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Original article

Effect of zinc gluconate, sage oil on inflammatory patterns and hyperglycemia in zinc deficient diabetic rats

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ABSTRACT

Background: The relationship between zinc homeostasis and pancreatic function had been established. In this study we aimed firstly to configure the inflammatory pattern and hyperglycemia in zinc deficient diabetic rats. Secondly to illustrate the effect of two selected agents namely Zinc gluconate and sage oil (*Salvia Officinalis*, family *Lamiaceae*).

Methods: Rats were fed on Zinc deficient diet, deionized water for 28 days along with Zinc level check up at intervals to achieve zinc deficient state then rats were rendered diabetic through receiving one dose of alloxan monohydrate (120 mg/kg) body weight, classified later into 5 subgroups.

Results: Treatment with sage oil (0.042 mg/kg IP) and Zinc gluconate orally (150 mg/kg) body weight daily for 8 weeks significantly reduced serum glucose, C-reactive protein (CRP), Tumor necrosis factor alpha (TNF- α), interleukins-6 1 β, inflammatory8 (IFN 8), pancreatic 1L1-β along with an increase in serum Zinc and pancreatic Zinc transporter 8 (ZNT8). Histopathological results of pancreatic tissues showed a good correlation with the biochemical findings.

Conclusions: Both sage oil and zinc gluconate induced an improvement in the glycemic and inflammatory states. This may be of value like the therapeutic agent for diabetes.

1. Introduction

Type one diabetes mellitus (T1DM) is a complex autoimmune disease, mostly attributed to β-cells destructions by auto reactive T-cells leading to severe insulin deficiency [1].

The early Diagnosis of diabetic individuals usually demonstrates higher level of serum inflammatory biomarkers. This indicates that the inflammatory response is activated during the early stages of the disease [2]. Zinc is one of the most important trace elements in biological systems [3]. It is found in plasma bound to albumin and α- macroglobulin, achieving less than 1% of the Zinc total body contents while the remaining (99%) are located intracellularly [4].

Zinc protects biological structures against free radical inducing damage through the maintenance of metallothioneins. The latter is an essential component of superoxide dismutase enzyme (SOD) which protects against oxidative stress [5].

Zinc also plays an important role in pancreatic islets as a specific structural component of the insulin molecule(Zinc- insulin) complex and also in insulin secretion [6]. It is involved in many processes within the islets like glucagon secretion, insulin packaging, signaling and secretion as well as the digestive enzyme activity [6].

Therefore, deregulation of Zinc metabolism may impair many key processes including glycemic control [7], pancreatic cancer [8,9] and chronic pancreatitis [10].

It is clear now that Zinc is co-secreted with insulin into the islet extracellular space, its release from insulin usually occurs at higher pH of the blood. Furthermore, Zinc ions provide an off-switch for glucagon release from the α-cells during glucose deprivation through closure of α-cell KATP channel [11,12].

Zinc is actually transported into pancreatic β-cells via certain transporters, ZnT8 is an example of a protein which transports Zinc into insulin granules. Release of auto antibodies to ZnT8 and its polymorphism is associated with the onset of Diabetes [13].

The Genus Salvia and its different species are commonly known as sage. Salvia Officinalis species which is referred to as common sage is native to the Mediterranean region and has been used as flavoring species as well as traditional herbal medicine [14]. Sage oil has been reported to have many biological activities such as antioxidant,

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antibacterial [15], hypoglycemic [16] additionally anti-inflammatory properties [17].

Therefore, the present study aims to first illustrate the profile of Zinc deficient state in alloxan-diabetic-rats put on Zinc deficient diet. Secondly, the effect of sage oil and zinc gluconate supplementation individually on certain inflammatory and Zinc transporter biomarkers of the present animal model.

This may demonstrate their potentials as effective antidiabetic, antioxidant and anti-inflammatory properties.

2. Materials and methods

Thirty adult male albino rats weighing 170 ± 20 g were housed under environmentally-controlled conditions and were allowed one week acclimatization at room temperature with a 12 h' dark/light cycle before beginning the experimental work. Rats were fed rodent chow and allowed free access of drinking water. The animals were maintained, used in accordance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals (The University of Zagazig, Egypt). Rats were fed on zinc deficient diet for 28 days and consist of:

Diet contents	Quantity (g/kg)
Egg albumin	200
Dextrin	631
Maiz oil	100
Vitamin mixture	11.7
Salt mixture (free of zinc)	31.3
Cellulose powder	20

Deionized water was allowed as drinking water for all rats [18]. Single dose (120 mg/kg) of freshly prepared solution of Alloxan Monohydrate which was purchased from the ACROS; ORGANICCHEMICALS COMPANY (New Jersey, USA)(Dissolved in Normal Saline, Citrate buffer, pH 4.5) was administered intraperitoneal to overnight fasting rats for the induction of diabetes mellitus. Control rats were similarly injected with normal saline. Fasting blood glucose level was checked after 48–72 h. Rats which achieved a blood sugar level > 200 mg/dl were selected as diabetics [19]. Seven experimental groups (n = 6) were used: normal rats (NC) which received no treatment and served as Normal group, Diabetic control,normal rats and received alloxan only (DC), Zinc deficient group (znd) which received zinc deficient diet and deionized Water only and diabetic zinc deficient group (Dz.d) which received alloxan and kept on Zinc deficient diet and deionized water. The last group was subclassified into Three groups. The first one (S.O group) diabetic zinc deficient rats, injected by sage essential oil (0.042 mg/kg body weight) intraperitonially [20], daily for 8 weeks. The second one received Zinc gluconate. (Z.G group) 150 mg/serving water for 8 weeks daily [21] while the third one received no drugs and referred to as Diabetic rats kept on Zinc deficient diet and deionized water and referred to as control for comparison with the drugs used. During the experimental period (8 weeks), body weight, blood glucose, food and water consumption and physical examinations were determined at regular intervals. The dosage was adjusted weekly in response to any changes in body weight.

2.1. Blood sampling

At the end of the treatment periods (8 weeks) rats were fasted overnight, blood samples were taken for each rat individually and directed to serum preparation. Samples were processed instantly for determination of glucose, IL-6, CRP, and TNF-α, IFN-γ, IL1-β and Zn.

2.2. Tissue collection

After blood collection, rats were killed by decapitation, pancreatic tissues were removed instantly, placed in cold saline solution, trimmed of adipose tissue and homogenized instantly on ice using buffer. Tissue homogenate stored at −20 °C for determination of pancreatic IL1-β and ZNT8.

2.3. Histopathological examination of pancreatic tissues

A slice of pancreas was fixed in 10% formalin for 1week at room temperature, the specimens were dehydrated in a graded series of ethanol cleared in xylene, and embedded in paraffin wax Tissue blocks were sectioned to 4-μm thick using a rotary microtome. Sections were stained by hematoxylin and eosin. Stained sections were examined by light microscope [22].

2.4. Analytical methods

Blood glucose determination was done using commercial kits supplied by Spinreact Kits, Barcelona, Spain [23]. Serum IL-1beta was determined using commercialELISA kit supplied by R & D quantikine (USA) [24]. C-reactive protein (CRP) wasdetermined using BD Biosciences ELISA Kit, USA [25]. Serum IL-6 was determined using commercial ELISA kitsupplied by R & D quantikine (USA) [26]. Serum TNF-α was determined according to the method of Juhasz, 2013 [27] using R & D Quantikine ELISA Kit (USA). Serum INFγ was determined according to the method of [28] using Platinum ELISA Kit (USA). Serum ZN was determined by colorimetric method according to [29] using QCA –QuímicaClínicaAplicada S.A., Spain. Znt-8 was determined using AnticropsELISA Kit (Aachen, Germany).

2.5. Statistical analysis

All results were expressed as Means ± SD. Statistical analysis and correlation were performed using Graph Pad Prism software. Student "*t*" test of unpaired data and the analysis of variance (one-way ANOVA) followed by Tukey's post hoc test were used for comparison between groups. The correlations between the studied parameters were done using the Pearson's correlation coefficient (r). Statistical significance was defined at $P < 0.05$ [30,31].

3. Results

3.1. Biochemical parameters

Zinc deficient (Znd), diabetic control (DC) and diabetic zinc deficient group (Dz.d) demonstrated significant increase in serum glucose, IL-6, CRP, and TNF-α, IFN-γ, IL1-β and in pancreatic IL1-β along with significant decrease in serum zinc and pancreatic ZNT8 content in comparison to normal group (Table 1). Dz.d group demonstrated also significant increase in serum glucose, IL-6, CRP, and TNF-α, IFN-γ, IL1-β and in pancreatic IL1-β along with significant decrease in serum zinc and pancreatic ZNT8 in comparison to diabetic group (DC) (Table 1). Treatment with S.O (sage oil) and zinc gluconate for 8 weeks resulted in significant decrease of serum glucose and the inflammatory markers as compared to the Dz.d group (control group, Table 2), The sage oil potential showed characteristic pattern than zinc gluconate as hypoglycemic and anti-inflammatory agent. Histopathological findings demonstratedgood correlation with the Biomarkers results (Fig. 2).

3.2. Zinc modulation parameters

Serum zinc and pancreatic ZNT8 showed Negative correlation with serum glucose (Fig. 1a, Fig. 1b) but positively correlated with each other (Fig. 1c).