Potential neuroprotective effect of Androst-5-ene-3β, 17β-diol (ADIOL) against experimentally-induced Parkinson's disease in rats

A thesis submitted for partial fulfillment of Doctor of Philosophy degree in Pharmaceutical Sciences
(Pharmacology and Toxicology)

By

Rania Mohamed Kamel Hassan Mohamed Salama

M.SC. in Pharmaceutical Sciences,
Ain Shams University, Egypt, 2012
Assistant Lecturer of Pharmacology and Toxicology
Faculty of Pharmacy, Misr International University

Under the supervision of

Prof. Dr. Ahmed M. Abdel-tawab

Professor of Pharmacology
Faculty of Medicine, Ain Shams University

Dr. Mona F. Schaalan

Acting Head of Clinical Pharmacy Department
Assistant Professor of Biochemistry
Faculty of Pharmacy, Misr International University

Dr. Nevine B. Soliman

Assistant Professor of Histology
Faculty of Medicine, Ain Shams University

Dr. Mariane G. Tadros

Assistant Professor of Pharmacology and Toxicology
Faculty of Pharmacy, Ain Shams University

Faculty of Pharmacy - Ain Shams University
2016

Examination Board Approval Sheet

Name of Candidate

Rania Mohamed Kamel Hassan Mohamed Salama

Title of Thesis

Potential neuroprotective effect of Androst-5-ene-3β, 17β-diol (ADIOL) against experimentally-induced Parkinson's disease in rats

Submitted to Faculty of Pharmacy
Ain Shams University
Department of Pharmacology and Toxicology

Approved by the committee in charge:

- **Prof. Ahmed Mohey-eldin Abdel-tawab** ----------
  Professor of Pharmacology
  Faculty of Medicine
  Ain Shams University

- **Prof. Gouda Kamel Abdel Bary** ----------
  Dean of Faculty of Pharmacy
  Professor of Pharmacology and Toxicology
  Azhar University "Boys"

- **Prof. Sawsan Aboul-Fotouh El-Said** ----------
  Professor of Pharmacology
  Faculty of Medicine
  Ain Shams University

- **Assistant Prof. Mariane George Tadros** ----------
  Assistant Professor of Pharmacology and Toxicology
  Faculty of Pharmacy
  Ain Shams University

Acting Head of Pharmacology and Toxicology Department
Assistant Prof. Samar Saad-eldin Azab

Date: 12 / 6 / 2016

Pre-requisite Postgraduate Courses

Besides the work presented in the thesis, the candidate has attended pre-requisite postgraduate courses including the following topics:

Special Courses:

- Clinical Pharmacology
- Pharmacology
- Selected topics
- Clinical Toxicology

The candidate has successfully passed the examination in these courses and the comprehensive exam with general grade *"**Excellent**"*.

Acting Head of Pharmacology and Toxicology Department
Faculty of Pharmacy, Ain Shams University
Assistant Prof. Samar Saad-eldin Azab

Acknowledgements

First and foremost, all praise to Almighty Allah who gave me courage and patience to carry out this work. At the end of my thesis, I would like to take this opportunity to show my gratitude to those who have assisted me in a myriad of ways.

I would like to express my sincere gratitude to my supervisor Prof. Ahmed Abdel-tawab whose attention to every detail and academic precision provided me the necessary direction and focus for my study.

I am also extremely indebted to my supervisor Assistant Prof. Mona Schaalan whose constructive comments and suggestions throughout the experimental and thesis works have contributed to the success of this research.

I would also like to thank my supervisor Assistant Prof. Nevine Soliman for her guidance, understanding and priceless advices throughout the duration of this study and the preparation of this thesis.

I would also like to thank my supervisor Assistant Prof. Mariane Tadros who helped in every step of the way. She never hesitated to provide relentless support and motivation at all times. Her timely and efficient contribution helped me shape this into its final form.

Besides my supervisors, I gratefully acknowledge Prof. Amani Khalifa for her encouragement and personal attention. My thanks are due to her valuable suggestions and constant support throughout my PHD journey.

A big thank to my brother for his support and encouragement. I wish to thank my parents-in-law for supporting me throughout this phase of life and for helping me in the successful completion of this thesis.

I'm forever indebted to my late mother, who raised me, supported me, taught me and loved me. Although she is not here to give me strength and support, I always feel her presence that used to urge me to strive to achieve my goals in life.

Father... Today I completed my PHD thesis, fulfilling your wish before your departure two years ago. I cannot directly address to you anymore, but deeply in my mind I always acknowledge and remember you. I wish your soul roots in peace and solace in the heaven. Dad, this is for you.

Rania Salama

التأثير الواقي المحتمل للخلايا العصبية لدواء أندروستينديول (أديول) ضد مرض الشلل الرعاش المُحدَث تجريبياً في الجرذان

رسالة مقدمة لإستكمال متطلبات الحصول على درجة الدكتوراة في العلوم الصيدلية
(الأدوية والسموم)

مقدمة من

رانيا محمد كامل حسن محمد سلامة

ماجستير العلوم الصيدلية، جامعة عين شمس، مصر، ٢٠١٢
مدرس مساعد بقسم الأدوية والسموم
كلية الصيدلة، جامعة مصر الدولية

تحت إشراف

أ.د/ أحمد محي الدين عبد التواب
أستاذ الأدوية، كلية الطب، جامعة عين شمس

أ.م/ منى فرج شعلان
القائم بأعمال رئيس قسم الصيدلة الإكلينيكية
أستاذ مساعد الكيمياء الحيوية، كلية الصيدلة،
جامعة مصر الدولية

أ.م/ نيفين بهاء الدين سليمان
أستاذ مساعد الأنسجة، كلية الطب،
جامعة عين شمس

أ.م/ ماريان جورج تادرس
أستاذ مساعد الأدوية و السموم، كلية الصيدلة، جامعة عين شمس

كلية الصيدلة، جامعة عين شمس
(٢٠١٦)

Contents

Page

- **List of Abbreviations**……..…..…..i
- **List of Figures**……...……....iv
- **List of Tables**……...…….....ix
- **Abstract**……...……..……...…….....xi
- **Literature Review**……..……..……...…….....1

1. Introduction to Parkinson's disease…….....1
   1.1. The history of Parkinson's disease…….....1
   1.2. Prevalence and epidemiology of Parkinson's disease…….……...1
      1.2.1. The global burden of Parkinson's disease……...……...1
      1.2.2. Parkinson's disease in Egypt…….....…3
   1.3. Clinical manifestations of Parkinson's disease……..……..……...4
   1.4. Pathophysiology of Parkinson's disease……..……...5
   1.5. Diagnosis of Parkinson's disease……...….....8
   1.6. Etiology of Parkinson's disease……10
   1.7. Pathogenesis of Parkinson's disease……...…...13
   1.8. Pharmacological treatment of Parkinson's disease……....20
      1.8.1. Currently available medications……...…20
      1.8.2. Future trend in the treatment of Parkinson's disease….....23
2. Animal models of Parkinson's disease…….....26
   2.1. Neurotoxin-induced models…….…..26
      2.1.1. 6-Hydroxydopamine……..…...26
      2.1.2. 1-methyl-1,2,3,6-tetrahydropyridine……....27
   2.2. Pesticide/Herbicide-induced models…….….....28
      2.2.1. Paraquat……..….…28
      2.2.2. Rotenone……..….....29
   2.3. Genetic models……..……31

3. The drug used in the current study

Androst-5-ene-3β, 17β-diol …….....32

3.1. Pharmacokinetics of Androst-5-ene-3β, 17β-diol ……....33

3.2. Pharmacodynamics of Androst-5-ene-3β, 17β-diol…….……34

3.2.1. The established mechanisms of Androst-5-ene-3β, 17β-diol.....34

3.2.2. The potential neuroprotective mechanism of Androst-5-ene-3β, 17β-diol ……..35

- **Aim of the work**……...…….…….....**39**
- **Materials and Methods**……..……..…....**41**

I. Materials…….…….……..…41

II. Methods…….……..….....48

1. Experimental design……..…….....48
2. Behavioral tests……...…50

2.1. Catalepsy test…….……....50

2.2. Postural instability test……..……..…….....51

2.3. Open field test ……..…….…….....52

3. Biochemical parameters…….……...……...53

3.1. Determination of dopamine and its metabolites …….....53

3.2. Determination of adenosine triphosphate …….....62

3.3. Determination of nuclear factor-kappa B …….....67

3.4. Determination of inducible nitric oxide synthase, interleukin-6 and B-cell activating factor ……....70

3.5. Determination of caspase-3……..…….…….…...75

3.6. Determination of Bcl2-associated X protein …….....78

3.7. Determination of B-cell lymphoma 2……..…….…….....81

3.8. Determination of protein content……...84

4. Histopathology and Immunohistochemistry…….……...86
5. Statistical Analysis……...……...88

- **Results**……..…….....**89**

- **Discussion**……...……...……..……....**148**
- **Summary and Conclusions**……..…....**159**
- **References**……..…….……...…...**164**
- **Arabic Summary**……...…….…..**196**

List of Abbreviations

AD	Alzheimer's disease
ADIOL	Androst-5-ene-3β, 17β-diol
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BAFF	B-cell activating factor
Bax	Bcl2-associated X protein
BBB	Blood–brain barrier
Bcl-2	B-cell lymphoma 2
BID	BH3 interacting-domain death agonist
BSA	Bovine serum albumin
CCD	Charge-coupled device
cDNA	Complementary deoxyribonucleic acid
C_{max}	Maximum plasma concentration
CMC	Carboxymethyl cellulose
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
COMTI	Catechol-O-methyltransferase inhibitor
ΔCT	Mean cycle threshold
ΔΔCT	Comparative cycle threshold
CtBP	C-terminal binding protein
C.V.	Coefficient of variation
DA	Dopamine
DAT	Dopamine transporter
DDC	Dopa decarboxylase
DDCI	Dopa decarboxylase inhibitor
DHBA	3,4-Dihydroxybenzylamine hydrobromide
DHEA	Dehydroepiandrosterone
DNA	Deoxyribonucleic acid
DOPAC	Dihydroxyphenylacetic acid
EAE	Experimental auto-immune encephalomyelitis
ECD	Electrochemical detection
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EM	Electron microscopy
ER	Estrogen receptor
ESC	Embryonic stem cells
GADPH	Glyceraldehyde-3-phosphate dehydrogenase
GSH	Reduced glutathione
H&E	Hematoxylin and eosin
H_2O_2	Hydrogen peroxide
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
HSD	Hydroxysteroid dehydrogenase
HVA	Homovanillic acid
ICH	International Conference on Harmonisation
IL	Interleukin
IMP	Inosine 5′-monophosphate
iNOS	Inducible nitric oxide synthase

i.p.	Intraperitoneal
IQR	Interquartile ranges
IS	Internal standard
i.v.	Intravenous
KCl	Potassium chloride
KH_2PO_4	Monobasic potassium phosphate
KO	knockout
LB	Lewy body
L-DOPA	L-3,4-dihydroxyphenylalanine
LM	Light microscopy
LN	Lewy neurite
LOD	Limit of detection
LOQ	Limit of quantification
LPS	lipopolysaccharide
LRRK 2	Leucine-rich repeat kinase-2
Maneb	Manganese ethylene-1,2-bisdithiocarbamate
MAO	Monoamine oxidase
MAOBI	Monoamine oxidase-B inhibitor
MPP+	1-methyl-4-phenylpyridinium
mPTP	mitochondrial permeability transition pore
MPTP	1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine
MS	Multiple sclerosis
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NE	Norepinephrine
NF-κB	Nuclear factor-kappa B
NINDS	National Institute of Neurological Disorders and Stroke
NO	Nitric oxide
Nrf2	Nuclear factor erythroid 2-related factor 2
O_2^-	Superoxide
OD	Optical density
6-OHDA	6-Hydroxydopamine
PBS	Phosphate buffered saline
PD	Parkinson's disease
PINK1	PTEN-induced putative kinase 1
rER	Rough endoplasmic reticulum
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
s.c.	Subcutaneous
S.D.	Standard deviation
SEO	Sumy Electron Optics
SN	Substantia nigra
SNpc	Substantia nigra pars compacta
SNpr	Substantia nigra pars reticulata
TEM	Transmission electron microscope
TH	Tyrosine hydroxylase
TLR	Toll-like receptor
TMB	Tetramethylbenzidine
TNF-α	Tumor necoris factor-alpha

UCH-L1	Ubiquitin C-terminal hydrolase L1
UKPDSBB	United Kingdom Parkinson Disease Society Brain Bank
UPS	Ubiquitin-proteasome system
USP 30-NF 25	United States Pharmacopeia and National Formulary
UV	Ultra-violet

List of Figures

Figure		Page
Figure i:	Clinical manifestations and time course of PD progression.	**5**
Figure ii:	Neuropathology of PD.	**7**
Figure iii:	Different pathways to neurodegeneration in PD.	**16**
Figure iv:	Inflammation in PD.	**19**
Figure v:	Schematic Illustration of sites of action of available medications for the treatment of motor symptoms of PD.	**22**
Figure vi:	Mechanisms of actions of different toxins used to induce PD.	**30**
Figure vii:	Chemical structure of Androst-5-ene-3β, 17β-diol (ADIOL).	**32**
Figure viii:	Direct metabolism of DHEA by 17β-HSD produces ADIOL.	**33**
Figure ix:	Anti-inflammatory effect of ADIOL via ERβ-mediated repression.	**38**
Figure x:	Performing the postural instability test.	**51**
Figure 1:	Standard curve of DA	**57**
Figure 2:	Standard curve of DOPAC	**59**
Figure 3:	Standard curve of HVA	**61**
Figure 4:	Standard curve of ATP	**66**
Figure 5:	Standard curve of NF-κB	**69**
Figure 6:	Standard curve of caspase-3	**77**

Figure 7:	Standard curve of Bax	**80**
Figure 8:	Standard curve of Bcl-2	**83**
Figure 9:	Standard curve of protein content	**85**
Figure 10:	Box plots for **catalepsy test (A) Bar** and **(B) Grid**, carried out in groups of rats receiving vehicle (control), ADIOL (3.5 mg/kg/day; s.c.), rotenone (1.5 mg/kg/day; i.p.) and ADIOL (0.35, 3.5 and 35 mg/kg/day; s.c.) in addition to rotenone for 30 days.	**92**
Figure 11:	Box plots for **postural instability test** carried out in groups of rats receiving vehicle (control), ADIOL (3.5 mg/kg/day; s.c.), rotenone (1.5 mg/kg/day; i.p.) and ADIOL (0.35, 3.5 and 35 mg/kg/day; s.c.) in addition to rotenone for 30 days.	**93**
Figure 12:	Box plots for **open field test**, showing **(A) Number of squares**, **(B) Number of stops**, **(C) Activity index**, **(D) Rearing frequency**, **(E) Active sitting** and **(F) Inactive sitting** of groups of rats receiving vehicle (control), ADIOL (3.5 mg/kg/day; s.c.), rotenone (1.5 mg/kg/day; i.p.) and ADIOL (0.35, 3.5 and 35 mg/kg/day; s.c.) in addition to rotenone for 30 days.	**97**
Figure 13:	Percentage change of **(A) DA concentration**, its metabolites; **(B) DOPAC** and **(C) HVA**, and **(D) DA turnover** in the **striatum** of groups of rats receiving vehicle (control), ADIOL (3.5 mg/kg/day; s.c.), rotenone (1.5 mg/kg/day; i.p.) and ADIOL (0.35, 3.5 and 35 mg/kg/day; s.c.) in addition to rotenone for 30 days.	**101**

Figure 14: Percentage change of **ATP concentration** in the **(A) striatum** and **(B) SN** of groups of rats receiving vehicle (control), ADIOL (3.5 mg/kg/day; s.c.), rotenone (1.5 mg/kg/day; i.p.) and ADIOL (0.35, 3.5 and 35 mg/kg/day; s.c.) in addition to rotenone for 30 days. **104**

Figure 15: Percentage change of **NF-κB concentration** in the **(A) striatum** and **(B) SN** of groups of rats receiving vehicle (control), ADIOL (3.5 mg/kg/day; s.c.), rotenone (1.5 mg/kg/day; i.p.) and ADIOL (0.35, 3.5 and 35 mg/kg/day; s.c.) in addition to rotenone for 30 days. **107**

Figure 16: Relative gene expression of **(A) iNOS**, **(B) IL-6** and **(C) BAFF** in the **striatum** of groups of rats receiving vehicle (control), ADIOL (3.5 mg/kg/day; s.c.), rotenone (1.5 mg/kg/day; i.p.) and ADIOL (0.35, 3.5 and 35 mg/kg/day; s.c.) in addition to rotenone for 30 days. **111**

Figure 17: Relative gene expression of **(A) iNOS**, **(B) IL-6** and **(C) BAFF** in the **SN** of groups of rats receiving vehicle (control), ADIOL (3.5 mg/kg/day; s.c.), rotenone (1.5 mg/kg/day; i.p.) and ADIOL (0.35, 3.5 and 35 mg/kg/day; s.c.) in addition to rotenone for 30 days. **115**

Figure 18: Percentage change in the levels of **(A) Caspase-3**, **(B) Bax**, **(C) Bcl-2** and **(D) Bax/Bcl-2 ratio** in the **striatum** of groups of rats receiving vehicle (control), ADIOL (3.5 mg/kg/day; s.c.), rotenone (1.5 mg/kg/day; i.p.) and ADIOL (0.35, 3.5 and 35 mg/kg/day; s.c.) in addition to rotenone for 30 days. **119**

Figure 19: Percentage change in the levels of **(A) Caspase-3**, **(B) Bax**, **(C) Bcl-2** and **(D) Bax/Bcl-2 ratio** in the **SN** of groups of rats receiving vehicle (control), ADIOL (3.5 mg/kg/day; s.c.), rotenone (1.5 mg/kg/day; i.p.) and ADIOL (0.35, 3.5 and 35 mg/kg/day; s.c.) in addition to rotenone for 30 days. **123**

Figure 20: Micrograph showing **H&E** stained-sections of **striatal** neurons in rats. **126**

Figure 21: Percentage change of the mean number of **viable neurons** in the **striatum** of groups of rats receiving vehicle (control), ADIOL (3.5 mg/kg/day; s.c.), rotenone (1.5 mg/kg/day; i.p.) and ADIOL (0.35, 3.5 and 35 mg/kg/day; s.c.) in addition to rotenone for 30 days. **128**

Figure 22: Micrograph showing **H&E** stained-sections of **nigral** neurons in rats. **131**

Figure 23: Percentage change of the mean number of **viable neurons** in the **SNpc** of groups of rats receiving vehicle (control), ADIOL (3.5 mg/kg/day; s.c.), rotenone (1.5 mg/kg/day; i.p.) and ADIOL (0.35, 3.5 and 35 mg/kg/day; s.c.) in addition to rotenone for 30 days. **133**

Figure 24: Micrograph showing **α-synuclein immunoreactivity** in the **SNpc** of rats. **136**

Figure 25: Percentage change of the **α-synuclein density** in the **SNpc** of groups of rats receiving vehicle (control), ADIOL (3.5 mg/kg/day; s.c.), rotenone (1.5 mg/kg/day; i.p.) and ADIOL (0.35, 3.5 and 35 mg/kg/day; s.c.) in addition to rotenone for 30 days. **138**