+2.14%**
A/G ratio	**1.29±0.13**	**0.86^{*}±0.03**	**$1.05^{n.s}$ ±0.01**
%		**-33.33 %**	**-18.6%**

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.

Table (5): ALT and AST activities in control, diabetic and C.roseus treated on male albino rats.

	Control	Diabetic	*C.roseus*
ALT (U/ml)	**8±0.32**	**10.2^{**}±0.37**	**9.6^{*}±0.51**
%		**+27.5 %**	**+20%**
AST (U/ml)	**7.4+0.51**	**11.2^{**}±0.37**	**10.2^{**}±0.58**
%		**+ 51.35 %**	**+37.84%**

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.

Table (6): Creatinine and Urea activities in control, diabetic and C.roseus treated on male albino rats.

	Control	Diabetic	*C.roseus*
Creatinine (mg/dl)	**0.62±0.04**	**0.82^{**}±0.04**	**$0.66^{n.s}$±0.02**
%		**+32.25 %**	**+6.45 %**
Urea(mg/dl)	**18.4±0.5**	**28.4^{**}±0.51**	**22.6^{**}±1.03**
%		**+ 54.35 %**	**+22.83 %**

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.

Table (7) RBCs and WBCs count, Hct value and percentage of lymphocyte and granulocyte in control, diabetic and *C.roseus* treatment on male albino rats.

	Control	Diabetic	*C.roseus*
RBCs count	**8.94±0.29**	**8.35 n.s±0.26**	**8.25n.s±0.16**
%		**-6.59 %**	**-7.72%**
Hct	**42.92±1.52**	**44.2n.s±1.15**	**43.8n.s±1.15**
%		**+ 2.98 %**	**+2.05 %**
WBCs	**9±0.35**	**7.78*±0.16**	**8n.s±0.23**
%		**-13.56 %**	**-11.11%**
Lymph	**90.02±1.42**	**82.58**±1.62**	**87.86n.s±1.76**
%		**-8.26 %**	**-2.39%**
GRAN	**5.64±0.31**	**6.82*±0.35**	**5.58 n.s±0.26**
Lymph	**90.02±1.42**	**82.58**±1.62**	**-1.06%**

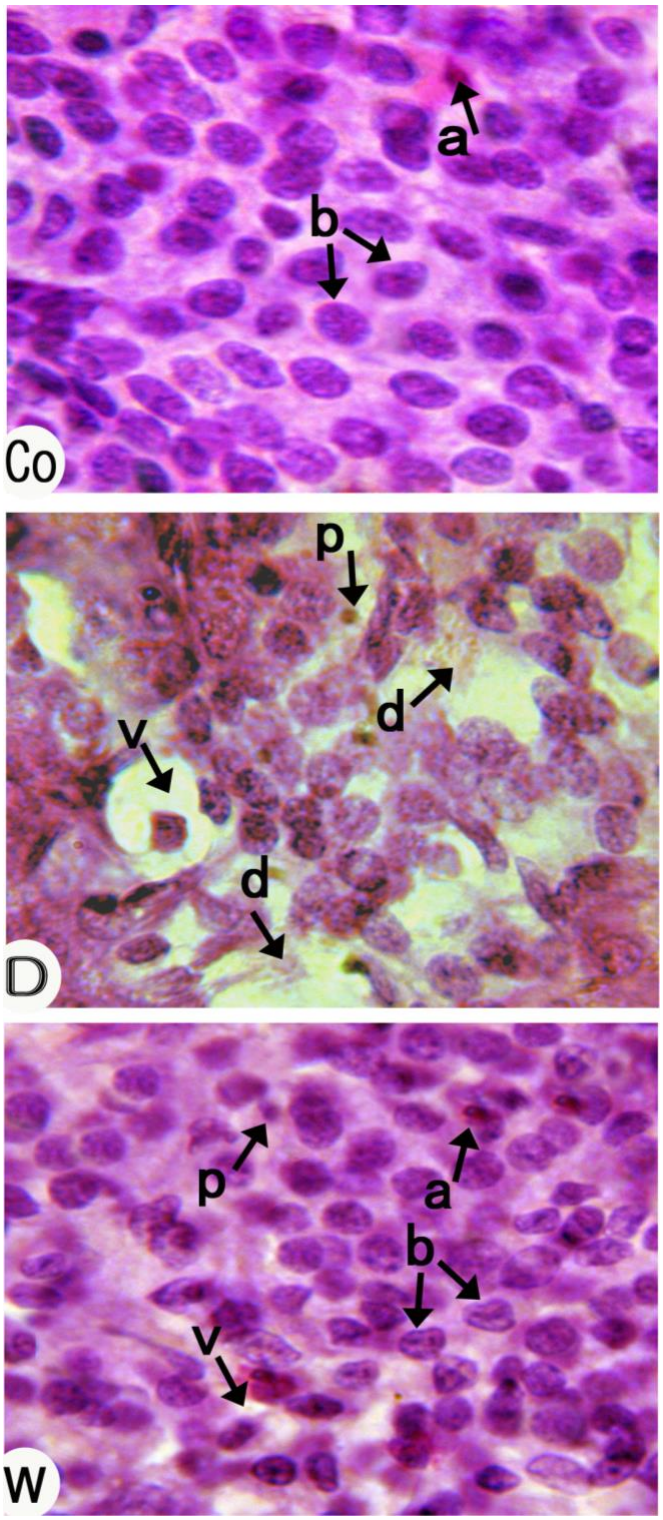
Data expressed as: Mean ± standard error, % = percentage of change, *=P≤0.05, **= P≤0.01, n.s. = non significant, (+) = Increased from control, (-) = Decreased from control.

Table (8): Number of A-cell, B-cell and D-cell in pancreas of the control, diabetic and *C.roseus* treated male albino rats.

	Control	Diabetic	*C.roseus*
A-cell count	**3.67±0.33**	**1.67**±0.33**	**2.67 n.S±0.33**
%		**-54.5 %**	**-27.25%**
B-cell count	**91.67±0.88**	**57.33**±0.88**	**79**±1.15**
%		**-37.46 %**	**-13.82%**
D-cell count	**4.67±0.33**	**2.33**±0.33**	**3.67n.s±0.33**
%		**-50.1 %**	**-21.41 %**

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.

Plate (1)



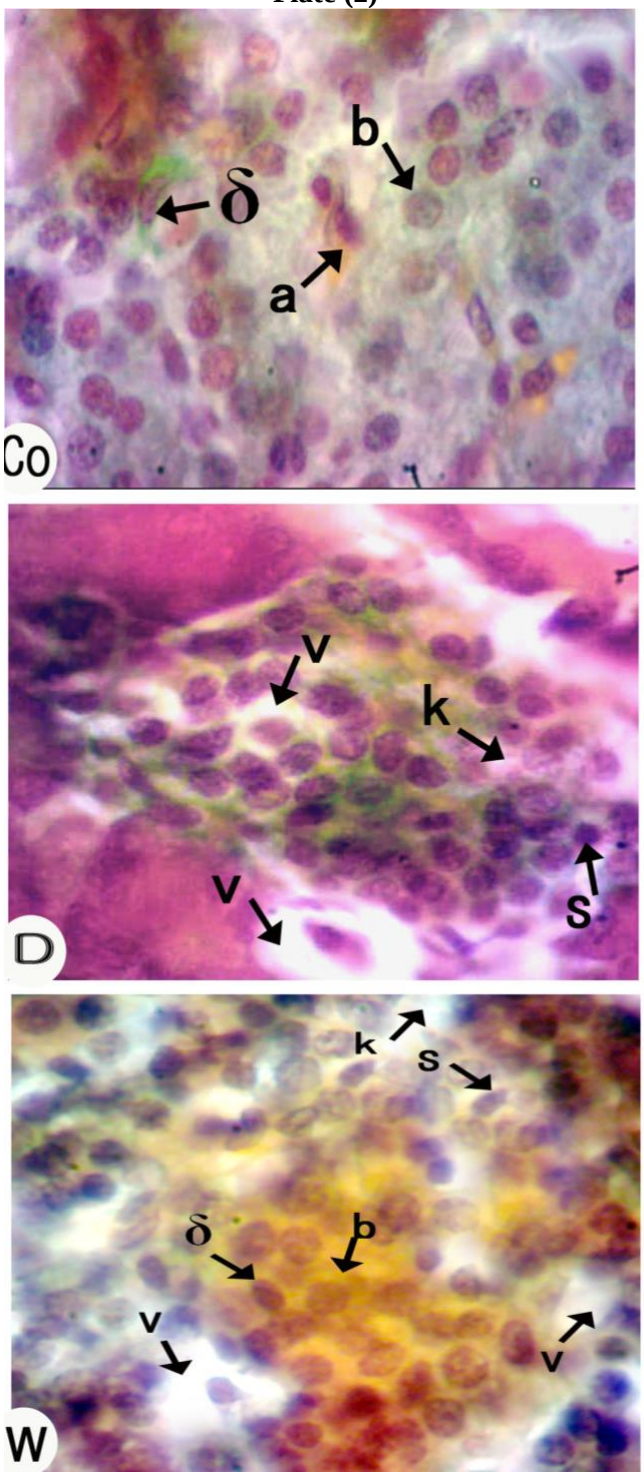
Photomicrographs of pancreas of the control (Co.), diabetic (D) and *C.roseus* (W) treated rats.

Where:

a:normal alpha cell
b: normal beta cell
d: degenerated beta cell
P: pyknotic beta cell

v: vacuolated beta cell **(Hx & E X 1000)**

Plate (2)



Photomicrographs of pancreas of the control (Co.), diabetic (D) and *C.roseus* (W) treated rats

Where:

a:normal alpha cell
b:normal beta cell
k: beta cell karyorrhexis
v:vacuolated beta cell
δ: normal delta cell
S: atrophied and pyknotic nucleus of beta cell

(Modified aldehyde Fuchsin stain X 1000)

DISCUSSION

According to the International Diabetes Association 260 million people (7% of the world population) have diabetes. Only 30 million people worldwide were diagnosed with diabetes in 1985, but by 2000 that number had increased to 150 million. By 2025, the number is expected to reach 380 million **(Stanfield, 2011)**.

Various medicinal plants and their extracts have been reported to be effective in the treatment of diabetes **(Marles and Fransworth, 1995)**. Plants are rich sources of antidiabetic, antihyperlipedemic and antioxidant agents such as flavonoids, gallotannins, amino acids and other related polyphenols **(Muruganandan *et al.*, 2005; Miyake *et al.*, 2006 and Ashok-Kumar *et al.*, 2012)**.

A scientific investigation of traditional herbal remedies for diabetes may provide valuable treatment for the development of alternative drugs and therapeutic strategies. Alternatives are clearly needed because of the inability of current therapies to control all of the pathological aspects of diabetes and the high cost and poor availability of current therapies for many rural populations, particularly in developing countries **(Marles and Farnsworth, 1995)**.

The present results, revealed loss in body weight gain in diabetic rats when compared with the control one. This loss may be due to an excessive amount of glucose and an insufficient amount of insulin in the bloodstream. This triggers the release of triglycerides from adipose tissue and catabolism of amino acids in the muscle tissue. A loss of both fat and lean mass, leading to a significant reduction in the total body weight gain and may be a result to fluid loss especially in untreated diabetic patient **(Morley *et al.*, 2006)**. While the improvement in body weight gain after *C.roseus* treatment may be due to its stimulation effect on most aspects of carbohydrate metabolism, including rapid up take of glucose by the cells, increased rate of absorption from the gastrointestinal tract and even increased insulin secretion with its resultant secondary effects on carbohydrate metabolism **(Guyton and Hall, 2000 ; Stanfield, 2011)**.

Serve hyperglycemia in diabetic rats recorded in the present work can be considered as a direct reflex to the marked hypoinsulinemia caused by the selective destructive cytotoxic effect of alloxan on β cells of the pancreas which has a direct effect on their membrane permeability by causing failure of ionic pumps and increased cell sizes. It also inhibits intercellular energy generation; insulin secretion causes sudden activation of quiescent cell for a high level of protein synthesis and produced rapid and massive beta cell death which leading to a decrement in β cells number **(Majno and Joris, 1999)**

The destructive effect of alloxan on β-cells may be also attributed to the ability to inhibit enzymes of tricarboxylic acid cycle and Ca^{+2} dependants dehydrogenises in β-cell mitochondrion, causing ATP deficiency, cessation of insulin production and cell necrosis **(Shafrir, 2003)**.

The present results also showed β-cells with vacuolated cytoplasm in the diabetic group. Vacuolation of the islet cells is the most prominent lesion associated with functional islet abnormality and development of hyperglycemia **(Bolaffi *et al.*, 1986; Kessler *et al.*, 1999)**. Also, the vaculation may be due to the diabetogenic action of alloxan which induced highly reactive oxygen radicals, which are cytotoxic to β-cells **(Fischer and Homburger, 1980)**.

C.roseus treatment led to insignificant decrease in serum glucose and insulin levels, liver glycogen content and HOMA-IR when compared with the control group .This action is possibly due to enhanced insulin secretion, decreased insulin resistance and glycogen synthesis activation. It seems to have a direct action on insulin secretion through stimulation of secretion of the Golgi complex **(Oliver-Bever and Zahand, 1979)** or it may stimulate insulin secretion through the B-cell receptor and possibly through a direct effect on

intracellular calcium transport **(Campbell *et al.*, 1991)**.

The present results elucidated that total lipids, triglycerides, total cholesterol, LDL-cholesterol and VLDL-cholesterol were increased significantly and HDL-cholesterol concentration showed very highly significant decrease in serum of diabetic rats. In agreement with these results, **Battell *et al.* (1998) and Abdel-Moneim *et al.* (2002)** found marked increase of serum triglycerides, cholesterol and LDL-cholesterol levels in the diabetic animals. This decrease may be due to the decrease in lipoprotein lipase (LPL) activity secondary to insulin deficiency **(Minnich and Zilversmit, 1989)** and agrees with those of **Moustafa *et al.* (2009)** and **Osman and Kandil (1991)** who demonstrated marked decrease of HDL-cholesterol in serum of IDDM patients and alloxan diabetic rats. **Stanfield (2011)** reported that diabetes increase lipid transport to cells as well as the production of reactive oxygen species and free radicals that contribute to the development of atherosclerosis. Specifically, diabetes increases the number of LDLs that transport lipids including cholesterol to the cells and decreases the number of HDLs that transport lipids and cholesterol to the liver.

The elevated level of serum triglycerides in diabetic animals of the present study may be attributed to decreased clearance and increased production of the major transporters of endogenously synthesized triglycerides **(Rawi *et al.*, 1998)**. Also, the expansion of cholesterol pool in diabetes might be explained by a higher input into system through acceleration of intestinal cholesterol synthesis or an increment of the rate of intestinal cholesterol absorption **(Mathe, 1995)**. LDL-cholesterol in serum of diabetic rats showed a significant increase. This abnormality certainly plays a role in the increased risk of cardiovascular disease. Increased LDL-cholesterol may be due to overproduction of VLDL by the liver or decreased removal of VLDL and LDL from the circulation **(Tsustsumi *et al.*, 1995)**.

Otherwise, alloxan diabetic rats treated with the *C.roseus* extract showed no significant change in lipids level when compared with control group. These observations indicate that the treatment with *C.roseus* were ameliorated these toxic effects generally and turn back all lipids profile to normal values. This result is in agreement with those of **Antia and Okokon (2005)** who reported that leaf juice of *C. rosea* produced a significant decrease in serum total cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol of rats.

The present study showed that serum total proteins, albumin concentrations and A/G ratio were significantly decreased ($p \leq 0.01$) in alloxan diabetic rats, while globulin concentration showed a significant increase ($p \leq 0.05$) when compared with those of non-diabetic ones. **Helal *et al.* (2012)**; **Abdel-Moneim (2002)** found a marked decrease in serum total proteins and albumin in the diabetic animals. This decrease in total serum protein content of diabetic rats may be due to the decreased amino acids uptake **(Garber, 1980)** greatly decreased concentration of a variety of essential amino acids **(Brosnan *et al.*, 1984)**, increased conversion rate of glycogenic amino acids to CO_2 and H_2O **(Mortimore and Mandon, 1970)**, reduction in protein synthesis which in turn may be due to a decrease in the amount and availability of mRNA **(Peavy *et al.*, 1985 ; Wool *et al.*, 1986)** and a reduction in ribosomal protein synthesis as a result of insulin deficiency **(Jefferson *et al.*, 1983)**.

Otherwise, treatment of alloxan diabetic rats with the *C.roseus* produced a significant decrease ($p \leq 0.05$) in serum total proteins, albumin concentrations and showed no significant change in A/G and globulin when compared with the control group. This improvement in proteins profiles is in harmony with increased serum insulin level. This explanation is in agreement with those of **Flaim *et al.* (1985)** who showed that the decrease of serum total proteins and albumin in diabetic animals was restored to control rates by insulin treatment. Insulin injection accelerates amino acids transport through uptake of amino acids by cells **(Werner, 1983)** and angmenting incorporation of certain amino acids into proteins **(Granner, 1988)**.

The elevation of serum AST and ALT activity in the present work 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 **(Awadallah and El-Dessouky, 1977)**. This may be consistent with their greater need for gluconeogenesis substrates or may reflect damage of the hepatic cells due to hepatotoxic effect of alloxan **(Helal, 2000; Youssef and Osman, 2002)**.

The significant increase of serum urea and creatinine levels may be resulted from failure of the body to excrete the metabolic end products of proteins **(Guyton and Hall, 2000)**. Where, proteins metabolic rate increased in diabetic group as a result of gluconeogenesis increasing rate. And this result can be caused by the hyperglycemia, hypertension, or hyperlipidemia that occurs with diabetes **(Stanfield, 2011)**.The author added that, hyperglycemia causes kidney damage through glycosylation, activation of protein kinase C, release of several cytokines and increase activity of the polyol pathway **.**

The improvement of serum creatinine level while serum urea level showed highly significant increase in rats treated with *C.roseus* may be due to its effect on kidney function and decrease the excessive loss of albumin in urine of diabetic rats.

Results of the present study revealed that the decrease in the WBCs count as a result of decrease proliferative response of lymphocyte may be due to high glucose concentration which concomitancy inhibited the DNA synthesis of mitogen stimulated lymphocytes and so high glycemic in addition to the lack of insulin may participates in the reduced proliferation capacity of lymphocytes from diabetic rats **(Rosemari Otton *et al.*, 2002)**. While, *C.roseus* treated rats showed no change in lymphocytes, this result may be due to increase insulin level which stimulates DNA synthesis and amino acids uptake in human fibroblasts **(Hollenberg and Cuatrecasas, 1975)**. Also, insulin was found to enhance the response of lymphocytes to Concanavalin A (Con. A), these cells were activated with short-term pulse of Con. A; insulin was capable of replacing the requirement of Con. A for the continuation of the proliferative response **(Snow *et al.*, 1980)**.

In conclusion, the present results showed that the damage of pancreas in alloxan-treated diabetic rats and regeneration of β-cells by *C.roseus* treatment was observed. *C.roseus* extract at this dose (300 mg/kg) was effective and showed normal results. This may be due to the possibility that some β-cells one still surviving to act upon by *C.roseus* extract to exert its insulin releasing effect. Histopathological studies reinforce the healing of pancreas, by *C.roseus* extracts, as possible mechanism of their antidiabetic activity.

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التأثيرالفسيولوجى لنبات ألوِنكا على الجرذان المصابة بالسكري

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يعتبر مرض السكري من أكبر مشاكل الصحة في العالم في هذه الآونه. تعد آسيا وأفريقيا من أكثر المناطق تأثراًمن باقي الدول.هناك العديد من الدراسات الحديثه تتجه إلى إستخدام النباتات الطبية في علاج الكثيرمن الأمراض المختلفة وذلك لفاعليتها وقلة آثارها الجانبيه.

و فى هذه الدراسه تم استعمال ٣٠ من الجرذان الذكور البالغة لدراسة الآثارالفسيولوجيه لنبات الوِنكا على الجرذان المصابة بالسكري، وتم تقسيم هذه الجرذان إلى ثلاث مجموعات كل منها يتكون من عشرة جرذان: مجموعة ضابطة ومجموعة مصابة بالسكر ولا تتلقى علاج، ومجموعة معالجة بالألوكسان وتعامل بالمستخلص المائي لنبات ألوِنكا ، وتم وزن كل جرذ في بداية التجربة وكذلك في نهايتها (بعد ٣٠ يوما) .

وقد أظهرت النتائج التى تم الحصول عليها من الدراسة حدوث ارتفاع ذو دلالة في مستوى الجلوكوز والدهون في الدم وكذلك وظائف الكبد والكلى كما حدث إنخفاض في مستوى البروتينات في الدم وكرات الدم البيضاء و HOMA-IRفي المجموعات المصابة بالسكري بالمقارنة بالمجموعة الضابطة. أما بالنسبة للدراسة الهستوباثولوجية أوضحت تغيراً باثولوجياً في خلايا بيتا ولم يلاحظ تغير في خلايا ألفا ودلتا.

التوصيه:
وقد لوحظ أن المعاملة بالمستخلص المائي للوِنكا أدت إلى ظهور تحسين ذو دلاله إحصائية في كل القياسات الفسيولوجية والهستوباثولوجية لخلايا بيتا في جزر لانجرهانزماعدا معدل نشاط وظائف الكبد ومستوى البروتينات في البلازما وزيادة مستوى اليوريا في الدم.