

Pharmacological Study Of Protective Mechanisms Of Thymoquinone In Experimentally Induced Lung Fibrosis

Thesis Presented By:

Dalia Abdelazim Ismail Ibrahim El-Khouly

B. Pharm. Sc. Ain Shams University (2004)

Demonstrator Of Pharmacology And Toxicology

Faculty Of Pharmacy, Future University

**Submitted For The Partial Fulfillment Of Master's Degree In
Pharmaceutical Sciences**

(Pharmacology And Toxicology)

Supervised By:

Prof. Dr. Hala Osman El-Mesallamy

Professor Of Biochemistry And Head Of The Department

Faculty Of Pharmacy, Ain Shams University

Prof. Dr. Ebtehal El-Demerdash Zaki

Professor Of Pharmacology And Toxicology

Faculty Of Pharmacy, Ain Shams University

Prof. Dr. Azza Sayed Mohamed Awad

Professor Of Pharmacology And Toxicology

Faculty Of Pharmacy, Al-Azhar University

Faculty Of Pharmacy- Ain Shams University

2012

ACKNOWLEDGMENT

First of all, no words can express my deep thanks to Allah; who without his help; this work would have never been accomplished and may this work add to our good deeds to gain his kind mercifulness.

*I wish to express my deep thanks and appreciations to my supervisor **Prof.Dr. Hala El-Mesallamy**, Professor of Biochemistry and Head of the Department, Faculty of Pharmacy, Ain Shams University, for her kind supervision and guidance throughout this work. It is my pleasure to acknowledge her for her support and kind help.*

*Actually, I would like to express my deep and sincere gratitude to my supervisor **Prof.Dr. Ebtahal El-Demerdash**, Professor of Pharmacology & Toxicology, Faculty of Pharmacy, Ain Shams University, for her keen supervision, her valuable guidance and encouragement throughout the whole study. Her wide knowledge and her logical way of thinking have been of great value for me. She did her best to provide me with financial support to carry out this work. I am profoundly grateful to her support and wealth of knowledge.*

*Indeed, I am greatly thankful to my supervisor **Prof.Dr. Azza Awad**, Professor of Pharmacology & Toxicology, Faculty of Pharmacy, Al-Azhar University, for her kind supervision, endless support, as well as valuable instructions and guidance throughout this work. A very special thanks is paid to her for providing me with a good environment and facilities to complete all the practical work throughout the whole study. I would like to express my deepest appreciation with sincere gratitude for her generous supervision that enabled me to reach my goals and accomplish this thesis. I owe her a special word of thanks.*

*I wish to express my warm and sincere thanks to **Dr.Wesam M. El-Bakly**, Lecturer of Pharmacology & Therapeutics, Faculty of Medicine, Ain Shams University, for her enthusiastic help and guidance leading to completion of this thesis. I do appreciate her effort and valuable time she sacrificed to me. I thank her for her help in carrying out the statistical analysis, and for her helpful comments and discussion during writing this thesis.*

*Great thanks are devoted to **Prof.Dr.Ashraf B. Abdel-Naim**, Professor of Pharmacology and Toxicology and Head of the*

Department, Faculty of Pharmacy, Ain Shams University for his kind help.

*I owe my loving thanks to my father **Prof.Dr. Abdelazim Ismail El-Khouly**, Professor of Physical Chemistry, Faculty of Science, Mansoura University, for his endless guidance. I am forever indebted to him for his encouragement and support. Finally, yet importantly, I would like to express my heartfelt thanks to **my beloved family** for their blessings, for their endless love and wishes for the successful completion of this research.*

During this work I have collaborated with many colleagues for whom I have great regard, and I wish to extend my warmest thanks to all those who have helped me with my work. I would like to express my deep thanks to all members of Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams and Al-Azhar Universities for their faithful co-operation. The financial support of both Universities is gratefully acknowledged.

Dalia El-Khouly



CONTENTS

SUBJECT	PAGE
List of abbreviations	I
List of tables	V
List of figures	VI
Introduction	1
1. Pulmonary Fibrosis	1
<i>1.1 Definition, Incidence And Prevalence</i>	<i>1</i>
<i>1.2 Oxidative Stress And Antioxidants In The Lung</i>	<i>5</i>
<i>1.3 Pathogenesis Of Pulmonary Fibrosis</i>	<i>11</i>
<i>1.4 Nuclear Factor Kappa B</i>	<i>15</i>
<i>1.5Diagnosis Of Pulmonary Fibrosis</i>	<i>19</i>
<i>1.6 Therapeutic Approaches For Pulmonary Fibrosis</i>	<i>21</i>

2. Bleomycin	25
2.1 Chemical And Physical Properties	25
2.2 Pharmacokinetics	26
2.3 Pharmacodynamics	29
2.4 Therapeutic Indications	37
2.5 Adverse Reactions	38
3. Thymoquinone	43
3.1 Background Information	43
3.2 Chemistry	46
3.3 Biological Activity Of Thymoquinone	47
3.3.1 Anti-Inflammatory	47
3.3.2 Antioxidant	48
3.3.3 Anticancer	49
3.3.4 Other Reported Effects Of Thymoquinone	50
3.3.5 Toxicological Properties Of Thymoquinone	51
Aim Of The Work	53
Materials And Methods	54

<i>1. Design Of The Work</i>	<i>54</i>
<i>2. Materials</i>	<i>56</i>
<i>3. Methods</i>	<i>69</i>
<i>3.1 Processing Of Bronchoalveolar Lavage Fluid</i>	<i>69</i>
<i>3.2 Preparation Of Lung Homogenate</i>	<i>69</i>
<i>3.3 Determination Of Lactate Dehydrogenase</i>	<i>71</i>
<i>3.4 Determination Of Total Protein</i>	<i>72</i>
<i>3.5 Determination Of Mucin</i>	<i>74</i>
<i>3.6 Determination Of Lipid Peroxides</i>	<i>76</i>
<i>3.7 Determination Of Nitric Oxide</i>	<i>79</i>
<i>3.8 Determination Of Superoxide Dismutase Activity</i>	<i>82</i>
<i>3.9 Determination Of Lung Glutathione-S-Transferase Activity</i>	<i>84</i>
<i>3.10 Determination Of Hydroxyproline Content In Lung Tissue</i>	<i>86</i>
<i>3.11 Immunohistochemical Detection Of Nuclear Factor-Kappa B</i>	<i>89</i>
<i>3.12 Histopathological Examination</i>	<i>91</i>
Statistical Analysis	92
Results	93
<i>1. Lung Injury Markers</i>	<i>93</i>

2. <i>Oxidative Stress Markers</i>	103
3. <i>Fibrosis Markers</i>	110
Discussion	122
Summary And Conclusions	130
References	133
الملخص العربي	١

LIST OF ABBREVIATIONS

Ab	Antibody
Ag	Antigen
AIDS	Acquired immune deficiency syndrome
ANOVA	Analysis of variance
AP-1	Activator protein -1
ATR	Angiotensin II type 1 receptor
BALF	Bronchoalveolar lavage fluid
BLM	Bleomycin
BSA	Bovine serum albumin
CAM	Complementary/ Alternative Medicine
CAT	Catalase enzyme
COX	Cyclooxygenase enzyme
CPFE	Combined pulmonary fibrosis and emphysema
DCs	Dendritic cells
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid

ECM	Extracellular matrix
EC-SOD	Extracellular Superoxide dismutase enzyme
ET-1	Endothelin-1
5-FU	5-Fluorouracil
GABA	gamma- Aminobutyric acid
GM-CSF	Granulocyte macrophage colony stimulating factor
GSH	Reduced glutathione
GPx	Glutathione peroxidase enzyme
GRd	Glutathione reductase enzyme
GST	Glutathione-S-transferase enzyme
H & E	Hematoxylin and eosin
HIV	Human immunodeficiency virus
ICAM-1	Intercellular Adhesion Molecule -1
IFN-γ	Interferon-gamma
IFO	Ifosfamide
IκB	Inhibitory protein Kappa B
IL-1	Interleukin- 1
IL-1β	Interleukin- 1beta
IL-6	Interleukin-6

IL-8	Interleukin-8
i.p.	Intraperitoneal
IPF	Idiopathic pulmonary fibrosis
LDH	Lactate dehydrogenase enzyme
LD50	Lethal dose, 50%
LPS	Lipopolysaccharides
LTB4	Leukotriene B4
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde
NAC	N-acetyl-L-cysteine
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NF-κB	Nuclear factor kappa-B
NHE	Na(+)/H(+) exchanger
NLS	Nuclear localization signals
NO	Nitric oxide
NOS2	Nitric oxide synthase 2 enzyme
iNOS	inducible nitric oxide synthase enzyme
PF	Pulmonary Fibrosis

PGD	Primary graft dysfunction
PGE-2	Prostaglandin E-2
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SD	Standard deviation
SH	Sulfhydryl
SOD	Superoxide dismutase enzyme
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBS	Tris buffered saline
TGF- β	Transforming growth factor- beta
TLR	Toll-like receptor
TNF-α	Tumor necrotic factor- alpha
TQ	Thymoquinone
TXB2	Thromboxane B2
VATS	Video-assisted thoracoscopic surgery
VCAM-1	Vascular cell adhesion protein -1
WBCs	White blood cells

LIST OF TABLES

TABLE		PAGE
1	Effect of thymoquinone on lung injury markers in bronchoalveolar lavage fluid of rats treated with bleomycin	96
2	Effect of thymoquinone on oxidative stress markers in lung tissues in rats treated with bleomycin	105
3	Effect of thymoquinone on hydroxyproline content in lung tissues in rats treated with bleomycin	111

LIST OF FIGURES

FIGURE		PAGE
1	Prevalence of idiopathic pulmonary fibrosis in United States, by age & sex	3
2	Prevalence and annual incidence of idiopathic pulmonary fibrosis in United States	4
3	Oxygen and nitrogen-based reactants which are generated in abundance during oxidative stress	10
4	Phases of wound healing	15
5	A schematic presentation of activation of the transcription nuclear factor kappa-B	18
6	Chemical structure of bleomycin	26
7	Structure of metallobleomycins and their domain organization	31

8	Formation of 'activated' bleomycin and cleavage of DNA	32
9	Chemical structure of bleomycin-Fe (II) complex	33
10	Products of bleomycin-induced double-stranded DNA cleavage and a proposed model for this process	34
11	Proposed mechanisms for generation of 'activated bleomycin' <i>in-vivo</i>	35
12	Schematic diagram illustrating the signaling pathways after bleomycin-induced lung injury	36
13	Leaves, flowers and seeds of <i>Nigella Sativa</i>	45
14	Chemical structures of thymoquinone, thymol, dithymoquinone, p-cymene and α -pinene	46
15	Standard calibration curve of MDA	78