جامعة الإسراء
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Investigating the Effects of Vanillin on the 6-Hydroxydopamine Model of Parkinson's Disease

Prepared by

Rasha Khaled Abuthawabeh

Supervisored by

Dr. Amjad Abuirmeileh

A Thesis

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i

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I

II

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This Thesis (Investigating the Effects of Vanillin on the 6-Hydroxydopamine Model of Parkinson's Disease) was Successfully Defended and Approved on 21/08/2019

Examination Committee	Signature
Dr. Amjad Abuirmeileh (Associate Professor- Isra University) (Chairman)	----------
Dr. Ahmed Talhouni (Assistant Professor - Isra University) (Internal Member)	----------
Prof. Karem Alzoubi (Professor - JUST University) (External Member)	----------

Dedication

My sincere gratitude first and foremost goes to the lord of the world, **God** for giving me the strength, knowledge, and the opportunity to move through this project successfully.

I dedicate this thesis to **my lovely parents**, who have always loved me unconditionally; this accomplishment would not have been possible without them.

My brothers Dr. Mahde and Eng. Laith

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Our challenges don't define us, our actions do

Rasha Abuthawabeh

List of Contents

List of Tables

List of Figures

List of Abbreviations

MPTP	1methyl-4-phenyl-1,2,3,6 tetrahydropyridine
6-OHDA	6-hydroxydopamine
AAV	Adeno-associated virus
AC	Adenylyl cyclase
α-syn	Alpha-synuclein
AD	Alzheimer's disease
Bcl-2	B-cell lymphoma-2
COMT	Catechol-*O*-methyl transferase
CNS	Central nervous system
CT	Computed tomography
CRF	Corticotropin-releasing factor
DBS	Deep brain stimulation
DOPA	Dihydroxyphenylanaline
DA	Dopamine agonists
DAT	Dopamine transporter
Ex-4	Exendin-4
Fe^{2+}	Ferrous iron
Fe^{+3}	Ferric iron
GDNF	Glial cell derived neurotrophic factor
GPi	Globus pallidus interna
GLP-1	Glucagon-like peptide 1
GSH	Glutathione
HPLC-ECD	High-Performance Liquid Chromatography-electrochemical detector
H_2O_2	Hydrogen peroxide
•OH	Hydroxyl radical
iPSC	Induced Pluripotent Stem Cells
IL	Interleukin

I.P	Intraperitoneal
LRRK-2	Leucine-rich repeat kinase 2
L-DOPA	Levodopa
LBs	Lewy bodies
LPS	Lipopolysaccharide
LUHMES	Lund human mesencephalic
MRI	Magnetic resonance imaging
MFB	Medial forebrain bundle
MAO-B	Monoamine oxidase –B
NINDS	National Institute of Neurological Disorders and Stroke
NIH	National Institutes of Health
NTN	Neurturin
NO	Nitric oxide
NMDA	N-methyl-D-aspartate receptor
NAT	Noradrenaline transporter
NFκB	Nuclear Factor Kappa B
PD	Parkinson's disease
PC12	Pheochromocytoma 12
Pc19	Pheochromocytoma 19
PET	Positron emission tomography
PSI	Proteasome inhibitor 1
ROS	Reactive oxygen species
Se	Selenium
SPECT	Single photon emission tomography
S.C	Subcutaneous
SN	Substantia nigra
SNpc	Substantia nigra para compacta
STN	Subthalamic nucleus
O_2•	Superoxide anion radical
SOD	Superoxide dismutase

UPS	The ubiquitin–proteasome system
TTP	Tocopherol transfer protein
TCS	Transcranial sonography
TNF-α	Tumor necrosis factor-α
TH	Tyrosine hydroxylase
UOP	University of Petra
UCN	Urocortin

Investigating the Effects of Vanillin on the 6-Hydroxydopamine Model of Parkinson's Disease

By

Rasha Khaled Abuthawabeh

Supervisor

Dr. Amjad Abuirmeileh

ABSTRACT

Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder belonging to a group of conditions called motor system disorders. PD is primarily characterized by loss of structure in dopaminergic neurons in the substantia nigra para compacta (SNpc). In recent years; neuroinflammation became one of the significant characteristics that may play a role in the progression of PD. Vanillin is a candidate compound for neuroprotection in PD, it has antioxidant, anti-inflammatory properties and an ability to cross the BBB.

Aim: The aim of this study is to investigate the effects of vanillin on the 6-hydroxydopamine (6-OHDA) rodent model of PD. This was done by comparing 6-OHDA lesioned rats in the presence and absence of vanillin. Also, 6-OHDA is very commonly used to induce rodent model of PD, where it produces pathogenesis, progression, motor impairments and creates degeneration of dopaminergic neurons in the SNpc with similarities to human PD. As an animal model of PD, 6-OHDA proved its ability in validating and understanding the role of cell death. Our supposed work demonstrated that vanillin attenuates 6-OHDA-induced cytotoxicity.

Method: Male rats weighing between (200-250) g were randomized and divided into groups of 6 rats per group. Group A was considered as control and injected with vehicle only, group B animals were subjected to 6-OHDA alone, and group C was given 6-OHDA + intraperitoneal (i.p) vanillin (20mg/kg) and 3 days later 6-OHDA surgery, group D was subjected to 6-OHDA + (i.p) vanillin 7 days after surgery. On the other hand, group E where 6-OHDA lesioned rats were treated with oral vanillin and 3 days later 6-OHDA surgery, and finally group F where 6-OHDA lesioned rats were treated with oral vanillin 7days after surgery. The lesion severity was assessed behaviorally by apomorphine rotation test, neurochemically, and through immunohistochemistry.

Results: Seven days after intracerebral injection of 6-OHDA and following apomorphine challenge, 6-OHDA lesioned rats receiving vanillin showed significantly lower tight contralateral circling in comparison to 6-OHDA only group. Consistent with these observations, striatal tissue dopamine concentrations were significantly higher in 6-OHDA + vanillin treated rats versus 6-OHDA only group, the assay of TH immunoreactivity production was greatly reduced in the substantia nigra (SN) of 6-OHDA rats. While rats co-administered with vanillin presented an increase of TH-positive cell bodies.

Conclusion: Initial expectations for vanillin (20mg/kg) showed a promising results aspect as a neuroprotective or neuropreservative and may have potential in the management of PD. In conclusion, our work identified that vanillin act as neuroprotective compound with a capacity to slow down the degeneration of dopaminergic neuron, regenerate lost dopaminergic neurons and protect dopaminergic neurons that have not yet been affected by the disease which if translated therapeutically would offer a significant advance in PD treatment and prevention.