The Journal of Basic & Applied Zoology (2015) 71, 20–31

HOSTED BY

ELSEVIER

The Egyptian German Society for Zoology

The Journal of Basic & Applied Zoology

www.egsz.org
www.sciencedirect.com

Improvement in beta-islets of Langerhans in alloxan-induced diabetic rats by erythropoietin and spirulina

N.I. El-Desouki [a,*], G.A. Tabl [a], K.K. Abdel-Aziz [b], E.I. Salim [a], N. Nazeeh [b]

[a] *Dept. of Zoology, Faculty of Science, Tanta University, Tanta 31527, Egypt*
[b] *Dept. of Zoology, Faculty of Science, Damnhour University, Egypt*

Received 26 January 2015; revised 1 April 2015; accepted 1 April 2015
Available online 27 April 2015

KEYWORDS

Diabetes;
Erythropoietin;
Spirulina;
Oxidative stress;
Histology;
IHC;
Rats

Abstract The present study was undertaken to assess the effect of erythropoietin (EPO) and/or spirulina to treat alloxanized-diabetic rats. Eighty male albino rats were equally divided into eight groups; Group I: Normal control rats, Group II: Non-diabetic rats treated with EPO (40 U/kg) injected subcutaneously three times weekly for 3 weeks, Group III: Non-diabetic rats administered orally with spirulina (2 g/kg/d) for 21 days, Group IV: Non-diabetic rats treated by EPO (40 U/kg) together with spirulina (2 g/kg/d) as mentioned in groups II & III, Group V: Alloxanized-diabetic rats. Group VI: Diabetic rats treated with EPO (40 U/kg) as in group II, Group VII: Diabetic rats administered with spirulina (2 g/kg/d) as in group III, Group VIII: Diabetic rats were given with EPO (40 U/kg) and spirulina (2 g/kg/d) as in group IV. Diabetic rat group showed a significant increase in glucose and NO; and a significant decrease in insulin, SOD and CAT levels. Diabetic rats treated with EPO or/and spirulina recorded a significant decrease in the glucose and NO levels; and a significant increase in insulin, SOD and CAT levels when compared with the diabetic group. Histopathologically, diabetic rats treated with EPO or spirulina showed a slight improvement of pancreatic islets and acinar cells, diabetic rats treated with EPO & spirulina together showed an obvious recovery to approximately normal status. IHC, the expression of insulin producing cells (β-cells) of diabetic rats was improved in the three treatment groups with a lesser affinity for EPO than spirulina while with both together showed marked recovery into normal status. In conclusion, all the changes were minimized in spirulina administered group more than EPO group, however, the co-treatment of EPO and spirulina exerted stronger anti-hyperglycemic effects than treatment with each agent alone.

Introduction

Diabetes mellitus is a universal metabolic disorder characterized by hyperglycemia, hyperlipidemia, hyperaminoacidemia

* Corresponding author.
E-mail address: nabiladesoky@yahoo.com (N.I. El-Desouki).
Peer review under responsibility of The Egyptian German Society for Zoology.

http://dx.doi.org/10.1016/j.jobaz.2015.04.003

and hypoinsulinemia that leads to reduction in both insulin secretion and insulin action (Altan, 2003). There is always a greater risk of all manifestations of atherosclerosis along with diabetes mellitus (Khuwaja et al., 2004), as well as association with a reduced quality of life and increase in risk factors of mortality and morbidity (Shaw et al., 2009). Type 2 diabetes mellitus is a chronic disorder of insulin insufficiency resulting in the dysregulation of glucose homeostasis, hyperglycemia and vascular complications. Diabetes has distinct pathogenic insufficient functional pancreatic β-cell mass that is required to maintain euglycemia (Kahn, 2003; Rhodes, 2005). Thus, one of the overarching goals in the treatment of diabetes is the preservation and growth of β-cells.

Erythropoietin (EPO), is a glycoprotein hormone with a molecular mass of 30.4 kD that controls erythropoiesis, it is produced by interstitial fibroblasts in the kidney in close association with peritubular capillary and tubular epithelial cells (Obara et al., 2008). It is also produced in perisinusoidal cells in the liver. While liver production predominates in the fetal and perinatal period, renal production is predominant during adulthood. EPO is the hormone that regulates red blood cell production. It also has other known biological functions (Hand and Brines, 2011; McGee et al., 2012). For example, it plays an important role in the brain's response to neuronal injury (Siren et al., 2001). EPO is also involved in the wound healing process (Haroon et al., 2003).

Recent studies have shown that the EPO protects against diabetes through direct effects on pancreatic cells. The EPO receptor (EPO-R) is present in nonerythroid tissues, including the pancreatic islets of human and rodents (Fenjves et al., 2003; Choi et al., 2010). In particular, several studies have shown the efficacy of EPO in providing cytoprotection in experimental models of tissue injury (Brines and Cerami, 2006).

EPO overexpression in human pancreatic islets has been shown to prevent cytokine-induced cell death (Fenjves et al., 2004). EPO deficiency and a higher incidence of anemia have been shown in individuals with diabetes, suggesting potential beneficial effects of EPO in the setting of diabetes (McGill and Bell, 2006; Thomas, 2006). EPO clinical trial for non-diabetic individuals with chronic renal failure was associated with a significant increase in the incidence of hypoglycemia which raises the intriguing possibility of a direct effect of EPO on pancreatic β-cells (Drüeke et al., 2006).

EPO-R belongs to the cytokine class I receptor superfamily and utilizes a similar signal transduction pathway as the receptors for growth hormone and prolactin, knockouts of these show defects in β-cell mass and function (Freemark et al., 2002; Liu et al., 2004). Collectively, these data raise the possibility that EPO signaling may have significant biological effects on β-cells and thus may be relevant to diabetes (Brines and Cerami, 2006; Choi et al., 2010).

Spirulina, refers to the dried biomass of the cyanobacterium, *Arthrospira platensis*, and is a whole product of biological origin. Spirulina is a name used to describe mainly two species of Cyanobacteria, *A. platensis* and *Arthrospira maxima* that are commonly used as food and as dietary supplement (Mühling et al., 2006). The number of research articles discussing the beneficial effects of spirulina is increasing every year. Spirulina is rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols and some more vital elements such as calcium, iron, zinc, magnesium, manganese and selenium. It is a natural source of vitamin B12, vitamin E, ascorbic acid, tocopherols and a whole spectrum of natural mixed carotene and xanthophylls phytopigments.

Some of the early health effects of spirulina were in its role in diabetes management and its significant plasma triglycerides reduction effects (total- and LDL-cholesterol), blood pressure lowering, improving the antioxidant status, as well as inflammatory effects (Eun et al., 2008). Recent reports note the importance of spirulina for its immunomodulatory, anti fatigue, radio protective and antioxidant effects particularly on the biochemical parameters such as SOD and CAT levels (Mendiola et al., 2010). Spirulina protects against diabetes through direct effects on pancreatic β-cells (Khursheed et al., 2012). Recent studies have shown an insulin-like protein extracted from spirulina to have the same molecular mass, immunoreactivity and retention time, detected by reversed-phase chromatography (RP-HPLC), to be similar to that of the bovine insulin (Anwer et al., 2012).

The present study aimed to assess the impact of treatment with erythropoietin and the natural extract of the marine algae (spirulina) separately or in combination on the physiological and histopathological parameters of experimentally-induced diabetes in rats.

Materials and methods

Animals and housing conditions

Eighty male albino rats (*Rattus rattus*) weighing 150 ± 5 g were used in the current study. They were obtained from the Breeding Unit of the Egyptian Organization for Vaccine and Biological Preparation, Cairo, Egypt. All rats were kept under the same environmental conditions for 2 weeks before the study. The animals were fed *ad Libitum* with a standard pellet diet and allowed free access of water and they were housed in metal cages in a well-ventilated animal room. All protocols and procedures adopted for the present investigation were in accordance with the approval of the Institutional Animal Ethics Committee of National Research Center and in accordance with recommendation of the proper care and use of laboratory animals.

Induction of diabetes mellitus in rats

The diabetes was induced in the animals by three intraperitoneal (i.p.) injections of alloxan monohydrate (Sigma Aldrich, USA) dissolved in acetate buffered-saline (Merck). The 1st dose was at a dose of 150 mg/kg as recommended by Bromme et al. (2000). The 2nd dose was at a 100 mg/kg of alloxan after 2 days. Finally, the 3rd dose of alloxan (100 mg/kg) was applied 5 days after the 2nd one. Note, the 2nd and 3rd injections were used to ensure the insult of diabetes through the experimental duration.

The rats were fasted overnight, collection of blood samples and sera glucose determination were drawn from their tail tips. Sera glucose estimation was done by one touch electronic glycometer using glucose test strips, and the glucose level more than 250 mg/dl was used in the present study.