Increased beneficial outcome of insulin by medical ozone against experimentally diabetic rats

Abstract

The present research aimed to determine the effective dose of medical ozone on some blood parameters in experimental diabetic rats.48 adult female rats with (180 - 200 g) weight average were randomly divided in to (6) groups (n=8): control (T1), Medical ozone (MO) (T2), diabetic (T3), ozone-treated diabetic (T4), insulin-treated diabetic(T5), and MO, insulin-treated diabetic (T6). . Diabetes was induced by an intraperitoneal injection of single dose of Alloxan (150 mg/kg of the body weight) . The diabetic rats were left for 2 days to assure the stability of diabetes in them, before continuing experiments. The results of RBCs in groups (T2 , T4,T5 and T6) showed significant increase compared with diabetic group and control group , Hb and WBCs parameters showed significant decrease in (T3,T4 and T5)When compared with other groups .The results of platelet count there was significant increase in (T3,T4 and T5) compared to control group. Clotting time and calcium ions recorded significant decrease in all treated diabetic groups (T3,T4,T5 and T6) when compared with (T1 and T2), at the same time, the results of bleeding time recorded significant decrease in (T3 and T4) compared with the other groups while (T4 and T5) showed non-significant difference compared to (T1 and T2). The current study concluded that the treatment combination of medical ozone and insulin has ameliorative effects on some blood parameters except clotting time and calcium ion.

Key words: Medical Ozone , Insulin , Alloxan , Hematological Parameters

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder in which the body's ability to produce or respond to the hormone insulin is impaired, resulting in abnormal metabolism of carbohydrates and elevated levels of glucose in the blood and urine. DM have been diagnosed in canine and feline family after human .The clinical features described and investigated are rarely observed in other domestic large animals such as horse, cattle, buffalo, swine and other small ruminants (1-3).The classification of diabetes differs for the large and small animals, although it has similarity to human. The common and general forms of DM are known to be insulin dependent DM (IDDM) Type-1 and non-IDDM (NIDDM) Type-2 in animals. Also, the secondary DM or Type-3 has also been identified,which is the complication of the insulin antagonisms. This occurs due to the pancreatic islet damage by the pancreatic necrosis, tumor progression, and pancreatitis. Metabolic DM is the specific experimental expression of this form, primarily described in dogs and cats (2,4)

Ozone, a gas composed of three atoms of oxygen with a cyclic structure, was initially discovered as an oxidant and a disinfectant in 1834, exerting medical affectivity firstly for gunshot gangrene(5). Evidence supports ozone has been used for the treatment of cutaneous wounds with satisfactory healing results (6) .Ozone was used in many methods , tent, bag, even injection, and systematic applications referring to rectal insufflation as well as autohemotherapy.(7)

Ozone has been used as a therapeutical agent and beneficial effects have been observed. However, so far only a few biochemical and pharmacodynamic mechanisms have been elucidated. many study demonstrated that controlled ozone administration may promote an oxidative preconditioning or adaptation to oxidative stress, preventing the damage induced by ROS (1). Taking into account that diabetes is a disorder associated with oxidative stress, we postulate that ozone treatment might protect antioxidant systems and maintain, at a physiological level, other markers of damage associated with diabetic complications.

Materials and Methods

The study was carried out of (48) adults female albino rats weighing(180-200g). Animals were housed in temperature and light- controlled rooms and allowed free access to normal diet pellet and tap water .Medical Ozone (MO) was generated with (Medical Ozone generator Aqua plus ,model No:AOT-MD-520). MO given to each animal was adjusted to a final dose of 1.1 mg/kg BW intraperitonal (8) .Huminsulin R India was used at a dose of 0.75 IU/100 g body weight (9)

Induction of diabetes mellitus:

Diabetes mellitus (DM) was induced in overnight fasting rat by a single injection of alloxn (alloxan monohydrate) at dose 150 mg / kg body weight into intraperitonal(I.P) . after injection alloxan .Rats were given 5% glucose solution for 24hrs with drinking water to prevent initial drug-induced hypoglycemic mortality .

Experimental Design-:

The mature female rats were randomly divided into six groups each containing eight animals as follows :

T1	Animals daily administered (I.P) with citrate buffer (0.1 M, pH 5). daily for six weeks.
T2	Animals daily administered 1.1 mg/kg of MO (I.P) ,daily for six weeks.
T3	Animals Untreated diabetic group will induced with single (I.P) injections of alloxan (150 mg / kg)
T4	Diabetic rats will receive 1.1 mg/kg of MO (I.P), for six weeks.
T5	Diabetic rats will receive the dose of Mixtard insulin will injected subcutaneously at a dose of 0.75 IU/100 g body weight , for six weeks.
T6	Diabetic rats will receive 1.1 mg/kg of MO (I.P) and Mixtard insulin will injected subcutaneously at a dose of 0.75 IU/100 g body weight , respectively, for six weeks

Determination of Bleeding Time (BT)

The rat tail was warmed for one minute in water at 40℃ and then dried. A small cut was made in the middle of the tail with a scalpel. Bleeding time started when the first drop touched the circular filter paper. It was checked at 30 sec intervals until the paper no longer stained with blood. The time taken for bleeding to stop was recorded for each rat and the average was taken as bleeding time (10).

Clotting time estimation (CT)

Clotting time was determined using capillary glass tube method. A standard incision was made in the skin of the ear and the blood was taken into a capillary glass tube and the time of collection was noted. Pieces of capillary glass were broken from one end at every thirty seconds and the appearance of fibrin threads was used as the end point and the time was noted in seconds**(**11).

Collection of Blood Samples

On the 60th day of the experiment, Each of the rats were anesthetized . 10 ml blood samples were collected from each sacrificed rat by cardiac puncture and 5 ml of blood discharged into blood tube EDTA K for hematological analysis and the residual amount discharged into gel tube to obtain the serum for biochemical analysis .Hematological parameters that were analyzed include Red Blood Cells (RBC_s), White Blood Cells (WBC_s), Haemoglobin (Hb), Platelets. They were determined using Automated Hematology Analyzer Genex(COUNT 60).A calcium test kit is used to measure in serum(BIOLABO kit , 052010A)

Table (1) effects of Medical Ozone on some hematological parameters of diabetic female rats .

Groups n=8	Parameters		
	RBCs (10^6/µL)	Hb(g/dl)	WBCs(10^3/µL)
T1	7.8642 ±0.2364 [b]	13.8250 ± 0.2246 [b]	7.71 ± 0.47[ab]
T2	9.0783± 0.1595 [a]	14.4250 ± 0.1605 [a]	8.05 ± 0.47[a]
T3	6.0575± 0.2153 [c]	10.1917 ± 0.1367 [d]	6.00 ± 0.48[c]
T4	7.4225 ± 73.976 [b]	10.6583 ± 0.1389 [cd]	6.37 ± 0.66[c]
T5	7.3983 ± 73.9788 [b]	10.3583 ± 0.2224[d]	6.26 ± 0.62[c]
T6	7.8892 ± 0.1807 [b]	10.9000 ± 0.1692 [c]	7.52 ± 0.73[b]
LSD	0.60	0.50	0.47

Values expressed in small letters mean significant differences at p. value < 0.001

According to the results obtained from the current study ,recorded a significant (p >0.001) rise in RBCs count in the (T2) treated group when compared with the control group and the rest of the diabetic groups. as well as noticed that there was no significant difference in the number

of RBC between the groups (T1, T4, T5 , and T6), also a significant decrease was recorded in the diabetic group when it was compared with the control group. As for the results of white blood cells, no statistically significant difference was recorded between(T2) group and control group, As for the treated diabetes groups, no statistical difference was recorded when comparing them, except (T6) group, which came close to the value of the control group.

The results of hemoglobin value in the current study, noticed the T2 group recorded a statistical increase in the level of hemoglobin when compared with the T1 group. While the groups of diabetics were given doses of (MO)T2 or (Insulin) T4 , they recorded a significant increase when compared with other diabetic groups

Table (2) Effects of Medical Ozone on Hemostatic Parameters of Diabetic Female Rats .

Groups n=8	Parameters			
	Platelets count x 10^3/Ml	Clotting Time	Bleeding Time	Calcium
T1	457.0833 ±55.9 [d]	2.0333± 0.22293 [a]	3.5833±0.41090 [a]	7.1917± 0.69473 [a]
T2	473.5000 ± 48.9[cd]	1.8833± 0.15859 [a]	3.2792 ± 0.33345 [b]	7.3833± 0.67532 [a]
T3	769.4167± 58.6 [a]	0.9542±0.13049 [d]	1.9583± 0.37005 [c]	5.0375±0.55478 [c]
T4	571.0000 ± 39.6 [b]	1.1058± 0.16082 [c]	2.0117± 0.42000 [c]	5.1083 ±0.46993 [c]
T5	553.8333 ± 46.6 [b]	1.9833± 0.14035 [b]	3.2750± 0.249930[b]	5.1500± 0.61274 [c]
T6	493.0000 ± 30.6 [c]	1.8750±0.171230 [b]	3.2708±0.33671[b]	5.6417 ± 0.47760 [b]
LSD	38.8	0.13	0.29	0.47

Values expressed in small letters mean significant differences at p. value >0.001

The platelet count results showed a significant increase in the (T3)group compared to the other groups, while the (T2) group did not show any significant difference when compared with the control group. At the same time, there was no significant difference between (T4) and (T5) , but compared to(T3)there was a significant decrease. while (T6)group recorded a significant decrease compere to (T3) group In addition to being close to reading of the (T2) group.

Results showed a significant decrease (p >0.001) in bleeding time in the (T2) and (T3) groups when compared to the (T1), at same time had showed (T4) group non-significant different in bleeding time compared to (T3) group while the (T5and T6) recorded significant increase when compared to (T3) group.

The results of clotting time appeared a significant decrease in the (T2) group when compared with the control group, while the (T3) group showed a significant decrease compared to the control group.

On the other hand, all treated diabetic groups recorded a significant increase in clotting time compared to the (T3) group and at the same time both groups (T5and T6)were had approached to the reading of (T2) group .The obtained results in tab. (2) revealed non-significant change Ca++in serum of female rats when compered between(T2) group and (T1) group while the results showed a significant decrease in serum

Ca++of diabetic group (T3) compared with control group. At the same time, there was non-significant difference between (T4) , (T5) compared to (T3) group.

Discussion

In a study by Lopez (12) that used medical ozone autohemotherapy in horses, a significant increase in red blood corpus was detected; this result is similar to observations in the present study .Ozone therapy acts as a form of oxygen therapy that stimulates mitochondria and gives them bath of fresh air. Oxygen is the most vital element required for human life and it is the key to good health ,the best way to optimize health is to oxygenate every cell in the body (13).

The results of present study supported by Bocci (20) ,Mahmoud *et al*., (13) found that ozone therapy increase the oxygen level in blood, oxygenates the tissues in the whole body and can activate biochemical pathways in erythrocytes, haemoglobin and leukocytes without acute or chronic toxicity. during prolonged ozone therapy, the bone marrow may release a cohort (about 0.9% of the pool) of new erythrocytes with improved biochemical characteristics (15).

The appreciable decrease in RBCS count, Hb and WBCS count in the untreated alloxan induced diabetic rats correlates with the findings of Mansi (16) and Mounce and AL-Saeed, (17). Reactive oxygen species have also been implicated in the mechanism of RBCS damage(18). The cytotoxic action of diabetogenic agent such as alloxan is mediated by reactive oxygen species (19).

In diabetic rats red blood cells (RBC) count was found decreased significantly, due to the non-enzymatic glycosylation of RBCS membrane protein which is directly associated with the hyperglycemic condition. Because high glucose level leads to the generation of toxic products alteration which causes many such as reduced production of bone marrow (20). Hyperglycemia results in glycosylated haemoglobin, thus total haemoglobin level is decreased in alloxan induced diabetic rats (21). Reduction in haemoglobin may be accompanied by a fall in the red blood corpus count and packed cell volume (22).

Hyperglycemic situation leads to the production of lipid peroxides. These peroxides increases membrane stiffness, decreases cellular deformability, decreases life span of RBCs and lipid fluidity and also causes hemolysis of RBCs and low haemoglobin (23). Previous study recorded WBCs which play a pivotal role for initiating immune showed significant decrease ($P>0.05$ and $P>0.01$ respectively) In untreated diabetic rats(24) .

According to the current results, observed that the addition of MO with daily doses of insulin does not affect the action of insulin, as recorded a significant increase in the number of RBCs and WBCs .The combination of metabolic actions of insulin and the ability of ozone to enhance antioxidant endogenous systems, in addition to oxidative stress by oxidative preconditioning mechanisms, resulted in reduction in lipid and glucose oxidation, and increased NO oxidation caused by insulin alone plus antioxidant supplementation of ozone can normalize all parameters (25). Ozone may increase the endogenous antioxidant defense mechanism in rats, thereby protecting them from cranial and spinal tissue neuropathies .Moreover, the combination of ozone and Insulin therapy has better prospectus for treating DM (26).

Diabetes associated reductions in the antioxidant state can be ameliorated by insulin combined with antioxidant therapy. These results indicate that the combination of insulin treatment with ozone in calculated doses leads to a more significant enhancement in metabolic oxidative states and vascular complications in diabetic rats (27) .

Administration of insulin and exogenous antioxidant significantly normalized the RBCs count because of ability of antioxidant scavenges the peroxides, reduces membrane damages, simultaneously insulincompetes for the regulation of glucose metabolism and hence prevents glycosylation of proteins (28).

Ozone causes a decrease in blood and plasma viscosity (plasma macromolecules are decreased). Moreover ozone may induce a hypo-coagulatory state thus decreasing the tendency to clot (29), This agrees with the results of the current study.

In the present study diabetes rats (DM) group showed increment in platelet count. It is reported that platelets hyperactivity in patients with hyperglycaemia resulting from a dysregulated signaling pathways that

lead to an increased tendency to activate and aggregate response to a given stimulus (30), Platelets activation therefore triggers thrombus formation, microcapillary embolization and facilitates the development of other cardiovascular diseases. Akingbami *et al*.,(31) added that diabetes mellitus is characterized by enhanced platelets activation and coagulation proteins and reduced fibrinolytic activity which usually precede the development of cardiovascular complications.

As shown in table(2),It was observed that alloxan decreased the bleeding time and clotting time .The increased platelets counts in the diabetic rats may have been responsible for the observed decrease in bleeding and clotting times and may increase the risk of intravascular blood clotting and associated diseases (28). As for the results of the calcium level in the serum, a significant decrease was recorded in the diabetic groups when it was compared with the (control and MO) groups this is consistent with a previous study(32).

In many research studies, a relationship between trace elements and diabetes mellitus was observed, in different study an alteration in the metabolism of these trace elements (calcium and magnesium were noted (33). Calcium ion plays an important role in glycemic control by affecting the biosynthesis and release of insulin from beta cells of the pancreas. There is a significant correlation between diabetes and hypocalcemia (34).

Current study found that after ozone therapy there are no statistically significant changes in haemostatic parameters ,at same time MO plus insulin might provide a simple, safe, and more effective means for preventing and ameliorating chronic complications of DM than each given alone perhaps because their action as antioxidant.

The combination of metabolic actions of insulin and the ability of ozone to enhance antioxidant endogenous systems, in addition to oxidative stress by oxidative preconditioning mechanisms, resulted in up regulation of NOS isoforms and reduction in lipid and glucose oxidation, and increased NO oxidation caused by insulin alone plus antioxidant supplementation of ozone can normalize all parameters (35),(36).

Conclusion

Finally, from the previous discussion of these results and according to reports of other investigators in similar studies: It can be explained that ozone therapy has a significant effect in improvement of diabetic patients undergoing insulin treatment as evidenced by the improvement in hematological and hemostatic parameters. The results of the current study would introduce a scientific applicable protocol to help, physicians of dealing with diabetic, organize a plan of care to overcome this problem and prevent the complications of it and the development of associated morbidities.

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