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Medical Ozone Treatment Alleviate Blood Oxidative Stress And Pancreas Damage In an Alloxan-Induced Diabetes Model in Rats

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***Abstract*— Diabetic mellitus (DM) is a prevalent systemic disease affecting a human and animals. The effects of diabetes are devastating and well documented. The increased production and/or ineffective scavenging of reactive oxygen species (ROS) may play a critical role in the development of diabetic complications. Therefore, it seems reasonable that antioxidants can play an important role in the improvement of diabetes. So, use medical ozone to study if it can regulate the oxidative complications of DM .The study was carried out on forty eight albino females rats included six groups Group I (Control) : 8 female rats were injected IP with citrate buffer (0.1 M, pH 5) daily for 6 weeks ,Group II (MO): 8 female rats were injected IP 1.1 mg/kg b.w of MO daily for 6 weeks ,Group III (DM): 8 female rats induce diabetes by injection IP with freshly prepared alloxan 150 mg/kg b.w in citrate buffer (pH 5), Group IV(DM+MO): 8 diabetic female rats were injected IP 1.1 mg/kg b.w of MO daily for 6 weeks, group V(DM + Insulin): 8 diabetic female rats were injected subcutaneously 0.75 IU/100 g b.w. of Insulin daily for 6 weeks ,Group VI(DM +MO +Insulin):8diabetic female rats were injected IP 1.1 mg/kg b.w of MO and Insulin injected subcutaneously at a dose of 0.75 IU/100 g b.w , respectively daily for 6 weeks. experimental study parameters include antioxidant enzymes assay and histology the study for pancreas. The results of study recorded Medical Ozone injection at a dose of 1.1 mg save and showing a non-significant difference when compared (MO) group with the control group for the following parameters: Glucose level ,body weight , .At the same time, CAT, SOD and GSH , recorded a significant increase in the (MO)group compared to the control group, On the other hand, a significant decrease was recorded in MDA. Histology (MO) group showed normal pancreatic parenchyma; where normal islets of Langerhans and normal acinar also results of (DM+MO) group there was displaying nearly normal structure with some focal necrosis of islets of Langerhans on ,the other hand (DM+MO+Insulin) group Section of pancreas showed marked improvement with Langerhans normal pancreatic parenchyma; normal islets of Langerhans and normal acinar cell ,so that both Medical Ozone and Insulin used separately or together, can be useful in improves survival of some pancreatic cells.**

***Keywords* - Medical Ozone, Oxidative Stress, Pancreas, Alloxan and female Rats**

I. INTRODUCTION

In the past two decades, Medical Ozone therapy has received more attention. Research and scientific experiments have increased the possibility of using it as a treatment for many veterinary diseases, and by veterinarians specialized in this field[1]. The Ozone molecule was discovered in the year 1840, and the name Ozone is derived from the Greek word “Ozein”, which means smell, because of the unpleasant and distinctive smell [1,2]. Ozone (O3) is one of the unstable molecules in nature, as it consists of three atoms that share the same electron. Naturally, Ozone is generated in two ways, either as a result of the electrical discharge that occurs to atmospheric oxygen after the lightning process or through the emission of ultraviolet radiation, which in turn stimulates the regeneration of the natural Ozone layer[3]. Industrially, Medical Ozone is produced using a special generator that uses pure oxygen, after being subjected to an electric arc, similar to what happens in nature during an electrical discharge. The material used in the manufacture of the Ozone device is a material that is resistant to the influence of Ozone, thus ensuring the measurement of Ozone concentrations generated. Given that the Ozone molecule is unstable, it must be prepared before each use. In fact, ozone therapy is a cost-effective treatment that can be applied medically to animals and humans. [4-6]. According to what was indicated by [6,7], the principle of using Ozone as a treatment for many diseases depends mainly on its ability to

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inhibit oxidative stress. As it presents viricida, bactericidal, fungicidal and anti-inflammatory effects [5]. Also, [8], indicated that Medical Ozone therapy has been applied in veterinary science and is still in the emergency phase, Medical Ozone acts as a second messenger, thus mediating hydrogen peroxide induced lipid peroxidation and oxidative stress. As mentioned earlier, the principle of action of Ozone is that it is an efficient regulator of oxidative stress by stimulating the antioxidant system.

Diabetes is known as a metabolic disease that occurs due to the abnormal metabolism of carbohydrates, which leads to a high level of glucose in the blood and urine. Diabetes mellitus is diagnosed in the canine and feline family after humans, and the clinical signs described are rarely observed and investigated in other small and large domestic animals. [9,10]. The classification of diabetes varies for animals, although it has similarities to humans. The prevalent forms of DM are known to be insulin dependent DM or type -1 or and non-IDDM or type 2 in animals. Also, due to complications of the insulin antagonisms secondary DM or type -3 has also been identified, this occurs due to pancreatic islet damage [11].

The aim of this study: The research was directed to study the effect of Medical Ozone on experimental animals after the inducement of Diabetes mellitus by using alloxan and in order to evaluate the synergy between Medical Ozone and Insulin and the possibility of using Medical Ozone as a complementary treatment in diabetes mellitus.

II. METHOD AND MATERIALS

A. Experimental Animals

The study was carried out on forty eight albino female rats (at 180± 20 gm average weight). were purchased from the animal house of the biology department, college science, Thi-Qar University. They were placed in individual cages. The rats were fed with standard rodent chow and provided with tap water. The experiment was performed under controlled conditions (temperature ,humidity and a 12- hours light-dark cycle) .

B. Medical Ozone preparation

Ozone was generated with (Medical Ozone generator Aqua plus, model No:AOT-MD-520).The Ozone generating method is German corona discharge. The Ozone given to each animal was adjusted to a final dose of 1.1 mg/kg b.w [12].

C. Induced experimental diabetes.

Diabetic mellitus (DM) was induced in eighty four female rats .The acclimated rats were fasted for 12 h with free access to water, then injected with a single intraperitoneal injection of a freshly prepared solution of alloxa[13]. At a dose of 150 mg/kg. b.w [14].The rats were then kept for the next 24 h in 5% glucose solution bottles in their cages to prevent hypoglycemia , Three to five days after injection, diabetic symptoms such as polydipsia, polyuria , serum glucose level were monitored by a glucometer.

D. Experimental groups and design

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

Group I (Control): 8 female rats were injected IP citrate buffer (0.1 M, pH 5). daily for six weeks.

Group II (MO): 8 female rats were injected IP 1.1 mg/kg b.w of Medical Ozone ,daily for six weeks.

Group III (DM) : 8 female rats induce diabetes by injection IP freshly prepared Alloxan 150 mg/kg b.w in citrate buffer (pH 5)

GroupIV(DM+MO): 8 Diabetic female rats were injected IP 1.1 mg/kg of Medical Ozone, daily for six weeks.

GroupV(DM+Insulin):8 Diabetic female rats were injected subcutaneously Insulin at a dose of 0.75 IU/100 g b.w, daily for six weeks

Group VI(D M+MO +Insulin) : 8 Diabetic female rats were injected IP 1.1 mg/kg of Medical Ozone and Insulin injected subcutaneously at a dose of 0.75 IU/100 g b. w , respectively, daily for six weeks.

E. Experimental studied parameters

Determination of serum glucose concentration (mg/dl)

A standard enzymatic method by [15] was used for the determination of glucose spectrophotometrically at λmax 500 nm using a commercial kit from Biolab Company Germany.

F. Determination of body weight

The body weight of each animal was measured on the day of commencement of treatment and on the 60 day using electronic weighing balance Digital Precision Weighing Balance (JCS-QC03) – Chin 3.6. Animal Sacrificing and Collection of Blood Samples

After 6 weeks, Each of the rats was anesthetized by placing it in a glass jar with a gauze moistened with chloroform and observed for signs of decreased motility and unsteady gait for about(20) seconds, each rat was dissected by making a midline incision on the ventral aspect from the thoracic region to the abdomen. 5 ml blood samples were collected in a gel tube from each sacrificed animal by cardiac puncture to obtain the serum, the serum was prepared by centrifugation of blood at (3000) rpm for (10) minutes and frozen at (-20C º) until it was used to determine the oxidants and antioxidant levels between groups. And pancreas was removed for histopathological study .

G. Enzymatic Antioxidants and Malondialdehyde Serum Catalase(CAT)

Serum catalase was measured using an ELISA kit (Elabscence kit , catalog No :E-BC-K031-S) and the method based on the following principle [16]

Serum Superoxide Dismutase (SOD)

Serum SOD was measured using an ELISA kit (Elabscence kit , catalog No :E-EL-H1113) and the method was based on the following principle[17].

Serum GSH concentration:

The serum Thiol concentration was measured according to the Ellman method [18].

Serum Malondialdehyde (MDA)

The concentration of MDA in serum was determined according to Buege and Aust method [19].

H. Histopathological examination

Following the sacrifice, the pancreas was removed from the dead rats and then washed with a physiological saline solution (0.9%NaCl) for the removal of the blood, which might obstruct the process of fixation. Steps for preparation and staining of histological sections according to[20]. Fixation, Dehydration ,Cleaning, Wax Infiltration, Embedding sample in wax ,Sectioning ,de-waxing and Staining (Haematoxylin and Eosin).

I. Statistics analysis

All data of the present study were statistically analyzed by using Microsoft windows EXCEL (version2019) and SPSS version 26, based on using Paired sample t test, One Way ANOVA, Least Significant Difference and Non-parametric Chi-Square at p. value < 0.0001.

III. RESULTS

A. Effects of Medical Ozone on Blood glucose of diabetic female rats

Glucose concentration (mg/dl) is an index of diabetes and hyperglycemia in animals. The results of glucose concentrations of rats are shown in table (1). The glucose levels of experimental rats were not significant different between Medical Ozone (MO) group and control group . As for the groups in which diabetes was induced by alloxan, noticed a significant increase in glucose levels in these groups(DM, DM +MO DM+Insulin and DM+MO+Insulin) compared to the normal group and (MO) group. The best response was noted in the (DM+MO+Insulin)group, which was treated with both (MO) and Insulin together. When comparing the treatment with (MO) and Insulin separately (group DM +MO and DM+Insulin), it was noted that the Insulin group was more responsive, as it recorded a significant decrease in the level of glucose compared with (DM) group in table (1).

TABEL I . EFFECTS OF MEDICAL OZONE ON BLOOD GLUCOSE

Groups N= 12	Mean ± SD
	Glucose mg/dl
Control	84.8 ± 3.51e
Medical Ozone (MO)	82.7 ± 3.36e
Diabetic mellitus (DM)	488.9 ± 18.5a
DM +MO	318.0 ± 35.9b
DM+Insulin	203.7 ± 18.4c
DM+MO+Insulin	185.7 ± 14.5d
LSD	15.6

Values expressed in small letters mean significant differences at p. value less than 0.001, N: number of animals

Effects of Medical Ozone on body weight of diabetic female rats

The initial body weights were similar in normal and diabetic groups, whereas DM,DM+MO and DM+Insulin groups showed a significant reduction in body weight when compared to the control and MO goups .while DM+MO+Insulin ameliorates this depression as compared to other diabetic group .

TABAL II . Effects of Medical Ozone on body weight of diabetic female rats

Groups N= 12	Mean ± SD		Mean Difference
	Inatial B.W	Final B.W	
Control	186.8 ± 1.94^{a}	199.0 ± 0.90^{a}	12.5
Medical Ozone (MO)	180± 1.94^{a}	201.7 ± 2.59^{a}	21
Diabetic mellitus(DM)	175.5± 1.94^{a}	147.7 ± 6.54^{b}	27.8
DM +MO	190 ± 1.94^{a}	51.7± 6.54^{b}	38.3
DM+Insulin	189 ± 1.94^{a}	150 ± 6.54^{b}	39.1
DM+MO+Insulin	188 ± 1.94^{a}	199.0 ± 0.90^{a}	11

Values expressed in small letters mean significant differences at p. value less than 0.001, N: number of animals

TABLE III . effects of Medical Ozone on Serum levels of CAT , SOD , GSH and MDA of diabetic female rats

Groups N=12		Mean ± SD		
	CAT $_{U/mL}$	SOD $_{ng/ml}$	GSH $_{\mu mol/l}$	MDA $_{\mu mol/l}$
Control	2.94 ± 0.41^{b}	0.405 ± 0.051^{b}	238.7 ± 34.6^{b}	2.24 ± 0.40^{c}
Medical Ozone (MO)	3.54 ± 0.36^{a}	0.508 ± 0.084^{a}	348.3 ± 8.38^{a}	1.84 ± 0.45^{c}
Diabetic mellitus(DM)	1.69 ± 0.43^{d}	0.178 ± 0.060^{d}	109.6 ± 5.00^{e}	4.53 ± 0.94^{a}
DM +MO	2.19 ± 0.11^{c}	0.281 ± 0.120^{c}	125.7 ± 7.41^{d}	3.40 ± 0.83^{b}
DM+Insulin	2.07 ± 0.39^{c}	0.221 ± 0.502^{cd}	120.3 ± 4.20^{de}	3.56 ± 1.08^{b}
DM+MO+Insulin	2.31 ± 0.38^{c}	0.301 ± 0.064^{c}	165.0 ± 6.04^{c}	3.02 ± 0.29^{b}
LSD	**0.29**	**0.063**	**12.4**	**0.59**

Values expressed in small letters mean significant differences at p. value less than 0.001, N: number of animals

B. Histopathology Features of pancreas Tissue

The histopathology of rat pancreas was shown in Figure(1) Microscopic investigation of pancreas sections of Control group showed the normal appearance of islets of Langerhans and normal pancreatic parenchyma; Similar findings were obtained in the (MO) group in Figure (2) showed normal pancreatic parenchyma; where normal islets of Langerhans and normal acinar ;However, The histological sections in Figures (3 A and B) showed several

changes in rats pancreas (DM) group that treated with 150 mg/kg b.w of alloxan the diabetic rats showed pathological changes of both exocrine and endocrine components vacuolation, necrosis of islets of Langerhans cells, and fatty change were observed, In addition to these changes, congestion and edema were reported. On the other hand, (DM+MO) group there was displaying nearly normal structure with some focal necrosis of islets of Langerhans as in Figure (4 A and B) and there was avery scanty inflammatory cell infiltration . Figure(5) clarified a Section of pancreas in group (DM +Insulin) showed normal pancreatic tissue; normal exocrine part and normal islets cells, Similar findings were obtained in the (DM+MO+Insulin) group Section of pancreas showed marked improvement with Langerhans normal pancreatic parenchyma; normal islets of Langerhans and normal acinar cell Figure (6).

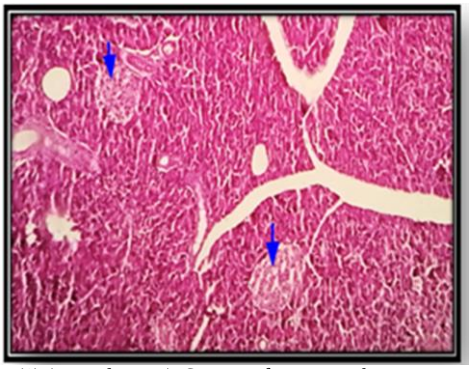


Fig. (1) (control group). Section of pancreas showing normal pancreatic parenchyma; normal islets of Langerhans (blue arrows) and normal exocrine tissue

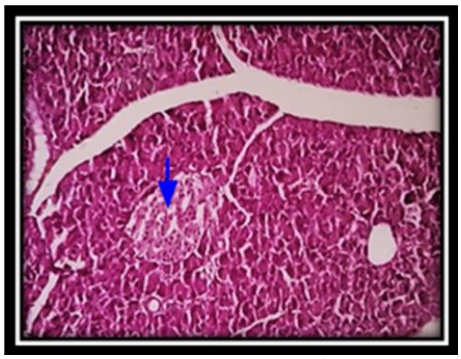
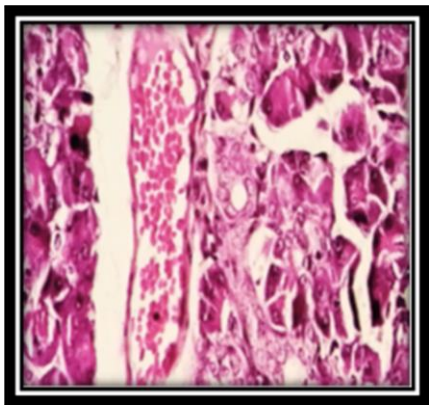
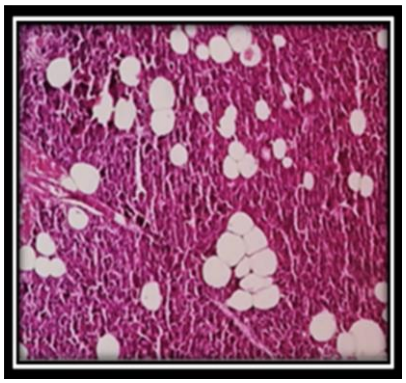


Fig. (2) Medical Ozone(MO). Section of pancreas showing normal pancreatic parenchyma; normal islets of Langerhans (blue arrows) and normal exocrine tissue.

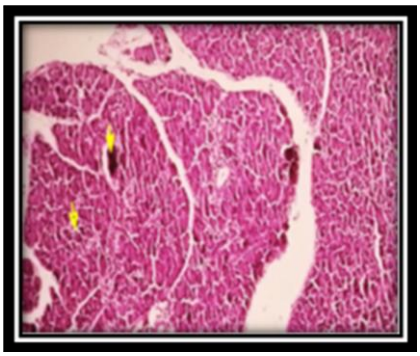


(A)

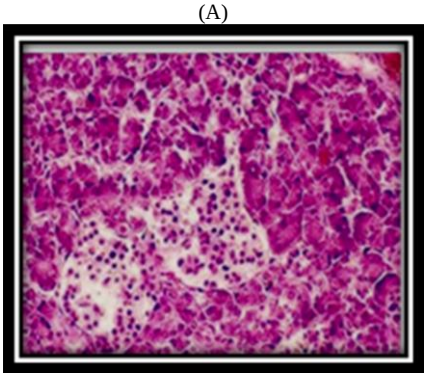


(B)

Fig. (3) Diabetic group (DM). **A** Section of pancreas showing congestion and edema. H&E, 400X. **B** Section of pancreas showing fatty pancreas disease. H&E, 100X



(A)



(B)

Fig. (4) (DM+MO). A Section of pancreas showed focal necrosis of islets of Langerhans (yellow arrows). H&E, 100X. B Section of pancreas showed inflammatory cell infiltration, H&E, 100X

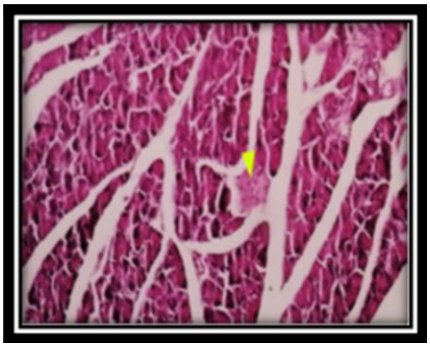


Fig.(5) D+Insulin . Section of pancreas showing normal pancreatic tissue; normal exocrine part and normal islets cells (yellow arrow). H&E, 100X

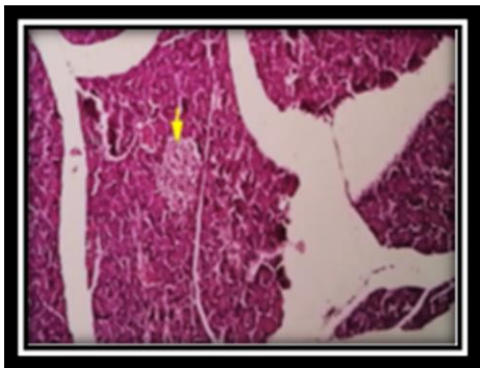


Fig. (6) (DM+MO+Insulin). Section of pancreas showing normal pancreatic parenchyma; normal islets of Langerhans (yellow arrow) and normal acinar cells. H&E, 100X

IV. DISCUSSION

Medical Ozone (MO) did not change the blood glucose level of the non-diabetic rats, this result is interesting because it might indicate that used doses of medical ozone in current study are therapeutically safe, The results of present study supported by[21]. Alloxan induces diabetes through ROS that leads to a rapid destruction of pancreatic beta cells causing hyperglycemia [22]. Hyperglycemia in turn increases the generation of free radicals by glucose auto-oxidation[23]. Administration of MO (1.1 mg/kg b.w) for 60 days led to a significant decrease in blood glucose level this effect produced by MO treatment seems to be associated with the antioxidant properties of Ozone. This is in agreement with [24] found a direct link between the presence of oxidative stress and impaired glucose uptake. In adipocytes, glucose uptake is rapidly decreased in the presence of hydrogen peroxide (H_2O_2), an effect that was reversed by MO)treatment in preclinical studies it is of critical importance to maintain the antioxidant potential of the pancreatic cell in order to ensure both its survival and insulin secretory capacity during times of increased oxidative stress. On the other hand, the pancreas is the main target of alloxan. The antioxidant–prooxidant balance, associated with the control of oxidative stress was favored by Ozone treatment, Ozone reduced hyperglycemia and it increased the antioxidant defenses of the pancreas. There is evidence that hyperglycemia can lower both the activity of a number of antioxident enzymes presumably by glycation[25]. [26]observed that diabetic patients have lowered antioxidant defenses, both enzymatic and non-enzymatic so that increased oxidative damage .Therefore, these results suggest that MO protective effects on antioxidant endogenous defenses improve glucose metabolism. In the study by[27] administration of MO (1mg/kg b.w.) for 15 days revealed a significant decrease of serum glucose when compared to the corresponding values in diabetic-non treated rats. This indicated that Ozone therapy potentially improved the glycemic control during diabetes.[28] recorded a significant increase in β-cell number in diabetic ozone-treated group when compared with diabetic non-treated group. [29] observed a significant decrease in the percentage of damaged islets for diabetic rats treated with Ozone with regard to diabetic group. Ozone therapy appears to be an effective method for DM treatment. The reason is in Ozone mechanisms when it can perform a number of processes, which provide its positive effect. First, Ozone improves the penetration of cellular membranes for glucose. It is achieved by stimulating pentose phosphate pathway and aerobic glycolysis that in case of DM are inhibited. It promotes hyperglycemia decrease due to better transport of glucose into tissues. Second, Ozone activates glucose metabolism that results in increasing content of 2,3 diphosphoglycerate in erythrocytes which provides better oxygen supply into the tissues. Patients with diabetes mellitus have the so called glycosylated hemoglobin forming very strong bonds with oxygen, thus, inducing hypoxia and determining the severity of the disease. That is why hypoxia control with the help of Ozone therapy is of the key importance in the course of treatment. After the course of ozone therapy the patients had significant decrease in the levels of urine, cholesterol and fibrinogen[30]. Both Ozone and insulin may act synergistically to reduce blood glucose level, Both of them independently exert hypoglycemic effects[12]. Ozone therapy may be considered as an adjuvant to insulin in the treatment of diabetes to prevent or alleviate diabetes induced complication[27]. loss of body weight have been associated with diabetes mellitus[31,32]. Alloxan induced diabetes significantly decreases body weight of the diabetic untreated rat as the study duration increase compared with the diabetic treated and control rats. Alloxan induced diabetes causes a significant loss in body weight while treatment with (MO) improvement the body weight. Diabetes is accompanied with increased glycogenolysis, lipolysis,gluconeogenesis and these biochemical activities result in muscles wasting and loss of tissue protein. (MO) is seen to prevent these changes and thus restore the body weight of the diabetic treated rats. This finding is similar to previous studies ,In diabetic individuals, the lack of insulin causes inhibition of protein synthesis and increased degradation which increases amino acid levels in the blood to be subsequently used for gluconeogenesis [33,34]. 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[35].

There is emerging evidence that diabetes leads to the depletion of the cellular antioxidant defense system and increased levels of ROS. The concept of oxidative stress, being an important in the onset and progression of diabetes and its complications, may offer a unique therapeutic option

for the treatment of diabetes and its complications using antioxidants or nutrients with high antioxidant capacity[36].This study evaluated the antioxidant potentials of Ozone in the alloxan diabetic model, Ozone revealed that had efficacy or capable of being synergistic or potentiating the effect of Insulin .There is much evidence from experimental studies that the formation of ROS is a direct consequence of hyperglycemia .The oxidative stress due to ROS generation may play an important role in the initiation of the pathophysiological cascade of events leading to vascular and other diabetic complications . In this way, loss of antioxidant-pro-oxidant balance represents a linking between diabetes and its complications [37]. Diabetes mellitus causes a significant increase in plasma MAD and a significant decrease in serum CAT , SOD and GSH. The high glucose level causes an increase in a product of oxidative damage, namely, MAD because of accelerated metabolism by the thermic effect of food and increased mitochondrial respiration and release of superoxide[38]. Glucose auto-oxidation and protein glycation are important additional sources of free radicals during hyperglycemia [39].[40,41] reported a decrease in total antioxidant status in diabetes mellitus, thus indicating the delicate balance between oxidants and antioxidants, whereas hyperglycemia can result in the generation of free radicals through several biochemical pathways such as nonenzymatic glycation, the poly pathway, and glucose auto-oxidation[42]. Free radicals can result in the destruction of antioxidant defences and enhanced susceptibility to lipid peroxidation. the current study, Medical Ozone(MO) has an antioxidant effect so it play this role through lowering the MAD level so (MO) tends to bring the peroxide back to near normal levels, which indicates that it may enhance antioxidant endogenous systems. These results were in agreement with those of [38], showed that (MO) treatment reduced oxidative stress in alloxan -induced diabetic rats and decreased MAD production. These results were also supported by [24] who showed that with (MO) treatment, there was a reduction in total peroxides and the concentrations of MAD and an increase in antioxidant systems. Insulin therapy alone resulted in a significant reduction in plasma MAD. These results were also supported by[43], who showed that insulin therapy normalizes the activities and protein expression of all antioxidant enzymes. According to[44], Insulin infusion could maintain the plasma antioxidant defenses. Insulin is involved in the regulation of fatty acid metabolism. It exerts inhibitory effects on lipolysis and leads to a reduction in free fatty acid production. Free fatty acid metabolism maintains the generation of free oxygen species as their conjugated double bonds can interact with hydroxyl radicals and hydrogen peroxide[45].[46] identified that Ozone, with its natural oxidizing capacity, can generate mild or moderate oxidative stress. This causes the body to respond by increasing the action of Nrf2, and thus, generate some beneficial effects, such as increased levels of enzymes that detoxify oxidants, such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx)

Histopathological change of pancreas

Pancreas is a glandular organ measured about 22 centimeters long and it possesses about 1 to 2 million islets of Langerhans in which β-cells have occupied 60 % of them[47]. Islets of Langerhans collectively considered the endocrine part of pancreas. Islets cell types include insulin-producing β-cells, glucagonproducing alpha cells, somatostatin-secreting delta cells and pancreatic polypeptide-secreting gamma cells[48]. Insulin is secreted from β- cells in response to rising concentrations of glucose in the blood, such as those occurring after a meal[49]. Histopathological data obtained from the current study were in consistent with[50] reported alloxan is widely used to induce diabetes in experimental animals by generation of ROS that causes damage of pancreatic β-cells. Moreover, the action of ROS with a simultaneous massive increase in cytosolic calcium concentration cause rapid destruction of pancreatic β-cells and thus hyperglycemia is occurred Inhibition of glucokinase enzyme is involved in the toxic action of alloxan on pancreatic β cells. According to a study conducted by[51] on a group of diabetic dogs showed the pancreas of the diabetic group showed hemorrhage , aerea of vacuolation and destruction of langerhans island cells which surrounded by fibrous capsule. The efficacy of ozone in several pathologic conditions has been extensively described such as The efficacy of ozone in several pathologic conditions has been extensively described such as studies of [52], that showed the efficacy of ozone treatment in oxidation- and inflammation-related pathologies. Ozone has been proved to have beneficial effects in rats during myocardial reperfusion[53] and, more recently, in rats subjected to prolonged high-intensity physical exercise, reducing muscular fatigue and improving cardiac performance [54].Ozone treatment rescued the diabetic pancreatic tissue, reducing the tissue degeneration evidenced by the partial restoration of normal cellular population size of islets of Langerhans and absence of islet damage, ozone treatment improves pancreatic cell survival. This was exerted through the increase of the endogenous Nuclear Factor Erythroid 2-Related Factor (Nrf2), and glutathione-s-transferase (GST) enzymes levels in pancreatic tissues. This reduction results in higher levels of insulin the consequent improvement of glucose metabolism[55].

V. Conclusions

On the basis of the mechanisms of action, Ozone therapy appears to be a safe, and economical . Medical Ozone has excellent therapeutic effects on alloxan toxicity.

Medical Ozone possesses antioxidant activity and improved the redox balance .The combination of Medical Ozone and insulin should be considered as a new strategy for diabetic patients, so this combination reduces the complications of diabetes

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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