Evaluating the severity of 6-hydroxydopamine

induced Parkinson's disease in diabetic rats

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I

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Committee Decision

This Thesis (Evaluating the severity of 6-hydroxydopamine induced Parkinson's disease in diabetic rats) was Successfully Defended and Approved on 18/8/2020

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III

<u>Dedication</u>

In the beginning I thank the lord of the world, **God** for giving me the strength and knowledge to complete this work successfully.

I dedicate this thesis to **my wonderful parents** for their constant love and and support

My brothers Eng-Subhi, Eng-Mohammed, lovely Hamzeh

My sisters Dr-Saja and Ph-Bayan

<u>Acknowledgement</u>

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List of Abbreviation

MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MPP+	1-methyl-4-phenylpyridinium
5-HT	5-hydroxytryptamine
2-h PG	2-Hour Plasma Glucose
α-syn	Alpha-synuclein
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
Aβ	β-amyloid peptide
BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
CSF	Cerebrospinal fluid
DCCT	Control and Complications Trial
CRP	C-reactive protein
Cox-2	Cyclo-oxygenase
DM	Diabetes mellitus
DLB	Dementia with lewy bodies
FPG	Fasting Plasma Glucose
GDNF	Glial cell line neurotrophic factor
GLUT2	Glucose transporter 2
HPFS	Health Professionals Follow up Study
HNF4-α	Hepatocyte nuclear factor 4-α
hNPC	Human neuronal progenitor cells
H2O2	Hydrogen peroxide
·OH	Hydroxyl radicals
IGF-1	insulin- like growth factor
IL	Interleukin

LB	Lewy bodies
LUHMES	Lund human mesencephalic
MAO-B	Monoamine oxidase-B
NHS	*National Health Service*
NAD+	Nicotinamide adenine dinucleotide
NMDA	N-Methyl-D-aspartate
PDD	PD with dementia (PDD
PD	Parkinson's disease
PGC-1α	Peroxisome proliferator-activated receptor (PPAR)-g coactivator-1α
PAI-1	Plasminogen activator inhibitor-1
PARP-1	Poly (ADP-ribose) polymerase-1
PET	Positron emission tomography
ROS	Reactive oxygen species
RA	Retinoic acid
SPECT	single photon emission tomography
STZ	Streptozocin
SNc	Substantia nigra pars compacta
O2·−	Superoxide anion
TNFα	Tumor necrosis factor-alpha
TH	Tyrosine hydroxylase
Vit	Vitamin
VGCC	voltage gated calcium (Ca++) channels
WHO	*World Health Organization*

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BY

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Abstract

Introduction: Parkinson's disease and Diabetes Mellitus are both age related diseases. A debate related to the association between them started in the early sixties. Today, this possible link is still under study and of interest. Several studies described that the symptoms of Parkinson's disease get worse after the onest of Diabetes Mellitus. Inflammation, mitochondrial dysfunction and oxidative stress are shared pathological mechanisms between neurodegenerative disorders (parkinson's disease) and diabetes mellitus.

Aim: The aim of this study is to understand the relationship between Parkinson's disease and diabetes Mellitus. This may be achieved by examination the ability of 6-OHDA ,which is neurotoxic to damage dopaminergic neurons in presence of diabetes.

Method: Male rats weighing between (220-250) were divided into four groups. Group A was a control and injected with vehicle only. Group B animals were given intraperitoneal streptozocin to induce diabetes. Group C was injected with intracerebral 6-OHDA alone and group D was subjected to 6-OHDA + streptozocin

three days before surgery. The lesion severity was assessd neurochemically and behaviorally.

Result: Fourteen days after intracerebral injection of 6-OHDA and after apomorphine rotation test, 6-OHDA lesioned rats that receive streptozocin showed significantly higher number of rotations in comparison to 6-OHDA alone group. Additionally, striatal dopamine concentration was lower in 6-OHDA+ streptozocin treated rats versus other groups .

Conclusion: This work suggests that diabetes mellitus accelerate neurodegeneration and put subjects at high risk for Parkinson'

Chapter One

Introduction

1.1 Parkinson disease

Parkinson disease is the second-most common neurodegenerative disorder (Poewe *et al.*, 2017). Pathological features include loss of neurons and dopaminergic degeneration within the substantia nigra pars compacta (SNc) leading to dopamine reduction within the corpus striatum (Politis *et al.*, 2010), as well as intra cytoplasmic inclusions called Lewy bodies within the remaining cells. Causes of Lewy body formation and dopamine depletion remain unclear in PD (Politis *et al.*, 2010; Dickson, 2018).

The pathological changes in parkinsonism are seen not solely within the SNc, but also additionally within the pedunculo pontine nucleus, locus coeruleus, ridge nucleus, dorsal motor nucleus of the cranial nerve, neural structure, autonomic neurons, cortex, and therefore the Mynert nucleus. Extensive neuropathology within the cortical regions and brain stem are accountable for numerous motor and non-motor symptoms of PD (Smith and Parr-Brownlie, 2019; Braak *et al.*, 2003). The movement of the population affected by PD and their quality of life is altered (Abuirmeileh *et al.*, 2009; Gubellini and Kachidian, 2015).

1.1.2 Prevalence of PD

Parkinson's disease (PD) is an age-related neurodegenerative disorder, affecting more than 1% of the persons over sixty five years of age (Cheong *et al.*, 2020). While the bulk of PD cases are idiopathic, about 10% have been related to genetic mutations with inheritance among families (Lill, 2016). The global number of PD patients was more than doubled between 1990 and 2016, from 2.5 million to 6 million. A relatively expected doubling of the numbers of patients over following thirty years would yield over twelve million patients worldwide by 2050 (Sadek *et al.*, 2019).

1.1.3 Signs and symptoms

PD is characterized by resting tremor, rigidity, bradykinesia or slowness, body instability and gait disturbance, as well as non-motor symptoms including cognitive symptoms; sleep disorders and behavioral symptoms (Casarrubea *et al.*, 2019; Barone *et al.*, 2009)**.**

1.1.4 Pathology

The pathologic feature that correlate with signs and symptoms of PD is neuronal loss in the substantia nigra with dopaminergic degeneration of the striatum (Dickson, 2018). Cellular and molecular processes contribute to neurodegeneration in PD (Zeng *et al.*, 2018).

1.1.4.1 Cellular pathways

Defects in cellular system activates the initiation of neuronal death, this include oxidative stress, mitochondrial dysfunction, neuroinflammation and excitotoxicity (Zeng *et al.*, 2018).

1.1.4.1.1 Role of mitochondrial damage and oxidative stress in PD

The most promising oxidative stress theory in PD research suggests that the mitochondria is the "hot-spot" for degenerative progressions. Defects in activity of complex-I in mitochondria has been detected, which directly affects cellular ATP production, causing cell death. α- synuclein might play direct and indirect role in modifying complex I action, directly inhibiting complex I action by transporting inside mitochondria and indirectly by physical interaction with Cardiolipin which is required for the formation of the mitochondrial complex I, III, and IV. Therefore, electron transfer could be disrupted (Rocha, De Miranda and Sanders, 2018).

In addition, breakdown of brain monoamines, like dopamine and 5-hydroxytryptamine (5-HT) by monoamine oxidase-B (MAO-B) when combined with O2, leads to the formation of reactive oxygen species (ROS). The higher tendency of dopaminergic neurons in the substantia nigra pars compacta (SNc) for producing ROS including hydroxyl radicals ($\cdot$OH), superoxide anion ($O_2\cdot^-$) and hydrogen peroxide (H_2O_2) in the metabolism of dopamine supported the importance of oxidative stress in the pathogenesis of PD (Dickson, 2012; Lotharius and Brundin, 2002; Xu *et al.*, 2018).

1.1.4.1.2 Neuroinflammation involvement in PD

Many studies suggest that activation of microglia and secretion of cytokines such as interleukin1α (IL-1α), IL-1β, IL-6**,** IL-8 is accoured after administration of 6-hydroxydopamine (6-OHDA) , 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and endotoxin lipopolysaccharide (LPS) leading to increase cyclo-oxygenase (cox-2) which causes DA-neuronal injury (Gelders, Baekelandt and Van der Perren, 2018; Maiti, Manna and Dunbar, 2017). Also, astrocytes can be activated by α- synuclein aggregates triggering degeneration of DA neurons (Joe *et al.*, 2018).

1.1.4.1.3 Role of excitotoxicity and prion hypothesis in PD

In PD, the SN becomes more active due to deficiency of dopamine neurons, leading to exaggerated production of neurotransmitter glutamate (Rodriguez, Obeso and Olanow, 1998). Excessive glutamate opens the voltage gated calcium channels (VGCC) by binding *N*-Methyl-D-aspartate (NMDA) or α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) inotropic receptors which causes Calcium excitotoxicity. Extra Calcium load can destroy the mitochondria and produce ROS, leading to oxidative stress (Mark *et al.*, 2001; Dong, Wang and Qin, 2009).

In addition, environmental toxins makes DA neurons susceptible to neurodegeneration because they increase production of glutamate, leading to Calcium excitotoxicity (Perier and Vila, 2012; Bjørling-Poulsen, Andersen and Grandjean, 2008; Bjørling-Poulsen, Andersen and Grandjean, 2008).

1.1.4.2 Molecular mechanisms of Parkinson's disease

1.1.4.2.1 Aggregation of alpha-synuclein (α-syn)

Accumulation of lewy bodies (LB) in dopamine neurons of SNc is one of the important diagnostic pathologies of PD (Melki, 2018).

Many genetic, molecular and biochemical studies affirmed that post-morten brains of patients who were diagnosed PD with dementia (PDD) and dementia with lewy bodies (DLB) are seen to have a mixture of multi misfolded aggregates such as D-tau, SNCA and β-amyloid peptide (Aβ) (Stefanis, 2012).

1.1.4.2.2 Role of gene mutation in PD

Parkin, PINK1and Dj1 are the most common PD-related genes

(Alberio, Lopiano and Fasano, 2012).

Table 2.1: Proteins and genes names related to parkinson's desease (Alberio, Lopiano and Fasano, 2012).

PARK locus	Gene name	Gene product	Alias
PARK1∕PARK4	SNCA	Alpha-synuclein	a-synuclein
PARK2	PARK2	PARKE3 ubiquitin-protein ligase parkin	Parkin`
PARK6	PINK1	Serine∕threonineproteinkinasePINK1	PINK1
PARK7	PARK7	Protein DJ-1	DJ-1
PARK8	LRRK2	Leucine-rich repeatserine∕threonine-protein kinase 2	LRRK2
PARK9	ATP13A2	Probable cation-transporting ATPase 13A2	ATP13A2
PARK11	GIGYF2	PERQ amino acid-rich with GYF do F2 main-containing protein 2	GIGYF2
PARK13	HTRA2	Serine protease	HtrA2
PARK14	PLA2G6	85 kDa calcium-independent phospholipase A2	IPLA2
PARK15	FBXO7	F-box only protein 7	FBXO7
PARK17	VPS35	Vacuolar protein sorting-associated protein 35	hVPS35

1.1.5 Diagnosis of PD

The diagnosis of (PD) usually takes place once the pathological process is advanced and most of dopamine neurons have already died (Sulzer *et al.*, 2018). Evidence suggests that cerebrospinal fluid (CSF) and blood biomarkers such as α-synuclein species, tau pathology, lysosomal enzymes, and neurofilament light chain reflect prognostic and diagnostic value in pathophysiology of Parkinson's disease. (Parnetti *et al.*, 2019). Imaging markers including ligand-based imaging methods, such as single photon emission tomography (SPECT) or positron emission tomography (PET) is used to measure reduction of dopaminergic nerve terminals within the striatum (Kalia, 2019).

1.2 Diabetes mellitus

Diabetes is classified as a metabolic diseases, characterized by high blood sugar levels over a prolonged time resulting from defects in insulin secretion action or insulin resistance which result from destruction of pancreatic cells (Punthakee, Goldenberg and Katz, 2018; Association, 2010)**.** Diabetes is the ninth major cause of death in the world (Zheng, Ley and Hu, 2018)**.** In 2010**,** 220 million people were affected by diabetes (Yang *et al.*, 2017). This number increased to 463 million in 2019 and expected to be 584 million by 2030 (Saeedi *et al.*, 2019)**.**

1.2.1 Signs and symptoms

Symptoms include weight loss, polyphagia, polyuria, polydipsia and blurred vision (Pafili, Papanas and Ziegler, 2018). The chronic symptoms of diabetes disease is related

to damage and failure of various organs ,especially the kidneys, eyes, nerves, heart and blood vessels (Wanner *et al.*, 2018).

1.2.2 Pathology of diabetes

 World Health Organization (WHO) classification of diabetes include the current major two types : type 1 DM and type 2 DM. WHO 2019 classification does not divided subtypes of T1DM and T2DM but consider new types "unclassified diabetes and hybrid types of diabetes" (Organization, 2019).

Beta cell dysfunction is common characteristic to all forms of diabetes. Many mechanisms can contribute to a destruction or decrease in function of a nonrenewable B cell . These mechanism include insulin resistance, genetic abnormalities , metabolic disorder, auto-immunity and environmental factors (Egan and Dinneen, 2019).

1.2.2.1 Genetic factors

In a case-control study within *National Health Service* (NHS) and the Health Professionals Follow up Study (HPFS) , a genetic factor score was considered on the basis of ten polymorphisms in nine loci (Cornelis *et al.*, 2009).

Genetic predisposition is The primary risk factor for beta-cell autoimmunity, mostly happening in persons with either HLA-DR3-DQ2 or HLA-DR4-DQ8 haplotypes or both (Pociot and Lernmark, 2016) . Type 1 diabetes (T1D) pathogenesis can be divided into three stages according to beta-cell autoimmunity, level of glycemia and appearance of symptoms (Insel *et al.*, 2015), and the genetic associate with each stage in different way (Pociot and Lernmark 2016). A first study based on both family data and case-control consisting of 867 DM patients and 72 control subjects, and in a family data set consisting of 1690 DM patients and 2340 control assess the relative effect of HLA, INS,

and PTPN22 on the onest of DM in wide range of age people (Bonora and DeFronzo, 2018).

1.2.2.2 Metabolic pathways in DM

dysregulation of adipose tissue in insulin resistance patients considered them by subclinical proinflammatory condition. Overweight ,infiltration of adipose tissue by macrophages leading to secretion of pro-inflammatory cytokine such as tumor necrosis factor-alpha (TNFα) and (IL-6) (Hu *et al.*, 2004).The inflammatory process is also connected with Endothelial and liver dysfunction through secretion of C-reactive protein (CRP) from liver and producing Plasminogen activator inhibitor-1 (PAI-1) by endothelial cells. These markers are significantly associated with DM (Bonora and DeFronzo, 2018).

1.2.2.3 Environmental and social factors in DM

Exposure to gaseous pollutants (carbon oxides, sulfer compounds) and radiations are responsible for DM development through ROS generation and inflammatory response leading to direct B-cell damage (Fleisch *et al.*, 2014; Dong *et al.*, 2019). In addition to this, poor life style like Alcohol intake causes significant inhibition of serum anti-inflammatory factors including neutrophic CD4 (Li *et al.*, 2016; Lee *et al.*, 2017) .

Engum et al presented that people with stress and depression were 1.8 times at higher risk to DM than normal persons (Chen *et al.*, 2016) . Another study performed in united states of 1,262,900 people showed that the risk of DM reduced by 36% while doing exercise for 300 min/week (Smith *et al.*, 2016).

1.2.3 Diagnosis 0f DM

Table 1.2 :Criteria for diagnosis of diabetes mellitus (Herold *et al.*, 2015).

Test	Value	Recommendation
Fasting Plasma Glucose (FPG)	≥126 mg/dl 7.0 mmol/L	Fasting for at least 8 hours
2-Hour Plasma Glucose (2-h PG)	≥200 mg/dl 11.1 mmol/L	Test must be performed according to WHO, using a glucose load of 75g anhydrous glucose dissolved in water
A1C	≥6.5% 48 mmol/mol	A method should be NGSP certified and standardized to the Diabetes Control and Complications Trial (DCCT) assay
Hyperglycemic crisis	≥200 mg/dl 11.1 mmol/L	Classic symptoms of hyperglycemia

Chapter Two

Literature Review

2.1 Parkinson's disease and diabetes mellitus

Early studies propose that up to 50%-80% of patients with PD have irregular glucose metabolism (Sandyk, 1993). Additionally, information from many of prospective studies recommend that patients with T2DM patients have nearly a 40% risk of developing PD (Xu *et al.*, 2011 ; Bosco *et al.*, 2012). Case-control studies from Taiwan, China, and Scandinavian nation additionally indicate that DM may be a risk for developing PD in these populations (Wahlqvist *et al.*, 2012; Schernhammer *et al.*, 2011)**.**

Researchers at University college London, studied records of over eight million individuals admitted to British hospitals over a 12-year period, they found that individuals with T2DM have a 30 % greater risk of developing PD than people without   DM. However, that   increases   to   forty   nine percent higher risk for individuals experiencing   complications   from   their DM   and four hundred percent for younger individuals (ages 25-44) with the disease (De Pablo-Fernandez *et al.*, 2018).

2.2 Shared pathological mechanisms between diabetes mellitus and Parkinson's disease.

 Insulin Resistance, mitochondrial dysfunction, inflammation, oxidative stress and vitamin D all play a critical role in dopaminergic neurodegeneration in both Parkinson's and diabetes mellitus diseases (Nasrolahi *et al.*, 2019).

2.2.1 Insulin Resistance in PD and DM

Traditionally, insulin secretion was thought only to be a peripherally acting internal secretion accountable for energy metabolism. However, accumulating verifications indicates that insulin can cross the blood-brain-barrier and influence multiple of processes within the brain including dopaminergic transmission, control growth, cells survival and maintenance of synaptic connection (Grillo *et al.*, 2019). Recent findings have confirmrd that there is a link between PD pathological process and the mechanisms of insulin resistance (Arnold *et al.*, 2018).

In conjunction, researches established that comorbidity to insulin resistance have found in patients with PD even those without DM. Incidence of defects in signaling pathway is the important contributor to progress the pathogenesis of PD. Thus, insulin signaling might be a completely unique target for therapy (Yang *et al.*, 2018).

Insulin and therefore the closely related insulin- like growth factor (IGF-1) also are created by hippocampus, pyramidic neurons within the cortex, and neural structure (Boucher, Kleinridders and Kahn, 2014). Insulin and IGF-1 play a good type of biological processes by working on 2 closely connected tyrosine kinase receptors (Santiago and Potashkin, 2013). Receptor activation initiates a cascade of phosphorylation events that ends up in the activation of enzymes that organize several aspects of metabolism and growth.

Insulin/IGF-1 signal contains many various points of regulation or vital nodes, controlled each positively and negatively. Disorder in these signal pathways will result in insulin resistance (Boucher, Kleinridders and Kahn, 2014). Increased phosphorylation at serine acid residues are seen within the

dopamine depleted corpus striatum within the 6-hydroxdopamine model of PD and transgenic rat overexpressing alpha-synuclein (Dilan Athauda and Foltynie, 2016).

2.2.2 Mitochondrial dysfunction in PD and DM

Brain is damaged by mitochondrial dysfunctions because it needs a lot of energy (Rango and Bresolin, 2018). Burbulla et al arranged a toxic sequence of events beginning from mitochondrial oxidative stress and lysosomal dysfunctions causes dopamine oxidation , then inhibition of glucocerebrosidase action, and finally α-synuclein accumulation resulting in SN dopaminergic neurodegenerative in PD brains (Parker Jr, Parks and Swerdlow, 2008 ; Burbulla *et al.*, 2017). In addition, mitochondrial dysfunction linked to various types of DM since it controls the secretion of insulin from B-cells in pancreas (Burbulla *et al.*, 2017)**.**

2.2.3 The role of inflammation in PD and diabetes

Many investigations registered the role of inflammatory mediators containing proinflammatory cytokines , microglia activation, T cell infiltration and disrupted blood brain barrier (BBB) in the pathology of PD (Gelders, Baekelandt and Van der Perren, 2018). Similarly, pancreatic islets of DM patients showed an elevation in the levels of cytokines and immune cells. Inflammation causes B cell failure and insulin resistance through lipotoxicity, glucotoxicity and oxidative stress (Esser *et al.*, 2014; Hotamisligil and Erbay, 2008). Anti-inflammatory drugs reduce the release of pro-inflammatory cytokines (Kempuraj *et al.*, 2016). They can decrease insulin resistance and relieve the loss of dopamine in diabetic rats (Sun *et al.*, 2011).

2.2.4 Oxidative stress in PD and DM

The nigrostriatum is a susceptible area to oxidative damage, because it contains high concentrations of iron ions that produce hydroxyl radicals and low levels of antioxidant glutathione that protects neurons from oxidation (Chinta and Andersen, 2008). Sustained hyperglycemia activates monoamine oxidase in dopaminergic neurons, causing dopamine auto oxidation and production of free radicals which in turn induced nigrostriatal dopaminergic neurons death (Renaud *et al.*, 2018; Goldstein, Kopin and Sharabi, 2014).

2.2.5 Vitamin D in PD and DM

Low serum level of vitamin D play a role in insulin resistance and advancement of DM (Yoon *et al.*, 2015). Also, satostal et al have reported that vitamin D insufficiency are related to PD intensity, as well as other degenerative diseases (Sato *et al.*, 2005). In addition 1-alpha-Hydroxylase enzyme which, activates vitamin D are found in dopaminergic neurons in the brain. The expression of vitamin D receptors and 1α-hydroxylase in PD patients is different compared with healthy people. Interestingly, vitamin D null mice have impaired locomotor activity (Nasrolahi *et al.*, 2019).

Vit D suppress the production of free radicals and promotes the manufacturing of antioxidants. Also, it controls the action of the glial derived neurotrophic factor (GDNF), which exacerbates motor symptoms in PD. Thus, vit D intake may reverse the progression of motor symptoms related to parkinsonism (Gill *et al.*, 2003).
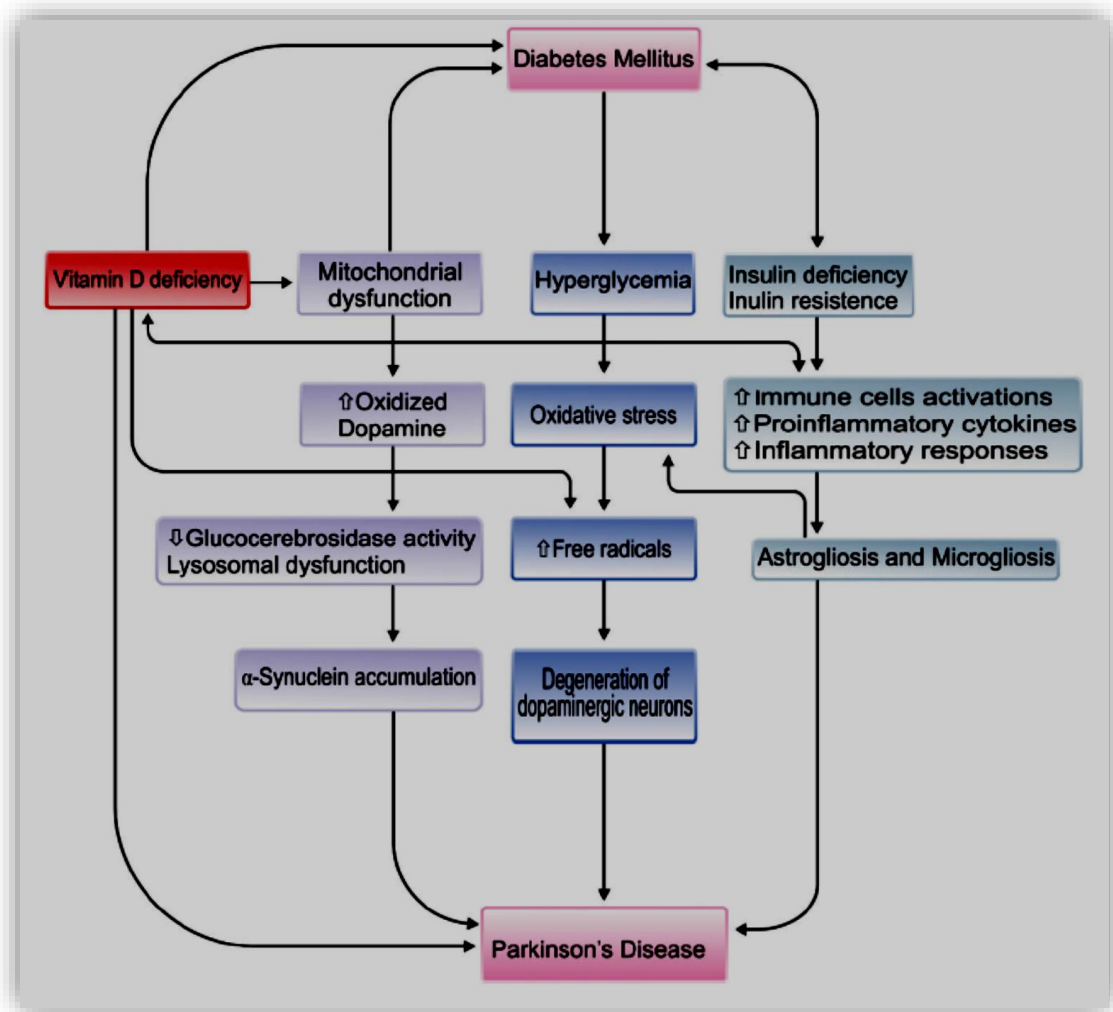
Figure 2-1: Shared pathological mechanisms between diabetes mellitus and parkinson's disease (Nasrolahi *et al.*, 2019).

2.3 Environmental factors lead to diabetes and PD

Many studies have shown that exposure to environmental factors, together with significant metals and pesticides like rotenone, will increase an individual's risk for PD (Cao *et al.*, 2019). Particularly, iron, manganese, and copper usually exist in high concentrations within the blood of PD patients (Ajsuvakova *et al.*, 2019). Similarly, heavy metal exposure is related to beta cells dysfunction and disease development in diabetic patients. Additionally, pesticides, and bisphenol which are endocrine disrupting factors, correlates

with hyperglycemia and islet dysfunction in DM patients (Zarean and Poursafa, 2019).

2.4 Metabolic dysregulation may lead to diabetes and PD

Many studies show a link between diet and neurodegeneration. High fat intake may lead to defects in insulin signaling pathway, lipid accumulation, misfolding of alpha synuclein and oxidative stress (Keshk *et al.*, 2020; Belvisi *et al.*, 2019). Hyperglycemia quickens the loss of dopamine via reducing endoplasmic reticulum folding capacity and oxidative stress (Liu *et al.*, 2020 ; Su *et al.*, 2020). The relationship between diabetes and neurodegeneration is better understood by explaining the transmission of signals in the brain and peripheral organs including pancreas, liver and muscle as shown in figure (2-2).
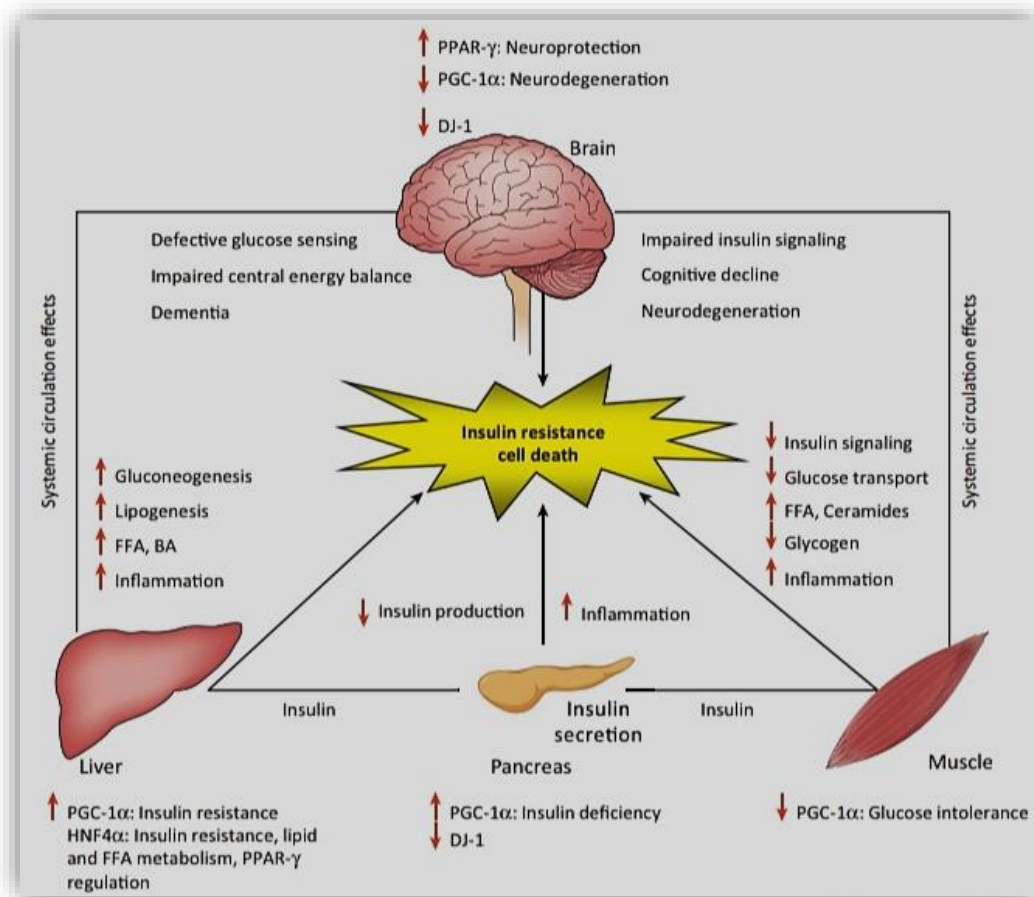
Figure 2-2: Metabolic dysregulation among insulin-sensitive organs. As a result of impaired insulin signaling, gluconeogenic response is altered in the liver and more glucose is released.So that the pancreas become unable to cope with the demand for insulin to inhibit glucose levels. In skeletal muscle defects in insulin signaling altered glucose transport and synthesis of glycogen. Defective glucose sensing and impaired insulin signaling in the brain are associated with cognitive decline and neurodegeneration. peroxisome proliferator-activated receptor (PPAR)-g coactivator1α; (PGC-1 α) and hepatocyte nuclear factor 4-α(HNF4-α) transcriptional coactivators play a role in neuroprotection, neurodegeneration, and insulin resistance (Santiago andPotashkin, 2013).

2.5 Dopamine

Dopamine is found as monoamine neurotransmitter in the dopaminergic neurons of the hypothalamus. Dopamine is produced in in the ventral tegmental area of the substantia nigra, the arcuate nucleus and mid brain (Treat and Ayano, 2016).

several physiological functions in the brain and periphery are controlled by dopamine while acting on its receptors D1, D2, D3, D4 and D5. Dopamine receptors which are G protein–coupled receptors are involved in the organization of motor activity and a number of neurological disorders such as schizophrenia, Parkinson's disease (PD), Alzheimer's disease, and attention-deficit (Mishra, Singh and Shukla, 2018). In patiens with PD, loss of dopamine neurons of the SNc in patients causes deficits and slowness in the initiation of movement (Mishra, Singh and Shukla, 2018).

2.6 PD models

To understand the pathology of PD, and to develop effective therapeutic agents for management, it is important to produce relevant disease models (Shimohama *et al.*, 2003). There are many models of PD, these can be divided into *in vivo* and *in vitro* (Lopes *et al.*, 2017 ; Duty and Jenner, 2011).

2.6.1 *In vitro* models

Dopamine neuron-dervied cell lines are useful for describing fully mechanisms of PD neuronal death and for examining new pharmacological agents (Shimohama et al., 2003).

2.6.1.1 Cell lines

Cell lines are derived from a multicellular organisim from people (Maqsood et al. 2013). Immortalizing of these cells artificially or naturally leading to disruption in normal cellular aging. Into preclinical studies this model is widely used to identify the most promising neuroprotective drugs for further in vivo studies. Beside this, efficient transfection is easy through producing genetically cell lines by molecular biological techniques such as knockout (Radio and Mundy 2008; Bal-Price et al.2010).

Toxin based or genetic models can be created to mimic PD pathology, firstly as mentioned above overexpression of related PD genes is possible, then 6-hydroxy dopamine or 1-methyl-4-phenylpyridinium (MPP+) can be used causing cell death in dopaminergic cell lines (Lopes *et al.*, 2017).

Cell lines such as PC12, MN9D and N27 models have high proliferation and reproducibility, but dopaminergic cell lines of human origin such as SH-SY5Y and Lund human mesencephalic (LUHMES) are most widely used (Thomas *et al.*, 2013 ; Scholz *et al.*, 2011 ; Lopes *et al.*, 2010).

2.6.1.2 SH-SY5Y cell line

The human neuroblastoma SH-SY5Y cell line was isolated in 1973 from metastasis biopsy of a girl (Xicoy, Wieringa and Martens, 2017 ; Popova, Karlsson and Jacobsson, 2017)**.** This cell line has ability to release dopamine is due to expression of some dopaminergic markers including tyrosine hydroxylase (TH), dopamine transporter and dopamine-b-hydroxylase, thus mostly used as a replacement for dopaminergic neurons in PD studies (Cheung *et al.*, 2009 ; Lopes *et al.*, 2010 ; Filograna *et al.*, 2015).

Treating of SH-SY5Y cells with differentiation agents like brain-derived neurotrophic factor (BDNF) and retinoic acid (RA) (Encinas *et al.*, 2000 ; Påhlman *et al.*, 1984),

(Agholme *et al.*, 2010) causing alteration in cellular morphology by developing cytoplasmic projections (Schönhofen *et al.*, 2015 ; Lopes *et al.*, 2010 ; Filograna *et al.*, 2015). Also**,** the differentiation process causing a phenotype which more closely look a lot like a neurons and they show an increase in the appearance of synaptic function genes like DNM1 and CLTC (Lopes *et al.*, 2017).

2.6.1.3 LUHMES cell line

LUMES is a tetracycline subclone which was isolated from an 8- week old fetus ventral mesencephalic brain tissue (Lotharius *et al.*, 2005).Unlike SH-SY5Y, over expression of dopamine markers require differentiation by adding neurotrophins and antibiotics. After differentiation dopamine release is increasing and these cells become more similar to neurons (Zhang, Yin and Zhang, 2014). MPP+ are commonly used to produce cytotoxic models of PD in this cell line (Stępkowski *et al.*, 2015 ; Stępkowski *et al.*, 2015; Schildknecht *et al.*, 2013).

2.7 *In vivo* models

Animal models of Parkinson's disease (PD) have demonstrated effectiveness in the finding of new treatments for motor disabilities of PD and in the search for evidences to the main reason of the illness (Rijntjes, 2019).

Accessible rodent models ranges from acute pharmacological models, like the haloperidol or reserpine managed rats that show one or more Parkinson's symptoms to models displaying destructive nigro-striatal pathway of dopamine such as the traditional (6-OHDA) and (MPTP) models. Furthermore, although administration of the pesticides, paraquat and rotenone toxic models of nigro-striatal degradation helps

in understanding the pathogenesis of disease, these are not so frequently used for drug improvement (Duty and Jenner, 2011).

All poisons that are used to produce rodent models of PD and to trial neuroprotective agents have mechanisms of action mimicking PD pathogenesis, including inflammogens such as LPS (Farfara *et al.*, 2019), proteasomal inhibitor like epoximycin (Jiang *et al.*, 2019), mitochondrial complex I inhibitors like MPTP and ROS producer as 6-OHDA (Zeng, Geng and Jia, 2018).

2.8 6-hydroxy dopamine model

The 6-OHDA which is an analogue of dopamine and extensively used to induce parkinsonism in rats was investigated in 1959 (Hamadjida *et al.*, 2019). It does not cross blood brain barrier, so direct injection into SNc and medial for bundle to produce full lesion or in the striatum to develop partial lesion is required (Duty and Jenner, 2011).

After its injection, 6-OHDA is transported via the dopamine transporter into the dopaminergic neurons and gradually increased in mitochondria causing inhibition of complex I and IV, also autooxidation creates ROS such as hydrogen peroxide and hydroxyl that reduce levels of glutathione or superoxide dismutase, which are antioxidant enzymes. This process causing oxidation to lipids, protiens and DNA subsequently results in mitochondrial dysfunction and oxidative stress (Zeng, Geng and Jia, 2018).

Nigral cell death starts within twelve hours after 6-OHDA injection and degeneration still continues to proceed for a least a further 14 days (Zuch*et al.*, 2000 ; Hernandez-Baltazar, Zavala-Flores and Villanueva-Olivo, 2017). Administration of desipramine 30-60 minutes before injection protect noradrenergic neurons from damage , moreover

pretreatment of pargyline is possible to decrease breakdown of 6-OHDA by MAO-B (Deumens, Blokland and Prickaerts, 2002 ; GeBlesa and Przedborski, 2014).

2.9 Diabetes models

Animals models of diabetes can reflect the pathology and the complications of diabetes (McNeill, 2018). Experimental Pancreatectomized dog was the first animal model of diabetes (Yorek, 201 ; Lenzen and Panten, 1988). Many animals including dogs, cats, primates and pigs are used in diabetes research but nowadays experiments are mostly performed on rats, covering over 80% of these models due to their small size and compatibility (Wilson and Islam, 2012).

Streptozocin and alloxan are the most frequently used chemicals to induce diabetes. These compounds are mostly used for type 1 diabetes induction, because they cannot directly induce type 2 diabetes (Gvazava *et al.*, 2018).

Streptozocin is a natural antibiotic produced by streptomycetes achromogens. It is a nitrosurea analogue (Bagaméry *et al.*, 2020). In 1963 Rakietenin formed STZ as diabetogenic (Lenzen, 2008). It enters B-cell due to its similar structure to glucose. Aditionally, it is a hydrophilic compound and it can cross cell membrane via Glucose transporter 2 (GLUT2) transporters (Vinayagam *et al*., 2018).

The STZ develops diabetes through three varied mechanisms that display DNA damage as a final result (Lenzen, 2008). DNA alkylation is the first and significant mechanism of B cell death, primary transmission of methyl group from STZ to DNA in B- cells over stimulated poly (ADP-ribose) polymerase-1 (PARP-1), furthermore minimizing cellular ATP and nicotinamide adenine dinucleotide NAD+ concentration (Plumb, 2018). The second mechanism is intracellular nitric oxide release after metabolism of STZ causing oxidative stress. Finally, STZ is capable of producing ROS such as

superoxide during hypoxanthine metabolism which speed up B cell apoptosis (Banerjee *et al.*, 2020).

After application of STZ , the progression of diabetes is very fast, especially in rats , 24 hours post injection of STZ the rats demonstrate all symptomps of diabetes including hyperglycemia, polyuria, lower pancreatic and plasma insulin level and glucosuria (Sviglerova, Kuncova and Stengl, 2017),but changes in insulin and glucose levels continues for 48 hours, this means that 72 hours is the best time for glucose measurement. Furthermore, In diabetes experiments STZ-induced diabetes can be used for both long-term and short-term researches and it is the most stable model (Radenković, Stojanović and Prostran, 2016).

Chapter Three

Materials and Method

3.1 Materials

Streptozocin, desipramine hydrochloride, pargyline, apomorphine, 6-OHDA, white phosphate buffered saline and crystalline l-ascorbic acid were all purchased from Sigma-Aldrich (Pool, UK). perchloric acid and water (HPLC grade) were obtained from fulltime (China) and Lab Chem (NJ, USA). Methanol was purchased from Chem-Lab analytical (Zedelgem, Belgium).

3.2 Methods

3.2.1 Animals

Male Wister rats weighning (220-250) g (n=24) were bought from Jordan University of Science and Technology (JUST) and were placed in hygenic animal house and supplied with food and water. Animals were grouped as three rats for diabetic groups and six rats for nondiabetic groups per cage. Room temperature was set at (24°C) and 12 hr light/dark cycle. Ethical guides for the use of laboratory animals were followed in all experiments.

3.2.2 Experimental design

Animals were divided into four groups (each group six rats). Group A was control and was administred to vehichle only, group B was injected with intraperitoneal (50mg/kg) STZ (Yang and Kang, 2018) , group C was injected with intracerebral (2μg/1μl) 6-OHDA (Gong *et al.*, 2012). Finally, group D was injected with intracerebral (2μg/1μl) 6-OHDA + intraperitoneal (50mg/kg) STZ .

Table 3-1: The following diagram presents the sequence of events as per the study protocol in. Group A was injected by normal saline only, group B was injected by STZ, group C was injected by 6-OHDA and group D injected by STZ before 6-OHDA administration. Apmorphine rotation test and killing were done on day 17.

Day (1)   Day (3)   Day (17)

Group A	Vehichle	Vehichle	Apmorphine+ killing
Group B	STZ	Vehichle	Apmorphine+ killing
Group C	Vehichle	6-OHDA	Apmorphine+ killing
Group D	STZ	6-OHDA	Apmorphine+ killing
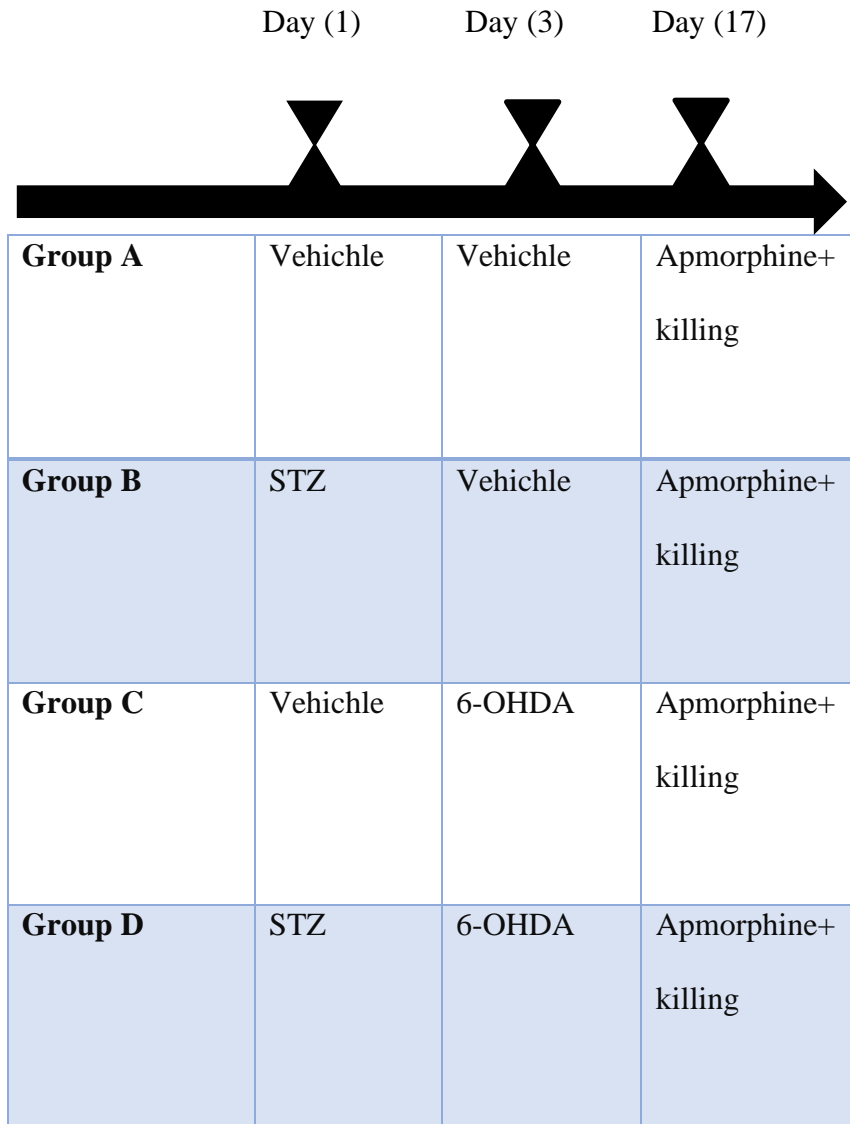
3.2.3 Adminstration of streptozocin to induce diabetes

Using an intraperitoneal injection of STZ (50mg/kg) dissolved into sodium citrate buffer with pH modified to 4.5 before using, diabetes was induced in rats (Yang and Kang, 2018). Animals were fasted 12 hours before injection of STZ (El-Far *et al.*, 2016). Three days after injection, fasting blood glucose levels were measured in fresh blood samples obtained from the eye using a (D-smart) blood glucose meter. Rats with blood glucose level ≥200 mg/dl were considered diabetic and included in the experiments (Qinna and Badwan, 2015).

Table 3-2: Animal groups and treatment.

Group number	Administrated drug	Dose of drug	Route of administration
A	Vehicle	-	-
B	STZ	50mg/kg	Intraperitoneal injection
C	6-OHDA	2μg/1μl	Intracerebral injection
D	STZ+6-OHDA	50mg/kg + 2μg/1μl	Intraperitoneal injection + Intracerebral injection

3.2.4 Stereotaxic surgery

Rats brain was administered by 6-hydroxy dopamine using stereotaxic surgery. Fourty minutes before 6-hydroxy intracerebral injection, rats were given desmethylimipramine and pargyline. Isoflurane was used for anesthetization (3% for induction, 1-1.5% for maintenance). Then, animals were fixed onto a vernier stereotaxic frame, a scalpel blade was used to make an incision along the longitudinal midline of the scalp and the scalp was swapped with an iodine solution. After that, bregma was identified by a marker, then the skull was drilled at an suitable location, and using a microsyringe (10μl) ,animals was injected with 5μl (2μg/1μl) 6-OHDA dissolved in normal saline including 0.2% ascorbic acid into right striatum (from bregma A 0.2mm, L 3mm, V 8mm).

The solution was placed in dark place on ice until injection. The needle was left in place for extra seven minutes before being slowly withdrawn to block flow back to the needle.
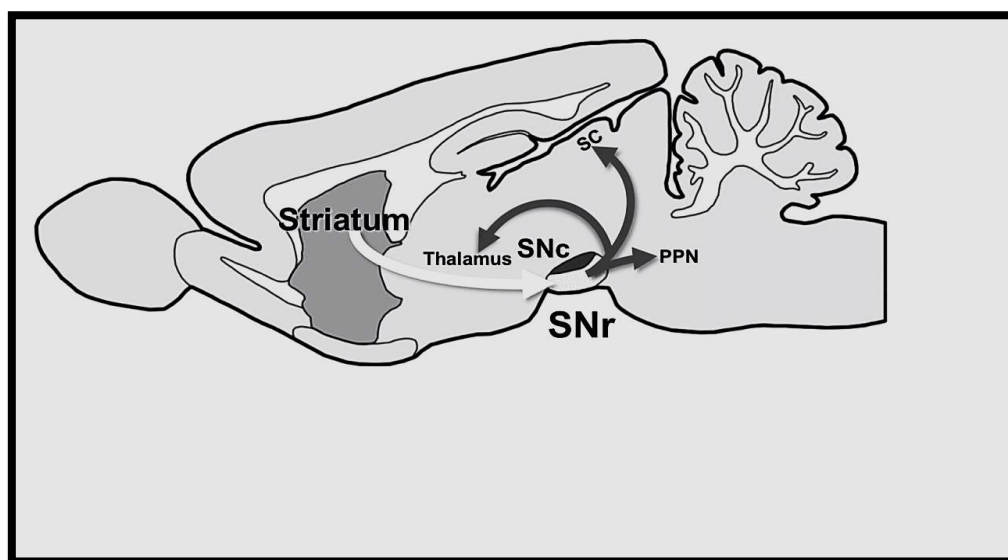

Figure 3- 2: Clarification of the dopaminergic fibers connecting the SN and striatum (Yamada *et al.*, 2016).

3.2.5 Tissue dopamine assay

Animals were sacirificed after finishing all treatments and behavioral estimation at day (17). Brains were removed from the skull and dropped into dry ice to freeze, once it's frozen, it was transferred to a -80 (Hair Biomedical) freezer and stored for later analysis using High-Performance Liquid Chromatography- electrochemical detector (HPLC-ECD).

At the day of tissue analysis, striata were dissected out and placed on ice bath, and homogenized with 1 ml cold phosphate buffer. The homogenates were centrifuged at 1000 rpm for 10 minutes at 4 C. Afte r that, 500 μl of the supernatant was prepared with 125 μl (0.2 M per chloric acid) to remove cell debris and centrifuged again using Hettich universal 30 RF cold centrifuge (Wiltshire, UK). The resulting supernatant was filtered through a 0.45 µm syringe and were put into 1ml eppendrof tube and whole tissue dopamine evaluation is done using HPLC.

3.2.5.1 System Component of HPLC

Dionex Ultimate 3000 as a part of Thermo Fisher Scinentific, Inc HPLC-ECD (Germany) was used for the optimum high-performance simultaneous assay of dopamine. The HPLC-ECD system consisted of an an autosampler (model-WPS-3000), a delivery pump (model-ISO-3100BM), highly selective electrochemical detector (model-ECD-3000RS) fitted with a VTO3 from cell (Vcell +625mV filtered to 5 abu with range set on 0.5 nA/volt for a full scale deflection), Chromatographic separation was done on a Venusil XBP (L) C18 (5μm particle size, 4.6×250mm) reverse phase column from Agela Technologies (Torrance, USA). Also, temperature of the column and the autosampler was kept at 4˚C, 25˚C respectively.

The mobile phase was made up of 35mM citric acid ,90mM sodium acetate trihydrate, 0.34mM EDTA ,0.06Mm of an ion paring reagent chem cruz 1-octane-sulfonic acid sodium salt, 5.5% methanol and pH was adjusted to 4.2 by citric acid. The flow rate was maintained at 0.65ml/min with 20µl sample injection volume.

3.2.6 Apomorphine rotation test

Fourteen days after surgery, a dopamine receptor agonist, apomorphine (0.5 mg/kg) was injected subcutaneously to all animals to detect lesion severity and motor defects between the right and left hemisphers. Fifteen minutes after injections, animals were placed in a circular test and full rotation were recorded using stop watch for 120 seconds.

3.2.7 Statistical Analysis

Data were analysed using one way ANOVA followed by Bonferonni's post hoc using statistical package Prizm 5 software package. All data are presented as mean ± SEM for six rats in each group and $P < 0.05$ was considered significant.

Chapter Four

Results

4.1 Introduction

The present study is aimed to evaluate the severity of 6-hydroxydopamine induced Parkinson's disease in diabetic rats. All relevant data were collected from neurochemical and behavioral evaluations. The results showed in this chapter were demonstrated as dopamine analytical process validation. Also, the data obtained from apomorphine-induced rotation. Finally, quantitative results were shown from tissue dopamine concentrations.

4.2 External Standard

The analytical technique in this project for dopamine using HPLC-ECD was first recognized through comparing its retention time with dopamine standard. After that, peak area ratio value is measured by software to quantify it and then connecting these values to a dopamine amount.

Many experiments were made to quantify dopamine as perfectly as possible. Typically, the instability of the dopamine with heat and light led to an increased rate of degradation and impacted the result. Therefore, all fresh solutions were prepared in optimum condition by working in a dark and cold room as well as all solutions were enclosed by aluminum foil to protect them from light.

As shown in Fig (4-1) a typical demonstrative sample of dopamine where X-axis shows run time in minutes that indicates the retention time in the range between (7-8) minutes, and Y-axis indicate concentration shown in terms of peak area. Chromeleon software version 6.8 was used to make Chromatogram.
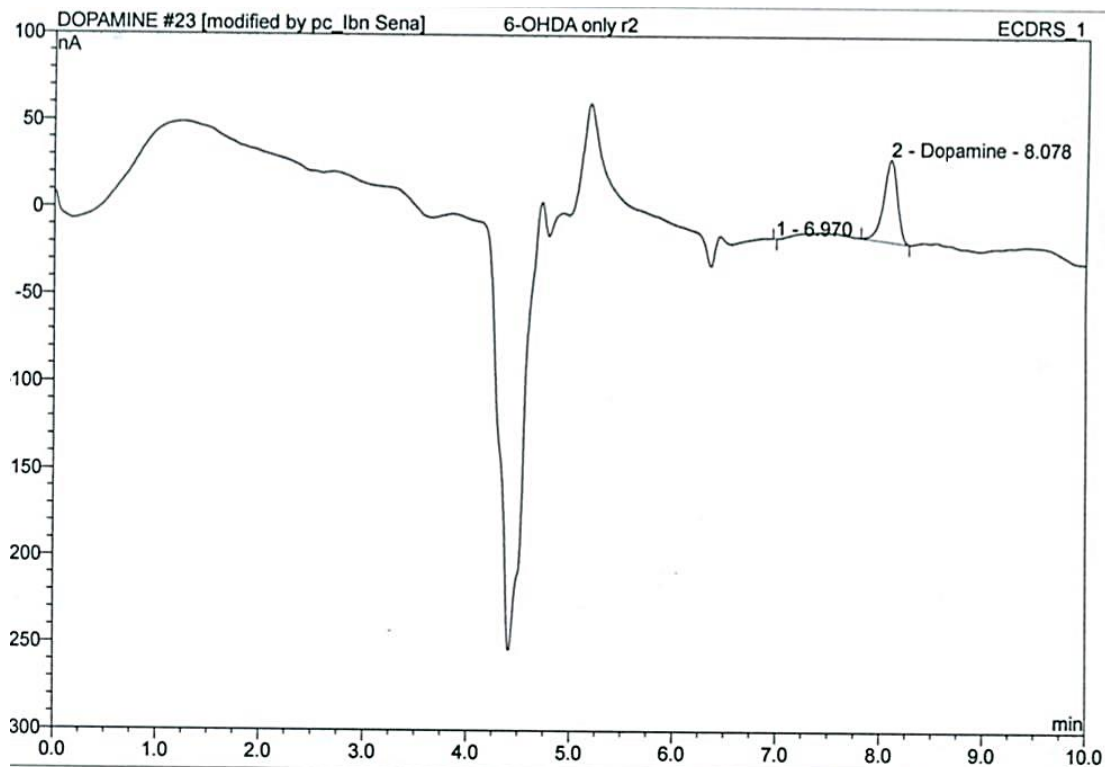
Figure 4-1: An example of a typical chromatogram of the striatum sample using HPLC-ED.

Figure 4-2 explains calibration curve for dopamine where X-axis represent concentration of dopamine in (mol/L), and Y-axis elucidates peak area .
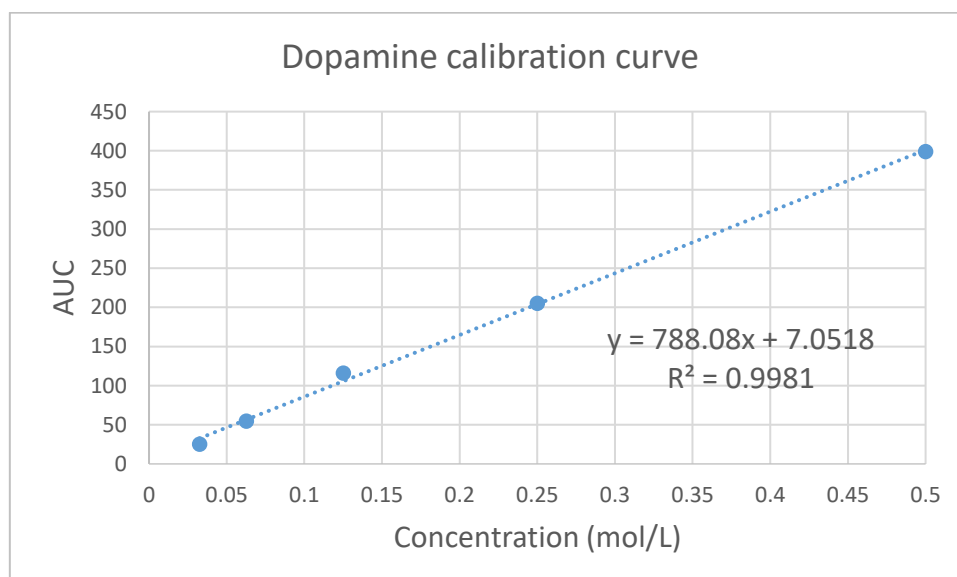
Figure 4-2: Beer-Lamberts calibration curve for dopamine concentration vs. peak area.

The analysis of the dopamine calibration curve resulted in the equation as
y=788.08x+7.0518, with R^2=0.9981.
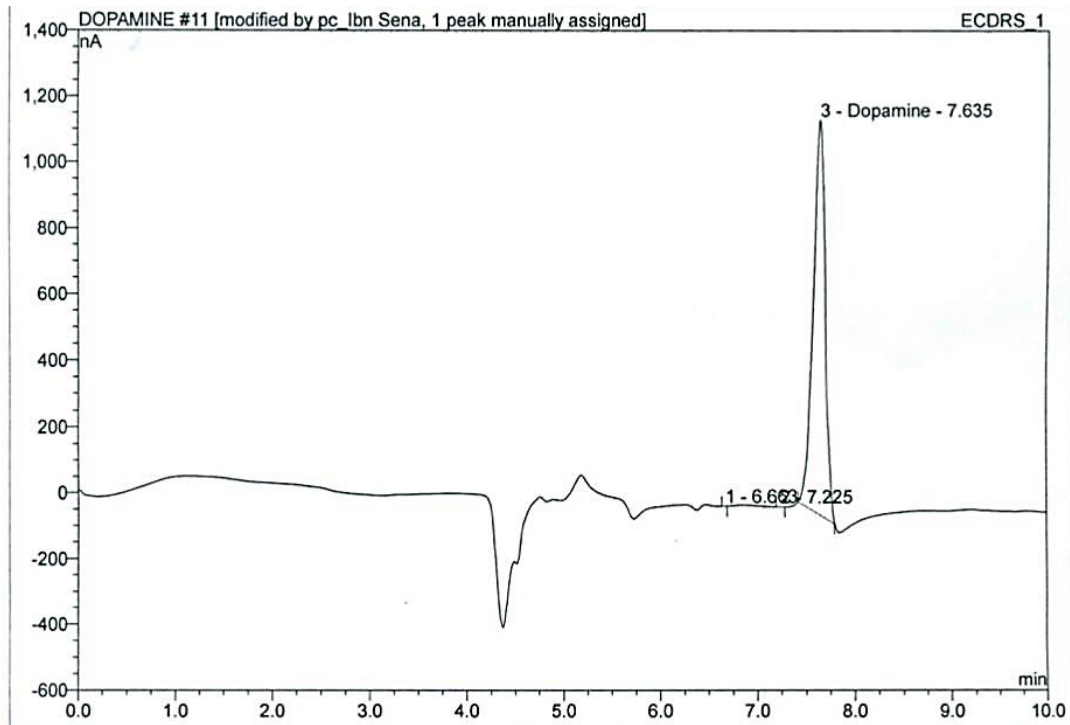
Figure 4-3: An example of one of the concentrations used to build-up the calibration curve.

4.3 Apomorphine challenge

Apomorphine test can discover motor deficits and lesion extent in unilateral PD rat models. Fourteen days after intracerebral injection of 6-OHDA, rats displayed rotational behavioral towards the opposite side of the lesion after subcutaneous injection of apomorphine where our findings revealed that tight contralateral circling, around 40 turns in 120 seconds.

On the contrary, control and diabetes only groups rats showed no rotations, as shown in figure (4-4). Additionally, our data indicates significant difference in rats 6-OHDA lesion group comparing with control and diabetes only groups.

Our result affirmed that Diabetes + 6-OHDA rats group significantly have higher number of rotations than 6-OHDA rats group and other groups.
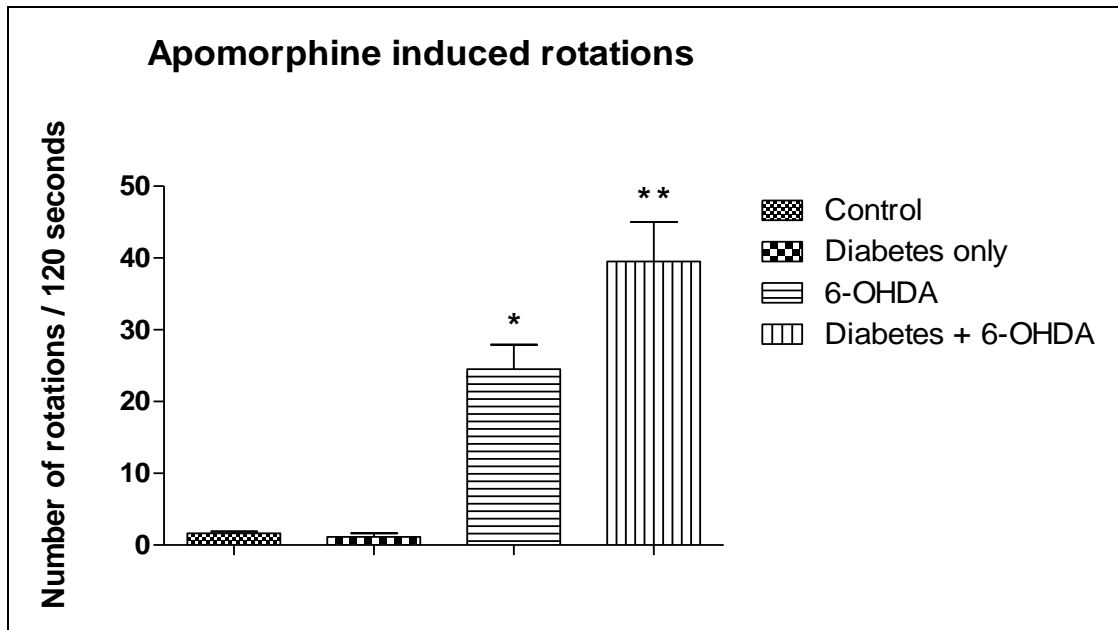
Figure 4-4: Effect of apmorphine-induced rotational behavior in 6-OHDA and Diabetes + 6-OHDA lesioned rats.

Animals were tested for denervation supersensitivity by administration of subcutaneous apomorphine (0.5mg/kg) in 6-OHDA and Diabetes + 6-OHDA lesioned rats, where Y axis indicates number of rotations as shown in figure (4-4). STZ was administered to induce diabetes in diabetes group only. Diabetes induced before surgery to Diabetes + 6-OHD group. Circling was measured after Fourteen days from toxin injection for 120 seconds, 15 min after apomorphine injection. * indicates significant difference than control and ** indicates significant difference than control and 6-OHDA ($p < 0.05$, n = 6 per group).

4.4 Determinations of tissue dopamine concentrations

Figure (4-5) represents striatal tissue dopamine concentration for control group, Diabetes alone group, 6-OHDA alone group and Diabetes + 6-OHDA group. 6-OHDA only treated group observed a significant decline in dopamine concentrations compared with control group and its dopamine concentration is also less than Diabetes alone group. In contrast, 6-OHDA alone group revealed higher concentration of dopamine than Diabetes + 6-OHDA group.

Diabetes alone group that was treated with STZ before surgery exhibited a decrease in dopamine concentrations than control group. Additionally, as seen in figure (4-5) Diabetes alone group exhibited an increase in dopamine concentrations that was significantly different than Diabetes + 6-OHDA group.

Diabetes + 6-OHDA group that was injected with STZ before surgery induced reduction in dopamine concentrations that was significantly different than control and diabetes alone group. As seen in figure (4-5) Diabetes + 6-OHDA group presented less dopamine concentrations comparing to 6-OHDA alone group.
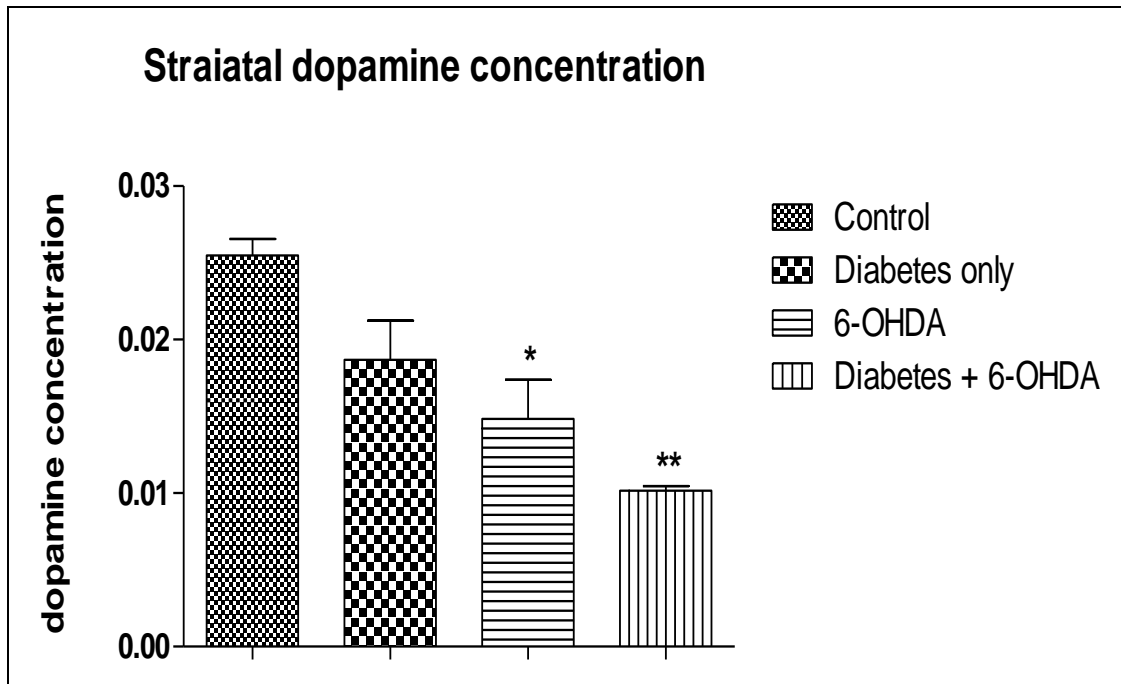
Figure 4-5: The figure shows the difference in total dopamine concentration between different groups.

Control group was only treated with vehicles and Diabetes + 6-OHDA treated groups were subjected to STZ (50mg/kg) before surgery. * indicates a significant difference than control and ** indicate a significant difference than control and diabetes only.

4.5 Determination of Blood Glucose Levels

Diabetes alone and Diabetes + 6-OHDA groups was injected with intraperitoneal freshly prepared solution of STZ to induce diabetes. Our resullt shows that normal rats have blood glucose levels around (70-88). Also, all diabetic rats have blood glucose levels $\geq$200mg/dl and range from (200-225).

Table 4-1: Blood glucose levels in normal rats before administration of STZ in Diabetes alone Diabetes + 6-OHDA groups.

Group	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
Control	72	70	75	80	75	85
Diabetes alone	83	77	75	79	85	72
6-OHDA + Diabetes	88	77	75	71	87	79

Table 4-2: Blood glucose levels in Diabetes alone and Diabetes + 6-OHDA three days after administration of STZ.

Group	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
Diabetes alone	225	213	215	209	200	217
6-OHDA + Diabetes	220	215	203	207	212	201

Fig 4-6: Effect of streptozocin induced diabetes in Diabetes + 6-OHDA and Diabetes alone groups.

Our result indicates that there is no significant difference between 6-OHDA and

Diabetes + 6-OHDA groups. * indicates significant difference than control (p$\leq$ 0.05).
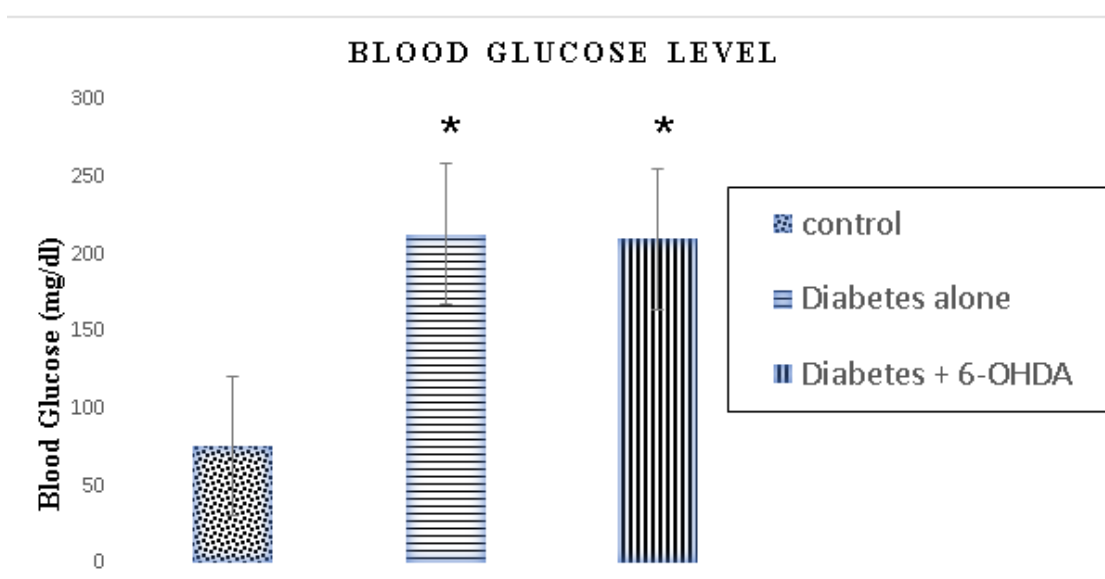
Chapter five

Discussion

5.1 Discussion

A lot of studies support a link between diabetes and Parkinson's disease (De Pablo-Fernandez *et al.*, 2011; Xu *et al.*, 2011; Bosco *et al.*, 2012; Cheong *et al.*, 2020).Beside this, based on 7 observational cohort studies that analyze the effect of diabetes on the risk of PD in over 1,761,000 persons, it was noted that, patients with diabetes were associated with a 38% increase in the risk of developing PD (Yue *et al.*, 2016). We therefore, we looked up to find the relationship between them in an animal model. Rats were subjected to sreptozocin three days before injecting 6-hydroxy dopamine into the striatum. Our goal was to determine whether diabetes whould exacerbate 6-OHDA induced dopamine depletion in to the nigrostriatum. To determine whether diabetic animals were actually more sensitive to 6-hydroxy dopamine, normal rats were injected by equal amount of 6-OHDA. We observed that the diabetic group exhibited larger levels of dopamine depletion in striatum.

Oxidation load on neurons in the SN is high due to enzymetic and nonenzymetic dopamine metabolism which generates ROS (Martin and Teismann, 2009; Morris *et al.*, 2010) and in this way , dopamine cause both intracellular and extracellular damage to neuron (Morris *et al.*, 2010). Also, iron content is very high in the SN, which may furthermore trigger oxidative damage and reactive radicals generation by reacting with byproducts of dopamine metabolites (Trist, Hare and Double, 2019) ,so it is possible that diabetes may increase dopamine depletion because it is already highly susceptible to damage (Roca *et al.*, 2011).

Unilateral injection of 6-OHDA into striatum induce neurotoxicity by increasing oxidative stress and mitochondrial dysfunction, closely mimicking events that occour

in PD (Hernandez-Baltazar, Zavala-Flores and Villanueva-Olivo, 2017; Stępkowski *et al.*, 2015), in this manner, our results show that 6-OHDA lesioned rats have striatal dopamine concentration significantly less than control .On the other hand many studies have shed light on shared pathological mechanisms between DM and PD neurodegenerative disorder (Nasrolahi *et al.*, 2019 ; Maluf, Feder and de Siqueira Carvalho, 2019). As mentioned in chapter 2 Oxidative stress (Chinta and Andersen, 2008) ; Goldstein, Kopin and Sharabi, 2014), mitochondrial dysfunction (Parker Jr, Parks and Swerdlow, 2008 ; Burbulla *et al.*, 2017), metabolic disorders (Liu *et al.*, 2020 ; Su *et al.*, 2020 ; Keshk *et al.*, 2020) as well as inflammation (Kempuraj *et al.*, 2016) are similar dysregulatory pathways between DM and PD leading to *α*-synclein accumulation, production of free radicals and finally loss of dopaminergic neurons (Esser *et al.*, 2014 ; D Athauda and Foltynie, 2016 ; Goldstein, Kopin and Sharabi, 2014 ; Renaud *et al.*, 2018). According to this, Our study indicate that striatal dopamine concentration in Diabetes + 6-OHDA group is significantly much lower than control group.

The fact that diabetes animals demonstrated significantly dopamine depletion than control animals support a connection between insulin resistance and Parkinson's disease. Insulin control dopamine transmission and neuronal survival (Speed *et al.*, 2011; Grillo *et al.*, 2019) . Also, IGF protect dopamine from 6-OHDA neurotoxicity in rats (Santiago and Potashkin, 2013) as it protect neurons from apoptosis (Wang *et al.*, 2010 ; Wang *et al.*, 2010) .A study confirm that dopamine neuronal loss and amphetamine-induced rotation decreased significantly seven days after human neuronal progenitor cells (hNPC) releasing IGF-1 transplantation (Björklund *et al.*, 1997). It has been suggested that (insulin /IGF-1) signaling is low in presence of diabetes (Santiago

and Potashkin, 2013), in addition to this researchers found that insulin level and glucose dysregulation trigger neuroinflammation production of cytokines and then apoptosis of dopaminergic neurons (Song and Kim, 2016; Esser *et al.*, 2014).

Consequently, if we look attentively at figure (4-5), we noticed that dopamine loss and possibly neuoronal damage produced by 6-OHDA in diabetic rats is the most severe.

Yang et al. and many studies demonstrated that DM increased the risk of PD and loss of dopaminergic neurons (Yang *et al.*, 2017; Biosa *et al.*, 2018; Schernhammer *et al.*, 2011; Cheong *et al.*, 2020). we recognize this in diabetic rats in our work. Therefore , long term hyperglycemia cause production of ROS and oxidative stress which in turn implicate in the neurodegeneration in the nigrostriatum (Renaud *et al.*, 2018; Goldstein, Kopin and Sharabi, 2014; Nasrolahi *et al.*, 2019). Moreover, risk factors that place individuals in danger for PD additionally place them in danger for DM disease, such as exposure to pesticides (Cao *et al.*, 2019); Zarean and Poursafa, 2019 ; Ajsuvakova *et al.*, 2019) and high fat intake (Keshk *et al.*, 2020 ; Belvisi *et al.*, 2019).

Apomorphine induced rotation test was used as quantitative predictive marker to estimate dopaminergic neurons defects. Apomorphine injected subcutaneously fourteen days after 6-OHDA injection causes contralateral turning by stimulating both D1 and D2 receptors (Miyanishi *et al.*, 2019). Our result in (figure 4-4) demonstrated that Diabetes + 6-OHDA group rotates significantly higher than 6-OHDA alone group and other groups. This means that diabetic rats is more vulnerable to neurodegenerative damage induced by 6-hydroxydopamine.

The dopaminergic nigrostriatal pathway of the rat brain is neurochemically asymmetric. The probability of an asymmetry in the D1 and D2 receptors density have been functionally linked to apomorphine rotational behavior and rats circiling (Monnot *et al.*, 2017 ; Glick *et al.*, 1988). Based on this, our result shows significant difference between 6-OHDA and 6-OHDA+STZ behaviorly but this difference was non significant neurochemically.

Several researches have confirmed a relationship between DM and neurodegeneration. A study shows that cholinergic and dopaminergic neurons are affected within the tissue layer in amacrine cells. The results confirmed that the speed of retinal cell programmed cell death is raised by diabetes disease, dopaminergic neuropathy was elevated as early as two weeks once the onset of DM disease, suggesting that cell loss is an immediate results of diabetic pathophysiology instead of a consequence of spreading vascular disease (J, 2006), as well as, oxidative stress was observed in Alzheimer's disease neurodegeneration, striking impairments in spatial learning and memory, and severe damage to hippocampal neurons was apparent in the rat induced by ICV-STZ (BadruzzamanKhan *et al.*, 2012).Our findings support these previous studies.

A recent study show that 3.4 -9.1% of DM patients suffering from PD, where as in normal people the prevalence of PD 1-2 per 1000 (Maluf, Feder and de Siqueira Carvalho, 2019). Finally, our data suggest that diabetic rat develop striatal dopaminergic neurodegeneration giving new insights into the pathology of a probable relationship between Diabetes Mellitus and Parkinson's disease.

Conclusion and Future Recommendations

This work reports novel data here that DM is a risk factor for development of Parkinson's and neurodegenerative diseases. Diabetic animals revealed greater dopamine depletion than control. Consequently, realization of risk factors such as obesity, family history, environmental toxins and age may prevent DM improvement and then decreases burden of neurodegeneration.

Disruption of shared pathways involving oxidative stress, metabolic defects and mitochondrial dysfunction put subjects at risk of both Parkinson's and Diabetes Mellitus diseases. Thus, according to this research results, further consideration is required to explain the relationship between PD and DM, then reducing the future risk of neurodegeneration and propose suitable disease modifying agents for these chronic diseases.

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Appendix 1

ملخص البحث

المقدمة: مرض الباركنسون الرعاشي ومرض السكري كلاهما مرضان مرتبطان بتقدم العمر ، بدأت دراسة الارتباط بين هذين المرضين في مطلع الستينات وما زال الأمر تحت الدراسة الى يومنا هذا. أثبتت العديد من الدراسات أن أعراض مرض الباركنسون تزداد سوءا بعد بدء الإصابه بمرض السكري. يتشارك مرض الباركنسون الرعاشي ومرض السكري بالعديد من الآليات التي تؤدي الى التحطم العصبي مثل الالتهاب ، الإجهاد التأكسدي و أمراض الميتوكندريا.

الهدف : تهدف هذه الدراسة الى فهم العلاقه بين مرض الباركنسون ومرض السكري، عن طريق تقييم مدى خطورة تحطم الخلايا العصبية الدوبامينية الناتج عن الحقن بالمادة السامة ٦-هيدروكسي دوبامين بوجود ممثل لمرض السكري مرض السكري .

منهجية البحث : تم تقسيم نموذج مثال القوارض من نوع " ويستر " البالغ وزنهم ما بين (٢٢٠-٢٥٠) ال٤ مجموعات عشوائيا ، حيث تحتوي كل مجموعة على ٦ قوارض مصممة على النحو الآتي ، المجموعة الأولى : مجموعة التحكم، المجموعة الثانية : المادة المسببه لمرض السكري ستريبتوزوسين ، المجموعة الثالثة: المادة السامة ٦-هيدروكسي دوبامين لوحدها، المجموعة الرابعة : ٦-هيدروكسي دوبامين مع الستريبتوزوسين قبل ٣-أيام من الجراحة عن طريق البطن.

النتائج : بعد ١٤ يوم من حقن مادة ٦-هيدروكسي دوبامين ، وبعد اجراء فحص الأبومورفين لوحظ أن القوارض المحقونة بالمادة السامة مع مادة الستريبتوزوسين ارتفاعا كبيرا بعدد لفات الدوران مقارنة مع المجموعة التي حقنت بالمادة السامة فقط.
كما أن تركيز الدوبامين في الجسم المخطط في القوارض التي حقنت ب مادة ٦هيدروكسي دوبامين + ستريبتوزوسين كان أقل من المجموعات الأخرى والتحطيم العصبي كان أكثر خطورة.

المناقشة : تشير نتائج بحثنا على أن وجود مرض السكري يسرع التحطم العصبي بواسطة ٦-هيدروكسي دوبامين ويزيد خطر الإصابة بمرض الباركنسون الرعاشي.