A PHYSIOLOGICAL STUDY TO EVALUATE THE ANTIOXIDATIVE ACTIVITY OF QUERCETIN AGAINST THE ISONIAZID-INDUCED ADVERSE REACTIONS IN MALE ALBINO RATS

A THESIS

Submitted For the Ph. D. Degree in Zoology
(Comparative Physiology)

Presented By

Ghada Mohammed Abul-Fotouh Mohammed Adly

(M. Sc. in Zoology)

Faculty of Science

Cairo University

2011

Cairo University
Faculty of Science
Department of Zoology

APPROVAL SHEET

Title of the PhD Thesis

A Physiological study to evaluate the antioxidative activity of quercetin against the isoniazid-induced adverse reactions in male albino rats.

Name of the Candidate:

Ghada Mohammed Abul-Fotouh

Submitted to:
Faculty of Science, Cairo University

Supervision Committee:

1- Professor Dr. Osama Abdel-Ghaffar Youssef
Professor of Comparative Physiology
Zoology Department, Faculty of Science, Cairo University

2- Professor Dr. Salwa Thabet Mahmoud
National Organization for Drug Control and Research, Giza, Egypt

Head of Zoology Department

Professor Dr. Abdel-Rahman Bashtar

ACKNOWLEDGEMENT

The first and foremost thanks are due to God, the most beneficent and merciful.

I would like to express my great appreciation to Professor Dr. Osama Abdel-Ghaffar, Professor of Comparative Physiology, Zoology Department, Faculty of Science, Cairo University, for his suggesting the point, planning the work, instructive guidance and valuable remarks. I am always thankful for his kind and tremendous assistance, valuable time and help.

Also, my appreciation and gratitude to Professor Dr. Salwa Thabet Mahmoud, Professor of Physiology, National Organization for Drug Control and Research (NODCAR), for her great efforts, care and valuable support to produce and present this thesis. Her continuous supervision and encouragement were of great value throughout the course of this work.

My deep acknowledgements are also due to the dear professors and colleagues in the Department of Zoology, Faculty of Science, Cairo University and in the Department of Physiology, National Organization for Drug Control and Research (NODCAR) for their helpful guidance and valuable assistance.

To whom I love very much

and I am in debt to them for my

happiness in this life: my great parents,

lovely sisters and faithful husband.

ABSTRACT

Student Name: Ghada Mohammed Abul-Fotouh Mohammed

Title of the thesis: A Physiological study to evaluate the antioxidative activity of quercetin against the isoniazid-induced adverse reactions in male albino rats.

Degree: PhD (Comparative Physiology)

The daily administration of INH to the male albino rats for five weeks caused a marked anemia evidenced with reduced red cell count, packed cell volume and hemoglobin content. It was assorted as macrocytic hypochromic anemia, due to the oxidative stress of INH on the red cell membranes. Also, INH treatment resulted in a reduction in the total leukocyte count. As regards the biochemical parameters, INH treatment caused significant decrements in the levels of serum glucose, total protein, albumin, and HDL-cholesterol. Nevertheless, there were marked increments in the levels of serum total cholesterol, LDL-cholesterol, triglycerides and uric acid, as well as in the activities of serum aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and alkaline phosphatase (ALP). Regarding the markers of the oxidative stress, the serum malondialdehyde (MDA) levels were increased while the reduced glutathione (GSH) content and superoxide dismutase (SOD) activity were decreased in liver of INH-treated rats. All the INH-induced hematological and biochemical alterations were markedly ameliorated in the animal group treated with Qc prior to INH. Furthermore, no side effects were observed in animal group treated with Qc alone.

Keywords: Isoniazid, Quercetin, Physiology, Hematology.

Supervisors: Signature:

1- Prof. Dr. Osama Abdel-Ghaffar Youssef …….
2- Prof. Dr. Salwa Thabet Mahmoud ……..…

Prof. Dr. Abdel-Rahman Bashtar

Chairman of Zoology Department
Faculty of Science- Cairo University

CONTENTS

INTRODUCTION ……. 1

MATERIALS AND METHODS ……..… 11

ANALYTICAL TECHNIQUES ….……..…… 14

I. Hematological Parameters ...……..……..... 14
I.1. Enumeration of erythrocytes ……..... 14
I.2. Determination of hematocrit …….. ……..…….…… 14
I.3. Determination of hemoglobin content …… 14
I.4. Calculation of red cell indices. ..……..…….. 15
I.5. Enumeration of leukocytes……. 16

II. Biochemical Parameters……..……...…….. 16

II.1. Determination of serum glucose concentration……. 16
II.2. Determination of serum cholesterol concentration…… 17
II.3. Determination of serum HDL- and LDL-cholesterol ……... 18
II.4. Determination of serum triglyceride concentration……..……. 19
II.5. Determination of total protein concentration in serum ……. 20
II.6. Determination of serum albumin concentration……. 20
II.7. Determination of serum globulin concentration…….**.....** 21
II.8. Determination of serum urea concentration…….…… 21
II.9. Determination of serum uric acid concentration…….…….. 22
II.10. Determination of serum creatinine concentration…….**.....** 22
II.11. Determination of lipid peroxidation in serum ……. 23
II.12. Determination of reduced glutathione concentration in liver….. 24
II.13. Determination of serum aspartate aminotransferase activity….. 25
II.14. Determination of serum alanine aminotransferase activity …… 26
II.15. Determination of serum alkaline phosphatase activity……..…. 27
II.16. Determination of superoxide dismutase activity in liver ….**.....** 28

IV. STATISTICAL ANALYSIS ……..…… 30

RESULTS …….. 31
I. The Hematological Study …….. 31
II. The Biochemical Study ……..……..……..…….. 51

DISCUSSION ……. 127
I. The Hematological Study …….. 127
II. The Biochemical Study ……..……..…….. 131

SUMMARY …… 147

REFERENCES ……. 154

ARABIC SUMMARY

LIST OF TABLES

Table No.	Title	Page No.
(1)	Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on red blood cell count ($X10^6$) of male albino rats.	32
(2)	Analysis of variance in red blood cell count of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks.	34
(3)	Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on packed cell volume (%) of male albino rats.	35
(4)	Analysis of variance in packed cell volume of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks.	38
(5)	Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on hemoglobin content (g/dl) of male albino rats.	39
(6)	Analysis of variance in hemoglobin content of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks.	41
(7)	Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on mean corpuscular	

volume (MCV) (fl) of male albino rats. 43

(8) Analysis of variance in mean corpuscular volume of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 45

(9) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on mean corpuscular hemoglobin (MCH) (pg) of male albino rats. 46

(10) Analysis of variance in mean corpuscular hemoglobin of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 48

(11) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on mean corpuscular hemoglobin concentration (MCHC) (g/dl cells) of male albino rats. 49

(12) Analysis of variance in mean corpuscular hemoglobin concentration of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 52

(13) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on total leukocyte count ($X10^3$) of male albino rats. 53

(14) Analysis of variance in total leukocyte count of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 55

(15) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum glucose concentration (mg/dl) of male albino rats. 56

(16) Analysis of variance in serum glucose concentration of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 59

(17) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum total cholesterol concentration (mg/dl) of male albino rats. 60

(18) Analysis of variance in serum total cholesterol concentration of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 62

(19) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum HDL-cholesterol concentration (mg/dl) of male albino rats. 64

(20) Analysis of variance in serum HDL-cholesterol concentration of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 66

(21) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum LDL-cholesterol concentration (mg/dl) of male albino rats. 67

(22) Analysis of variance in serum LDL-cholesterol concentration of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one 69

hour prior to isoniazid and alone, for five weeks.

(23) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum total cholesterol/HDL-cholesterol ratio of male albino rats. 71

(24) Analysis of variance in serum total cholesterol/HDL-cholesterol ratio of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 73

(25) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum LDL-cholesterol/ HDL-cholesterol ratio of male albino rats. 74

(26) Analysis of variance in serum LDL-cholesterol/ HDL-cholesterol ratio of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 77

(27) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum triglyceride concentration (mg/dl) of male albino rats. 78

(28) Analysis of variance in serum triglyceride concentration of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 80

(29) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum total protein concentration (g/dl) of male albino rats. 81

(30) Analysis of variance in serum total protein concentration of

male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 84

(31) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum albumin concentration (g/dl) of male albino rats. 85

(32) Analysis of variance in serum albumin concentration of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 87

(33) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum globulin concentration (g/dl) of male albino rats. 89

(34) Analysis of variance in serum globulin concentration of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 91

(35) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum albumin/globulin ratio of male albino rats. 92

(36) Analysis of variance in serum albumin/globulin ratio of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 94

(37) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum urea concentration (mg/dl) of male albino rats. 96

(38) Analysis of variance in serum urea concentration of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 98

(39) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum uric acid concentration (mg/dl) of male albino rats. 99

(40) Analysis of variance in serum uric acid concentration of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 101

(41) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum creatinine concentration (mg/dl) of male albino rats. 102

(42) Analysis of variance in serum creatinine concentration of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 104

(43) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum malondialdehyde (MDA) concentration (nmol/ml) of male albino rats. 105

(44) Analysis of variance in serum malondialdehyde concentration of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 108

(45) Effect of daily oral administration of isoniazid (INH) (27

mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on liver glutathione concentration (mg/g wet tissue) of male albino rats. 109

(46) Analysis of variance in liver glutathione concentration of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 111

(47) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum aspartate aminotransferase (ASAT) activity (U/L) of male albino rats. 113

(48) Analysis of variance in serum aspartate aminotransferase (ASAT) activity of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 115

(49) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum alanine aminotransferase activity (U/L) of male albino rats. 116

(50) Analysis of variance in serum alanine aminotransferase activity of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 118

(51) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum alkaline phosphatase activity (U/L) of male albino rats. 120

(52) Analysis of variance in serum alkaline phosphatase activity of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one

hour prior to isoniazid and alone, for five weeks. 122

(53) Effect of daily oral administration of isoniazid (INH) (27 mg/kg b. wt.), and quercetin (Qc) (100 mg/kg b. wt.), one hour prior to isoniazid and alone, on serum superoxide dismutase activity (U/dl) of male albino rats. 123

(54) Analysis of variance in serum superoxide dismutase activity of male albino rats as influenced by the daily administration of isoniazid (INH) and quercetin (Qc), one hour prior to isoniazid and alone, for five weeks. 126