بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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The Possible Effect of Grape Seed Extract on the Adrenal Cortex in Streptozotocin-Induced Diabetes in Rats: A Histological Study.

Thesis
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Esraa Tarek Fahmy Mohammaden

List of Contents

List of Abbreviations

ABC	Streptavidin biotin peroxidase complex
ACTH	Adrenocorticotropin
AGE	Advanced glycation end-product
AlkB	Alpha-ketoglutarate-dependent dioxygenase
ALT	Alanine aminotransferase
APIM	AlkB homologue 2 PCNA interacting motif
11B-HSD	11- beta hydroxysteroid dehydrogenase
Bax	BCL2 associated X
Bcl-2	B-cell lymphoma 2
BER	Base excision repair
COX	Cyclooxygenase
CRP	C-reactive protein
DAB	Diaminobenzidine tetrahydrochloride
DM	Diabetes mellitus
DPX	Distyrene, a plasticizer, and xylene
D.W.	Distilled water
eNOS	Endothelial nitric oxide synthase
GLUT2	Glucose transporter 2
GMP	Guanosine monophosphate
GSE	Grape seed extract
H&E	Hematoxylin and eosin
H_2O_2	Hydrogen peroxide
HPA	Hypothalamic-pituitary-adrenal axis
IDDM	Insulin dependent diabetes mellitus
IL-1β	Interleukin one beta

IL-6	Interleukin 6
iNOS	Inducible nitric oxide synthase
I.P	Intraperitoneally
LDL	Low density lipoprotein
5-LOX	5-lipoxygenase
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemotactic protein 1
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NIDDM	Non-insulin dependent diabetes mellitus
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
PBS	Phosphate buffer saline
PCNA	Proliferating cell nuclear antigen
PIP	PCNA-interacting peptide
PKC	Protein kinase C
RAGE	Receptor for advanced glycation end products
ROS	Reactive oxygen species
STZ	Streptozotocin
TNF- α	Tumor necrotic factor alpha
Wnt	Wingless/Integrated
ZF	Zona fasciculata
ZG	Zona glomerulosa
ZI	Zona intermedia
ZR	Zona reticularis
zU	Undifferentiated zone

List of Tables

List of Histograms

Abstract

Introduction: Diabetes is a disease affecting many body systems including the endocrine system. Induction of diabetes in rat models using streptozotocin (STZ) had led to increased activity of the hypothalamic-pituitary-adrenal (HPA) axis which negatively affects the adrenal gland. Management of diabetes without side effects remains a challenge and therefore new strategies need to be examined. Grape seed extract (GSE) was observed to have beneficial effects on different metabolic and inflammatory diseases.
Aim: To study the structural changes of the adrenal cortex in diabetic rats and the beneficial effect of GSE on the adrenal cortical injury.
Materials and methods: 35 adult male albino rats were divided randomly into 4 groups. *Group I* (control). *Group II* (GSE), rats received GSE orally (200 mg/kg), daily for 21 days. *Group III* (STZ), rats were injected with STZ (40 mg/kg) given intraperitoneally once. *Group IV* (STZ + GSE), rats received STZ as in group III. After rats were confirmed to be diabetic, they were treated with GSE as in group II. After 21 days, the adrenal glands from all groups were dissected and processed for light microscopic examination. Blood glucose, aldosterone and cortisol levels were measured. Morphometric and statistical analysis were also done.
Results: H&E-stained sections of the adrenal cortex of **Group III (STZ)** showed decrease in the ratio of the zona glomerulosa & zona fasciculata. Many cells appeared ballooned and vacuolated while others were shrunken and deeply acidophilic. Nuclear affection included pyknosis, karyrrhexis and karyolysis. Congested blood capillaries and focal inflammatory cellular infiltration were also observed. **Masson's trichrome stain** showed increase in the

capsular thickness and **Wilder reticular stain** showed dense trabeculae and disorganized reticular fibers. Meanwhile, immunohistochemical results showed significant increase in the reaction for **caspase-3**, **PCNA and iNOS** antibodies. Blood glucose level, aldosterone and cortisol levels were significantly increased. **Group IV (STZ + GSE)** showed restoration of the cortical structure & organization nearly similar to that of control group.

Conclusion: GSE showed a protective effect on the structure of the adrenal cortex in diabetic rats as detected by microscopic, biochemical and morphometric studies

Keywords: Diabetes, GSE, adrenal gland, Caspase-3, PCNA, iNOS.

Introduction

The adrenal gland is an important organ. This importance arises from the fact that the adrenocortical zones synthesize and secrete steroid hormones, which fall into three major categories. Mineralcorticoids, exemplified by aldosterone, are secreted by the zona glomerulosa. Aldosterone is an important regulator of salt homeostasis and fluid balance and it is a major control unit of acid/base balance **(Bielohuby *et al.*, 2007)**.

The zona fasciculata secretes glucocorticoids, exemplified by cortisol, which is essential for life. It has a major role in responding to environmental stimuli; it decreases protein synthesis, mobilizes fatty acids and glycerol from adipose cells and elevates blood glucose by stimulating the enzymes involved in gluconeogenesis in the liver. It also has anti-inflammatory effects. The zona reticularis secretes small amounts of androgens **(Fawcett and Jensh, 2002)**.

The global prevalence of diabetes among adults has nearly doubled since 1980 till present. It has been proven that diabetes is associated with disturbance in the endocrine system including the adrenal glands. This has been attributed to the generation of oxidative stresses which negatively affect the adrenal cortex **(Repetto *et al*, 2019)**.

On the other hand, grapes are considered one of the extensively consumed fruits in the world. Grape seed has many active ingredients, the most popular being proanthocyanidin **(Rauf *et al*., 2019)**.

It was hypothesized that proanthocyanidin extract acts as a free radical scavenger and an antioxidant. Furthermore, grape seed extract modifies many biological reactions, and it has anti-inflammatory **(Cádiz-Gurrea *et al*., 2017)**, anticarcinogenic **(Huang *et al*., 2012)** and antiaging effects **(Solanki *et al*., 2015)**; and therefore, it is considered a cytoprotective agent **(Khalaf *et al*., 2017)**.

In literature, few studies have investigated the beneficial effects of GSE on adrenal gland injuries.

Aim of work

The aim of the current study was to investigate the structural changes of the adrenal cortex in stretptozotocin diabetic rats. In addition, the beneficial effect of GSE on the suprarenal cortical damage was evaluated.

Review of Literature

Diabetes Mellitus

Diabetes mellitus is a metabolic disorder that is characterized by elevated blood glucose level (hyperglycemia) due to insufficiency in production or action of insulin produced by the pancreas. Diabetes is classified into two major types: Type I diabetes (Insulin dependent) and Type II diabetes (Non-Insulin dependent) **(Maritim *et al*., 2003)**.

However, according to the **American Diabetes Association (2004)**, diabetes mellitus is further classified into *type I diabetes* that is due to immune mediated destruction of beta cells leading to insulin deficiency, *idiopathic diabetes* is the type I diabetes with unknown etiologies, *type II diabetes* that is due to insulin resistance and lastly *gestational diabetes* that is any form of intolerance to glucose with first onset during pregnancy.

Diabetes mellitus prevalence is rapidly increasing as a result of population ageing, urbanization rate and associated lifestyle changes. The number of people with diabetes mellitus worldwide has more than doubled over the past three decades. In 2010, approximately 285 million people worldwide had diabetes mellitus, 90% of them had type II diabetes mellitus. The global number of people with diabetes mellitus is expected to increase to 439 million by 2030, which represents 7.7% of the total adult population of the world aged from 20 to 79 years (**Chen *et al*., 2012)**. Most recently, the WHO (2021) stated that 422 million people currently have diabetes, mostly in low- and middle-income countries.