A study on the Potential Protective Effect of Wogonin Against Cisplatin-Induced Nephrotoxicity in Rats

Thesis presented by
Alaa Mahdy Taha El-Said Badawy
B.Pharm.Sc. Ain Shams University (2013)
Quality control specialist,
Department of Pharmacology,
National Organization for Drug Control and Research

Submitted for the Fulfillment of Master's Degree
in Pharmaceutical Sciences
(Pharmacology and Toxicology)

Under the Supervision of

Prof. Dr. Hala Mahmoud Fawzy
Professor and Head of General Division of Pharmacology Department, National Organization for Drug Control and Research

Dr. Mariane George Tadros
Associate Professor in the Department of Pharmacology and Toxicology
Faculty of Pharmacy
Ain Shams University

Dr. Reem Nabil Mohamed Ali Abou El-Naga
Associate Professor in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University

Faculty of Pharmacy
Ain Shams University
2019

Examination Board Approval Sheet

- **Title of the Master's Degree thesis in Pharmaceutical Science (Pharmacology and Toxicology):**

A study on the potential protective effect of wogonin against cisplatin-induced nephrotoxicity in rats

- **Name of candidate:**

Alaa Mahdy Taha El-Said Badawy

- **Submitted to:**

Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams University.

- **Approved by the committee in charge:**

Dr. Hala Mahmoud Fawzy .....
Professor and head of Pharmacology Department, National Organization for Drug Control And Research (NODCAR).

Dr. Hanaa Abd El-fattah .....
Professor and former head of Pharmacology Department, National Organization for Drug Control And Research (NODCAR).

Dr. Gouda Kamel Helal .....
Professor of pharmacology and Toxicology Department, Dean of Faculty of Pharmacy, Heliopolis University.

Dr. Mariane George Tadros .....
Associate Professor of Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams University.

Dr. Reem Nabil Abou El-Naga .....
Associate Professor of Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams University

Head of Pharmacology and Toxicology Department

Professor. Ebtehal El-Demerdash

Date: 17/9/2019

"وَعَلَّمَكَ مَا لَمْ تَكُن تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيماً "

(النساء ١١٣)

List of Contents

Acknowledgement

First and foremost, praise is to Allah, the most gracious, and the source of all knowledge by whose abundant grace this work has come to completion.

It would not have been possible to write this doctoral thesis without the help and support of the kind people around me, to only some of whom it is possible to give particular mention here

I would like to express my sincere gratitude and everlasting thanks to **Professor Dr. Hala Mahmoud Fawzy,** Professor of Toxicology, The National Organization for Drug Control and Research for her kind supervision, great encouragement, valuable advice, endless cooperation, helpful instruction, generous support, revising the thesis, interest and concern about my progress.

My deepest thanks, heartfelt appreciation and endless gratitude to **Dr. Mariane George Tadros ,** *Associate Professor* of Pharmacology and Toxicology, Faculty of Pharmacy, Ain shams University, for her active supervision, enlightening thoughts, useful comments, efforts, kind relation and valuable time that she sacrificed for me during this work, without which I would have never been able to produce such a work.

I'm heavily indebted to **Dr. Reem Nabil Abou El-Naga,** *Associate Professor* of Pharmacology and Toxicology, Faculty of Pharmacy, Ain shams University, my dearest supervisor for providing with the idea of this study, her tremendous effort, deep experience and the valuable time that she sacrificed for me during the exhaustive process of organizing, revising, proof reading this thesis, openness of mind and above all the patience with which she supervised my work always helped me a lot in sustaining my enthusiasm in carrying out this work till its fruition.

I would like to express my deepest appreciation and endless gratitude to **Dr. Amany Mohamed Ahmed Gad,** Researcherof Pharmacology, National Organization for Drug Control and Research for her tremendous support ,indispensable help in the practical work and thesis writing , her active participation to me at all difficult circumstances and giving me a boost when I

have fallen down, continuous support, confidence, motivation, kindness, understanding that helped me to forwards to continue. In fact, she is more than a doctor for me. I am considering her as a friend and older sister.

My deepest thanks and appreciation to **Professor Dr. Adel B. Khelosy,** Professor of Pathology, Faculty of Veterinary Medicine, Cairo university for his professional aid and meticulous guidance in the histopathological aspects of this thesis.

Grateful thanks are to **all my colleagues** and **all staff members** of the Pharmacology Department in the National Organization for Drug Control and Research and to **all my colleagues** and **all staff members** of the Pharmacology Department in the Faculty of Pharmacy, Ain shams University for their generous support and helpful advice throughout the thesis.

Finally, but of great importance, I wish to express my deep gratefulness and thanks to **my family members** for their love and prayers, which are and will always, be invaluable, listening to my frustrations and celebrating my accomplishments, calming me down when I need it and giving me a boost when I have fallen down, and for their continuous support to achieve my goal.

Alaa Mahdy Taha

Abstract

Cisplatin, a platinum chemotherapeutic agent, is used in a diversity of malignancies; however, the excessive nephrotoxicity following cisplatin treatment is the dose-limiting destructive reaction. In fact, numerous mechanisms are anticipated to induce cisplatin nephrotoxicity. Indeed, the mechanisms underlying cisplatin-mediated nephrotoxicity are not absolutely understood. The current study was designed to explore the possible nephroprotective impact of wogonin, a forceful anti-oxidant, anti-inflammatory, and anti-tumor agent, in a rat model of cisplatin-induced renal injury. The potential mechanisms of this nephroprotective effect were additionally investigated. Wogonin was given at a dose of 40 mg/kg via intraperitoneal injection, 7 days before giving a single dose of cisplatin (7mg/kg). Acute nephrotoxicity was indicated by a significant rise in blood urea nitrogen (BUN), and serum creatinine levels in cisplatin-injected rats. Also, cisplatin enhanced the lipid peroxidation, diminished the reduced glutathione (GSH), catalase (CAT), and peroxisome proliferator-activated receptor-gamma (PPAR-γ**)** levels. Additionally, statistics of cisplatin-injected rats confirmed an apparent pro-inflammatory response as evidenced by a significant rise in tissue levels of interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Besides, cisplatin induced a marked elevation in the activity of the apoptotic caspase-3 enzyme. Pre-treatment with wogonin notably ameliorated the nephrotoxic outcomes, oxidative stress, inflammation and apoptosis prompted by means of cisplatin, in addition to the up-regulation of PPAR-$_{\gamma}$ expression. The involvement of Wingless-type and beta-catenin (Wnt/β-catenin) pathway was debatable; however, our findings showed that it was significantly elevated by cisplatin, while wogonin pre-treatment markedly attenuated this elevation. In conclusion, these findings imply that wogonin improves the therapeutic index of cisplatin via being an anti-oxidant, anti-inflammatory, and a PPAR-$_{\gamma}$-inducing agent. Also, Wnt/β-catenin pathway is partially involved in the pathogenesis of cisplatin nephrotoxicity.

Keywords: Cisplatin; Nephrotoxicity; Wogonin; PPAR-γ; Wnt/β-catenin pathway

List of Abbreviations

A	Absorbance
AAP	Aminophenazone
ABCAI	ATP-binding cassette transporter A1
c-Abl	Abelson murine leukemia
Ac-DEVD-pNA	Acetyl-Asp-Glu-ValAsp p-nitroanilide
ACE-I	Angiotensin-converting enzyme inhibitors
Ag I	Angiotensin I
AgII	Angiotensin II
AKI	Acute kidney injury
ALD	Alcoholic liver disease
ANOVA	One-way analysis of variance
aP2	Adipocyte Protein 2
APC	Adenomatous polyposis coli
APoA-I	Apolipoprotein A-I
ApoE	Apolipoprotein E
ARB	Angiotensin II receptor blockers
ARF	Acute renal failure
ARI	Acute kidney injury
ATF-2	Activating transcription factor
ATT	Rad3-related protein
AUC	Area under the curve
BUN	Blood urea nitrogen
Ca2+	Calcium
CAT	Catalase
Cl−	Chloride
CO_2	Carbon dioxide
COX-2	Cyclooxygenase-2
Ctr1	Copper transporter-1
Cyt c	Cytochrome c
DHBS	3,5-dichloro -2-hydroxybenzene sulfonic acid
DNA	Deoxyribonucleic Acid
DSH	Dishevelled
EMT	Epithelial-mesenchymal transition
ER	Endoplasmic reticulum

ERK	Extracellular signal-related kinase
ELISA	Enzyme-linked immunosorbent assay
EtOH	Ethanol
FA	Fatty acid
FDA	Food and Drug Administration
FFA	Free fatty acid
FZ6	Frizzled 6
FZD	Frizzled receptor
GABA	Gamma-aminobutyric acid
GFR	Glomerular filtration rate
GLUT4	Glucose transporter type 4
GSH	Reduced glutathione
GSK3	Glycogen synthase kinase 3
H^+	Cationic form of atomic hydrogen
H2O2	Hydrogen peroxide
HBV	Hepatitis B virus
HCO_3^-	Hydrogen carbonate
HDL	High-density lipoprotein
HO●	Hydroxyl radical
HONOO	peroxinitrous acid
HRP	Horseradish Peroxidase
ICAM	Intercellular adhesion molecule
IL-4	Interleukin -4
IL-6	Interleukin -6
IL-1β	Interleukin 1β
i.p	Intraperitoneal injection
iNOS	Inducible nitric oxide synthase
JNK	c-Jun N-terminal kinase
K^+	Potassium
LBD	Ligand binding domain
LD	Lethal Dose
LEF	Lymphocyte enhancement factor
LRP	Low-density lipoprotein receptor-related protein
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemo-attractant protein-1

MDA	Malondialdehyde
MMP-7	Matrix metalloproteinases-7
MRT	Mean resi-dence time
mRNA	Messenger Ribonucleic Acid
mTOR	Mammalian target of rapamycin
Na^+	Sodium
NaClO	Sodium hypochlorite
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NF-κB	Nuclear factor kappa-light-chain enhancer of activated B cells
NH_4^+	Ammonia
NO•2	Nitrogen dioxide radical
NOS	Nitric oxide synthase
Nrf-2	Nuclear factor erythroid 2–related factor-2
NRs	Nuclear hormone receptors
NSAID	Nonsteroidal anti-inflammatory drugs
O2●	Superoxide anion
OCT	Organic cation transporter
OCT2	Organic cation transporter-2
OCT3	Organic cation transporter-3
OD	Optical density
ONOO•	Peroxynitrite
p38	protein 38
p53	Protein 53
p73	Protein 73
PCP	Planar cell polarity
PKD	Polycystic kidney disease
$_P$NA	p-nitroaniline
Porc	Porcupine
PPAR	Peroxisome Proliferator Activated Receptors
PPARα	Peroxisome Proliferator-activated receptor alpha
PPAR-γ	Peroxisome Proliferator-Activated Receptor Gamma
PPIs	Proton-pump inhibitors
PPREs	Peroxisome Proliferator hormone response elements