The Possible Beneficial Effect of Melatonin Co-administration to Perindopril on Left Ventricular Dysfunction Induced by Doxorubicin in Hypertensive albino Rats.

Thesis submitted for partial fulfilment of Master degree in Pharmacology & Therapeutics.

By

Takwa Mohammed Abdul Salam Elsaid

M.B., B.Ch. (2010), demonstrator in Department of Pharmacology & Therapeutics.

Faculty of Medicine, Ain Shams University

Supervised by

Prof. Dr. / Ahmed Al-Sayed Badawy
Professor of Pharmacology& Therapeutics.
Faculty of Medicine Ain-Shams University

Ass. Prof. Dr. / Amany Helmy Mohamed
Assistant Professor of Pharmacology & Therapeutics.
Faculty of Medicine Ain-Shams University

Ass. Prof. Dr. / Wessam Mostafa El-Bakly
Assistant Professor of Pharmacology & Therapeutics.
Faculty of Medicine Ain-Shams University

Faculty of Medicine

Ain Shams University

2016

Acknowledgement

First of all, all gratitude is due to ***God*** *almighty for blessing this work, until it has reached its end, as a part of his generous help, throughout my life.*

It is a great pleasure to express my sincere thanks and gratitude to ***Prof. Dr. Ahmed Al-Sayed Badawy,*** *Professor of Pharmacology and Therapeutics, Ain Shams University, for his consistent supervision, continuous enthusiastic encouragement and valuable advice. It is a great honor to work under his guidance and supervision.*

I am also indebted to ***Ass. Prof. Dr. Amany Helmy Mohamed,*** *Assistant Professor of Pharmacology and Therapeutics, Faculty of Medicine, Ain Shams University, who gave me much of her time, for her patience, valuable suggestions, continuous everlasting help and valuable scientific guidance and support through this work.*

I want also to my sincere appreciation and gratitude to ***Ass. Prof. Dr. Wessam Mostafa El-Bakly*** *Assistant Professor of Pharmacology and Therapeutics, Faculty of Medicine, Ain Shams University, for her tremendous effort and time ,valuable scientific* *help and guidance and for her continuous directions and support throughout the whole work..*

I would like to thank **Ass. Prof. Dr. Mona Hussein Raafat** *Assistant Professor of Histology, faculty of medicine, Ain Shams University, for providing her time and support to accomplish the histopathological studies.*

I would like also to thank **Dr. Ahmed Mohammed Abdella***, Researcher at Medical Research Centre, Ain Shams University Hospitals for his great help in the practical part of this work.*

I'm really indepted to **Prof. Dr. Ahmed Abd El-Salam** *Professor of Pharmacology and Therapeutics, Faculty of Medicine, Ain Shams University for supplying us with the Perindopril powder as a gift as well known about Prof. Dr. Ahmed Abd El-Salam to help and support the young candidates in need.*

Last but not least, I dedicate this work to my family for their continuous devoted support and encouragement.

Takwa Mohammed Elsaid

List of contents

List of Tables

List of Tables (cont.)

List of Figures

List of Figures(Cont.)

List of Figures(Cont.)

Table of Figures(Cont.)

List of Abbreviations

ACE	Angiotensin converting enzyme
Ang-II	Angiotensin II
DOX	Doxorubicin
eNOS	Endothelial nitric oxide synthase
HF	Heart failure
L-NAME	N-Nitro-L-arginine methyl ester hydrochloride
LVD	Left ventricular dysfunction
LVEDP	Left ventricular end diastolic pressure
MDA	Malondialdehyde
MT	Melatonin
NO	Nitric oxide
RAAS	Renin angiotensin aldosterone system
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SOD	Superoxide dismutase
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor- α

Introduction

Left Systolic ventricular dysfunction is a pathological condition at which there is impaired myocardial contractility, with structural changes of the myocardium accompanied by increase in interstitial fibrosis and ventricular remodeling ***(Copete et al., 2015)***. It is considered a major global health problem, with huge economic burden ***(Cook et al., 2014)***.

The pathophysiology of failing of human heart is characterized by neurohormonal activation and autonomic imbalance with increase in sympathetic activity and withdrawal of vagal activity which initially able to compensate for the depressed myocardial function. However, their long-term activation has deleterious effects on cardiac structure and performance, like interstitial growth and remodeling that increase myocardial mass leading to cardiac decompensation and heart failure (HF) progression ***(Lymperopoulos et al., 2013).***

Considerable progress in identification and description of the pathophysiological mechanisms that can lead to HF in humans has taken place over the past few decades ***(Kemp and Conte, 2012)***.

Unfortunately though, this progress couldn't give rise to significant advances in management for the causes of this syndrome and chronic ventricular dysfunction, treatment is still largely symptomatic, aiming at prolonging life expectancy of the patient (without necessarily improving quality of life) ***(Georgiopoulou et al., 2012)***.

Therefore, it is absolutely crucial that future research in the field of HF continues to focus on its pathological mechanisms, especially at the molecular, biochemical, and cellular levels, for the hope of discovering a cure for this detrimental syndrome ***(Copete et al., 2015).***

Renin Angiotensin System (RAS) is activated in patients with heart failure. Angiotensin converting enzyme (ACE) inhibitors are the principle key element of drug therapy for chronic heart failure because they reduce mortality and the risk of hospitalization for heart failure at all degrees of heart failure ***(Gradman and Papademetriou, 2009).***

Perindopril which acts to inhibit ACE was confirmed to decrease sympathetic modulation and increase parasympathetic effect of the heart, and improve left ventricular function ***(Fox et al., 2007)*** and has shown antiinflammatory, antiatherosclerotic, antioxidant, and profibrinolytic effects ***(Krysiak and Okopie, 2008).*** Considering its properties and the gathered clinical evidence on efficacy and tolerability, perindopril fulfils the criteria for better hypertension and cardiovascular disease management ***(De Backer et al., 2003).***

The ability of Melatonin to combat molecular damage to the heart becomes an interesting therapeutic strategy in the treatment of myocardial injury and fibrosis ***(Yang et al., 2014).*** Moreover, numerous studies suggest that melatonin plays a significant role in HF due to its antiflammatory, antioxidant and its antifibrotic properties (***Dominguez-Rodriguez et al., 2014).***

- ***Aim of the work:***

The aim of the present study was to investigate the beneficial effect of co-administration of melatonin to perindopril regarding cardiac functions, myocardial damage, oxidative stress, inflammation and fibrosis, together with identification of histopathological changes resulted from an animal model of left ventricular dysfunction induced by doxorubicin in (L-NAME) hypertensive rats.

- ***Induction of left ventricular dysfunction:***

Left ventricular dysfunction was induced by daily oral administration of L-NAME (40 mg/kg/day) for 8 weeks by gastric gavage ***(Raja, 2010)*** with intraperitoneal injection of doxorubicin hydrochloride (2.5mg/kg twice per week) ***(Spivak et al., 2013)*** in the last 3 weeks of the experiment which lasted for 8 weeks.

- ***Study design:***

30 male albino rats (weighting 200-250 g) were divided randomly into 5 groups, 6 animals each.

1- **Control group (naïve) animals.**

2- **L-NAME/DOX untreated rats:** L-NAME (40mg/kg/day) for 8 weeks by oral gavage and doxorubicin (2.5 mg/kg/twice per week) for the last 3 weeks by intraperitoneal injection.

3- **Perindopril treated rats**:L-NAME-doxorubicin intoxicated rats treated with perindopril (5mg/kg/day) for the last 3 weeks ***(Louise et al., 2005)*** by daily oral gavage

4- **Melatonin treated rats:** L-NAME-doxorubicin intoxicated rats treated with melatonin (10mg /kg) for last 3 weeks ***(Simko et al., 2014b)*** by daily oral gavage.

5- **Perindopril/Melatonin treated rats:** L-NAME-doxorubicin intoxicated rats treated with perindopril (5mg/kg/day) and melatonin (10mg /kg) for the last 3weeks by daily oral gavage.

The duration of the whole study lasted for 8 weeks. Drugs were administered daily from the 6thweek.

Melatonin dose was selected from previous works were shown to lower increased blood pressure and protect the cardiovascular system against deleterious effect of hemodynamic overload (***Mukherjee et al., 2012).*** Moreover, the higher dose of melatonin was shown to have sedative effects. Thus, the tolerable pharmacological dose of melatonin (10 mg/kg/day) may be sufficient to induce the potential protective effects of tested substances ***(Simko et al., 2014b)***.