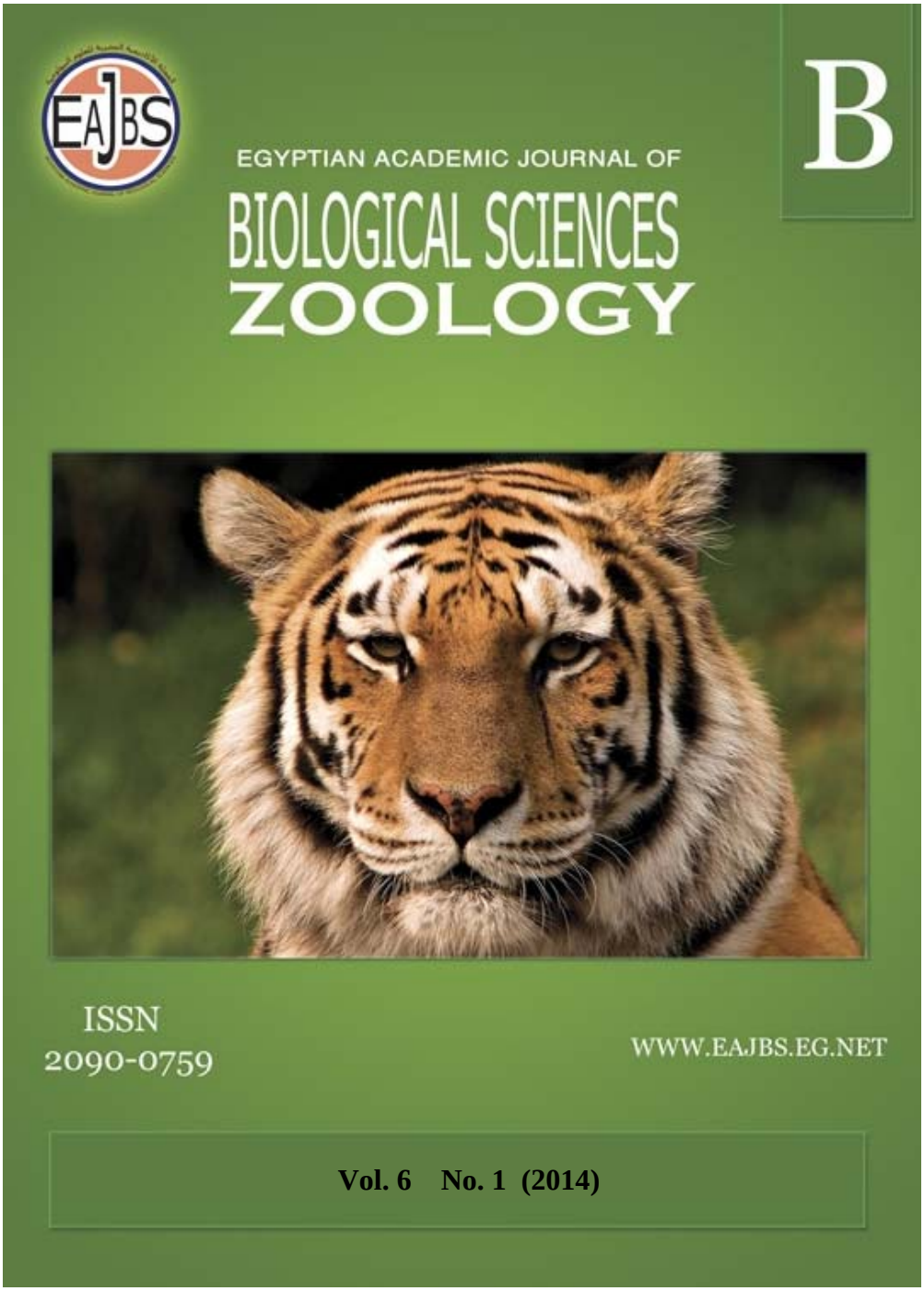

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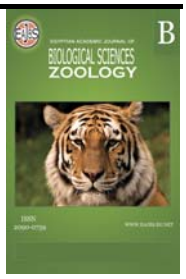

Impact of manganese as an important element in pancreatic secretion on diabetes mellitus

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ABSTRACT

Chronic hyperglycemia may cause significant alterations in the status of some micronutrients (such as manganese cations) some of which can directly modulate glucose homeostasis. In the meantime, it was reported that people with diabetes have low levels of manganese in the blood. This abnormality is also associated with exocrine pancreatic dysfunction evaluated by reduced concentrations of digestive enzymes. The purpose of this study is to investigate the interaction between endocrine and exocrine pancreatic functions, indicating the importance of manganese ions in pancreatic disorder. The present study composed of 40 rats that were divided into 4 groups; control (saline), control ($MnCl_2$), alloxan-induced diabetic and Mn^{2+}-treated diabetic rats. Fasting blood glucose, manganese (Mn^{2+}), calcium (Ca), lipase, a-amylase, malondialdehyde (MDA), glutathione reductase (GR), nitric oxide (N_2O_3) and superoxide dismutase (SOD) were measured in all groups. In this study, serum manganese, calcium, lipase, a-amylase, GR and SOD levels were decreased in diabetic groups compared to control groups. There was a clearly difference in serum pancreatic enzymes at $P > 0.05$ in Mn^{2+}-treated diabetic group compared to untreated diabetic group. It can be concluded that exocrine pancreatic insufficiency (EPI) could be one of the mechanisms underlying (DM). Accordingly, it can be suggested that therapy of diabetes should address both endocrine and exocrine pancreatic functions.

Keywords: Hyperglycemia, pancreatic enzymes, oxidative stress, antioxidants and exocrine pancreatic insufficiency.

INTRODUCTION

The pancreas is a glandular organ in the upper abdomen; it serves as two glands, a digestive exocrine gland and a hormone endocrine gland which has a complex anatomical and functional interaction (Akos, 2004). Though the exocrine part of the pancreas is influenced by the islet hormones mainly which insulin has effects on the regulation of the pancreatic digestive enzymes biosynthesis and secretions.

One of the major disorders of endocrine part is diabetes mellitus (DM) that impaired carbohydrates, fats, proteins metabolism and increased oxidative stress in pancreatic tissue (Viswanathaswamy, 2011). Furthermore, it is often associated with exocrine pancreatic insufficiency (EPI) that is the syndrome of maldigestion resulting from disorders of

pancreatic enzyme activity (Junqueira & Carneiro, 2005) and might be a consequence of underlying pancreatic diseases. Both of these disorders; DM and EPI cause abnormalities in the concentrations of digestive enzymes and some micronutrient elements such as manganese (Kazi *et al*., 2008) and calcium cations (Pittas, 2007), which modulated glucose homeostasis directly.

Alloxan-induced diabetes has been commonly employed as an experimental model of insulin dependent diabetes mellitus (IDDM) by stimulating reactive oxygen species (ROS) formation such as nitric oxide (N_2O_3), superoxide ($O2\cdot-$), hydrogen peroxide (H_2O_2), peroxynitrite (ONOO–) (Ines, 2010), that resulting in increased oxidative stress (OS) contributing to the development and progression of diabetes and its complications (Ceriello, 2000; Whiting PH, 2008).

Supplementation with antioxidant micronutrient like manganese (Dallatu, 2009), may increase activities of some antioxidant enzymes and decreased of free radicals activities. Manganese (Mn^{2+}) is dynamically involved in fats and carbohydrates metabolism, regulating blood sugar level that is required for normal insulin synthesis and secretion (Junqueira & Carneiro, 2005). It exerts direct effects on pancreatic enzyme synthesis and secretion (Murray, 1983) by stimulating calcium efflux from pancreas. In addition, it acts as a very powerful antioxidant which is a cofactor for superoxide dismutase that helps protect the cell membrane and tissues from degeneration (Nicoloff,2004; Syed *et al*., 2012).

The present study investigates the interaction between endocrine and exocrine pancreatic functions. In addition this study shows the hypoglycemic and antioxidant property of manganese supplement in alloxan-induced diabetic rats.

MATERIAL AND METHODS

Chemicals

All the reagents used for the study were of analytical grade. Alloxan, $MnCl_2.4\ H_2O_2$ were purchased from Oxford Chemical Co. and kits for the assay of MDA, N_2O_3, SOD and GR were purchased from Biodiagnostics, Dokki, Egypt.

Experimental animals

Male albino rats weighing (100-120 g) were housed under similar standard cages at 25±2°C, with 12-hr light/dark cycle. The animals were fed on basal diet and water was provided *ad libitum*. The study was approved by the SC University Ethics Committee. All experiments were performed in accordance with the National Institute of Health guidelines of care and use of laboratory animals.

Induction of experimental diabetes

Experimental rat model of diabetes was induced by a single intraperitoneally injection of 120 mg/kg alloxan (Kumawat, 2010). Alloxan solution was freshly prepared by dissolving alloxan monohydrate in normal saline 0.9% NaCl. Injecting after overnight fasted rat, the blood glucose level were determined at 72 hr. The animal with blood glucose level above 200 mg/dl only was taken in the study (Mamatha, 2011).

Experimental Design

All experimental rats were divided into 4 groups each containing 10 animals; group I control received saline 0.9% NaCl single intraperitoneal (IP) injection, group II received $MnCl_2$ 10 mg/kg single (IP) injection, group III alloxan induced diabetes that received alloxan 120 mg/kg single (IP) injection and group IV received Alloxan + $MnCl_2$ (IP) injection. The experiment lasted for 10 days and after the last day; the animals were fasted overnight and anesthetized with ethyl ether. Blood samples were taken from the cardiac puncture and collected into non-heparinized tubes. Blood samples were allowed to clot and then centrifuged at 4000 rpm for 10 minute. Serum was separated and then frozen at -20 until analysis.

Measurement of Biochemical Analysts

The fasting blood sugar level (FBS) was determined in mg/dl using a digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Germany). The manganese concentration by acid digestion (Mamatha, 2011) and the calcium concentration (Gindler & King, 1972) were measured colorimetrically. Lipase and a-amylase activities were measured using the method of reduction starch by iodine (Hopkins, 1954). N_2O_3 was measured by enzymatic conversion nitrite by nitrate reductase (Montgomery, 1961). MDA level was assayed based on MDA reaction with thiobarbituric acid (TBA) (Botsoglou, 1994). SOD activity was assayed using the autoxidation of hematoxylin (Martin, 1987).

Statistical analysis

The data were expressed as means ± SD. Statistical analyses were performed with the SPSS statistical package (version17.0; SPSS Inc.). Statistical significance for intergroup differences was assessed by unpaired Student's t-test and followed by Pearson's correlation at ($P < 0.05$, $P < 0.01$).

Abbreviations:

Antioxidants Enzymes (AOE)
Calcium (Ca)
Diabetes Mellitus (DM)
Exocrine Pancreatic Insufficiency (EPI)
Fasting blood sugar (FBS)
Glutathione Reductase (GR)
Insulin-Dependent Diabetes Mellitus (IDDM)
Malondialdehyde (MDA)
Manganese (Mn^{2+})
Nitric Oxide (N_2O_3)
Oxidative Stress (OS)
Superoxide Dismutase (SOD)

RESULTS

Results of the present study are presented in Tables 1 and 2. The effect of alloxan injection revealed a significant increase in the fasting blood sugar level at ($P < 0.01$), while the serum nitric oxide (N_2O_3) and malondialdehyde (MDA) concentrations increased at ($P<0.05$) compared to control groups. As well as, the serum manganese (Mn^{2+}), calcium (Ca) and superoxide dismutase (SOD) concentrations were significantly decreased at ($P<0.05$), besides glutathione reductase (GR) and lipase activities at ($P<0.01$) in alloxan-induced diabetic group compared to control groups.

Table 1: The variation of the means of FBS, Mn^{2+}, Ca, lipase and a-amylase parameters in controls, untreated diabetic and treated diabetic rats.

Parameters	Group (1) Saline control	Group (2) $MnCl_2$ control	Group (3) untreated diabetic rats	Group (4) treated diabetic rats
FBS mg/dl	140 ± 12	172 ± 17	220 ± 4 [b]	130 ± 20 [d]
Manganese conc. ug/l	3.6 ± 0.8	3.5 ± 1.3	2 ± 0.8 [a]	3.5 ± 1.4 [d]
Calcium conc. mg/dl	10 ± 2	11 ± 1.6	7.6 ± 0.3 [a]	10 ± 1.4 [d]
Lipase activity U/L	123 ± 9	117 ± 9	96.3 ± 5 [b]	109 ± 27
Amylase activity U/L	63 ± 0.6	63 ± 12.6	56 ± 16	66.5 ± 21

Values are expressed as mean ± SE (n=10/group).
[a] $P < 0.05$, [b] $P < 0.01$, vs. control group; [c] $P< 0.05$, [d] $P < 0.01$, vs. diabetic group using student's unpaired t-test.

Table 2: The variation of the means of N_2O_3, MDA, SOD, GR parameters in controls, untreated diabetic and treated diabetic rats.

Parameters	Group (1) Saline control	Group (2) $MnCl_2$ control	Group (3) untreated diabetic rats	Group (4) treated diabetic rats
N_2O_3 µmol/L	33 ± 2	36 ± 4	42 ± 3 [a]	29 ± 12 [c]
MDA nmol/ml	1.94 ± 0.02	1.94±0.1	3 ± 0.6 [a]	1.93±0.02 [c]
SOD U/ml	170 ± 35.5	189.5 ± 42	82 ± 17 [a]	134 ± 48.5 [c]
GR U/L	324 ± 4.5	339 ± 40	170 ± 11 [b]	300 ± 6 [c]

Values are expressed as mean ± SE (n=10/group).
[a] $P < 0.05$, [b] $P < 0.01$, vs. control group; [c] $P< 0.05$, [d] $P < 0.01$, vs. diabetic group using student's unpaired t-test.

The treatment with manganese has caused a significant increase in the Mn^{2+} and Ca levels at ($P<0.01$), and the SOD and GR activities at ($P<0.05$) compared to the untreated diabetic group. Furthermore, the FBS level was significantly decreased at ($P<0.01$) while the N_2O_3 and MDA concentrations at ($P<0.05$) compared to control groups. There was no statistically significant increase in the serum lipase and a-amylase concentrations between untreated diabetic versus treated diabetic groups.

DISCUSSION

Diabetes mellitus causes alterations in trace elements status such as manganese and calcium, and these nutrients might have specific roles in the pathogenesis and progress of this disease (Webb, 2007*)*. In this study, the results revealed a parallel significant decline in Mn^{2+} and Ca Conc. in alloxan-induced diabetic group compared to control group at ($P<0.05$). This result agreed with (Kazi *et al*., 2008) which suggested that the homeostasis of the trace element can be disrupted by diabetes mellitus.

It is thus evident that Mn^{2+} regulates Ca concentration with an impact on regulating fasting blood glucose level (Syed *et al*., 2012). The loss of these elements might be caused by impaired renal function (Abou-Seif, 2004) or by excess production of free radicals and decreased antioxidants enzymes (AOE) which manganese acts as micronutrient antioxidant (Wali, 2011) as progression of diabetes complication.

The digestive enzymes activities decreased by (22, 12%) respectively in untreated diabetic rats versus control group. The deficiency of these enzymes as indicator of occurance of exocrine pancreatic insufficiency (EPI) in diabetic rats (Icks, 2001; Rathmann, 2001; Dallatu, 2009) that was associated with the pathogenesis of impaired insulin action (Nakajima, 2011). Furthermore insulin acts as a trophic factor on acinar tissue and also increases the enzyme output from cultivated islets (Ewald, 2011). So that alterations in these enzymes may reflect impaired exocrine endocrine relationship in the pancreas.

Increased oxidative stress (OS) has been proposed to be one of the major causes of hyperglycemia induced diabetic complication, and hyperglycemia in an organism stimulates reactive oxygen species (ROS) formation from variety of sources (Valko, 2007). In alloxan-induced diabetic group, there were significant increase in OS indicators as N_2O_3 and MDA at ($P<0.05$) and significant decrease in AOE as GR ($P<0.01$) and SOD ($P<0.05$) versus control group.

The administration of manganese into diabetic group showed a highly significant decrease in FBS level at ($P<0.01$) and a significant increase in Mn^{2+} and

Ca concentrations at ($p<0.01$) compared to untreated diabetic group. This reduction of FBS confirmed the role of manganese in controlling diabetes mellitus by reduction in blood glucose levels and subsequent increase storage of glycogen in the liver (Philip, 1984). In addition, supplement of Mn element could decrease digestive enzymes activities compared to the untreated diabetic rats. This indicates that Mn^{2+} may stimulate Ca influx to the pancreas that can enhance exocytosis and secretion of the digestive enzymes from pancreas.

In the present study, the supplementation with antioxidant micronutrient Mn^{2+} showed a significant decrease in OS indicators, and a significant increase in AOE activities compared to untreated diabetic group at ($P<0.05$). These findings agreed with (Margaritis *et al*., 2003). An increase in the activities of antioxidants in diabetic group may be due to the antioxidant effect of the $MnCl_2$ that might actually stimulate cell survival through strengthening the defense systems.

CONCLUSION

The supplementation with antioxidant micronutrients may cause increase activities of some antioxidant enzymes, decreased concentration of some oxidative indicators and improves the concentration of digestive enzymes in alloxan-induced diabetic rats. We recommend further research in this area, for possible consideration of micronutrients supplementation in the routine treatment of patients with diabetes mellitus. It can be concluded that pancreatic exocrine insufficiency could be one of the mechanisms underlying diabetes mellitus and vice versa. Accordingly, it can be suggested that therapy of diabetes should address both endocrine and exocrine pancreatic functions.

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