Ayman Mohamed

The Metabolic Effects of Echinochrome Pigment Extracted from Sea Urchin on Diabetic Rats

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A THESIS
Submitted to the Faculty of Science,
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In Partial Fulfillment of the
Requirements for
the Degree of Ph.D.
(Molecular and integrated physiology)

BY

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ABSTRACT

Student Name: Ayman Saber Mohamed

Title of the thesis: Metabolic effects of Echinochrome pigment extracted from sea urchin on diabetic rats

Degree: Ph.D. in Zoology (Molecular and Integrated Physiology)

Diabetes mellitus is one of the most public metabolic disorders. It is mainly classified into type 1 and type 2. Echinochrome (Ech) is a pigment from sea urchins that has antioxidant, anti-microbial, anti-inflammatory and chelating abilities. The present study aimed to investigate the anti-diabetic mechanisms of Ech pigment in streptozotocin-induced diabetic rats. Thirty-six male Wistar albino rats were divided into two main groups (18 rats/group). Each group was divided into 3 subgroups (6 rats/subgroup); control, diabetic and Ech subgroups. Diabetic models were induced by a single dose of streptozotocin (60 mg/kg, i.p) for type 1 diabetes and by a high fat diet for 4 weeks before the injection of streptozotocin (30 mg/kg, i.p) for type 2 diabetes. Diabetic groups were treated orally with Ech (1 mg/kg body weight in 10% DMSO) daily for 4 weeks. Ech groups showed a reduction in the concentrations of glucose, globulins, triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), creatinine, urea, uric acid, malondialdehyde (MDA) and the activities of arginase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT). While, it caused general increase in the levels of insulin, total bilirubin (TB), direct bilirubin (DB), indirect bilirubin (IB), total protein (TP), albumin, nitric oxide (NO) and the activities of glucose-6-phosphate dehydrogenase (G6PD), hexokinase, glutathione-S-transferase (GST), superoxide dismutase (SOD) and glutathione reduced (GSH). The histopathological investigation showed partial restoration of pancreatic islet cells and clear improvement in the hepatic and kidney architecture. The results of this study clearly show that Ech has anti-diabetic potential in both types of diabetes. The possible anti-diabetic mechanisms of Ech involving improved glucose metabolism, restoration of β cells, improve insulin secretion, improve insulin signaling and antioxidant activity

Key words: Diabetes-Echinochrome-Oxidative stress-Pancreas-Liver-Kidney-Histopathology.

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List of abbreviation

Abbreviation	Meaning
A/G	Albumin/globulins
AAP	Amino-antipyrine
AAP	Aminophenazone
Ach	Acetylcholine
AchE	Acetylcholine esterase
AGEs	Advanced glycation endproducts
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
anti-GAD	Anti-glutamic acid decarboxylate
AR	Aldose reductase
AST	Aspartate aminotransferase
BCG	Bromocresol green
CAT	Catalase
CE	Cholesterol esterase
CO	Cholesterol oxidase
CVD	Cardiovascular disorders
DAG	Diacylglycerol
DB	Direct bilirubin
DCHB	Dichloro-2-hydroxybenzenesulfonic acid
DHBS	Dichloro-2-hydroxybenzene sulfonic acid
DM	Diabetes mellitus
DMSO	Dimethyl sulfoxide
DTNB	Dithiobis-2-nitrobenzoic acid
Ech	Echinochrome
G6PDH	Glucose-6-phosphate dehydrogenase
GGT	Gamma-glutamyltransferase
GLUT	Glucose transporter
GOD	Glucose oxidase
GSH	Reduced glutathione
GSH	Glutathione reduced
GST	Glutathione-S-transferase
H&E	Hematoxylin and eosin
HDL-C	High density lipoproteins cholesterol
HFD	High fat diet
HK	Hexokinase
HMOX	Heme oxygenase
IA-2	Insulinoma-associated protein-2
IB	Indirect bilirubin
ICA	Islet cell antibody

LDL-C	Low density lipoprotein cholesterol
MDA	Malondialdehyde
NAD+	Nicotinamide adenine dinucleotide
NEDA	N-(1-naphthyl)-ethylenediamine
NO	Nitric oxide
NOS	Nitric oxide synthase
PI3K	Phosphatidylinositol-3 kinase
PIs	Phosphatidylinositides
PKC	protein kinase C
PLC	Phospholipase C
PLD	Phospholipase D
POD	Peroxidase
PP	Pancreatic polypeptide
ROS	Reactive oxygen species
SDH	Sorbitol dehydrogenase
SOD	Superoxide dismutase
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TAGEs	Toxic advanced glycation endproducts
TB	Total bilirubin
TBA	Thiobarbituric acid
TC	Total cholesterol
TCA	Trichloroacetic acid
TG	Triglycerides
TP	Total protein
VLDL	Very low density lipoproteins

I. Introduction

Glucose is an essential metabolic substrate of all mammalian cells. Most of the energy needed to sustain life is delivered by oxidation of glucose **(Pischetsrieder, 2000)**. Although glucose is required by all cells, its main consumer is the brain in the fasting or postabsorptive phase, which accounts for approximately 50% of the body's glucose use. Another 25% of glucose disposal occurs in the splanchnic area (liver and gastrointestinal tissue), and the remaining 25% takes place in insulin-dependent tissues, including muscles and adipose tissues **(DeFronzo, 2004)**. Approximately 85% of endogenous glucose production is derived from the liver, with glycogenolysis and gluconeogenesis contributing equally to the basal rate of hepatic glucose production. The remaining ~15% of glucose is produced by the kidneys **(Mari *et al.*, 1994; DeFronzo, 2004)**.

The pancreas is considered as a doubled-entity organ, with both exocrine and endocrine components, reciprocally interacting with a composed system whose function is relevant for digestion, absorption, and homeostasis of nutrients **(Piciucchi *et al.*, 2015)**. Pancreatic islets composed of many types of cells, including insulin-producing β cells, glucagon-releasing α cells, somatostatin-producing δ cells, pancreatic polypeptide (PP)-containing cells and ghrelin containing ε cells **(Damasceno *et al.*, 2014)**. All of these hormones are involved in the regulation of nutrient metabolism and glucose homeostasis **(Assmann *et al.*, 2009)**.

Normally, following glucose ingestion, the increase in plasma glucose concentration triggers insulin release, which stimulates splanchnic and peripheral glucose uptake and suppresses endogenous glucose production. In healthy adults, blood glucose levels are tightly regulated within a range of 70 to 99 mg/dl, and maintained by specific hormones (e.g., insulin, glucagon,

incretins) as well as the central and peripheral nervous system, to meet metabolic requirements **(Wardlaw and Hampl, 2007)**.

Various cells and tissues (within the brain, muscles, gastrointestinal tract, liver, kidney, and adipose tissue) are also involved in blood glucose regulation by means of uptake, metabolism, storage, and excretion **(DeFronzo, 2004)**. The majority of glucose uptake in peripheral tissues occurs in muscles, where glucose may either be used immediately for energy or stored as glycogen **(Guyton and Hall, 2006)**. Transport of glucose into muscles is insulin-dependent, and thus requires insulin for activation of the major enzyme (glycogen synthase) that regulates production of glycogen **(Porte *et al*., 2003)**. While adipose tissue is responsible for a much smaller amount of peripheral glucose uptake (2%-5%), it plays an important role in the maintenance of total body glucose homeostasis by regulating the release of free fatty acids (which increase gluconeogenesis) from stored triglycerides, influencing insulin sensitivity in the muscles and liver **(DeFronzo, 2004)**. While the liver does not require insulin to facilitate glucose uptake, it needs insulin to regulate glucose output **(DeFronzo, 2004)**. So, for example, when insulin concentrations are low, hepatic glucose output rises **(Porte *et al*., 2003)**. Additionally, insulin helps the liver to store most of the absorbed glucose in the form of glycogen **(Guyton and Hall, 2006)**. The kidneys are increasingly recognized to play an important role in glucose homeostasis via release of glucose into the circulation (gluconeogenesis), uptake of glucose from the circulation to meet renal energy needs, and reabsorption of glucose at the proximal tubule **(Wright *et al*., 2007)**. The kidneys also aid in the elimination of excess glucose (when levels exceed approximately 180 mg/dL, though this threshold may rise during chronic hyperglycemia by facilitating its excretion in the urine **(ADA, 2008)**.

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both **(Kumar and Clark, 2002)**. Insulin deficiency, in turn, leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism **(Lindberg *et al*., 2004)**. DM is considered as one of the most dangerous metabolic disorders in the world **(Sosale *et al*., 2015)**. It is a complex and potentially debilitating disease that affects an estimated 8.3% of the adult population or 382 million people worldwide **(IDF, 2013)**. Egypt will have at least 8.6 million adults with diabetes and will be the tenth largest population of diabetics in the world **(Shaw *et al*., 2010)**. The eleventh most important cause of premature mortality in Egypt is diabetes mellitus (**Saad *et al*., 2013)**. It's responsible for 2.4% of all years of life lost. Also, diabetes is the six most important cause of disability burden in Egypt **(NICHP, 2004)**.

DM generally classified into type 1 (T1DM) and type 2 (T2DM) diabetes mellitus. Type 1diabetes (T1DM) is an autoimmune disease, which characterized by loss of insulin producing β cells and reliance on exogenous insulin for survival **(Simmons and Michels, 2015)**. T1DM is characterized by mononuclear infiltration of the pancreatic islets, followed by the destruction of insulin-producing β cells **(Mathis *et al*., 2001)**. The two main forms of clinical type 1 diabetes are type 1a (about 90% of type 1 cases in Europe) which is thought to be due to immunological destruction of pancreatic β cells, resulting in insulin deficiency; and type 1b (idiopathic, about 10% of type 1 diabetes), in which there is no evidence of autoimmunity **(Bastaki, 2005)**. Type 1a is characterized by the presence of islet cell antibody (ICA), anti-glutamic acid decarboxylate (anti-GAD) and insulinoma-associated protein-2 (IA-2) that identify the autoimmune process with β cells destruction **(Zimmet, *et al*., 2004)**. Autoimmune diseases such as Grave's disease, Hashimoto's thyroiditis and Addison's disease may be associated with T1a **(Atkinson and Maclaren, 1994)**.