

Comparison between the effect of ozone and vitamin C in treatment of diabetes mellitus

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ABSTRACT:

Objectives: There is strong evidence that diabetes results a state of oxidative stress and that reactive oxygen species contribute to the production of insulin resistance, β -cell dysfunction and both the microvascular and macrovascular long-term complications of diabetes. Antioxidants are used as supportive therapy in the treatment of DM, so, we use ozone and vitamin C to study if they can regulate the oxidative complications of DM.

Material and method: twenty male adult albino rats were divided into two groups; group 1: control group, group 2: alloxan induced diabetic rats which divided into three subgroups. subgroup1: diabetic untreated rats, subgroup2: diabetic treated with ozone and subgroup 3: diabetic rats treated with vitamin C. After thirty days of treatment the body weight gain was detected. Blood sample were collected to1- estimate biochemical parameters as: glucose, serum insulin, lipid and protein profiles and liver and kidney functions 2- estimate some hematological parameters. Also, liver samples collected to determine their glycogen content and pancreatic samples were obtained for microscopic and quantitative evaluation.

Results: in diabetic untreated rats the results showed reduction of gained body weight, hyperglycemia, and hypoinsulinemia, significant increase in liver and kidney functions and change in lipid and protein profiles and decreased liver glycogen content. While, O₃ and vitamin C treated rats reported an amelioration of the most toxic effect of alloxan and returned most of these parameters nearly normal. Microscopically pancreatic beta cells showed definite vaculation, degeneration, karyolysis and pyknosis in the diabetic group while pancreatic alpha and delta cells were not affected. The use of O₃ and vitamin C treatment in this study showed significant improves of such cellular changes when compared to diabetic untreated rats but still abnormal when compared with normal rats. **Conclusion:** it was recommended that the use of the O₃ or vitamin C as a supplementary agent to reduce oxidative stress damage of hyperglycemia and recommended to use variable doses and different periods of treatment to evaluate the best dose and period.

Key words: Alloxan; Hypoglycemia; Diabetic; antioxidant.

Introduction:

WHO estimates that more than 180 million people worldwide have diabetes. This number is likely to more than double by 2030 without urgent action. In 2005, an estimated 1.1 million people died from diabetes, almost 80% of them occur in low and middle-income countries, and half of them in people under the age of 70 years; 55% of diabetes deaths are in women[1].

In clinical and experimental research, attention is paid to the role of antioxidant defense systems in the prevention of human diseases such as cancer, diabetes mellitus, and cardiovascular pathologies [2]. During the progression of these diseases, oxidative stress events occur, and free radicals and reactive oxygen species (ROS) are generated. These free radicals and ROS are thought to contribute to lipid

peroxidation (LPO) [3], DNA damage [4], and protein degradation [5]. Host survival depends upon the ability of cells and tissues to adapt to or resist the stress and repair or remove damaged molecules and cells.

Presently available oral hypoglycemic agents do not show marked improvement in oxidative stress in diabetic patients [4]. Ozone (O₃) has been used as a therapeutic agent and beneficial effects have been observed. However, so far only a few biochemical and pharmacodynamic mechanisms have been elucidated. The possibility that ozone could induce a useful adaptation to chronic oxidative stress was described in 1996 [6]. Other studies [7-9] reported that controlled ozone administration may promote an oxidative preconditioning or adaptation to

oxidative stress, preventing the damage induced by ROS.

Several studies have reported lower concentration of non-enzymatic antioxidants, enzymatic antioxidants and as well as vitamin C level in type 2 diabetes mellitus. Ascorbic acid (vitamin C), an anti-oxidant vitamin plays an important role in protecting against free radical-induced damage [10-12]. It is structurally similar to glucose and can replace it in many chemical reactions and thus is effective for prevention of non enzymatic glycosylation of protein [7].

So that this study designed to compare the efficacy of both vitamin C and ozone as antioxidants in the treatment of diabetes and their side effects if present.

Material and methods:

Twenty adult male albino rats of local strain with body weight ranging between (120-140 gm) were divided into two groups as following: group I= control non diabetic rats; Group II = alloxan-induced diabetic rats which divided into three subgroups; subgroup 1= diabetic untreated rats, subgroup2=diabetic rats treated with the mixture of ozone and oxygen (O_3 and O_2) as intraperitoneal injection three times / week, at a dose 2 cm^3 of ozone concentration $50\ \mu\text{g}/\text{cm}^3$ [13] and subgroup3= diabetic rats treated orally with vitamin C (0.1 ml/ 100 gm b.wt./day i.e. 1000mg/ day).

Induction of diabetes mellitus by giving s.c freshly prepared alloxan solution 120 mg / kg dissolved in 0.5 ml acetate buffer (pH 5.5) to overnight fasting animals quoted from Helal *et al.* [14]. After 48 hours of alloxan injection, rats were fasted overnight (8-10hrs) and administered glucose (3g/kg b.wt.) by gastric intubation. After 2 hours of glucose administration, blood glucose level was determined by glucometer. The rats with blood glucose levels above 200mg/dl were used for the study.

After a month of treatment blood samples were collected from the orbital plexus using heparinized capillary tubes [15]. Part of blood was collected on EDTA for hematological studies and the other part collected in clean centrifuge tubes to separate the serum by centrifugation for 10

min. for 5000 rpm, and the supernatant serum was immediately separated for biochemical analysis. Samples were collected for histological examination. Livers were taken for glycogen determination. Samples from the pancreas were also taken, stained with Heamatoxylin and Eosin (HX & E) and modified aldehyde fuchsin [16] for histological study.

Statistical analyses:

Data were analyzed using student (t) test to compare between groups; data at ($P < 0.05$) were considered significant [17].

Results:

Percentage of body weights change:

Rats treated with alloxan only showed depression of body weight gain ($P < 0.01$) (Table1). Treatment with vitamin C and ozone ameliorates this depression as compared to diabetic non treated group.

Some parameters associated with type 2 diabetes mellitus:

The present study investigated some parameters associated of diabetes mellitus in order to evaluate whether O_3 or vitamin C treatment has antidiabetic effects. Where, diabetic animals showed the expected symptoms of insulin independent diabetes mellitus, i.e. hyperglycemia, hypoinsulinemia, depressed liver glycogen content and elevation the insulin resistant ($P < 0.01$) in table (2).

Treatment with O_3 and vitamin C ameliorate some of these markers where, both of them showed significant increase ($P < 0.01$) in liver glycogen content and serum insulin level when compared with diabetic group. And recorded significant decrease ($P < 0.01$) in blood glucose level when compared to diabetic rats but also still significant increase ($P < 0.01$) when compared with normal rats and showed no change in HOMA-IR when compared to the control group.

Lipids profile:

Hyperinsulinemia in untreated diabetic rats associated with hypertriglyceridemia, hypercholesterolemia, increased production of VLDL and decrease HDL levels. Rats treated with O_3 or vitamin C showed markedly reduction ($P < 0.01$) in hyperlipidemia and amelioration of HDL-C and HDL-C/LDL-C depression when compared with diabetic untreated group (table 3).

Proteins level:

In diabetic rats, there is an increased of gluconeogenesis rate associated with increased proteolysis rate and decreased protein synthesis so that, diabetic rats recorded a significant decrease ($P<0.01$) in total protein, albumin and A/G ratio, and a significant increase ($P<0.05$) in globulin level when compared to the control group.

On the other hand, O_3 and vitamin C treated groups showed no significant change in protein profiles except serum albumin level which recorded a significant decrease ($P<0.05$) in O_3 treated group (table 4) when compared to the control rats.

Some parameters of liver function:

Diabetes mellitus has toxic effects on liver tissues which can be indicated by increase ($P<0.01$) in ALAT and ASAT activities as showed in table (5). Otherwise, O_3 and vitamin C treated groups ameliorates these activities and returned it back nearly to their normal value.

Some parameters of kidney functions:

Hyperglycemia causes nephropathy which can be detected by increasing ($P<0.01$) serum creatinine and urea levels in diabetic rats. Otherwise, vitamin C treatment ameliorates this elevation of serum creatinine level but not serum urea level which showed significant increase ($P<0.01$) as compared to normal rats. While, O_3 treated group showed significant increase in creatinine and urea levels ($P<0.05$ and $P<0.01$ respectively) when compared with normal rats (table 6).

Some hematological parameters:**DISCUSSION:**

An antioxidant-based therapy combined with glucose control will give patients more of advantage and lessen the chance of complications with diabetes [18]. Supplementations of antioxidant to type 2 diabetes mellitus might support improve endogenous antioxidant capacity due to reducing blood glucose and lipid metabolites. Antioxidants have an important function in glucose metabolism [19].

Abnormalities in the defense mechanisms of diabetic rats have long been recognized as in the present result where WBCs and lymphocytes which play a pivotal role for initiating immune showed significant decrease ($P<0.05$ and $P<0.01$ respectively) while, other hematological parameters (RBCs count and Hct %) record no significant change when compared with normal rats (table 7). Otherwise, rats treated with O_3 or vitamin C showed no significant change in all these parameters when compared with normal rats.

Some Histological changes:

There is reduced β -cell mass in type 2 DM patients but the present study when evaluate the pancreatic islets cells number showed reduction ($p<0.01$) in the three types of the islet cells not β -cell only in diabetic untreated rats. While, O_3 and vitamin C treated rats showed no significant change in both α -cell and δ -cell number but showed significant decrease ($p<0.01$) in β -cell number when compared with control group, at the same time, it was recorded significant increase ($p<0.01$) when compared with diabetic group as shown in table (8).

Histological examinations of the pancreatic tissues of diabetic animals display shrinkage in the pancreatic islets, vacuolated cytoplasm with pyknotic nuclei. Pancreatic tissues from O_3 and vitamin C 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 (Plate 1&2).

An epidemiologic study clarified that plasma levels of the antioxidant vitamin C were inversely correlated with HbA1c in diabetic patients [20]. Researchers at the Harold Hamm Oklahoma Diabetes Center have found that cells have "a memory" that causes damage to continue even when blood sugar is controlled. By adding antioxidants like vitamin C, the cell memory disappeared and cell function and oxidation stress were normalized [21].

Ozone help to alleviate oxidative stress associated with diabetes mellitus

[22] so that, emphasis and attention has been focused on the use of medical ozone [23].

In light of the results, the situation in diabetic untreated rats showed a reduction in β -cell mass which associated not only by hypoinsulinemia but also by increasing insulin resistance. These abnormalities lead to alterations in the carbohydrates metabolism which cause severe hyperglycemia and decrease liver glycogen content ($p \leq 0.01$). Severe hypoinsulinemia recorded in diabetic untreated rats may be due to the selective destructive cytotoxic effect of alloxan on the β cells of the pancreas which attributed to its direct effect on their membrane permeability by causing failure of ionic pumps and increased cell size which inhibits intracellular energy generation by inhibiting enzymes of tricarboxylic acid cycle and Ca^{+2} dependants dehydrogenases in β -cell mitochondrion, causing ATP deficiency, cessation of insulin production and cell necrosis [24-25]. Which causing 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 [26].

While, the vaculation of most β -cells cytoplasm in untreated diabetic rats which showed in plates (1&2) in agreement with finding of **Kessler *et al.*** [27] who reported that vaculation of the islet in the most prominent with lesion associated with functional islet abnormality and development of hyperglycemia and also with **Fischer and Homburger** [28] who explained the vaculation by the diabetogenic action of alloxan which induced highly reactive oxygen radicals, which induced cytotoxic to β -cells.

The severe hyperglycemic effect of alloxan may be due to hypoinsulinemia as explained above or due to oppose the hepatic effects of insulin by stimulating glycogenolysis and gluconeogenesis resulted from intracellular straining sensation and inhibition of its effect on peripheral utilization of glucose. Thus, in the

diabetic rats with insulin deficiency and insulin resistance as showed in the present study which in agreement with **Defronzo and Goodman** [29] who reported that there is an increase in hepatic glucose production, a decrease in peripheral glucose uptake, and decrease ($P \leq 0.01$) in the conversion of glucose to glycogen in the liver. The expected result increase glucose level in blood and decrease its level intracellular.

Ozone and vitamin C improve the condition of diabetes mellitus in rats treated by any of them as indicated by parameters like serum glucose level, liver glycogen content, insulin level, HOMA-IR and islet cells count (tables 2 & 8) and histological observation in plates (1 & 2). This action is possibly due to regenerate of β -cells and improving insulin sensitivity which lower the concentration of glucose in blood by inhibiting hepatic glucose production and by stimulating glucose uptake and metabolism by muscle and adipose tissues which explain the lowering of the glucose level and increasing liver glycogen content. These results are in agreement with **Hamden *et al.*** [30] who found that combined vitamins (C and E) can be effective in inhibiting hyperglycemia, oxidative stress and cell damage in pancreas and liver, by enhancing antioxidant enzyme activity, scavenging ROS and eventually by contributing to the improvement of tissue dysfunction in diabetic rats. And also in agreement with **Al-Dalain *et al.*** [31] who repeated that administration of ozone in non-toxic doses might play a role in the control of diabetes and its complications.

In the present results insulin deficiency and resistance resulted from alloxan induction without treatment which led to alterations of the metabolism of lipids. Hypertriglyceridemia and hypercholesterolemia associated with increased production of VLDL and decreased HDL level which in agreement with **Goodman and Gilman's** [32] who explained the same result by 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. And also, agreement with **Harvey and Ferrier** [25] who reported that the metabolic abnormalities of type2 diabetes are a result of insulin resistance which lead to dislipidemia in the liver where, fatty acids are converted to triacylglycerol which are packaged and secreted in VLDL. Both lipid accumulations particularly triglycerides and reduction in antioxidant activity are contributed to the development of oxidative stress in diabetes [33]. Hyperglycemia was found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide dependent pathway resulting in the generation of free radicals [34].

The treatment by O₃ or vitamin C was ameliorating this dislipidemia produced by diabetes as recorded in table (3). This improvement of lipids profile may be attributed mainly to relatively 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. Or may be due to decrease lipid peroxidation and increased the antioxidant enzymes activities in type 2 diabetes mellitus as reported by **Kedziora-Kornatowska et al.** [35]. The treatment with vitamin C decreases the elevated levels of glucose, cholesterol, triglycerides and low-density lipoprotein (LDL) in T2DM [19]. **Paolisso et al.** [36] also reported beneficial effects of oral vitamin C (1000 mg/day for 4 months) on glucose, lipid metabolism, and free radicals in type 2 diabetes. Vitamin C is required for regeneration of α -tocopherol (vitamin E) and may thus prevent LDL oxidation, and transport of α -tocopherol in

HDL may enhance and preserve these protective antioxidant effects of HDL.

In untreated diabetic rats, the decrement of total protein, albumin and A/G ratio may be attributed to enhanced rate of gluconeogenesis which secondary effect of 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). These results are in agreement with **Shafir** [24] who explained 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 myofibrillar protease.

The improvement of protein profile in harmony with increased serum insulin level and tissues sensitivity by treatment with both of vitamin C and ozone 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 [32].

The reduction in body weight gain in diabetic untreated rats may be due to an excessive amount of glucose and an insufficient amount of insulin in the bloodstream, which triggers the release of triglycerides from adipose tissue (lipolysis) and catabolism of amino acids in muscle tissue (proteolysis) as discussed above, leading to a significant reduction in total body weight gain and may be a result to fluid loss [37].

While the amelioration of body weight gain depression resulted by treatment with O₃ and vitamin C may be due to inhibition of both lipolysis and proteolysis process as a result of increasing insulin level and decrease insulin resistance [38].

Alloxan has toxic effects on liver tissues which can be indicated by increase ALAT and ASAT activities [39] in agreement with result. This elevation of

serum ASAT and ALAT activities 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 [40]. And may be consistent with their greater need for gluconeogenesis substrates or may reflect damage of the hepatic cells due to hepatotoxic effect of alloxan [41].

Treatment with O₃ or vitamin C returned ALAT and ASAT activities to normal condition which may be a result to their antioxidant effect. This effect may be reduced the oxidative damage produced by alloxan or may be due to their effect on insulin level and insulin resistance which lead to inhibition of gluconeogenesis and improvement of carbohydrate metabolism which ameliorate liver damage [38].

In type 2 diabetes serum creatinine often rises due to renal arterial disease and/or cardiac failure rather than to diabetic nephropathy [42 & 43]. And also, this may be resulted from failure of the body to excrete the metabolic end products of proteins [43]. Where, proteins metabolic rate increased in diabetic as a result of gluconeogenesis increasing rate. And this result can be caused by the hyperglycemia, hypertension, or hyperlipidemia that occurs with diabetes [38]. Hyperglycemia causes kidney damage through glycosylation, activation of protein kinase C, release of several cytokines, and increase activity of the polyol pathway [38]. Diabetes has been demonstrated to be associated with oxidative stress and hyperglycemia, one of the most important indicators of oxidative stress.

The endogenous mechanisms of enzymes and antioxidants are able to destroy the reactive species and create a balance between antioxidant and free radicals. In diabetes, the oxidative stress is increased because of the deficiency in the antioxidant defense. The intake of antioxidants may reduce the oxidative stress associated with diabetes and hence help to

restore the antioxidant defense system [44]. The present study recorded an improvement in serum creatinine when compared with control one which, may be attributed to its improvement effect on kidney function and decrease the excessive loss of albumin in urine of diabetic rats. Therefore based on the findings, antioxidant therapy must be one of the most important strategies in combat with diabetic nephropathy progression [45]. There are reports that glomerulosclerosis decreased by vitamin C, alpha lipoic acid and vitamin E [46-47].

On the other hand, serum urea level still showed highly significant increase in rats treated with O₃ and vitamin C when compared with normal rats. So, other antioxidant must be tested with different doses & periods because they may be more effective.

Conclusion:

From the above discussion it concludes that treatment with O₃ and vitamin C exhibited significant antihyperglycemic activity in alloxan-induced diabetic rats. These results also showed improvement in parameters like body weight and lipids profile as well as regeneration of β -cells of pancreas and so might be of value in diabetes treatment. Further investigation is necessary to determine the mechanism of action to both of them as antidiabetic agent.

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Table (1) showed the percentage of body weight change in control, diabetic, O₃ and vitamin C treated male albino rats.

	Control	Diabetic	O ₃	Vitamin C
% Body weight change	21.89+0.93	13.26+1.03	19.72+0.7	19.8+0.73
a		**	n.s	n.s
b			**	**

Data expressed as:

*=P≤0.05 **= P≤0.01,

(a) = compared to control,

Mean + standard error,

n.s. = non significant,

(b) = compared to diabetic.

Table (2) showed some parameters associated with DM in normal, diabetic, O₃ and Vitamin C treated male albino rats.

	Control	Diabetic	O ₃	Vitamin C
Glucose (mg/dl)	121.8+1.11	286.4+3.36	170.6+3.32	170.8+1.55
%a		+135.14**%	+40.06**%	+40.23**%
%b			-40.43**%	-40.36**%
Insulin (µu/l)	4.06+0.04	3.01+0.09	3.49+0.04	3.41+0.04
%a		-25.86**%	-14.04**%	-16.01**%
%b			+15.95**%	+13.29**%
Glycogen content (mg/dl)	18.18+0.56	2.45+0.16	8.41+0.43	9.07+0.3
%a		-77.27**%	-53.74**%	-50.27**%
%b			+243.26**%	+270.2**%
HOMA-IR	1.22+0.07	2.11+0.08	1.5 +0.01	1.31 +0.05
%a		+72.95**%	+13.93^{n.s}%	+7.38^{n.s}%
%b			-28.91**%	-37.91**%

Data expressed as:

Mean + standard error,

*=P≤0.05, **= P≤0.01,

(+) = Increased from control,

(a) = compared to control,

% = percentage of change,

n.s. = non significant,

(-) = Decreased from control.

(b) = compared to diabetic.

Table (3) showed the lipids profile in normal, diabetic, O₃ and vitamin C treatment on male albino rats.

	Control	Diabetic	O ₃	Vitamin C
T. Lipid	303.8+2.08	436.2+1.36	407.6+2.06	276.4+2.11
%a		+43.58**%	+34.17**%	-9.2**%
%b			-6.56**%	-66.63**%
T. cholesterol (mg/dl)	109.4+1.96	199+1.87	118.6+1.21	110.2+1.46
%a		+81.9**%	+8.41**%	+0.73 ^{n.s} %
%b			-40.4**%	-44.62**%
Triglycerides (mg/dl)	85+1.37	92.2+1.02	86.6+1.89	75.4+1.86
%a		+8.47**%	+1.88 ^{n.s} %	-11.29**%
%b			-6.07**%	-18.22**%
HDL-C (mg/dl)	25.2+0.73	19.2+0.97	28.8+0.86	25.2+0.86
%a		-23.8**%	+14.29**%	0 ^{n.s} %
%b			+50**%	+31.25**%
LDL-C (mg/dl)	67.2+2.34	117.36**+1.22	71.88+2.16	76.52+1.04
%a		+74.64%	+6.96 ^{n.s} %	+13.87 ^{n.s} %
%b			-38.75**%	-42.47**%
(HDL-C)/ (LDL-C)	0.38+ 0.02	0.16+ 0.01	0.4 + 0.01	0.37 + 0.01
%a		-57.89**%	+5.26 ^{n.s} %	-2.63 ^{n.s} %
%b			+150**%	+131.25**%
VLDL-C (mg/dl)	17+0.28	64.4**+1.96	17.32+0.38	15.08+0.37
%a		+278.82%	+1.88 ^{n.s} %	-11.29**%
%b			-73.11**%	-76.58**%

Data expressed as:

Mean + standard error,

* = P ≤ 0.05, ** = P ≤ 0.01,

(+) = Increased from control,

(a) = compared to control,

% = percentage of change,

n.s. = non significant,

(-) = Decreased from control.

(b) = compared to diabetic.

Table (4) showed the proteins level in normal, diabetic, O₃ and vitamin C treated male albino rats.

	Control	Diabetic	O ₃	Vitamin C
Total protein (g/dl)	6.34+0.11	5.88+0.09	6.02+0.18	6.12+0.14
%a		-7.26**%	-5.05 ^{n.s} %	-3.47 ^{n.s} %
%b			+2.38 ^{n.s} %	+4.08 ^{n.s} %
Albumin (g/dl)	3.54+0.13	2.84+0.07	3.08+0.1	3.26+0.08
%a		-19.77**%	-12.99**%	-7.9 ^{n.s} %
%b			+8.45 ^{n.s} %	+14.79**%
Globulin (g/dl)	2.8+0.19	3.3+0.06	2.54+0.06	2.86+0.12
%a		+17.86**%	-9.29 ^{n.s} %	+2.14 ^{n.s} %
%b			-23.03**%	-13.33**%
A/G ratio	1.29+0.06	0.86+0.03	1.22+0.06	1.15+0.06
%a		-33.33**%	-5.43 ^{n.s} %	-10.85 ^{n.s} %
%b			+41.86**%	+33.72**%

Data expressed as:

Mean + standard error,

* = P ≤ 0.05, ** = P ≤ 0.01,

(+) = Increased from control,

(a) = compared to control,

% = percentage of change,

n.s. = non significant,

(-) = Decreased from control.

(b) = compared to diabetic.

Table (5) showed some parameters of liver function in control, diabetic, O₃ and vitamin C treated male albino rats.

	Control	Diabetic	O ₃	Vitamin C
ALAT (U/ml)	8+0.32	10.2+0.37	8.8+0.66	8.4+0.51
%a		+27.5** %	+10^{n.s}%	+5^{n.s} %
%b			+13.75^{n.s}%	-17.64* %
ASAT (U/ml)	7.4+0.51	11.2+0.37	8.6 +0.51	8.4 +0.4
%a		+ 51.35** %	+ 16.22^{n.s} %	+13.51^{n.s} %
%b			-23.21**%	-25**%

Data expressed as:

Mean + standard error,

*=P≤0.05, **= P≤0.01,

(+) = Increased from control,

(a) = compared to control,

% = percentage of change,

n.s. = non significant,

(-) = Decreased from control.

(b) = compared to diabetic.

Table (6) showed some parameters of kidney function in control, diabetic, O₃ and vitamin C treated male albino rats.

	Control	Diabetic	O ₃	Vitamin C
Creatinine (mg/dl)	0.62+0.04	0.82+0.04	0.78+0.05	0.6 +0.03
%a		+32.25** %	+25.81*%	-3.23^{n.s} %
%b			-4.88^{n.s} %	-26.83** %
Urea (mg/dl)	18.4+0.5	28.4+0.51	25.6+1.29	25.8+1.29
%a		+ 54.35** %	+ 39.13** %	+ 40.22** %
%b			-9.86^{n.s}%	-9.15^{n.s}%

Data expressed as:

Mean + standard error,

*=P≤0.05, **= P≤0.01,

(+) = Increased from control,

(a) = compared to control,

% = percentage of change,

n.s = non significant,

(-) = Decreased from control.

(b) = compared to diabetic.

Table (7) showed some hematological parameters in control, diabetic, O₃ and Vitamin C treatment on male albino rats.

	Control	Diabetic	O ₃	Vitamin C
RBCs count (X10⁶/μl)	8.94+0.29	8.35 +0.26	8.17 +0.28	8.24+0.23
%a		-6.59 ^{n.s} %	-8.61 ^{n.s} %	-7.83 ^{n.s} %
%b			-2.16 ^{n.s} %	-1.32 ^{n.s} %
Hct%	42.92+1.52	44.2 +1.15	42.22+1.49	42.28+1.34
%a		+ 2.98 ^{n.s} %	-1.63 ^{n.s} %	-1.49 ^{n.s} %
%b			-4.48 ^{n.s} %	-3.34 ^{n.s} %
WBCs (X10³/μl)	9+0.35	7.78+0.16	8.3+0.1	8.66+0.39
%a		-13.56 [*] %	-7.78 ^{n.s} %	-3.78 ^{n.s} %
%b			+6.68 [*] %	+11.31 ^{n.s} %
Lymph (X10²/μl)	90.02+1.42	82.58+1.62	85.82 +1.44	87.28+1.43
%a		-8.26 ^{**} %	-4.67 ^{n.s} %	-3.04 ^{n.s} %
%b			+3.92 ^{n.s} %	+5.69 ^{n.s} %

Data expressed as:

Mean + standard error,

*=P≤0.05, **= P≤0.01,

(+) = Increased from control,

(a) = compared to control,

Lymph= Lymphocyte

% = percentage of change,

n.s. = non significant,

(-) = Decreased from control.

(b) = compared to diabetic.

Table (8) showed some histological changes in control, diabetic, O₃ and vitamin C treated male albino rats.

	Control	Diabetic	O ₃	Vitamin C
α-cell count	3.67+0.33	1.67+0.33	2.67+0.33	3 +0.58
%a		-54.5 ^{**} %	-27.25 ^{n.s} %	-18.26 ^{n.s} %
%b			+59.88 ^{n.s} %	+79.64 ^{n.s} %
β-cell count	91.67+0.88	57.33+0.88	83.67 +1.2	78.67+1.86
%a		-37.46 ^{**} %	-8.73 ^{**} %	-14.18 ^{**} %
%b			+45.94 ^{**} %	+37.22 ^{**} %
δ-cell count	4.67+0.33	2.33+0.33	4.67+0.33	3.33 +0.67
%a		-50.1 ^{**} %	0 ^{n.s} %	-28.69 ^{n.s} %
%b			100.43 ^{**} %	+42.91 ^{n.s} %

Data expressed as:

Mean + standard error,

*=P≤0.05, **= P≤0.01,

(+) = Increased from control,

(a) = compared to control,

% = percentage of change,

n.s. = non significant,

(-) = Decreased from control.

(b) = compared to diabetic.

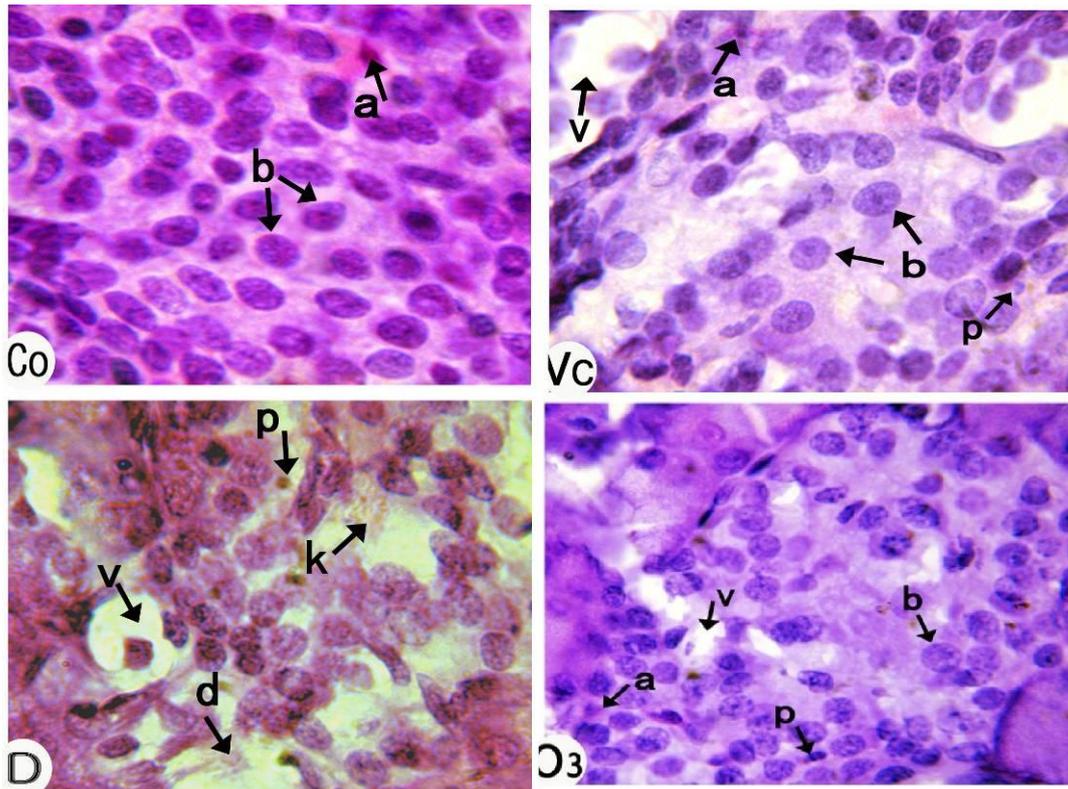


Plate (1): Photomicrograph of pancreatic islets of control (Co.), diabetic (D), ozone (O₃) and vitamin C (Vc) treated rats stained by heamatoxylin and eosin stain (X 1000).

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

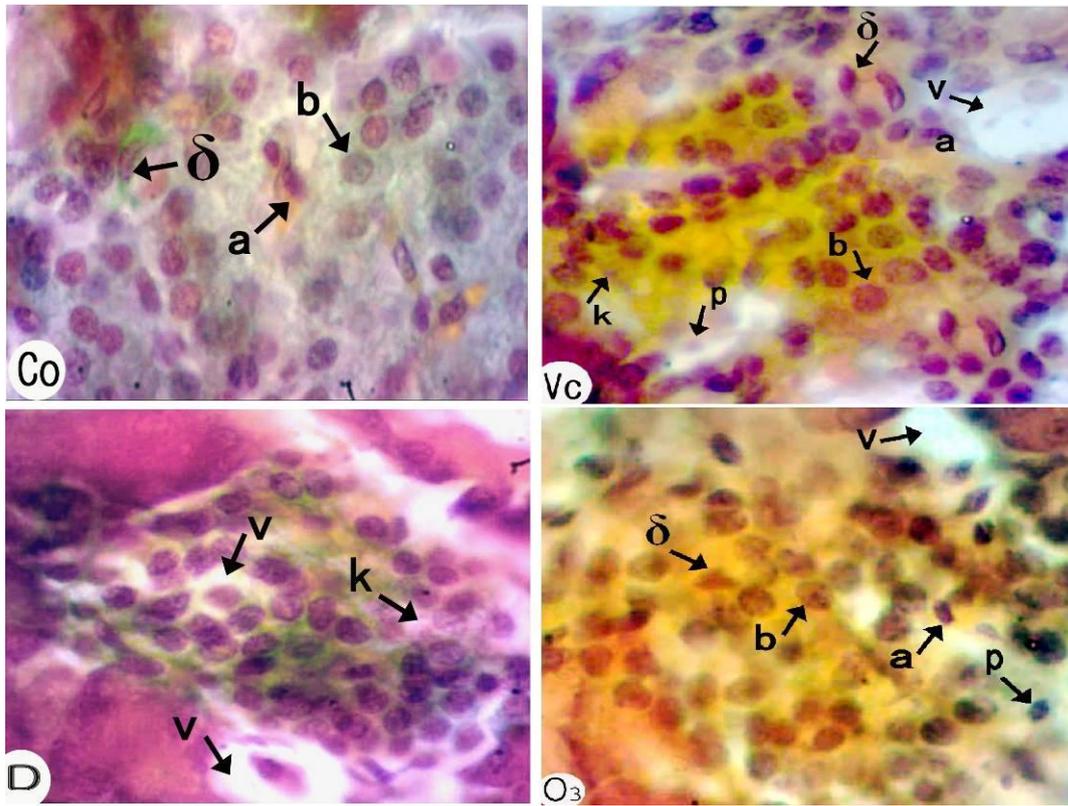


Plate (2): Photomicrograph of pancreatic islets of control (Co.), diabetic (D), ozone (O₃) and vitamin C (Vc) treated rats stained by Modified aldehyde Fuchsin stain (X 1000).

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