Possible Protective Effect of Thymoquinone (Active Ingredient of Nigella Sativa) in the Treatment of Experimental Diabetic Neuropathy

THESIS

Submitted In Partial Fulfillment For M.Sc.Degree In Basic Medical Sciences of Physiology

Presented By:

Nashwa Negm Eldin Kabil
(M.B.B.Ch)
Teacher Assistant of Physiology
German University in Cairo

Supervised By:

Prof. Dr. Maha Mohamed Gamal Eldin
Prof. of Physiology
Faculty of Medicine
Cairo University

Prof. Dr. Lobna Abdel Aal Kassem
Prof. of Physiology
Faculty of Medicine
Cairo University

Ass. Prof. Dr.Laila Ahmad Rashed
Ass. Professor of Biochemistry
Faculty of Medicine
Cairo University

Faculty of Medicine
CairoUniversity
2010

Abstract

Background Neuropathy is the most common and debilitating complication of diabetes and encompasses a variety of forms whose impact ranges from discomfort to death. Hyperglycemia induces oxidative stress in diabetic neurons and results in activation of multiple biochemical pathways such as the polyol pathway; the hexosamine pathway; excess/inappropriate activation of protein kinase C (PKC) isoforms; accumulation of advanced glycation endproducts (AGE). Thus the use of an antioxidant, will be promising as a preventive therapy against the deleterious effects of D.N., and therefore in the current study we chose TQ as a potent antioxidant and anti-inflammatory drug form a natural source.

Objective The objective of this work was to explore the possible protective effect of TQ against the deteriorating functional nerve parameters in D.N. In addition, to test the modulation of inflammatory pathways such as NF-κB and p38 M.A.P.K by TQ in the pathogenesis of D.N.

Methwds Our study was done on 60 male albino rats. These rats were divided into the following groups, each group consisted of 10 rats:

Group 1: Control group - **Group 2:** Thymoquinone group - **Group 3:** Diabetic Neuropathy group - **Group 4:** Diabetic Neuropathy treated with thymoquinone. - **Group 5:** Diabetic Neuropathy treated with insulin - **Group 6:** Diabetic Neuropathy treated with Insulin and Thymoquinone.

Furthermore, this study proofed that TQ exerts a partial protective effect on nerve injury in experimental D.N. Current results also demonstrated that a combination of both insulin and TQ resulted in the best protection against the deteriorating nerve functions in D.N. The synergistic effect of both drugs, targeting wide varieties of pathophysiological mechanisms in D.N. model has been observed.

Concluaion

It can be concluded form this study that TQ exerted a partial protective effect on nerve injury in experimental D.N., possibley through its anti- oxidant or anti-

inflammatory actions. Our data also demonstrated that the combined effect of both insulin and TQ offered the best protection against D.N. possibly due to their synergestic action against the pathophysiology of D.N.

Key word : Diabebtes / Neuropathy / Thymoqwonone

Contents:

Acknowledgment:

First and foremost, I would like to thank **ALMIGHTY ALLAH** for empowering me with the strength and patience to complete this work and reach this acheivment.

I would like to express my deep sense of gratitude to **Prof. Dr. Maha Mohamed Gamal,** Professor of Physiology, Cairo University for her tremendous motivation, expert advice, and extensive work during revising this thesis. A professor of such deep experience deserves all respect and appreciation.

I would also like to express my sincere gratitude to **Prof. Dr. Lobna Abdel Aal Kassem,** Professor of Physiology, Cairo University, for her continous support and encouragement, as well as her valuable technical advices during the experimental work and revision of this thesis.

I wish to express my thanks to **Ass. Prof. Laila Ahmed Rashed**, Assistnat Professor of Biochemistry, Cairo University, for her great support and effort during the experimental work of this thesis.

No words can express how deeply grateful I feel towards my **Parents** for their constant support and encouragement throughout my life. Their guidance and prayers helped me reach this acheivment. Many thanks also go to my sisters for their continous help and support.

Last, but not least, I would like to thank my friends and colleagues for their motivation, support, and their helpful advices in order to present this work.

List of Tables:

A. Tables of Review: Page

B. Tables of Material and Methods:

C. Tables of Results:

List of Figures:

List of Figures In Review: **Page:**

List of figures in Materials and Methods:

List of Figures in Results:

List of Abbreviations

- ADA: American Diabetes Association
- AGE: Advanced Glycation Endproducts
- ALEs: Advanced Lipoosygenation Endproducts
- ALT: Alanine aminotransferase
- AP: Alkaline Phosphatase
- AR: Aldose Reductase
- ARIs: Aldose Reductase inhibitors
- AST: Aspartate aminotransferase
- Bcl-2: B-cell lymphoma protein-2
- CCl4: Carbon tetrachloride
- CGRP: Calcitonin Gene Related Peptide
- CNS: Central Nervous System
- COX-2: Cyclooxygenase-2
- CRP: C-Reactive protein
- CV: Conduction Velocity
- CVD: Cardiovascular Disease
- DAG: Diacylglycerol
- DAN: Diabetic Autonomic Neuropathy
- DCs: Dendritic cells
- DKA: Diabetic Ketoacidosis
- DN: Diabetic Neuropathy
- DOX: Doxorubcin

- DPN: Diabetic Peripheral Neuropathy
- Drp 1: Dynamin related protein 1
- DSPN: Distal Symmetric Polyneuropathy
- DTQ: Dithymoquinone
- EAE: Allergic Encephalomyelitis
- EDHF: Endothelial Derived Hyperpolarizing Factor
- EGF: Epidermal Growth Factor
- EMG: Electromyography
- eNOS: Endothelial Nitric Oxide synthase
- ET-1: Endothelin 1
- FPG: Fasting Plasma Glucos
- GAD: Glutamate Decarboxylase
- GDM: Gestational Diabetes Mellitus
- GLA: Gamma linoleic acid
- GSH: Reduced glutathione
- Hb: Haemoglobin
- HbA1C: Glycosylated Haemoglobin
- HHcy: Hyperhomocysteinemia
- HLA: Human Leucocytic Antigen
- HSP: Heat Shock Protein
- i.p. Intraperitoneal
- ICAMs: Intracellular adhesion molecules
- IENF: Intraepidermal nerve fibers
- IFG: Impaired Fasting Glucose

- IGF-1: Insulin like Growth Factor 1
- IGT: Impaired Glucose Tolerance
- IIDM: Insulin Dependent Diabetes Mellitus
- IKK: Inhibitor for Nuclear Factor κ B kinase
- IL-6: Interleukin-6
- INF-γ: Interferon-γ
- iNOS: Inducible Nitric Oxide synthase
- IU: Internation Unit
- IκB: Inhibitor for Nuclear Factor κ B
- KBrO3: Potassium bromate
- LO: Lipooxygenase
- LPD: Lipid peroxide
- LPS: Lipopolysacchride
- LT: Leukotriens
- MAPKs: Mitogen Activated Protein Kinases
- MCAP: Maximal Compound Action Potential
- MHC: Major Histocompatability Complex
- MQ: Macrophage
- NCS: Nerve Conduction Studies
- NCV: Nerve Conduction Velocity
- NDDG: National Diabetes Data Group
- NF-κB: Nuclear Factor kappa B
- NGF: Nerve Growth Factor
- NGF: Nerve Growth Factor

- NIH: National Institute of Health
- NIIDM: Non-Insulin Dependent Diabetes Mellitus
- NK: Natural Killer cells
- NO: Nitric Oxide
- NS: Nigella Sativa
- NT-3: Neurotrophin 3
- OGTT: Oral Glucose Tolerance Test
- PAD: Peripheral Arterial Disease
- PAI-1: Platelet Activator Inhibitor-1
- PARP: Poly ADP-ribose polymerase pathway
- PBMC: Peripheral blood mononuclear cells
- PGE: Prostaglandins
- PKC: Protein Kinase C
- PKCi: Protein Kinase C inhibitors
- PNS: Peripheral Nervous System
- PZI: Protamine Zinc Insulin
- QST: Quantitative Sensory Testing
- RAGE: Receptors for Advanced Glycation Endproducts
- RBC: Red blood cells
- RNS: Reactive Nitrogen Species
- ROS: Reactive Oxygen Species
- RRP: Relative Refractory Period
- SOD: Superoxide dismutase
- STZ: Streptoztocin

- SWMT: Semmes-Weinstein Monofilament Test
- TGF-h: Transfoming Growth Factor h
- TGF-β: Transforming Growth Factor β
- THQ: Thymohydroquinone
- THY: Thymol
- TNF-α: Tumor Necrosis Factor-α
- TQ: Thymoquinone
- Trk: Tyrosine Kinase receptor
- VCAM: Vascular cell adhesion molecule
- VEGF: Vascular Endothelial Growth Factor
- VPT: Vibration Perception Threshold
- WBC: White blood cells