

***TARGETING miRNA EXPRESSION IN EARLY STAGE OF ZEBRAFISH
ASHWAGANDHA THROUGH PD MODEL***

Submitted in partial fulfillment of the requirements for the award of Bachelor
of Technology degree in Biotechnology

by

DHASHNI D (38230010)

RAJALAKSHMI G (38230026)



**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade “A” by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI 600119

APRIL 2022

(DEEMED TO BE UNIVERSITY)

Accredited with Grade “A” by NAAC

JEPPIAR NAGAR, RAJIV GANDHI SALAI, CHENNAI – 600 119

www.sathyabama.ac.in

DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Dhashni D (38230010)** who carried out the project entitled “**TARGETING miRNA EXPRESSION IN EARLY STAGE OF ZEBRAFISH .PD MODEL THROUGH ASHWAGANDHA**” under our supervision from September 21 to April 2022.

Internal Guide

(Dr.Jayshree nellore)

Head of the Department

Dr. Ramesh Kumar. V

_ Submitted for Viva voice Examination held on _____ 2020

Internal Examiner External Examiner

ii

DECLARATION

I, **Dhashni D(38230010)** hereby declare that the Project Report entitled “**TARGETTING miRNA EXPRESSION IN EARLY STAGE OF ZEBRAFISH .ASHWAGANDHA THROUGH PD MODEL**” done by us under the guidance of **Dr. JAYASHREE NELLORE** is submitted in partial fulfillment of the requirements for the award of Masters of Technology degree in Biotechnology.

DATE:

PLACE: SIGNATURE OF THE CANDIDATE 1.

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to Board of Management of SATHYABAMA for their kind encouragement in doing this project and for completing it successfully. I am grateful to them. I convey my thanks to , and Dr. Ramesh Kumar. V, Head of the Department, Dept. of Biotechnology for providing me necessary support and details at the right time during the progressive reviews. I would like to express my sincere and deep sense of gratitude to my Project Guide Dr. jayshree nellore for his valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work. I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

ABSTRACT

Dopamine (dA) is involved in many functions viz., reward, motivation memory, attention, and even regulating body movement in the mammalian central nervous system CNS. Mesencephalic dopaminergic mDA neurons are the main sources of dopamine. The development of mDA neurons involves complex events from proliferation and differentiation of neuron progenitors; and maturation to form mDA neurons. Multiple signaling pathways and specific transcription factors (TFs) guide these events at each differentiation stage which is used as the foundation for driving mDA neuron differentiation and maturation. In addition to these transcription factors, microRNAs and mitochondria have been identified as critical players to confer mDA identity. The cascade of transcriptional and posttranscriptional regulation from a gene network maintains the mitochondrial function that confers mDA identity by maintaining the motor function and regulates their function. Although loss of midbrain dopaminergic neurons is associated with one of the most common human neurological disorders, impairment of cognitive capabilities, however, these symptoms manifest only after 70-80% of mDA neurons have been degenerated. *Parkinson's Disease (PD) is one of the most common of the neurodegenerative disorders that affects the motor system, and includes cardinal motor symptoms such as resting tremor, cogwheel rigidity, bradykinesia and postural instability. Its prevalence is increasing worldwide due to the increase in life span. Although, two centuries since the first description of the disease, no proper cure with regard to treatment strategies and control of symptoms could be reached. One of the major challenges faced by the researchers is to have a suitable research model. Rodents are the most common PD models used, but no single model can replicate the true nature of PD. In this review, we aim to discuss another animal model, the zebrafish (Danio rerio), which is gaining popularity. Zebrafish brain has all the major structures found in the mammalian brain, with neurotransmitter systems, and it also possesses a functional blood-brain barrier similar to humans. From the perspective of PD research, the zebrafish possesses the ventral diencephalon, which is thought to be homologous to the mammalian substantia nigra. We summarize the various zebrafish models available to study PD, namely chemical-induced and genetic models. The zebrafish can complement the use of other animal models for the mechanistic study of PD and help in the screening of new potential microRNAs (miRNAs) are small (~22 nucleotide) non-coding RNAs that regulate gene expression at the post-transcriptional level, typically by inhibiting translation. The genes encoding these small RNAs are estimated to comprise approximately 2–3% of animal genomes yet potentially regulate a majority of protein-coding genes including those involved in cell specification and development. A key remaining question is to identify target mRNAs regulated by microRNAs. As a means to identify potential targets, we*

designed a sensitive microarray to analyze global miRNA expression patterns at twelve developmental stages in zebrafish of dopamine neuron is 18-96hpf. MicroRNAs (miRNAs) are evolutionarily conserved, 18-25 nucleotide non-protein coding transcripts that play an important function in post-transcriptional regulation of gene expression during development (1-4). However, the significance of miRNAs in postmitotic cells, such as neurons in the mammalian CNS, is less well characterized. Here we investigate the role of miRNAs in the terminal differentiation, function, and survival of mammalian midbrain dopaminergic neurons (DNs). We identify a miRNA, miR-133b, that is specifically expressed in midbrain DNs and deficient in Parkinson's disease midbrain tissue that has lost midbrain DNs. MiR-133b regulates the maturation and function of midbrain DNs within a negative feedback circuit that includes the paired-like homeodomain transcription factor Pitx3.

Table of contents

S.No.	Topic	Page No
1.	Introduction	1
2.	Review of literature	25
3.	Aim and objectives	33
4.	Materials and methods	34
5.	Results and Discussion	42
6.	Summary and Conclusion	

7.	References	46
-----------	-------------------	-----------

vi

LIST OF SYMBOLS AND ABBREVIATIONS

% - *percentage*

DA -*dopamine*

CNS -*central nervous system* AADC: aromatic L-amino acid decarboxylase

COMT: Catechol-o-methyltransferase

DOPAC:3,4-Dihydroxyphenylacetic acid

L-DOPA: levodopa or L-3,4-dihydroxyphenylalanine

HVA: homovanillic acid; MAO: monoamine oxidase

PMNT: phenylethanolamine N-methyltransferase,

TH: tyrosine hydroxylase.

INTRODUCTION

1.1. Overview

zebrafish (*Danio rerio*) have been extensively used in genetic and developmental biology studies. Low cost of housing, ease of handling and drug delivery, high mating rate, and gene homology to mammals makes them suitable model organism for human diseases. Unlike other lower model organisms like *Caenorhabditis elegans* and *Drosophila melanogaster* zebrafish are vertebrates, hence they resemble more closely to humans.^[1](Makhija & Jagtap, 2014) It is well-established fact that zebrafish also exhibits various neurobiological responses. Like other vertebrates, zebrafish exhibit cognitive, sensorimotor, and basic motor responses which are controlled by central nervous system. These features make them best candidates for neurochemical and behavioral studies. Parkinson's disease (PD) is the second-most common neurodegenerative disorder after Alzheimer's disease. It is known to affect nearly 1% of world population above 60 years of age. It is characterized by progressive loss of dopaminergic neurons in substantia nigra and pars compacta; which on later stages extends to other parts of brain including raphe nuclei (serotonergic neurons), locus coeruleus (noradrenergic neurons), and also to cortex. Some of the toxins like rotenone, paraquat, MPTP which are known to produce PD like symptoms in mammals, also cause dopaminergic loss in zebrafish. Anti-psychotics like haloperidol which act by temporary blockade of dopaminergic neurons are known to produce cataleptic movements in zebrafish leading to aberrant swimming patterns (upside down, circular, arrow like swimming toward bottom). Further, various proteins like parkin (shares 62% homology with human genes), LRRK2, DJ-1 (83% homologous to human counterpart) Pink1, Trap1, α -synuclein, Ubiquitin C-terminal hydrolase L1 (70% homologous to human genes); playing crucial role in pathology of PD are expressed in zebrafish.^[6,7,8] (Makhija & Jagtap, 2014)

1.2. DESCRIPTION OF SELECTED PLANTS

1.2.1 *Withania somifera*

TAXONOMICAL CLASSIFICATION

Kingdom – Plantae

Subkingdom – Tracheophyta

Class – Magnoliopsida

Order – Solanaceae

Family – Rubiaceae

Genus – withania

Species – W. somnifera

1.2.3 General information

Withania somnifera, known commonly as ashwagandha or winter cherry, is an annual evergreen shrub in the [Solanaceae](#) or nightshade family that grows in India, the Middle East, and parts of Africa. Several other species in the genus *Withania* are morphologically similar. Although thought to be useful as a [medicinal herb](#) in [Ayurveda](#) and sold in many countries as a [dietary supplement](#), there is insufficient scientific evidence that it is safe or effective for treating any disease.

1.2.4 Geographical distribution

. This species is a short, tender [perennial shrub](#) growing 35–75 cm (14–30 in) tall. [Tomentose](#) branches extend radially from a central stem. Leaves are dull green, elliptic, usually up to 10–12 cm (3.9–4.7 in) long. The flowers are small, green and bell-shaped. The ripe fruit is orange-red

1.2.5 Medicinal benefits

The plant has been used as an aphrodisiac, liver tonic, anti-inflammatory agent, and more recently to treat asthma, ulcers, insomnia, and senile dementia. Clinical trials and animal research support the use of ashwagandha for anxiety, cognitive and neurological disorders, inflammation, and Parkinson's disease. This herb is considered an adaptogen which is a nontoxic herb that works on a nonspecific basis to normalize physiological function, working on the HPA axis and the neuroendocrine system. Ashwagandha is effective for insomnia but does not act as a sedative. Its rejuvenative and nervine properties produce energy which in turn help the body to settle and sleep. Thus it helps the body to address a stress related condition rather than masking it with sedatives. (kumar et al., 2012)

Botanical Name : *Withania Somnifera* Family Name : Solanaceae

Common Name : Withania, Winter Cherry, Indian Winter Cherry, Indian Ginseng, Ashwagandha Part Used : Roots, Leaves Habitat : Cultivated throughout drier parts of India. Product offered : Roots (kumar et al., 2012)

1.2.6 Side effects;

Excessive doses of ashwagandha have been reported to cause abortions,

so pregnant women should avoid this herb. Do not take this herb with other sedatives or anti-anxiety drugs. Large doses can cause diarrhea, stomach upset and vomiting.

2.1 Parkinson diseases;

Parkinson's disease is a progressive nervous system disorder that affects movement. Symptoms start gradually, sometimes starting with a barely noticeable tremor in just one hand. Tremors are common, but the disorder also commonly causes stiffness or slowing of movement. In the early stages of Parkinson's disease, your face may show little or no expression. Your arms may not swing when you walk. Your speech may become soft or slurred. Parkinson's disease symptoms worsen as your condition progresses over time. Although Parkinson's disease can't be cured, medications might significantly improve your symptoms. Occasionally, your doctor may suggest surgery to regulate certain regions of your brain and improve your symptoms.

2.1.2 symptoms:

Parkinson's disease signs and symptoms can be different for everyone. Early signs may be mild and go unnoticed. Symptoms often begin on one side of your body and usually remain worse on that side, even after symptoms begin to affect both sides.

2.1.3 Parkinson's signs and symptoms may include:

Tremor. A tremor, or shaking, usually begins in a limb, often your hand or fingers. You may rub your thumb and forefinger back and forth, known as a pill-rolling tremor. Your hand may tremble when it's at rest. **Slowed movement (bradykinesia).** Over time, Parkinson's disease may slow your movement, making simple tasks difficult and time-consuming. Your steps may become shorter when you walk. It may be difficult to get out of a chair. You may drag your feet as you try to walk. **Rigid muscles.** Muscle stiffness may occur in any part of your body. The stiff muscles can be painful and limit your range of motion

2.1.4 Impaired posture and balance. Your posture may become stooped, or you may have balance problems as a result of Parkinson's disease.

2.1.5 Loss of automatic movements. You may have a decreased ability to perform unconscious movements, including blinking, smiling or swinging your arms when you walk. **Speech changes.** You may speak softly, quickly, slur or hesitate before talking. Your speech may be more of a monotone

rather than have the usual inflections. Writing changes.

2.1.63 Causes;

In Parkinson's disease, certain nerve cells (neurons) in the brain gradually break down or die. Many of the symptoms are due to a loss of neurons that produce a chemical messenger in your brain called dopamine. When dopamine levels decrease, it causes abnormal brain activity, leading to impaired movement and other symptoms of Parkinson's disease.

The cause of Parkinson's disease is unknown, but several factors appear to play a role, including:

- **Genes.** Researchers have identified specific genetic mutations that can cause Parkinson's disease. But these are uncommon except in rare cases with many family members affected by Parkinson's disease.
However, certain gene variations appear to increase the risk of Parkinson's disease but with a relatively small risk of Parkinson's disease for each of these genetic markers.
- **Environmental triggers.** Exposure to certain toxins or environmental factors may increase the risk of later Parkinson's disease, but the risk is relatively small.

Researchers have also noted that many changes occur in the brains of people with Parkinson's disease, although it's not clear why these changes occur. These changes include:

- **The presence of Lewy bodies.** Clumps of specific substances within brain cells are microscopic markers of Parkinson's disease. These are called Lewy bodies, and researchers believe these Lewy bodies hold an important clue to the cause of Parkinson's disease.
- **Alpha-synuclein found within Lewy bodies.** Although many substances are found within Lewy bodies, scientists believe an important one is the natural and widespread protein called alpha-synuclein (a-synuclein). It's found in all Lewy bodies in a clumped form that cells can't break down. This is currently an important focus among Parkinson's disease researchers.

3.1 Risk factors

Risk factors for Parkinson's disease include:

- **Age.** Young adults rarely experience Parkinson's disease. It ordinarily begins in middle or late life, and the risk increases with age. People usually develop the disease around age 60 or older.
- **Heredity.** Having a close relative with Parkinson's disease increases the chances that you'll develop the disease. However, your risks are still small unless you have many relatives in your family with Parkinson's disease.
- **Sex.** Men are more likely to develop Parkinson's disease than are women.
- **Exposure to toxins.** Ongoing exposure to herbicides and pesticides may slightly increase your risk of Parkinson's disease.

3.1.1 Complications

Parkinson's disease is often accompanied by these additional problems, which may be treatable:

- **Thinking difficulties.** You may experience cognitive problems (dementia) and thinking difficulties. These usually occur in the later stages of Parkinson's disease. Such cognitive problems aren't very responsive to medications.
- **Depression and emotional changes.** You may experience depression, sometimes in the very early stages. Receiving treatment for depression can make it easier to handle the other challenges of Parkinson's disease.
You may also experience other emotional changes, such as fear, anxiety or loss of motivation. Doctors may give you medications to treat these symptoms.
- **Swallowing problems.** You may develop difficulties with swallowing as your condition progresses. Saliva may accumulate in your mouth due to slowed swallowing, leading to drooling.
- **Chewing and eating problems.** Late-stage Parkinson's disease affects the muscles in your mouth, making chewing difficult. This can lead to choking and poor nutrition.
- **Sleep problems and sleep disorders.** People with Parkinson's disease often have sleep problems, including waking up frequently throughout the night, waking up early or falling asleep during the day.
People may also experience rapid eye movement sleep behavior disorder, which involves acting out your dreams. Medications may help your sleep problems.
- **Bladder problems.** Parkinson's disease may cause bladder problems, including being unable to control urine or having difficulty urinating.
- **Constipation.** Many people with Parkinson's disease develop constipation, mainly due to a slower digestive tract.

You may also experience:

- **Blood pressure changes.** You may feel dizzy or lightheaded when you stand due to a sudden drop in blood pressure (orthostatic hypotension).
- **Smell dysfunction.** You may experience problems with your sense of smell. You may have difficulty identifying certain odors or the difference between odors.
- **Fatigue.** Many people with Parkinson's disease lose energy and experience fatigue, especially later in the day. The cause isn't always known.
- **Pain.** Some people with Parkinson's disease experience pain, either in specific areas of their bodies or throughout their bodies.
- **Sexual dysfunction.** Some people with Parkinson's disease notice a decrease in sexual desire or performance.

4.1 Genes and genetics of parkinson diseases;

4.1.1 SNCA(PARK1-4);

SNCA was the first gene with mutations reported to cause autosomal-dominant PD. Patients with *SNCA* mutations usually have early-onset PD (EOPD, age of onset ≤ 50 yr) with an initially good response to levodopa treatment. However, the disease has a rapid progression and often presents with dementia and cognitive decline, and sometimes with atypical features such as central hypoventilation and myoclonus. Lewy bodies are present and spread through the substantia nigra, locus ceruleus, hypothalamus, and cerebral cortex ([Polymeropoulos et al. 1996](#))

4.1.2 LRRK2(PARK8);

Mutations in the *LRRK2* gene are the most frequent known cause of late-onset autosomal-dominant and sporadic PD, with a mutation frequency ranging from 2% to 40% in different populations ([Brice 2005](#); [Lesage et al. 2006](#); [Ozelius et al. 2006](#)). Clinically, *LRRK2*-linked PD usually shows mid-to-late onset and progresses slowly. Patients respond favorably to levodopa therapy, and dementia is not common. Neuropathological findings are mostly inconsistent, showing both Lewy body (and sometimes tau- and ubiquitin-containing inclusions) pathology and pure nigral degeneration without Lewy bodies, with or without neurofibrillary tangles ([Giasson et al. 2006](#)).

LRRK2 is a large gene that consists of 51 exons. It encodes the 2527-amino acid cytoplasmic protein leucine-rich repeat kinase 2 (LRRK2) that consists of a leucine-rich repeat toward the amino terminus of the protein and a kinase domain toward the carboxyl terminus with various conserved domains in between. There are more than 50 different missense and nonsense mutations reported in *LRRK2* to date ([Nuytemans et al. 2010](#))

and at least 16 of them (including the six recurrent mutations—p.R114C, p.R1441G, p.R1441H, p.Y1699C, p.G2019S, and p.I2020T) seem to be pathogenic. These pathogenic changes are clustered in 10 exons, mostly encoding the carboxy-terminal region of the protein. By far the most frequent and best-studied mutation is c.6055G >A (p.G2019S) that accounts for as many as 40% of cases of Arab descent ([Lesage et al. 2006](#)), about 20% of Ashkenazi Jewish patients ([Ozelius et al. 2006](#)), and 1%–7% of PD patients of European origin ([Clark et al. 2006](#); [Zabetian et al. 2006](#)). Interestingly, three different founders have been described for the p.G2019S mutation and at least 29 patients have been reported to carry the mutation in the homozygous state ([Klein and Lohmann-Hedrich 2007](#)). Owing to a founder effect, the p.R1441G is very frequent in Basques ([Simon-Sanchez et al. 2006](#); [Gorostidi et al. 2009](#)) and p.I2020T in Japanese patients ([Tomiya et al. 2006](#)). Whereas p.G2019S shows reduced penetrance, sometimes estimated to be as low as 24%, the p.R1441 mutation is highly penetrant (95% at the age of 75 yr) ([Haugarvoll et al. 2008](#)).

The pathogenic mechanism leading to PD caused by LRRK2 mutations is still uncertain. LRRK2 is a large protein with many domains capable of protein–protein interactions, and thus it is plausible that changes in these domains would influence the LRRK2’s relationship with other proteins, i.e., currently unknown interactors with which it forms complexes or which it phosphorylates. In addition, various mutations affect its kinase activity as shown for the p.G2019S and p.I2020T mutants ([MacLeod et al. 2006](#)).

4.1.3 Parkin (park2);

Parkin was the second identified PD gene and the first gene irrefutably causing an AR form of the disorder. The disease usually starts in the third or fourth decade of the patients’ life, and is usually slowly progressive with an excellent response to dopaminergic treatment. However, some of the *Parkin*-mutation carriers have an onset even in childhood, and homozygous mutations in *Parkin* are the most frequent cause of juvenile PD (age of onset ≤ 21 yr). The clinical phenotype of *Parkin*-, *PINK1*-, and *DJ-1*-linked PD is indistinguishable. Reported postmortem examinations indicate that the substantia nigra shows neuronal loss and gliosis, however, it is frequently lacking Lewy bodies.

A large number and wide spectrum of *Parkin* mutations have been detected, including alterations of all 12 exons, across various ethnic groups. *Parkin* mutations are the most common known cause of EOPD, accounting for up to 77% of the familial cases with an age of onset <30 yr ([Lucking et al. 2000](#)), and for 10%–20% of EOPD patients in general ([Klein and Lohmann-Hedrich 2007](#)). To our knowledge, 887 exonic mutations have been described to date, comprising 147 different changes. About a third (293/887) of all exonic mutations are single-nucleotide changes, 13% (119/887) are small deletions, and 54% (475/887) are deletions or duplications of one or several exons (A Grünwald and C Klein, in prep.). However, because methods for the detection of exon rearrangements used to be labor intensive and expensive in the first years after the identification of *Parkin*, they were frequently omitted. Thus, the number of exon rearrangements is likely even underestimated.

Parkin is the second largest gene in human genome and codes for a 465-amino acid protein with a modular architecture. The Parkin protein functions as an E3 ubiquitin ligase in the process of ubiquitination, a form of posttranslational modification that conjugates ubiquitin protein(s) to lysine residue(s) of target proteins, which in turn determines their cellular fate. The amino-terminal ubiquitin-like domain (UBL) of Parkin shares 62% homology with ubiquitin and plays an important role in stabilizing the structure and controlling the expression levels of Parkin. The carboxy-terminal domain consists of three RING (really interesting new-gene) domains (amino acids 145–215 [RING0], 237–292 [RING1], and 417–448 [RING3]) and one IBR (in-between-ring) domain (amino acids 327–378) and is responsible for the interaction with the ubiquitination machinery.

About half of the published changes affect the region spanning exons 2–4 (52%, 459/887) that codes for the UBL domain, the linker region, and the very beginning of the RING0 domain. Although the largest total number of mutations was identified in exon 3 (257/887), exon 1 is remarkable for the highest mutation density with 2.4 mutations per bp (followed by exon 4 with 1.3 mutations per bp). In exon 2, the largest diversity of mutations was found, comprising 27 different variations. A deletion of exon 3 is the most frequent mutation in the *Parkin* gene (88/887, reported in 17 different studies). The second most common change is the c.924C>T single-nucleotide mutation in exon 7 (RING1), which was detected 75 times in 13 different screens (A Grünewald and C Klein, in prep.).

From the beginning of 2004 until July 2009, screening results of 4841 patients, mostly EOPD cases, were published. *Parkin* mutations were detected in about 8% of these. More than half of all mutation-positive cases (55%, 207/378) carried a single heterozygous change. Twenty-five percent (94/378) of them were compound-heterozygous and the remaining 20% (77/378) harbored a homozygous mutation (A Grünewald and C Klein, in prep.)

Pink 1(PARK 6);

Mutations in the phosphatase and tensin homolog (*PTEN*)-induced putative kinase 1 (*PINK1*) gene are the second most common cause of AR EOPD. The frequency of *PINK1* mutations is in the range of 1%–9%, with considerable variation across different ethnic groups ([Healy et al. 2004](#); [Rogaeva et al. 2004](#); [Valente et al. 2004](#); [Bonifati et al. 2005](#); [Klein et al. 2005](#); [Li et al. 2005](#)).

Interestingly, in contrast to *Parkin*, the majority of *PINK1* mutations reported are either missense or nonsense mutations, and, to date only three families with whole-exon deletions (exons 4–8 [[Cazeneuve et al. 2009](#)], 6–8 [[Li et al. 2005](#)], and 7 [[Camargos et al. 2009](#)]) and one with a heterozygous whole-gene deletion have been reported ([Marongiu et al. 2007](#)). More than 60 different missense and nonsense mutations were found in >170 patients, affecting all 8 *PINK1* exons at nearly equal frequencies (in each of the exons 5–10 different mutations were reported). The largest total number of mutations was found in exon 7 (in >50 patients), and the most frequent mutation is p.Q456X. Although only a quarter of the *PINK1* mutations are truncating (vs.

3/4 missense), >40% of the total number of patients carry this type of mutation.

PINK1 is a 581 amino acid ubiquitously expressed protein kinase. It consists of an amino-terminal 34 amino acid mitochondrial targeting motif, a conserved serine–threonine kinase domain (amino acids 156–509; exons 2–8), and a carboxy-terminal autoregulatory domain. Two-thirds of the reported mutations in PINK1 are loss-of-function mutations affecting the kinase domain, demonstrating the importance of PINK1’s enzymatic activity in the pathogenesis of PD. Interestingly, recent studies provided evidence that PINK1 and Parkin function in a common pathway for sensing and selectively eliminating damaged mitochondria from the mitochondrial network. PINK1 is stabilized on mitochondria with lower membrane potential, and as such, it recruits Parkin from the cytosol. Once recruited to mitochondria, Parkin becomes enzymatically active and initiates the autophagic clearance of mitochondria by lysosomes, i.e., mitophagy ([Youle and Narendra 2011](#)).

4.1.4DJ-1 (PARK7)

DJ-1 is the third gene associated with AR PD, and it is mutated in about 1%–2% of EOPD cases ([Pankratz et al. 2006](#)). Given that *DJ-1*-linked PD seems to be rare, very few patients have been reported in the literature. However, about 10 different point mutations and exonic deletions have been described, mostly in the homozygous or compound-heterozygous state. Given that the mutations are so uncommon, there is not enough data to draw any conclusion about the possible role of heterozygous mutations in this gene.

The seven coding exons of the *DJ-1* gene code for a 189-amino acid-long protein that is ubiquitously expressed and functions as a cellular sensor of oxidative stress ([Canet-Aviles et al. 2004](#); [Junn et al. 2005](#)). The DJ-1 protein forms a dimeric structure under physiologic conditions ([Macedo et al. 2003](#)), and it seems that most of the disease-causing mutants (p.L166P, p.E64D, p.M26I, and p.D149A) heterodimerize with wild-type DJ-1 ([Takahashi-Niki et al. 2004](#)). In addition, the mutated proteins are frequently not properly folded, unstable, and promptly degraded by the proteasome. Thus, their neuroprotective function and antioxidant activity are reduced ([Anderson and Daggett 2008](#); [Malgieri and Eliezer 2008](#)).

4.1.5ATP13A2 (PARK9);

Homozygous and compound-heterozygous mutations in *ATP13A2* have been found to cause an AR atypical form of PD named Kufor-Rakeb syndrome ([Ramirez et al. 2006](#)). This syndrome has juvenile onset with rapid disease progression, accompanied by dementia, supranuclear gaze palsy, and pyramidal signs.

ATP13A2 is a large gene comprised of 29 exons coding for an 1180-amino acid protein. The ATP13A2 protein is normally located in the lysosomal membrane and it has 10 transmembrane domains and an ATPase domain ([Ramirez et al. 2006](#)). About 10 different pathogenic mutations have been found in the homozygous or compound-heterozygous state, directly or indirectly affecting transmembrane domains. Most of the mutations produce truncated proteins that are unstable and are retained in the endoplasmic reticulum

and subsequently degraded by the proteasome. No exonic deletions or deletions or multiplications of the entire gene have been found, to date. Several single heterozygous missense mutations are known, but their role in PD pathogenicity is currently not clear.

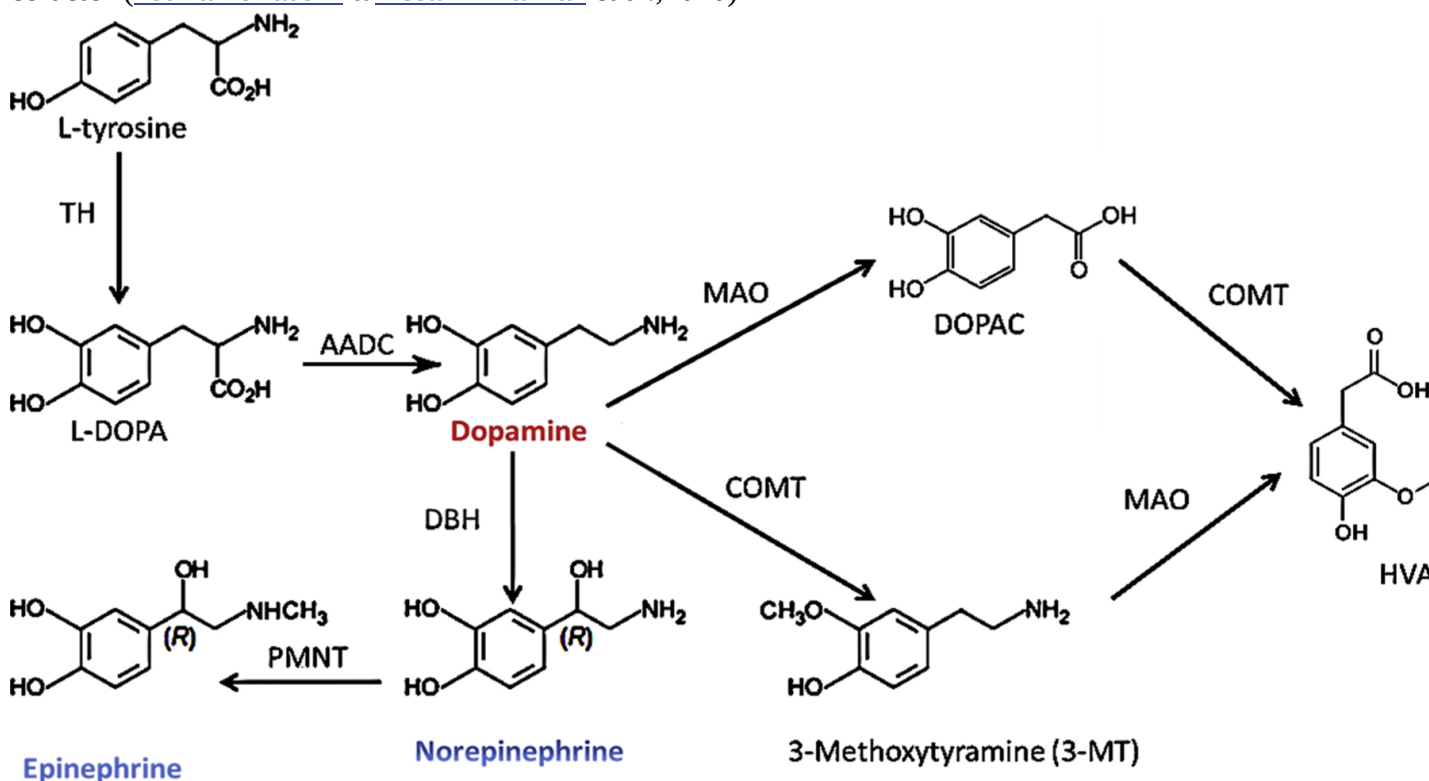
5.1 Brain dopamine system;

5.1.1 HISTORY OF DOPAMIN;

Dopamine (DA) was first synthesized by barger & ewins and mannich & jacobsohn in 1910 .the swedish pharmacologist arvid carlsson rediscovered dopamine in the 1950 and identified that it act as aneurotransmitter in brain (hornykiewicz et al.,2002) dopamine is also known as 3 dyhydroxy ,and from its initial discovery it was well characterized .it consists of a benzene ring with two side group of hydroxyls attached to one group of amine through an ethyl group .it produced from dopaminergic neurons by using precursor of tyrosine (ayano et al.,2016)

5.1.2 Dopamine biosynthesis;

Dopamine is synthesized in the cytoplasm from the non-essential amino acid L-tyrosine. A structural based summary of dopamine biosynthesis and breakdown is illustrated . In brief, tyrosine is converted to L-3,4-dihydroxyphenylalanine (levodopa, L-DOPA) by the tyrosine hydroxylase enzyme using tetrahydrobiopterin, oxygen (O₂), and iron (Fe²⁺) as cofactors in both the CNS and in a non-neuronal dopaminergic system within the gastrointestinal tract. In neurons, L-DOPA is quickly decarboxylated to dopamine by aromatic L-amino acid decarboxylase (AADC) enzyme . This oxidation reaction is a rate-limiting step in the production of both dopamine and other catecholamines. In noradrenergic neurons, L-DOPA is converted by the β -hydrolase enzyme into norepinephrine (NE) with O₂ and L-ascorbic acid as cofactors. In turn, NE can be converted into epinephrine by phenylethanolamine N-methyltransferase (PMNT) with S-adenosyl-L-methionine as the cofactor ([Louiza Belkacemi](#) & [Nissar A. Darman](#) et al.,2020)



Structurally based summary of primary pathways of biosynthesis and metabolism of dopamine.

Dopamine biosynthesis: L-tyrosine is converted to L-DOPA in the presence of the TH. In a second reaction, the enzyme AADC converts L-DOPA into dopamine. Dopamine is converted into norepinephrine by the enzyme DBH, with O₂ and L-ascorbic acid as cofactors. Norepinephrine is converted into epinephrine by the enzyme PMNT with S-adenosyl-L-methionine as the cofactor. *Dopamine metabolism:* Dopamine is broken down into inactive metabolites by a set of enzymes including MAO and COMT. Several breakdown pathways exist but the major end-product is the HVA, which has unknown biological activity.

Anatomy of dopamine neurons:

They contain GABA, display the ultrastructural features of interneurons and form axo-axonic synapses with putative cortical-like glutamatergic boutons. The local dendritic release of dopamine by neurons in the substantia nigra pars compacta and ventral tegmental also influences basal ganglia functions. Thus, the long-term belief that the effects of dopamine in the basal ganglia were solely mediated through the nigrostriatal system must be changed to take into account extrastriatal dopaminergic projections and intrastriatal dopaminergic neurons. (Y Smith & J Z Kieva et al., 2000)

1. Nigrostriatal
2. Mesolimbic
3. Mesocortical
4. Tuberoinfundibular / Incerto-hypothalamic tract

Nigrostriatal dopamine tract;

The nigrostriatal pathway originates in the zona compacta of the substantia nigra and sends its projections to the caudate nuclei and putamen of the striatum. The nigrostriatal DA system has been implicated in the control of procedural aspects of movement and motivated behaviors, as it reaches dorsal areas of the basal ganglia (Alcaro et al., 2007). DA is released in the dorsal striatum only after the male begins to copulate, suggesting that this release reflects motor activation rather than motivational aspects of copulation (Damsma et al., 1992). Bilateral lesions of the substantia nigra slowed the rate of copulation and decreased ejaculatory capacity (Brackett et al., 1986). Copulation did not induce Fos-ir in the dorsal striatum, in contrast to mesocorticolimbic areas like the NAc, VTA, and mPFC

Mesolimbic pathway and mesocortical pathway;

Mesocorticolimbic Circuits and rsFC;

The mesolimbic and mesocortical pathways, two of the brain's major dopaminergic pathways, have been implicated as key circuits that are disrupted in addictive behaviors (Blum et al., 2012). Both pathways originate primarily in the ventral tegmental area (VTA); the mesolimbic pathway projects to the nucleus accumbens, and is a part of complex circuits involving the amygdala, hippocampus, and the bed nucleus of the stria terminalis (Fig. 2A). In contrast, the mesocortical pathway projects primarily to the prefrontal cortex. Rats rapidly learn to press a lever to self-administer an electric pulse to regions along this pathway (Olds and Milner, 1954), and activation of dopaminergic neurons is necessary for this behavior (Garris et al., 1999). Some rats will even forego food and starve themselves to continue receiving stimuli to the VTA (Stutz et al., 1971). Together, these findings indicated that mesocorticolimbic dopamine (DA) circuitry contribute to the rewarding aspect of a stimulus. Subsequent findings showed that dopaminergic transmission in this system not only respond to rewards, but to the cues that reliably indicate that a reward will or will not arrive (Schultz et al., 1997).

Midbrain dopamine neurons development and their connectivity;

Identity of ventral midbrain dopaminergic;

neural precursors Once the appropriate patterning of the VM region has occurred, a developmental programme involving a sequential pattern of gene expression establishes the identity of VM DA neural precursors (NPs) that ultimately generate VM DA neurons (Fig. 1). The identity of these VM DA NPs has been the focus of intensive research in recent years, largely due to their potential to be used as a cell source to generate DA neurons for cell replacement therapy in PD (Kim, 2011; Morizane et al., 2008; Toulouse and Sullivan, 2008)

While the floor plate and isthmus organiser are critical determinants of VM patterning, they are also crucial for the induction of a VM DA phenotype. Their role in induction of a DA phenotype is dependent upon the interaction of floor plate-secreted Shh and isthmus-secreted FGF8 (Hynes et al., 1997; Ye et al., 1998). FGF8 The mechanism by which FGF8 regulates VM DA development is still under investigation, however a recent study suggested that FGF8 is required to induce the correct patterning of VM DA NPs, as the loss of FGFRs (FGF receptors) resulted in altered

patterning of the VM and failure of VM DA neuron maturation, with the DA domain adopting diencephalic characteristics (Lahti et al., 2012). In support of this, a similar study that mutated the FGFRs reported a reduction in the generation of VM DA NPs, and a disturbance in the maturation of VM DA neurons (Saarimäki-Vire et al., 2007). Another FGF, FGF2, has been shown to function in the regulation of SNc DA NPs proliferation, and also in the developmental cell death of mature SNc DA neurons (Ratzka et al., 2012). Sonic hedgehog (Shh)

signalling and Lmx1 expression;

The first sign of a DA phenotype in VM NPs is the initiation of expression of two key determinants of VM DA cell fate, the limhomeodomain factor Lmx1a and the homeodomain transcription factor Msx1, at ~E9 in the mouse (Alavian et al., 2008). Shh induces the expression of Lmx1a, which subsequently induces the expression of its downstream effector Msx1 (Andersson et al., 2006). The overexpression of Lmx1a in the anterior VM results in the ectopic generation of DA neurons, while reduced expression results in a loss of VM DA neurons (Andersson et al., 2006). Additionally, null mutation of Lmx1a or the spontaneous mutation of Lmx1a in dreher mice results in substantial reductions in the numbers of VM DA neurons generated (Deng et al., 2011; Ono et al., 2007). Lmx1a expression is maintained in post-mitotic VM DA neurons until postnatal day (P) 180 in mice (Zou et al., 2009); however Msx1 expression is confined to VM DA NPs (Andersson et al., 2006; Failli et al., 2002). This is surprising considering that Lmx1a is upstream of Msx1, suggesting that the post-mitotic repression of Msx1 expression somehow overrides the inductive effect of Lmx1a. Msx1 contributes to DA neurogenesis by inducing the expression of the proneural gene, neurogenin (Ngn) 2, and thus neuronal differentiation. Ngn2 expression in VM NPs also appears to be under the control of Otx2 expression, as conditional Otx2 mutant mice display a loss of Ngn2 expression in DA NPs (Vernay et al., 2005). This finding is not surprising, considering that Otx2 induces the expression of Lmx1a in VM floor plate cells, suggesting that the loss of Ngn2 expression in the Otx2 knockout mouse is due to a failure of Lmx1a induction and subsequently Msx1 expression, rather than a direct effect on Ngn2 expression, but this remains to be determined (Ono et al., 2007). This suggestion is supported by recent findings showing that in the absence of Otx2, VM NPs fail to activate the expression of Lmx1a, Msx1 and Ngn2, and therefore largely fail to differentiate into VM DA neurons (Omodei et al., 2008). Support for the role of Ngn2 in DA induction comes from studies showing that loss of Ngn2 in mice results in a severe reduction in the

expression of post-mitotic VM DA markers Nurr1 and tyrosine hydroxylase (TH) (discussed later), demonstrating its importance in the generation of mature VM DA neurons (Kele et al., 2006). However, the role of Ngn2 is likely to be generally proneural, rather than specific for VM DA neuronal differentiation. Fig. 1. Molecular factors involved in the development of VM DA neurons. Molecular interactions in the genesis of VM DA neurons: The sequence of appearance (see time-course arrow) of each of the factors involved in VM DA neuronal development, and their effects on each other. (Note: the molecules in black text are not shown at the time point at which they appear.) The arrows denote the effect on expression: purple¼positive regulation, green¼autoregulatory loop, orange¼cooperative regulation, and black¼negative regulation. The factors are colour-coded as per their role (listed above molecules). Otx2, Gbx2, En1/2, Lmx1b, Wnt1 and Pax2/5 play vital roles in the establishment of the midbrain/hindbrain region, including the isthmus organizer and VM floor plate, and the majority also play direct roles in VM DA neurogenesis. The diffusible signalling factors FGF8, Shh and Wnt1 induce VM DA neurogenesis in radial glial-like floor plate cells, through the induction of FoxA2, Lmx1a/1b, and Msx1 expression. Two autoregulatory loops, Shh–FoxA2 and Wnt1–Lmx1a, contribute to this process, with Hes1 being involved in the Shh–FoxA2 autoregulatory loop, and Lmx1b functioning cooperatively with Lmx1a. The expression of Nurr1 and Pitx3 promotes the differentiation of VM DA NPs into post-mitotic neurons. Nurr1 induces the expression of proteins that are key to the neurotransmitter phenotype of VM DA neurons. A number of factors facilitate the induction of TH by Nurr1 (listed in orange), and Pitx3 has been shown to cooperatively regulate a number of important genes involved in VM DA neurogenesis with Nurr1. 126 S.V. Hegarty et al. / Developmental Biology 379 (2013) 123–138 In support of this, overexpression of Ngn2 induces neurogenesis but not a DA phenotype in cultured VM NPs (Kim et al., 2007). These data suggest the existence of a developmental programme consisting of an inductive effect of floorplate-secreted Shh on Lmx1a expression which subsequently induces the expression of Msx1, which in turn induces the expression of Ngn2 which is required for neuronal differentiation of VM DA NPs. How and where Otx2 fits into this cascade is unclear, but it is known that Otx2 is required for the expression of Lmx1a. It has yet to be determined whether this is a parallel pathway cooperating with Shh or whether Otx2 is a master regulator of Shh-induced Lmx1a expression. In support of a role of Otx2 as a master regulator, studies involving the conditional knockout of Otx2 in the midbrain have suggested that Otx2 controls the fate of VM progenitors through the repression of Nkx2.2 and maintenance of Nkx6.1 expression (Puelles et al., 2004). FoxA2, involved in a feedback loop with Shh (discussed later),

induces Lmx1a expression and also inhibits Nkx2.2 (Lin et al., 2009). It is possible that Otx2 functions via a similar pathway to FoxA2, or indeed that FoxA2 may function downstream of Otx2 and Shh in the regulation of Lmx1a and Nkx2.2 expression during VM DA neurogenesis. Interestingly, a recent genome-wide gene expression profiling study has expanded the regulatory role of Lmx1a in this process by identifying novel transcription factors involved in the generation of the VM DA neuronal field. The Oc transcription factors, Oc1, 2 and 3, display similar expression profiles to that of Lmx1a in the developing VM, and their loss resulted in diminished generation of VM DA neurons (Chakrabarty et al., 2012). Whether these Oc transcription factors are regulated by Lmx1a, which is plausible given their overlapping expression patterns, or whether they act in parallel to regulate neuronal differentiation in the VM, will be an important question for future research. Similar to Lmx1a, the related protein Lmx1b has also been shown to promote VM DA neurogenesis (Deng et al., 2011; Lin et al., 2009; Yan et al., 2011). Lmx1a and Lmx1b are co-expressed in VM DA NPs, and have been shown to mediate the initial steps of NP DA specification (Andersson et al., 2006; Smidt et al., 2000). Furthermore, Lmx1a and Lmx1b are co-expressed in the P0 VM, suggesting that they may function in the maturation of VM DA neurons also (Zou et al., 2009). Similar to Lmx1a, Lmx1b can induce the ectopic production of VM DA neurons when ectopically expressed (Nakatani et al., 2010), and its loss results in a substantial reduction in the number of VM DA neurons (Deng et al., 2011; Smidt et al., 2000). A recent study using conditional knockout of Lmx1a and Lmx1b in mice demonstrated that Lmx1a and Lmx1b function cooperatively to regulate the proliferation of VM DA NPs and Ngn2 expression (Yan et al., 2011). This suggestion is supported by studies on Lmx1a null mice carrying one mutant Lmx1b allele (as double null mutations are embryonically lethal) which found that Lmx1a and Lmx1b function cooperatively in the generation of VM DA neurons. This study also showed that Lmx1b is involved in the generation of ocular motor neurons and red nucleus neurons in the VM (Deng et al., 2011) and it has been suggested that Lmx1b partially compensates for Lmx1a function in dreher mice, as only 46% of VM DA neurons are lost in these mutants (Ono et al., 2007). It will be important to understand whether Lmx1b exerts its effects in precisely the same way as Lmx1a, for example by modulating Msx1 expression or that of Oc1, 2 and 3 (Chakrabarty et al., 2012).

Nurr1;

Nurr1 is a member of the nuclear receptor superfamily of steroid-thyroid hormone-activated transcription factors (Law et al., 1992), which atypically lacks both a ligand cavity and a canonical coactivator-binding site (Wang et al., 2003). Nurr1 is expressed in the VM from E10.5 in the mouse, as VM DA NPs begin to become post-mitotic, one day before the appearance of TH (Zetterstrom et al., 1996), and Nurr1 expression is maintained into adulthood (Backman et al., 1999). Nurr1 expression levels show a sharp peak between E13 and E15 in the rat, at a stage of development when most VM DA neurons are undergoing terminal differentiation (Volpicelli et al., 2004). VM DA neurons in Nurr1- deficient animals do not express TH, l-aromatic amino acid decarboxylase (AADC), the vesicle monoamine transporter 2 (VMAT2) or the dopamine transporter (DAT), all markers of a DA neuron that has acquired its DA neurotransmitter identity (Castillo et al., 1998; Filippi et al., 2007; Smits et al., 2003). Nurr1 has been shown to play a direct role in regulating the expression of these genes, and a number of well-established signalling pathways in VM DA development cooperate with Nurr1 to mediate this induction. Specifically, Nurr1 has been shown to induce TH expression by binding to a NRBE (NGFI-B response element) sequence in the TH promoter (Kim et al., 2003; Sakurada et al., 1999), and is known to induce DAT expression via an NRBE-independent mechanism (Sacchetti et al., 2001). These effects of Nurr1 on the expression of these genes are enhanced by Wnt-activated β -catenin, which has been shown to promote Nurr1-induced TH promoter activation, by interacting with Nurr1 at NRBEs, causing the dissociation of transcriptional co-repressors and recruitment of transcriptional co-activators (Kitagawa et al., 2007). Similarly, nuclear FGFR1 has been shown to cooperate with Nurr1 to promote activation of the TH promoter (Baron et al., 2012a). Collectively these data show that Nurr1 functions as a master regulator in the induction of the neurotransmitter phenotypic identity of VM DA neurons, and controls the expression of the molecules that regulate the synthesis, vesicle packaging, axonal transport and reuptake of DA. Aside from this role in DA identity, Nurr1 has also been shown to be crucial for long-term VM DA neuron survival. In Nurr1- deficient animals, VM DA neurons adopt a correct ventral position and express the DA markers Lmx1b, Pitx3 and En1 (SaucedoCardenas et al., 1998; Wallen et al., 1999), demonstrating that Nurr1 is not required for all aspects of VM DA specification and differentiation. However, these Pitx3-expressing VM DA neurons are lost in Nurr1- deficient animals during later development (Saucedo-Cardenas et al., 1998), suggesting a role for Nurr1 in the survival and maintenance of VM DA neurons. In support of these findings, Nurr1 is expressed throughout the life of VM DA neurons and its heterozygous mutation increases the vulnerability of VM DA neurons to the parkinsonian toxin, 1-methyl-4-phenyl1,2,3,6-

tetrahydropyridine-HCl (MPTP) (Le et al., 1999). Furthermore, the conditional ablation of Nurr1 at a late stage of VM DA neuron development or in the adult brain results in loss of striatal DA, loss of VM DA markers and DA neurodegeneration, with SNc DA neurons more vulnerable than those of the VTA (Kadkhodaei et al., 2009). While Nurr1 has been shown to directly regulate the expression of TH and DAT, Nurr1 may promote VM DA neuron survival indirectly, by inducing the expression of genes essential for their survival. Nurr1 has been found to regulate the expression of the glial cell line-derived neurotrophic factor (GDNF) receptor, cRet, whose expression is lost in Nurr1-deficient animals (Castillo et al., 1998). GDNF is a well-known survival-promoting factor for VM DA

Pitx3;

Pitx3 is a bicoid-related, homeodomain-containing transcription factor that is exclusively expressed in the mouse VM from E11.5, at a time when VM DA neurons are beginning to appear (Smidt et al., 1997). VM DA neurons only begin to express Pitx3 when they arrive at their final ventral position, suggesting that Pitx3 is not involved in the early development or migration of VM DA neurons (Smidt et al., 2004). GDNF has been suggested to induce the expression of Pitx3 in the VM (Lei et al., 2011; Peng et al., 2011) which is interesting as Nurr1 is known to regulate the expression of the GDNF receptor cRet, and is expressed before Pitx3 in the VM. Nurr1 may therefore play an indirect, non-essential (Pitx3 expression is retained in Nurr1 null mutants) role in the induction of Pitx3 expression. Pitx3 and Nurr1 have been shown to function cooperatively in the regulation of target genes involved in VM DA neurogenesis (discussed later) (Chakrabarty et al., 2012; Hwang et al., 2009; Jacobs et al., 2009a; Jacobs et al., 2009b). Pitx3 is co-expressed in the TH-positive neurons of the VM (Smidt et al., 1997; Van Den Munckhof et al., 2003; Zhao et al., 2004). In aphakia mice, which lack Pitx3 expression due to deletions in the Pitx3 gene, there is unaltered VM DA development until E12.5, at which time a deficit is observable in the lateral population of VM DA

Role of mitochondria in DA neuron development;

Mitochondria are ubiquitous and multi-functional organelles involved in diverse metabolic processes, namely energy production and biomolecule synthesis. The intracellular mitochondrial morphology and distribution change dynamically, which reflect the metabolic state of a given cell type. A dramatic change of the mitochondrial dynamics has been observed in early development that led to further investigations on the

relationship between mitochondria and the process of development. A significant developmental process to focus on, in this review, is a differentiation of neural progenitor cells into neurons. Information on how mitochondria-regulated cellular energetics is linked to neuronal development will be discussed, followed by functions of mitochondria and associated diseases in neuronal development. Lastly, the potential use of mitochondrial features in analyzing various neurodevelopmental diseases (Geriumson et al., 2018)

Role of miRNA in DA neuron development ;

The molecular mechanisms underlying the neurogenesis and differentiation process of dopamine neurons are still largely unknown. In addition, emerging evidence shows that noncoding RNAs that are part of the post transcriptional machinery, including microRNAs, play an important role in modulating mRNA decay and translation rates in eukaryotes (Lim et al., 2003). Later, Abeliovich and colleagues demonstrate the importance of miRNA activity for midbrain dopamine neurons function (Kim et al., 2007)

miRNA consists of approximately 18-25 nucleotides with single-stranded small noncoding RNAs. miRNA (Kim et al., 2007). miRNA has an inhibitory function to modulate post transcriptional expression by partial or complete binding of the miRNA in the 3' untranslated region which is the mediation of miRNA translation inhibition or degradation. Based on the bioinformatics analysis, individual miRNA can bind up to 100 genes are regulated by miRNA. miRNA diversity and gene regulatory capacity and particularly important in brain and neurogenesis period

MicroRNAs regulating temporal fate specification of neural progenitors;

During retinal and cerebral cortex development, neural progenitor cells (retinal progenitors and radial glial cells, respectively) proceed through different competence states. This results in the successive emergence of distinct neuronal cell types, which are organized in a laminar pattern according to their neuronal birth order (corticogenesis: reviewed by Greig et al. [2013](#); retinogenesis: reviewed by Centanin and Wittbrodt [2013](#)). The shift in progenitor competence over time is controlled by several transcription factors and there is mounting evidence that miRNAs play a regulatory role as well (reviewed by Cremisi [2013](#)). Conditional deletion of Dicer during early mouse retinal development resulted in increased and prolonged production of early-born ganglion cells, while the production of late-born cell types was impaired (Georgi and Reh [2010](#)). Similarly, Dicer-null mouse cortical stem cells were only able to produce early-born deep layer projection neurons and failed to generate late-born upper layer neurons (Saurat et al. [2013](#)). The authors of this study proposed that the production of the late-born neurons might critically depend on an active miRNA system. Noteworthy to mention in this context is that the production of Cajal Retzius cells, which emit instructive cues for proper cortical development, is impaired in miR-9_2/9_3 double knock-out mice (Shibata et al. [2011](#)). Accordingly,

inhibition of miR-9 using an antisense oligonucleotide resulted in an abnormal development of cortical layers (Shibata et al. [2008](#), [2011](#)). For more information on the impact of miR-9 during cortical neurogenesis, please refer to the review by Abernathy and Yoo in this Special Issue.

During retinal development, a specific subset of miRNAs has been shown to modulate the fate of neural progenitors by ensuring the correct temporal expression of key transcription factors (reviewed by Cremisi [2013](#)). In *Xenopus*, the homeobox genes *Xotx2* and *Xvsx1* are necessary for the generation of bipolar neurons, the last neuronal cell type produced. Although the respective transcripts are already present in early retinal progenitors, *Xotx2* and *Xvsx1* protein is only detected at later stages due to the action of four miRNAs. These miRNAs, i.e., miR-129, miR-155, miR-214 and miR-222, are down-regulated during retinal development allowing the translation of *Xotx2* and *Xvsx1* (Decembrini et al. [2009](#)). Inhibition of these miRNAs by transfection of respective antisense oligonucleotides into the optic vesicle resulted in an ectopic generation of bipolar neurons. Interestingly, their expression level is coupled to progenitor cell cycle length, which increases during retinal development and might serve as an intrinsic timer of neural progenitor age (Ohnuma et al. [2002](#); Decembrini et al. [2009](#); Pitto and Cremisi [2010](#)). Lengthening of the cell cycle by inhibition of SHH signaling resulted in a decreased expression of miR-129, miR-155, miR-214 and miR-222, while speeding-up of cell cycle progression had the opposite effect (Decembrini et al. [2009](#)). Another group of miRNAs, i.e., let-7, miR-125 and miR-9 has been shown to promote the progression of murine retinal progenitors from early to late fates via targeting *Protogenin (Ptrg)* and *Lin28b*, two factors involved in early retinal progenitor competence (La Torre et al. [2013](#)). Overexpression of let-7, miR-125 and miR-9 accelerated progenitor fate progression and development of late-born neurons. In contrast, overexpression of their target genes—*Ptrg* and *Lin28b*—retained the progenitor cells at an early competence state. Similar to their function as developmental indicators of retinogenesis, recent findings have pointed to a role of let-7 and miR-125 during temporal fate specification in *Drosophila* mushroom body (MB) neurons (Wu et al. [2012](#); Kucherenko et al. [2012](#)). Here, let-7 and miR-125 contribute to the progressive down-regulation of *chinmo*, which controls MB subtypes specification in a concentration-dependent manner (Wu et al. [2012](#)). In addition to *chinmo*, let-7 and miR-125 also target *abrupt*, another temporal regulator of MB subtype specification (Kucherenko et al. [2012](#)). Very recently, it was shown that olfactory bulb (OB) interneurons generated during embryogenesis show no miR-125b expression, while OB interneurons generated during adult neurogenesis exhibit miR-125b expression (Akerblom et al. [2014](#)). Hence, the lack of miR-125b expression appears to distinguish OB interneuron subpopulations generated during different time periods suggesting that miR-125b might be implicated in regulating the temporal appearance of distinct neuronal subtypes. Interestingly, both let-7 and miR-125b regulate temporal fate progression of different lineages during *C. elegans* development (Olsen and Ambros [1999](#); Ambros et al. [2003](#); Ambros [2011](#)). Together, these findings indicate that, although the cellular context is different, similar factors, such as let-7 and miR-125b, take part in controlling developmental fate transitions.

Review of literature;

Hegarty, S. V., Sullivan, A. M., & O’Keeffe, G. W. (2013). Midbrain dopaminergic neurons: A review of the molecular circuitry that regulates their development. *Developmental Biology*, 379(2), 123–138. <https://doi.org/10.1016/j.ydbio.2013.04.014>

Alexander, G. E. (2004). Biology of Parkinson’s disease: Pathogenesis and pathophysiology of a multisystem neurodegenerative disorder. *Dialogues in Clinical Neuroscience*, 6(3), 259–280.

Kumar, K. P. S., Bhowmik, D., Duraivel, S., & Umadevi, M. (2012). *Traditional and Medicinal Uses of Banana*. 1(3), 51–63. Makhija, D. T., & Jagtap, A. G. (2014)

Studies on sensitivity of zebrafish as a model organism for Parkinson's disease: Comparison with rat model. *Journal of Pharmacology and Pharmacotherapeutics*, 5(1), 39–46. <https://doi.org/10.4103/0976-500X.124422>

Xi, Y., Noble, S., & Ekker, M. (2011). Modeling neurodegeneration in zebrafish. *Current Neurology and Neuroscience Reports*, 11(3), 274–282. <https://doi.org/10.1007/s11910-011-0182-2>

Dhanani, T., Shah, S., Gajbhiye, N. A., & Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry*, 10, S1193–S1199.

Makhija, D. T., & Jagtap, A. G. (2014). Studies on sensitivity of zebrafish as a model organism for Parkinson's disease: Comparison with rat model. *Journal of Pharmacology and Pharmacotherapeutics*, 5(1), 39–46. <https://doi.org/10.4103/0976-500X.124422>

Baitharu, I., Jain, V., Deep, S. N., Hota, K. B., Hota, S. K., Prasad, D., & Ilavazhagan, G. (2013). *Withania somnifera* root extract ameliorates hypobaric hypoxia induced memory impairment in rats. *Journal of Ethnopharmacology*, 145(2), 431–441. <https://doi.org/10.1016/j.jep.2012.10.063>

RajaSankar, S., Manivasagam, T., & Surendran, S. (2009). Ashwagandha leaf extract: A potential agent in treating oxidative damage and physiological abnormalities seen in a mouse model of Parkinson's disease. *Neuroscience Letters*, 454(1), 11–15. <https://doi.org/10.1016/j.neulet.2009.02.044>neuroethological perspective

<https://doi.org/10.1016/j.brainresrev.2007.07.014>Behavioral functions of the mesolimbic dopaminergic system: An affective neuroethologicalperspective

Julian Little, Julian P. T. Higgins, John P. A. Ioannidis, David Moher, France Gagnon, Erik von Elm, Muin J. Khoury, Barbara Cohen, George Davey-Smith, Jeremy Grimshaw, Paul Scheet, Marta Gwinn, Robin E. Williamson, Guang Yong Zou, Kim Hutchings, Candice Y. Johnson, Valerie Tait, Miriam Wiens, Jean Golding, Cornelia van Duijn, John McLaughlin, Andrew Paterson, George Wells, Isabel Fortier, Matthew Freedman, Maja Zecevic, Richard King, Claire Infante-Rivard, Alex Stewart, Nick Birkett ... See fewer authors
First published: 10 March 2009

<https://doi.org/10.1002/gepi.20410>

Mandhane SN, Chopde CT, Ghosh AK. Adenosine A2 receptors modulate haloperidol-induced catalepsy in rats. *Eur J Pharmacol* 1997;328:135-41.

. Berridge MV, Herst PM, Tan AS. Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. *Biotechnol Annu. Rev* 2005;11:127-52.

. Bové J, Prou D, Perier C, Przedborski S. Toxin-induced models of Parkinson's disease. *NeuroRx* 2005;2:484-94.

Singh N, Pillay V, Choonara YE. Advances in the treatment of parkinson's disease. *Prog. In Neurobiol* 2007;81:29-44.

. Schapira AH. Neuroprotection in PD: A role for dopamine agonists? *Neurology* 2003;61 (6 Suppl 3):S34-42.

Schapira AH, Olanow CW. Rationale for the use of dopamine agonists as neuroprotective agents in Parkinson's disease. *Ann Neurol* 2003;53 Suppl 3:S149-59.

Goldsmith P. Zebrafish as a pharmacological tool: The how, why and when. *Curr Opin Pharmacol* 2004;4:504-12.

Breitaud S, Lee S, Guo S. Sensitivity of zebrafish to environmental toxins implicated in Parkinson's disease. *Neurotoxicol Teratol* 2004;26:857-64

. Gerlai R, Lahav M, Guo S, Rosenthal A. Drinks like a fish: Zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. *Pharmacol Biochem Behav* 2000;67:773-82.

Swain HA, Sigstad C, Scalzo FM. Effects of dizoclipine (MK-801) on circling behavior, swimming activity and place preference in zebrafish (*Danio rerio*). *Neurotoxicol Teratol* 2004;26:725-9.

. Echevarria DJ, Hammack CM, Pratt DW, Hosemann JD. A novel behavioural test battery to assess global drug effects using the zebrafish. *Int J Comp Psychol* 2008;21:19-34.

. Iturriaga-Vasquez P, Osorio F, Riquelme S, Catro S, Herzog R. Zebrafish: A model for behavioural pharmacology. *Rev Farmacol Chile* 2012;5:27-32.

. Ghosh A, Dhumal VR, Tilak AV, Singh A, Pandey M, Bondekar AA. Effect of chronic administration of low dose of aspirin on haloperidol induced catalepsy in rats. *J. Pharmacol Pharmacother* 2011;2:198-9.

Aebi, Catalase in vitro, *Methods Enzymol.* 105 (1984) 121–126.

K. Aoyama, N. Matsumura, M. Watabe, T. Nakaki, Oxidative stress on EAAC1 is involved in MPTP-induced glutathione depletion and motor dysfunction, *Eur. J. Neurosci.* 27 (2008) 20–30.

K. Bone, Clinical applications of ayurvedic and chinese herbs. Monographs for the western Herbal practitioner, Phytotherapy Press, Australia, 1996, pp. 137–141.

X.C. Chen, Y.C. Zhou, Y. Chen, Y.G. Zhu, F. Fang, L.M. Chen, Ginsenoside Rg1 reduces MPTP-induced substantia nigra neuron loss by suppressing oxidative stress, *Acta Pharmacol. Sin.* 26 (2005) 56–62.

J.C. Crabbe, P. Metten, C.H. Yu, J.P. Schlumbohm, A.J. Cameron, D. Wahlsten, Genotypic differences in ethanol sensitivity in two tests of motor incoordination, *J. Appl. Physiol.* 95 (2003) 1338–1351.

M. Di Napoli, I.M. Shah, D.A. Stewart, Molecular pathways and genetic aspects of Parkinson's disease: from bench to bedside, *Expert Rev. Neurother.* 7 (2007) 1693–1729.

R.L. Eriksen, L. Petrucelli, Parkinson's disease—molecular mechanisms of disease, *Drug Discov. Today: Dis. Mech.* 1 (2004) 399–405. [8] P.O. Fernagut, E. Diguët, B. Labattu, F. Tison, A simple method to measure stride length as an index of nigrostriatal dysfunction in mice, *J. Neurosci. Methods* 113 (2002) 123–130.

A. Fredriksson, T. Archer, MPTP-induced behavioural and biochemical deficits: a parametric analysis, *J. Neural. Transm. Park. Dis. Dement. Sect.* 7 (1994) 123–132.

T. Gasser, Update on the genetics of Parkinson's disease, *Mov. Disord.* 22 (2007) S343–S350. [11] S. Genc, M. Akhisaroglu, F. Kuralay, K. Genc, Erythropoietin restores glutathione peroxidase activity in 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine-induced neurotoxicity in C57BL mice and stimulates murine astroglial glutathione peroxidase production in vitro, *Neurosci. Lett.* 321 (2002) 73–76.

B.I. Giasson, V.M. Lee, Are ubiquitination pathways central to Parkinson's disease? *Cell* 114 (2003) 1–8. [S.K. Gupta, A. Dua, B.P. Vohra, *Withania somnifera* (Ashwagandha) attenuates antioxidant defense in aged sp

J. Gu, S. Su, J. Guo, K. Wakamatsu, Anti-inflammatory and anti-apoptotic effects of the combination of *Ligusticum chuanxiong* and *Radix paeoniae* against focal cerebral ischaemia via TLR4/MyD88/MAPK/NF- κ B signalling pathway in MCAO rats, *J. Pharm. Pharmacol.* 70 (2) (2018) 268–277, <https://doi.org/10.1111/jphp.12841>.

B. Yu, M. Ruan, T. Liang, Y. Yu, Synergy between borneol and extract of *Ligusticum Chuanxiong* Hort against cortex and striatum ischemia, *Int. J. Pharmacol.* 16 (2) (2020) 104–119, <https://doi.org/10.3923/ijp.2020.104.119>.

D. Ji-Nan, Z. Yi, Z. Lian-Mei, L. Yue-Min, Z. Wei, B. Li-Gong, A. Qing-Long, L. YiDan, S. Jun, L. Di, Gastrodin inhibits expression of inducible NO synthase, cyclooxygenase-2 and proinflammatory cytokines in cultured LPS stimulated microglia via MAPK pathways, *PLoS One* 6 (7) (2011), e21891, <https://doi.org/10.1186/s12868-019-0512-x>

X.L. Wang, G.H. Xing, B. Hong, X. Li, M. Zou, Y. Zhang, M.X. Dong, Gastrodin prevents motor deficits and oxidative stress in the MPTP mouse model of Parkinson's disease: involvement of ERK1/2-Nrf2 signaling pathway, *Life Sci.* 114 (2) (2014) 77–85, <https://doi.org/10.1016/j.lfs.2014.08.004>.

G. Jiang, Y. Hu, L. Liu, J. Cai, C. Peng, Q. Li, Gastrodin protects against MPP(+)- induced oxidative stress by up regulates heme oxygenase-1 expression through p38 MAPK/Nrf2 pathway in human dopaminergic cells, *Neurochem. Int.* 75 (9) (2014) 7, <https://doi.org/10.1016/j.neuint.2014.06.003>

H. Kim, S. Ham, J.Y. Lee, A. Jo, G.H. Lee, Y.S. Lee, M. Cho, H.M. Shin, D. Kim, O. Pletnikova, J.C. Troncoso, J.H. Shin, Y.I. Lee, Y. Lee, Estrogen receptor activation contributes to RNF146 expression and neuroprotection in Parkinson's disease models, *Oncotarget* 8 (63) (2017) 106721–106739, <https://doi.org/10.18632/oncotarget.21828>

T. Tun, Y.S. Kang, Imperatorin is transported through blood-brain barrier by carrier-mediated transporters, *Biomol. Ther.* 25 (4) (2017) 441–451, <https://doi.org/10.1101/cshperspect.a009316>.

N. Bae, T. Ahn, S. Chung, M.S. Oh, H. Ko, H. Oh, G. Park, O.H. Yang, The neuroprotective effect of modified Yeoldahanso-Tang via autophagy enhancement in models of Parkinson's disease, *J. Ethnopharmacol.* 134 (2) (2011) 313–322, <https://doi.org/10.1016/j.jep.2010.12.016>.

C.S. Vidya, A.M. Mahalakshmi, Neuroprotective role of beta-asarone: a review, *Int. J. Res. Pharmaceut. Sci.* 12 (3) (2021) 1908–1914, <https://doi.org/10.26452/ijrps.v12i3.4792>

Z. Wang, C. He, Y. Peng, F. Chen, P. Xiao, Origins, phytochemistry, pharmacology, analytical methods and safety of Cortex Moutan (*Paeonia suffruticosa* Andrew): a systematic review, *Molecules* 22 (6) (2017) 946, <https://doi.org/10.3390/molecules22060946>

H. Tang, K. Li, S. Zhang, H. Lan, L. Liang, C. Huang, T. Li, Inhibitory effect of Paeonol on apoptosis, oxidative stress, and inflammatory response in human umbilical vein endothelial cells induced by high

glucose and palmitic acid induced through regulating SIRT1/FOXO3a/NF- κ B Pathway, *J. Interferon Cytokine Res.* 41 (3) (2021) 111–124, <https://doi.org/10.1089/jir.2019.0236>.

H.Q. Liu, W.Y. Zhang, X.T. Luo, Y. Ye, X.Z. Zhu, Paeoniflorin attenuates neuroinflammation and dopaminergic neurodegeneration in the MPTP model of Parkinson's disease by activation of adenosine A1 receptor, *Br. J. Pharmacol.* 148 (3) (2006) 314–325, <https://doi.org/10.1038/sj.bjp.0706732>

M.Z. Zheng, C.M. Liu, Y.J. Fan, P. Yan, D.F. Shi, Y.C. Zhang, Neuroprotection by paeoniflorin in the MPTP mouse model of Parkinson's disease, *Neuropharmacology* 116 (2017) 412–420, <https://doi.org/10.1016/j.neuropharm.2017.01.009>

I.S. Kim, H.M. Ko, S. Koppula, B.W. Kim, D.K. Choi, Protective effect of Chrysanthemum indicum Linne against 1-methyl-4-phenylpyridinium ion and lipopolysaccharide-induced cytotoxicity in cellular model of Parkinson's disease, *Food Toxicol.* 49 (4) (2011) 963–973, <https://doi.org/10.1016/j.fct.2011.01.002>

K. Tieu, A guide to neurotoxic animal models of Parkinson's disease, *Cold Spring Harbour Perspect. Med* 1 (1) (2011) 1–20, <https://doi.org/10.1101/cshperspect.a009316>. [24] G.E. Meredith, D.J. Rademacher, MPTP mouse models of Parkinson's disease S.N. Rai, P. Singh, Advancement in the modelling and therapeutics of Parkinson's disease, *J. Chem. Neuroanat.* 104 (2020), 101752, <https://doi.org/10.1016/j.jchemneu.2020.101752>.

[2] S.N. Rai, V.K. Chaturvedi, P. Singh, B.K. Singh, M.P. Singh, Mucuna pruriens in Parkinson's and in some other diseases: recent advancement and future prospective, *3 Biotech* 10 (12) (2020) 522, <https://doi.org/10.1007/s13205-020-02532-7>.

S.N. Rai, P. Singh, R. Varshney, V.K. Chaturvedi, E. Vamanu, M.P. Singh, B. K. Singh, Promising drug targets and associated therapeutic interventions in Parkinson's disease, *Neural Regen. Res.* 16 (9) (2021) 1730–1739, <https://doi.org/10.4103/1673-5374.306066>.

P. Rizek, N. Kumar, M.S. Jog, An update on the diagnosis and treatment of Parkinson disease, *Can. Med. Assoc. J.* 188 (16) (2016) 1157–1165, <https://doi.org/10.1503/cmaj.151179>

L.V. Kalia, A.E. Lang, Parkinson's disease, *Lancet* 386 (9996) (2015) 896–912, [https://doi.org/10.1016/S0140-6736\(14\)61393-3](https://doi.org/10.1016/S0140-6736(14)61393-3)

.Y. Zhang, Z.Z. Wang, H.M. Sun, P. Li, Y.F. Li, N.H. Chen, Systematic review of Traditional Chinese Medicine for depression in Parkinson's disease, *Am. J. Chin. Med.* 42 (5) (2014) 1035–1051, <https://doi.org/10.1142/S0192415x14500657>.

X. Li, Y. Zhang, Y. Wang, J. Xu, P. Xin, Y. Meng, Q. Wang, H. Kuang, The mechanisms of Traditional Chinese Medicine underlying the prevention and treatment of Parkinson's disease, *Front. Pharmacol.* (2017), <https://doi.org/10.3389/fphar.2017.00634>.

J. Li, Q. Zhu, Y.P. Lu, P. Zhao, Z.B. Feng, Z.M. Qian, L. Zhu, Ligustilide prevents cognitive impairment and attenuates neurotoxicity in D-galactose induced aging mice brain, *Brain Res.* 1595 (2015) 19–28, <https://doi.org/10.1016/j.brainres.2014.10.012>.

J. Gu, S. Su, J. Guo, K. Wakamatsu, Anti-inflammatory and anti-apoptotic effects of the combination of Ligusticum chuanxiong and Radix paeoniae against focal cerebral ischaemia via TLR4/MyD88/MAPK/NF- κ B signalling pathway in MCAO rats, *J. Pharm. Pharmacol.* 70 (2) (2018) 268–277, <https://doi.org/10.1111/jph.12841>

B. Yu, M. Ruan, T. Liang, Y. Yu, Synergy between borneol and extract of *Ligusticum Chuanxiong* Hort against cortex and striatum ischemia, *Int. J. Pharmacol.* 16 (2) (2020) 104–119, <https://doi.org/10.3923/ijp.2020.104.119>.

D. Ji-Nan, Z. Yi, Z. Lian-Mei, L. Yue-Min, Z. Wei, B. Li-Gong, A. Qing-Long, L. YiDan, S. Jun, L. Di, Gastrodin inhibits expression of inducible NO synthase, cyclooxygenase-2 and proinflammatory cytokines in cultured LPS stimulated microglia via MAPK pathways, *PLoS One* 6 (7) (2011), e21891, <https://doi.org/10.1186/s12868-019-0512-x>.

X.L. Wang, G.H. Xing, B. Hong, X. Li, M. Zou, Y. Zhang, M.X. Dong, Gastrodin prevents motor deficits and oxidative stress in the MPTP mouse model of Parkinson's disease: involvement of ERK1/2-Nrf2 signaling pathway, *Life Sci.* 114 (2) (2014) 77–85, <https://doi.org/10.1016/j.lfs.2014.08.004>.

G. Jiang, Y. Hu, L. Liu, J. Cai, C. Peng, Q. Li, Gastrodin protects against MPP(+)- induced oxidative stress by up regulates heme oxygenase-1 expression through p38 MAPK/Nrf2 pathway in human dopaminergic cells, *Neurochem. Int.* 75 (9) (2014) 7, <https://doi.org/10.1016/j.neuint.2014.06.003>.

H. Kim, S. Ham, J.Y. Lee, A. Jo, G.H. Lee, Y.S. Lee, M. Cho, H.M. Shin, D. Kim, O. Pletnikova, J.C. Troncoso, J.H. Shin, Y.I. Lee, Y. Lee, Estrogen receptor activation contributes to RNF146 expression and neuroprotection in Parkinson's disease models, *Oncotarget* 8 (63) (2017) 106721–106739, <https://doi.org/10.18632/oncotarget.21828>.

T. Tun, Y.S. Kang, Imperatorin is transported through blood-brain barrier by carrier-mediated transporters, *Biomol. Ther.* 25 (4) (2017) 441–451, <https://doi.org/10.1101/cshperspect.a009316>.

N. Bae, T. Ahn, S. Chung, M.S. Oh, H. Ko, H. Oh, G. Park, O.H. Yang, The neuroprotective effect of modified Yeoldahanso-Tang via autophagy enhancement in models of Parkinson's disease, *J. Ethnopharmacol.* 134 (2) (2011) 313–322, <https://doi.org/10.1016/j.jep.2010.12.016>.

C.S. Vidya, A.M. Mahalakshmi, Neuroprotective role of beta-asarone: a review, *Int. J. Res. Pharmaceut. Sci.* 12 (3) (2021) 1908–1914, <https://doi.org/10.26452/ijrps.v12i3.4792>.

Z. Wang, C. He, Y. Peng, F. Chen, P. Xiao, Origins, phytochemistry, pharmacology, analytical methods and safety of Cortex Moutan (*Paeonia suffruticosa* Andrew): a systematic review, *Molecules* 22 (6) (2017) 946, <https://doi.org/10.3390/molecules22060946>.

H. Tang, K. Li, S. Zhang, H. Lan, L. Liang, C. Huang, T. Li, Inhibitory effect of Paeonol on apoptosis, oxidative stress, and inflammatory response in human umbilical vein endothelial cells induced by high glucose and palmitic acid induced through regulating SIRT1/FOXO3a/NF- κ B Pathway, *J. Interferon Cytokine Res.* 41 (3) (2021) 111–124, <https://doi.org/10.1089/jir.2019.0236>.

H.Q. Liu, W.Y. Zhang, X.T. Luo, Y. Ye, X.Z. Zhu, Paeoniflorin attenuates neuroinflammation and dopaminergic neurodegeneration in the MPTP model of Parkinson's disease by activation of adenosine A1 receptor, *Br. J. Pharmacol.* 148 (3) (2006) 314–325, <https://doi.org/10.1038/sj.bjp.0706732>.

M.Z. Zheng, C.M. Liu, Y.J. Fan, P. Yan, D.F. Shi, Y.C. Zhang, Neuroprotection by paeoniflorin in the MPTP mouse model of Parkinson's disease, *Neuropharmacology* 116 (2017) 412–420, <https://doi.org/10.1016/j.neuropharm.2017.01.009>.

I.S. Kim, H.M. Ko, S. Koppula, B.W. Kim, D.K. Choi, Protective effect of *Chrysanthemum indicum* Linne against 1-methyl-4-phenylpyridinium ion and lipopolysaccharide-induced cytotoxicity in cellular model of Parkinson's disease, *Food Toxicol.* 49 (4) (2011) 963–973, <https://doi.org/10.1016/j.fct.2011.01.002>.

K. Tieu, A guide to neurotoxic animal models of Parkinson's disease, *Cold Spring Harbour Perspect. Med* 1 (1) (2011) 1–20, <https://doi.org/10.1101/cshperspect.a009316>. [24] G.E. Meredith, D.J. Rademacher, MPTP mouse models of Parkinson's disease: an update, *J. Parkinsons Dis.* 1 (1) (2011) 19–33, <https://doi.org/10.3233/JPD-2011-11023>.

B. Butler, K. Saha, T. Rana, J.P. Becker, D. Sambo, P. Davari, J.S. Goodwin, H. Khoshbouei, Dopamine transporter activity is modulated by α -synuclein, *J. Biol. Chem.* 290 (49) (2015) 29542–29554, <https://doi.org/10.1074/jbc.M115.691592>.

T.N. Martinez, J.T. Greenamyre, Toxin models of mitochondrial dysfunction in Parkinson's disease, *Antioxid. Redox Signal.* 16 (9) (2012) 920–934, <https://doi.org/10.1089/ars.2011.4033>. [27] R.L. Vaz, T.F. Outeiro, J.J. Ferreira, Zebrafish as an animal model for drug discovery in Parkinson's disease and other movement disorders: a systematic review, *Front. Neurol.* 9 (2018) 347, <https://doi.org/10.3389/fneur.2018.00347>

A.V. Kalueff, A.M. Stewart, R. Gerlai, Zebrafish as an emerging model for studying complex brain disorders, *Trends Pharmacol. Sci.* 35 (2) (2014) 63–75, <https://doi.org/10.1016/j.tips.2013.12.002>.

E. Rink, M.F. Wullimann, Development of the catecholaminergic system in the early zebrafish brain: an immunohistochemical study, *Brain Res. Dev. Brain Res.* 137 (1) (2002) 89–100, [https://doi.org/10.1016/S0165-3806\(02\)00354-1](https://doi.org/10.1016/S0165-3806(02)00354-1).

L. Wen, W. Wei, W. Gu, P. Huang, X. Ren, Z. Zhang, Z. Zhu, S. Lin, B. Zhang, Visualization of monoaminergic neurons and neurotoxicity of MPTP in live transgenic zebrafish, *Dev. Biol.* 314 (2008) 84–89, <https://doi.org/10.1016/j.ydbio.2007.11.012>

R. Perfeito, T. Cunha-Oliveira, A.C. Rego, Revisiting oxidative stress and mitochondrial dysfunction in the pathogenesis of Parkinson disease – resemblance to the effect of amphetamine drugs of abuse, *Free Rad. Biol. Med.* 53 (9) (2012) 1791–1806, <https://doi.org/10.1016/j.freeradbiomed.2012.08.569>. S. Dongjie et al. *Biomedicine & Pharmacotherapy* 148 (2022) 112706 10

J. Nellore, P. Nandita, Paraquat exposure induces behavioral deficits in larval zebrafish during the window of dopamine neurogenesis, *Toxicol. Rep.* 2 (2015) 950–956, <https://doi.org/10.1016/j.toxrep.2015.06.007>.

H.G. Kim, G. Park, Y. Piao, M.S. Kang, Y.K. Pak, S.P. Hong, M.S. Oh, Effects of the root bark of *Paeonia suffruticosa* on mitochondria-mediated neuroprotection in an MPTP-induced model of Parkinson's disease, *Food Chem. Toxicol.* 65 (2014) 293–300, <https://doi.org/10.1016/j.fct.2013.12.037>.

J.M. Beaulieu, R.R. Gainetdinov, The physiology, signaling, and pharmacology of dopamine receptors, *Pharmacol. Rev.* 63 (1) (2011) 182–217, <https://doi.org/10.1124/pr.110.002642>.

W. Boehmler, T. Carr, C. Thisse, B. Thisse, V.A. Canfield, R. Levenson, D4 dopamine receptor genes of zebrafish and effects of the antipsychotic clozapine on larval swimming behaviour, *Genes Brain Behav.* 6 (2) (2007) 155–166, <https://doi.org/10.1111/j.1601-183X.2006.00243.x>

3. AIM;

Targeting miRNA expression in early stage of zebrafish .pd model through ashwagandha

OBJECTIVES ;

Inducing a chemogenetic dopamine (DA) neuron ablation model in larval zebrafish (Administration of MPP+ to zebrafish embryos between 18hpf and 96hpf to create a model of PD)To assess the time specific change in nurr 1, transcription factor that regulates the dopamine neuron differentiation and survival in zebrafish pd model To asses the time specific change in the miRNA targeting nurr 1(computationally predicted)in zebrafish PD modelTo investigate how ashwagandha extract affect the miRNA response to DA injury evoked by MPP+ in

4. MATERIALS AND METHODS

4.1.SAMPLE COLLECTION

Sample are collected from the nearby Ayurveda shop

4.2.EXTRACT PREPARATION

Sample was extracted using of distilled water .take 10 g of ashwagandha powder add 100 ml of distilled water keep the sample in 40 c in hot air over.keep the sample in hot air oven through overnight and the next day the sample was taken and do phytochemical analysis.

4.3.ANIMAL

Early stage of zebrafish embryos were taken and Many experiments were done on zebrafish embryos.the collected embryos were immediately rinsed with water and transferred to 8 tissue culture flask with E3 medium with each 50 embryos and incubated at room temperature.Dead individuals were removed and E3 medium changed daily.

4.4.EXPOSURE PROCEDURES

Zebrafish embryos were raised in 8 tissue culture flask contain E3 medium with a volume of .For ashwagandha exposure standard room temperatures were used.To the larvae at 18hours post fertilization(Hpf) ashwagandha powder's solution were added.An untreated control group of larvae was examined in parallel.after this the flasks were incubated at room temperature.dead larvae were removed daily and solution of each group was disposed of completely and replaced with fresh E3 medium solution daily.the larvae were exposed to ashwagandha till 96 Hpf and ashwagandha powder extract were changed and replaced with freshly prepared daily.under safety protocols all the toxins were discarded.the developmental effects of embryos due to treatment with ashwagandha,observations were made at 24,48,72,96 Hpf and embryos

were raised to adult stage. Mortality and morphological deformities of embryos were recorded and photographed. dead embryos were removed immediately after observation...

4.5.BEHAVIORAL ANALYSIS

Zebrafish's locomotor activity were noted by placing the embryos on petri dish marked with 1cm grid lines rows and columns. the locomotion were observed for 1 minute by allowing it to move through rows and columns and calculate the distance traveled by the fish from 24hpf to 96hpf. in 24hpf the embryos tail movement was observed under microscope. at 48-96hpf, the embryos locomotor activity was studied in petri plate marked with 1cm grid lines. the embryos movement across the grid lines was observed for period of 1 minute from which the distance covered by the embryo was calculated.

RNA isolation

Principle

Guanidinium has powerful protein denaturant and property to inactivation of RNases and its association with acidic phenol to disrupt nucleoprotein complexes, allowing the RNA to release into aqueous supernatant for separation. Then RNA is selectively precipitated with isopropanol and purified by using 75% ethanol finally dissolved in nuclease free water.

Procedure

50 embryos were taken in micro centrifuge tube, Trizol was used for RNA isolation. 250µl of trizol is added to tube and triturated with embryos and mixed by vortexing followed by incubated at room temperature for 15 minutes. Then 100µl of chloroform was added and mixed by inverting 3 times then incubated for 15 minutes followed by centrifuged at 12,000rpm for 10 minutes at 4°C. then supernatant was removed and 500µl ice cold isopropanol And incubated at -20°C for overnight. Later the samples are centrifuged at 12000rpm for 10 minutes. after supernatant was discarded and pellet was washed with 75% ethanol followed by vortex and centrifuged at 7500rpm for 5 minutes. finally, RNA pellet was dried for 5-10 minutes and 30µl of nuclease free water is added.

Electrophoretic analysis of RNA:

The purity of the extracted RNA was checked by using 21 M formaldehyde denatured

agarose gel electrophoresis (Sambrook and Russell. 2001) 2% agarose gel was prepared by melting required amount of agarose in DEPC treated water, cooled to 60°C and formaldehyde was added to a final concentration of 1X and 2.2M respectively. The solution was poured into the tray and allowed to solidify for 30 min at room temperature. The RNA samples were prepared by mixing 2 µl RNA with 2 µl of 10X gel running buffer (MOPS), 4.0 µl of formaldehyde and 10 µl of formamide. The mixture was kept at 65°C for 15 min and immediately chilled on ice for 5 min. After a brief pre run of the gel for 5 min at 5 V/cm, the samples were loaded into the wells and was run in 1X MOPS gel running buffer for 2 hrs and viewed under UV- transilluminator. Good quality RNA shows two intact bands (16s and 24s rRNA) with smearing in between (mRNA)

DNA Isolation

Principle

The membrane and nucleus membrane lysed with the lysis solution and proteins and cell components were released into aqueous solution. combination of phenol and chloroform denature and separate proteins from DNA. Released DNA from disrupted cells was precipitated by cold isopropanol, after precipitate the salts were removed by washing 75% ethanol. the DNA isolation need to separate from the rest of cell components.

Procedure

Zebrafish embryos (50) were taken into micro centrifuge tube and homogenized with 250 µl of trizol and triturated embryos and mixed by vortexing followed by incubated at room temperature for 15 minutes. Then 100 µl of chloroform was added and mixed by inverting 3 times then incubated for 15 minutes followed by centrifuged at 12,000 rpm for 10 minutes at 4°C. then supernatant was removed. To the bottom layer 100 µl of 100% ethanol is added then mixed by inverting it and incubate at room temperature for 2-3 minutes followed by centrifuged at 2000g for 5 minutes at 4°C. resuspend the pellet in 500 µl of 0.1M sodium citrate and incubated for 30 minutes. again centrifuged at 2000g for 5 minutes at 4°C then the supernatant is discarded and again resuspend the pellet in 500 µl of 0.1M sodium citrate and incubated for 30 minutes. again centrifuged at 2000g for 5 minutes at 4°C. resuspend the pellet in 1ml of 75% ethanol and incubated for 10-20 minutes followed by centrifuged at 2000g for 5 minutes at 4°C and DNA pellet was air dried at 37°C. finally, the DNA pellet was dissolved in 30 µl of nuclease free water.

Polymerase Chain Reaction

Principle

The polymerase chain reaction (PCR) is a laboratory technique of molecular biology to amplify a target DNA sequence based on the thermal cycling conditions. It consists a repeated cycles of heating and cooling of the reaction mixture involves the denature of double standard DNA and followed by the binding complementary short nucleotide to target DNA along with the help of polymerase to synthesize new strand of DNA complementary to the target template. As PCR progress template DNA amplify (Pavlov et al., 2004).

Agarose gel electrophoresis

principle

Gel electrophoresis separates DNA fragments by size in a solid support medium (an Agarose gel). Agarose is a natural linear polymer extracted from seaweed that forms a gel matrix by hydrogen-bonding when heated in buffer. and allowed to cool. When in electrical field Agarose matrix allows the negatively charged DNA to move towards the cathode based on the size Finally DNA is visualized using an intercalating dye, Ethidium bromide under the ultraviolet light.

Procedure

1.5% Agarose solution was prepared in 50 mL of IX TBE and it ut boiled in microwave oven for 3 min. After few min the solution was brought to hand bearable temperature, 6µl of Ethidium bromide (10 mg/ml) was added and mixed followed by poured in to the gel casting tray. After complete solidification gel was transferred to the electrophoresis tank which contains IX TBE buffer and equipped with power supply. To the gel, 10µl of PCR amplified product was loaded into the appropriate well and 2µl of 100 bp Marker was added followed by electrical field (100V for 30 min) was appliedto separate the DNA.Gel images were captured in gel documentation system(Bio Rad,USA) (Ylmaz et al.,2012).

Purification of PCR product

After visualizing in gel documentation system, DNA fragment was excised with a clean, sharp scalpel.To the gel slice 400µl of Buffer QG was added and incubated at at 50°C for 10 min to complete dissolve the gel.Then the suspension was precipitated by adding the one volume of isopropanol and transferred to the QIA quick spin column.The spin

column was centrifuged at 12000 rpm for 1 minute. Then the column was washed with the 500 µl Buffer QG for 2 times at 12000 rpm for 1 minutes. To remove the droplets of buffer QG column was centrifuged at 14000 rpm for 3 minutes. Finally, DNA fragment was eluted by adding 40 µl of elution buffer followed by centrifugation at 12000 rpm for 1 minute and product was stored at -20°C for further use (Vogelstein and Gillespie, 1979).

miRNA

The isolated RNA is diluted using 200µl of DEPC water taken in centrifuge tube. The blue ring column is placed over the collection tube and RNA is added to it then centrifuged at 11,000 x g for 1 minute. Remove the blue ring column but don't discard, Take the supernatant flow through and add 300µl Buffer ML then vortex later centrifuge it at 11,000 x g for 3 minutes. Protein precipitate column is placed and centrifuged at 11,000 x g at 1 minute. 800µl Buffer MX is added to flowthrough then discard the column. Place the blue ring column again and add the whole mix into it then centrifuge for 30 seconds at 11,000 x g. First wash is done by adding 700µl Buffer MW2 to column and centrifuged for 30 seconds at 11,000 x g then discard the flowthrough. For the second wash 250µl Buffer MW2 is added to column and centrifuged for 2 minutes at 11,000 x g then discard the flowthrough. Place the column in a new column tube and 30l of RNase-free water is added. Then incubated for 1 minute at room temperature and centrifuge for 30 seconds at 11,000 x g.

ANALYSIS DURING EXPERIMENTAL TREATMENTS:

Toxicity assay

Medium was changed regularly for every 24 hour intervals with fresh E3 medium. Dead embryos were removed and the live embryos were continuously exposed with the respective temperature during development. The mortality, neurodegenerative phenotypes including a flat head, small eyes, pinched midbrain-hindbrain boundary, thin yolk extension, and curved-up body of embryos at 24 hpf, 48 hpf, 72 hpf and 96 hpf respectively in the specified range of Temperature will be analyzed by using stereo zoom microscope.

Locomotor activity

The swimming activity of the larvae i.e., 96 hpf were evaluated (Behra et al., 2002; Westerfield, 1993) A petri plate was marked with graph lines of 1cm distance. Embryos

were allowed to swim in the petri plate for 1 minute and the total distance crossed was noted. This was performed for 6 embryos from each samples at 24, 48, 72, and 96 hpf for each concentrations of each treatments The duration of the swimming movements was measured with a stopwatch from the recorded film. In these experiments, only the swimming movements were scored

Glutathione estimation- Antioxidant assay

The GSH content in the zebrafish embryos was determined by Moron et al (1979). To the supernatant, 5% Trichloroacetic acid (TCA) was added and centrifuged for 5000rpm for 10 minutes. The resulting supernatant was collected and used for GSH estimation. The reaction mixture contains a total volume of 3.6ml. The test sample contains 2.4ml of 0.2M Phosphate buffer, 0.6ml of 0.6mM DTNB [5,5-dithiobis (2- nitrobenzoic acid)] and 0.6ml of tissue homogenate and the blank contains 2.4ml of 0.2M Phosphate buffer, 0.6ml of 0.6mM DTNB [5,5-dithiobis (2- nitrobenzoic acid)] and 0.6 ml of 5% TCA. The yellow colour developed was read immediately at 412nm on a spectrophotometer. The GSH content was calculated as pmol DTNB conjugate formed/gram tissue using molar extinction coefficient of $13.6 \times 10^3 \text{ NA}^{-1} \text{ cm}^{-1}$. With the help of formula

$$\text{GSH} = \frac{\text{AO. D I min} \times \text{Vol. of assay} \times 100}{\text{-----}}$$

1.36 of mole GSH conjugate/gm tissue

4.6. PHYTOCHEMICAL QUANTITATIVE ANALYSIS

4.6.1. Detection of alkaloids

Add few drops of Mayer's reagent to 1 ml of the extract. Formation of yellow coloured precipitate indicates the presence of In 100g of sample i took 10g of ashwagandha powder and add 100 ml of distilled water keep that sample in

hot air

alkaloids.

4.6.2. Detection of carbohydrates

Add few drops of Benedict's reagent to the extract and heat it for 5 minutes.

Appearance of orange red colour indicates the presence of reducing sugars.

4.3.3. 4.6.3. Detection of glycosides

To 1 ml of the plant extract add 2 ml of ferric chloride and immerse the contents in boiling water bath for about 5 minutes. Cool the mixture and extracted with equal volume of benzene and treat it with equal amount of Ammonia solution. Formation of rose pink colour in the ammonical layer indicates the presence of anthranol glycosides.

4.6.4. Detection of saponins

Take 1ml of the plant extract and dilute it with distilled water to 5 ml and shake the contents continuously for five minutes. Formation of foam indicates the presence of saponins.

4.6.5. Detection of phytosterols

To 1ml of the extract add 1ml of chloroform and few drops of conc. Sulphuric acid. Shake the contents and allow it to stand for few minutes. Appearance of golden yellow colour indicates the presence of triterpenes.

16

4.6.6. Detection of phenols

Add 3-4 drops of ferric chloride solution to 1ml of the extract. Observe for the formation bluish black colour which then indicates the presence of phenols. **4.3.7.**

4.6.7. Detection of tannins

To 2ml of the extract add few drops of ferric chloride solution appearance of blue colour indicate the presence of blue colour hydrolysable tannins and green colour indicates the presence of condensed tannins.

4.6.8. Detection of flavonoids

To 2ml of the extract add 2ml of sodium hydroxide solution. Intense yellow colour formation indicates the presence of flavonoids.

4.6.9. Detection of proteins and amino acids

To 1ml of the extract add few drops of concentrated Nitric acid. Formation of yellow colour indicates the presence of proteins.

4.7. PHYTOCHEMICAL QUANTATIVE ANALYSIS

4.7.1. Estimation of phenols

S.No	Reagents
1.	ashwagandha-1mg/ml
2.	methonal

18

3.	folin ciocalteau reagent
4.	20% sodium carbonate

Table 4.7.1 represents the reagents required for estimation of phenol Procedure

100 µl of extract from 1 mg/MI solution 900µl methanol was added and then

1ml of 1:10 diluted folin ciocalteau reagent was added.

To this 1ml of 20% sodium carbonate solution was also added

And Shaken well and incubated at room temperature for 30 mins in dark.

Absorbance of supernatant was taken at 765 nm.

4.8. ANTIOXIDANT ASSAY

PREPARATION OF EXTRACT

About 1g of sample was soaked in Ethanol for 72 h at room temperature. Then, the supernatant was filtered and extracted .It was used for further analysis.

DPPH radical scavenging activity (Blois, 1958)

The DPPH radical scavenging activity was determined according to the method of Blois (1958). DPPH offers a convenient and accurate method for oxidizable groups of natural or synthetic antioxidants. DPPH solution was prepared at the concentration of 0.1mM in methanol. During the assay, 1mL of test solution (20-120 µg/mL) was mixed with 1mL of DPPH solution. The mixture was incubated at room temperature in dark for 30min. The absorbance was recorded at 517 nm by UV spectrophotometer. The percentage of DPPH free radical scavenging activity was calculated by following equation:

$$\text{RSA \%} = [(\text{Abs (control)} - \text{Abs (Sample)}) / \text{Abs (Control)}] \times 100$$

5. RESULTS AND DISCUSSION

5.1. Phytochemical analysis

The phytochemical screen of the seven plants are listed in the table below. From the table +ve represent the presence of the phytochemical whereas –ve represents the absence of the phytochemical. Proteins and carbohydrates are present in all the plants. Glycosides are absent in most of the plants except *Zingiber officinale* and *Catunaregum spinose*. Phenols are present in most plants except in *Calotropis gigantea* and *Calamus rotang*. Whereas phytoesters are present in most plants except *Zingiber officinale*, *Acacia Arabica* and *Catunaregum spinose*. Saponins are absent only in *Acacia Arabica*. The presence of flavonoids are detected in plants except *Terminalia arjuna* and *Acacia Arabica*. Tannins are present only in *Zingiber officinale*, *Cassia auriculata* and *Catunaregum spinose*. The presence of alkaloids is detected in plants except *Calotropis gigantea*, *Calamus rotang*, *Catunaregum spinose*. Screening of the fruits extract of *Catunaregum spinose* is showed out the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, cardiac glycosides, steroids, terpenoids, phlobatannins and alkaloids(Anand (2007)). The phytochemical composition of the *Z. officinale* has been extensively studied in the past studies. *Z. officinale* is reported to possess essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, terpenoids and tannin as the major phytochemical groups These phytochemicals plays an important role in the medicinal property of this plant (Otunola et al , Sasidharan et al, Adel e.at.(2010)). Anitha (2014) reported that *Cassia fistula* contains alkaloids, tannins, flavonoids, terpenes, sugars, and glucosides. Flavonoids have been shown to possess various biological properties related to the antioxidant, anti nociceptive and anti-inflammatory mechanisms by targeting

reactive oxygen species and prostaglandins which are involved in the late phase of acute inflammation and pain perception.

23

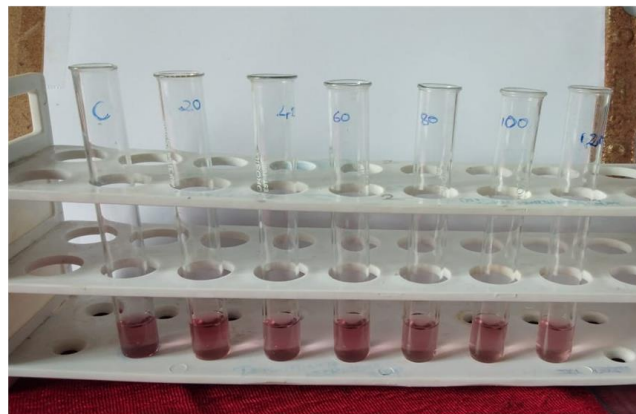
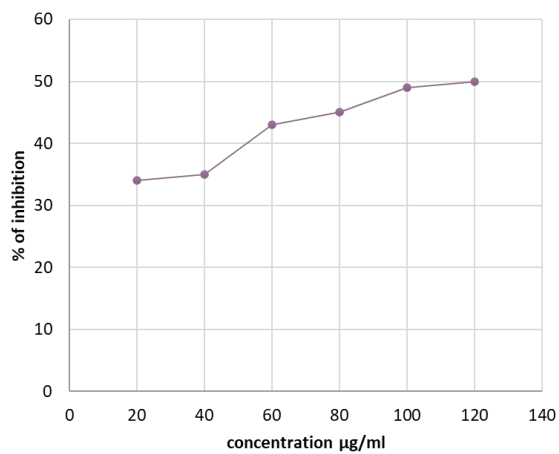
S.NO	PHYTOCHEMICALS	<i>Zingiber officinale</i>	<i>Terminalia arjuna</i>	<i>casia earicalata</i>	<i>calotropis gigantea</i>	<i>acacia arabica</i>	<i>calamus rotang</i>	<i>catunaregum spinose</i>
1	Carbohydrates	+VE	-VE	+VE	+VE	+VE	+VE	+VE
2	Glycosides	+VE	-VE	-VE	-VE	-VE	-VE	+VE
3	Phenol	+VE	+VE	+VE	-VE	+VE	-VE	+VE
4	Phytoesters	-VE	+VE	+VE	+VE	-VE	+VE	-VE
5	Saponins	+VE	+VE	+VE	+VE	-VE	+VE	+VE
6	Flavonoids	+VE	-VE	+VE	+VE	-VE	+VE	+VE
7	Tannins	-VE	+VE	+VE	-VE	-VE	-VE	+VE
8	Alkaloids	+VE	+VE	+VE	-VE	+VE	-VE	-VE
9	Proteins	+VE	+VE	+VE	+VE	+VE	+VE	+VE

Table 5.1 represents the phytochemical analysis of the seven plants.

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to anti-oxidative action. The total phenolic content of Terminalia arjuna was reported by Sundar. S. Mety & Pratima Mathad (2011). It was found to be 1.9543 ± 0.013 in ethanolic extract was reported by Satheesh Kumar et al. (2013). The present study for Terminalia arjuna was carried out and it was recorded 630µg/ml for T. arjuna. The result was compared with standard Gallic acid (5-100µg/ml) and was found to contain good amount of phenols in the extract.

S.NO	OD AT 765nm	GAE (µg/mg)
1	1.961	101

Figure 5.1 represents the result of estimation of phenol



DPPH RADICAL SCAVENGING ASSAY

S.NO

CONCENTRATIONS

OD VALUE

% OF INHIBITION

(µg)

1

Control

0.256

--

2

2

0.080

68.75

3

4

0.066

74.21

4

6

0.050

80.46

5

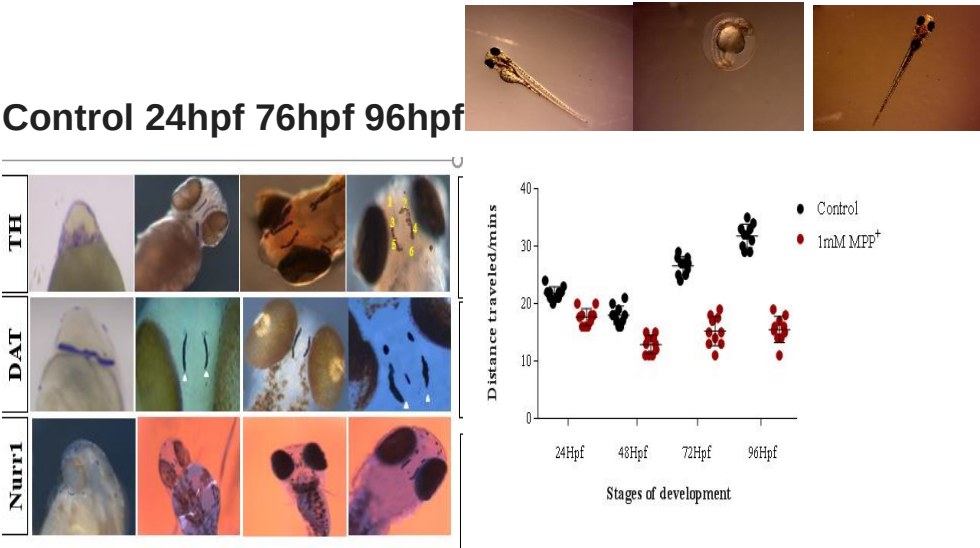
8

0.042

83.59

6	10	0.038	85.15
7	12	0.029	88.67

Figure 5.3 represents the concentration of Asboric acid



TF's	miRNA	24Hpf		48Hpf		72Hpf		96Hpf	
		Fold Change	p-Value	Fold Change	p-Value	Fold Change	p-Value	Fold Change	p-Value
Nurr1	miR-34c-5p	1.719101	0.04	1.712766	0.04	1.612796	0.04	1.520126	0.04
	miR-34a-5p	1.627273	0.04	1.807229	0.04	1.515094	0.04	-1.6415	0.04
	miR-449b-5p	1.983607	0.04	-1.91667	0.04	1.57619	0.04	-1.80328	0.04
	miR-449a	-1.61538	0.04	-1.63934	0.04	-5	0.03	-1.70732	0.04
	miR-183-5p.1	-1.97183	0.04	-1.49378	0.06	-1.8529	0.04	-1.57062	0.04
	miR-30e-5p	1.661157	0.04	1.882353	0.04	-1.52424	0.04	-1.92059	0.04
	miR-30c-5p	1.828829	0.04	1.5484	0.04	1.796748	0.04	-7.21429	0.04
	miR-30a-5p	-1.05263	0.07	-1.78431	0.04	-8.33333	0.03	-1.5556	0.04
	miR-30b-5p	-1.19608	0.04	-1.67647	0.04	3.69697	0.03	-1.82941	0.04
	miR-30d-5p	0	0	-1.8235	0.04	1.385093	0.06	-1.5864	0.04
	miR-373-3p	-1.7965	0.04	1.541176	0.04	1.99187	0.04	-1.6963	0.04
	miR-520a-3p	-1.8018	0.04	-1.8824	0.04	-1.991	0.04	-1.9547	0.04
	miR-520c-3p	-1.63333	0.04	-1.5128	0.04	1.403333	0.04	1.970451	0.04
	miR-520e	1.809091	0.04	-4.66667	0.03	1.67155	0.04	1.518667	0.04
	miR-206	-6.36842	0.03	-3.58333	0.03	1.51036	0.04	1.732965	0.04
	miR-1-3p	1.94467	0.04	1.804511	0.04	1.569106	0.04	1.58566	0.04
	miR-613	-1.54444	0.04	1.827068	0.04	1.911765	0.04	1.554328	0.04
	miR-132-3p	1.91791	0.04	1.648256	0.04	1.59722	0.04	1.655882	0.04
	miR-212-3p	-1.63889	0.04	-1.81667	0.04	1.5691	0.04	-1.6195	0.04
	miR-372-3p	1.631429	0.04	-8.44118	0.03	1.64815	0.04	1.801538	0.04
	miR-106a-5p	1.822674	0.04	-6.54688	0.03	1.542975	0.04	1.699784	0.04
	miR-519d-3p	1.506818	0.04	-5.60938	0.03	1.510681	0.04	1.52	0.04
	miR-520b	1.71084	0.04	-3.97674	0.03	1.80556	0.04	-1.74766	0.04
	miR-106b-5p	1.701754	0.04	1.582759	0.04	1.53678	0.04	-1.52342	0.04
	miR-20a-5p	1.738806	0.04	1.43871	0.06	1.506497	0.04	1.707447	0.04
	miR-33a-5p	1.176072	0.04	1.715447	0.04	1.5375	0.04	-4.7234	0.04
	miR-520d-3p	1.53488	0.04	1.71271	0.04	1.5161	0.04	1.743534	0.04
	miR-129-5p	1.548889	0.04	1.711111	0.04	1.61491	0.04	1.5190066	0.04

- **References;** Kumar GP, Khanum F. Neuroprotective potential of phytochemicals. *Pharmacognosy Reviews*. 2012;6(12):81-9
 - Kuboyama T, Tohda C, Komatsu K. Effects of ashwagandha (roots of *Withania somnifera*) on neurodegenerative diseases. *Biological and Pharmaceutical Bulletin*. 2014;37(6):892-897.
 - Kuboyama T, Tohda C, Komatsu K. Neuritic regeneration and synaptic reconstruction induced by withanolide A. *British journal of pharmacology*. 2005;144(7):961-971.
 - Guizani N, Waly MI, Ali A, et al. Papaya epicarp extract protects against hydrogen peroxide-induced oxidative stress in human SH-SY5Y neuronal cells. *Experimental Biology and Medicine*. 2011;236(10):1205-1210.
 - More SV, Kumar H, Kang SM, Song S-Y, Lee K, Choi D-K. Advances in Neuroprotective Ingredients of Medicinal Herbs by Using Cellular and Animal Models of Parkinson's Disease. *Evidence-based Complementary and alternative medicine*. 2013;2013:15.
 - . Kuboyama T, Tohda C, Komatsu K. Withanoside IV and its active metabolite, sominone, attenuate A β (25–35)-induced neurodegeneration. *European Journal of Neuroscience*. 2006;23(6):1417-1426.
 - . Essa MM, Guillemin G, Al-Rawahi A, Singh V, Guizani N, Memon A. Walnuts (*juglans regia* linn) and its health benefits. 2012.
 - . Guizani N, Essa MM, Al-Kharousi Z, Singh V, Soussi B, Guillemin G. Omega 3 fatty acids and its potential health benefits. 2012.
 - Singh V, Essa MM, Waly M, Ali A, Guizani N, Guillemin G. Mango (*Mangifera indica* linn.) and its health benefits. 2012.
 - Dries DR, Yu G, Herz J. Extracting β -amyloid from Alzheimer's disease. *Proceedings of the National Academy of Sciences*. 2012;109(9):3199-3200.
 - Mishra L-C, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review. *Alternative Medicine Review*. 2000;5(4):334-346.
 - . Kulkarni S, Dhir A. *Withania somnifera*: an Indian ginseng. *Progress in NeuroPsychopharmacology and Biological Psychiatry*. 2008;32(5):1093-1105.
- The Canadian Journal of Clinical Nutrition, Volume 5, Issue 2, June 2017
 ISSN 1927-8942 (Print Edition), ISSN 1927-8950 (Online Edition) The Canadian Journal of Clinical Nutrition is published by Global Science Heritage, (<http://www.globalscienceheritage.org>) Registered publisher by the Library and Archives/Government of Canada, (www.collectionscanada.gc.ca)
- Page 47 of 13. Dafni A, Yaniv Z. Solanaceae as medicinal plants in Israel.

Journal of ethnopharmacology. 1994;44(1):11-18. 14. Andallu B, Radhika B. Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (*Withania somnifera*, Dunal) root. Indian Journal of Experimental Biology. 2000;38(6):607-609.

- Rajeswara Rao B. Opportunities and challenges in the cultivation of Ashwagandha [*Withania somnifera* (L.) Du-nal]. Journal of Pharmacognosy, ISSN. 2012.
- Rātsch C. The encyclopedia of psychoactive plants: ethnopharmacology and its applications. Inner Traditions/Bear & Co; 2005.
- Elsakka M, Grigorescu E, Stănescu U, Dorneanu V. New data referring to chemistry of *Withania somnifera* species. Revista medico-chirurgicală a Societății de Medici și Naturaliști din Iași. 1990;94(2):385. Puri H. Simple Ayurvedic Remedies. UBS Publishers Distributors; 2002.
- . Verma SK, Kumar A. Therapeutic uses of *Withania somnifera* (Ashwagandha) with a note on withanolides and its pharmacological actions. Asian Journal of Pharmaceutical and Clinical Research. 2011;4(1):01-04.
- . Ghosal S, Lal J, Srivastava R, et al. Immunomodulatory and CNS effects of sitoindosides IX and X, two new glycowithanolides from *Withania somnifera*. Phytotherapy Research. 1989;3(5):201-206.
- . Bhattacharya S, Satyan KS, Ghosal S. Antioxidant activity of glycowithanolides from *Withania somnifera*. Indian Journal of Experimental Biology. 1997;35:236- 239.
- Malhotra C, Mehta V, Prasad K, Das P. Studies on *Withania ashwagandha*, Kaul. IV. The effect of total alkaloids on the smooth muscles. Indian journal of physiology and pharmacology. 1965;9(1):9-15.
- . Grandhi A, Mujumdar A, Patwardhan B. A comparative pharmacological investigation of Ashwagandha and Ginseng. Journal of ethnopharmacology. 1994;44(3):131-135.
- Sharma S, Dahanukar S, Karandikar S. Effects of long term administration of the roots of ashwagandha and shatavari in rats. 1985.
- . Bhattacharya S, Muruganandam A. Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress. Pharmacology Biochemistry and Behavior. 2003;75(3):547-555.
- Bhattacharya A, Muruganandam A, Kumar V, Bhattacharya S. Effect of poly herbal formulation, EuMil, on neurochemical perturbations induced by chronic stress. Indian Journal of Experimental Biology. 2002;40(10):1161-1163.
- . Dhuley JN. Retracted: Nootropic-like effect of ashwagandha (*Withania*

somnifera L.) in mice. *Phytotherapy Research*. 2001;15(6):524-528.

- Ahmad M, Saleem S, Ahmad AS, et al. Neuroprotective effects of *Withania somnifera* on 6-hydroxydopamine induced Parkinsonism in rats. *Human & experimental toxicology*. 2005;24(3):137-147.
- . Schliebs R, Liebmann A, Bhattacharya SK, Kumar A, Ghosal S, Bigl V. Systemic administration of defined extracts from *Withania somnifera* (Indian Ginseng) and *The Canadian Journal of Clinical Nutrition*, Volume 5, Issue 2, June 2017 ISSN 1927-8942 (Print Edition), ISSN 1927-8950 (Online Edition) The Canadian Journal of Clinical Nutrition is published by Global Science Heritage, (<http://www.globalscienceheritage.org>) Registered publisher by the Library and Archives/Government of Canada, (www.collectionscanada.gc.ca) Page48 Shilajit differentially affects cholinergic but not glutamatergic and GABAergic markers in rat brain. *Neurochemistry international*. 1997;30(2):181-190.
- Glotter E. Withanolides and related ergostane-type steroids. *Natural product reports*. 1991;8(4):415-440.
- Tohda C, Kuboyama T, Komatsu K. Dendrite extension by methanol extract of *Ashwagandha* (roots of *Withania somnifera*) in SK-N-SH cells. *Neuroreport*. 2000;11(9):1981-1985.
- Russo A, Izzo A, Cardile V, Borrelli F, Vanella A. Indian medicinal plants as antiradicals and DNA cleavage protectors. *Phytomedicine*. 2001;8(2):125-132.
- . Parihar M, Hemnani T. Phenolic antioxidants attenuate hippocampal neuronal cell damage against kainic acid induced excitotoxicity. *Journal of biosciences*. 2003;28(1):121-128.
- . Choudhary MI, Nawaz SA, Lodhi MA, et al. Withanolides, a new class of natural cholinesterase inhibitors with calcium antagonistic properties. *Biochemical and biophysical research communications*. 2005;334(1):276-287.
- Sehgal N, Gupta A, Valli RK, et al. *Withania somnifera* reverses Alzheimer's disease pathology by enhancing low-density lipoprotein receptor-related protein in liver. *Proceedings of the National Academy of Sciences*. 2012;109(9):3510- 3515.
- Kurapati KRV, Atluri VSR, Samikkannu T, Nair MP. *Ashwagandha* (*Withania somnifera*) Reverses β -Amyloid Induced Toxicity in Human Neuronal Cells: Implications in HIV-Associated Neurocognitive Disorders (HAND). 2013.
- . Nagashayana N, Sankarankutty P, Nampoothiri M, Mohan P, Mohanakumar K. Association of L-DOPA with recovery following Ayurveda medication in Parkinson's disease. *Journal of the neurological sciences*. 2000;176(2):124-

- Sankar SR, Manivasagam T, Krishnamurti A, Ramanathan M. The neuroprotective effect of *Withania somnifera* root extract in MPTP-intoxicated mice: An analysis of behavioral and biochemical variables. *Cellular & molecular biology letters*. 2007;12(4):473-481.
- Kumar P, Kumar A. Possible neuroprotective effect of *Withania somnifera* root extract against 3-nitropropionic acid-induced behavioral, biochemical, and mitochondrial dysfunction in an animal model of Huntington's disease. *Journal of medicinal food*. 2009;12(3):591-600.
- . Naidu PS, Singh A, Kulkarni SK. Effect of *Withania somnifera* root extract on reserpine-induced orofacial dyskinesia and cognitive dysfunction. *Phytotherapy Research*. 2006;20(2):140-146.
- Kulkarni SK, Naidu PS. Pathophysiology and drug therapy of tardive dyskinesia: current concepts and future perspectives. *Drugs Today (Barc)*. 2003;39(1):19-49.
- Kulkarni S, Sharma AC, Verma A, Ticku M. GABA receptor mediated anticonvulsant action of *Withania somnifera* root extract. *Indian Drugs*. 1993;30(7):305-312.
- Kulkarni S, George B, Nayar U. Amygdaloid kindling in rats: Protective effect of *Withania somnifera* (Aswagandha) root extract. *Indian Drugs*. 1995;32(1):37-49.
- The Canadian Journal of Clinical Nutrition, Volume 5, Issue 2, June 2017
ISSN 1927-8942 (Print Edition), ISSN 1927-8950 (Online Edition) The Canadian Journal of Clinical Nutrition is published by Global Science Heritage,
- (<http://www.globalscienceheritage.org>) Registered publisher by the Library and Archives/Government of Canada, (www.collectionscanada.gc.ca) Page 49
- Kulkarni S, George B. Anticonvulsant Action of *Withania somnifera* (Aswagandha) Root Extract against Pentyleneetetrazol-induced Kindling in Mice. *Phytotherapy Research*. 1996;10(5):447-449.
- . Kulkarni S, George B, Mathur R. Neuroprotection by *Withania somnifera* root extract against lithium-pilocarpine-induced seizures. *Indian Drugs*. 1998;35(4):208-215.
- . Handa S. Plants and plant products for mental health. *Decade of the brain*. Rockville. MD: US Department of Health and Human Services. pp 163-171. 1995.
- Panda S, Kar A. Changes in thyroid hormone concentrations after

administration of ashwagandha root extract to adult male mice. Journal of pharmacy and pharmacology. 1998;50(9):1065-1068.

- Bhattacharya A, Ghosal S, Bhattacharya S. Anti-oxidant effect of Withania somnifera glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. Journal of ethnopharmacology. 2001;74(1):1-6.
- Andrade C, Aswath A, Chaturvedi S, Srinivasa M, Raguram R. A double-blind, placebo-controlled evaluation of the anxiolytic efficacy of an ethanolic extract of withania somnifera. Indian journal of psychiatry. 2000;42(3):295.
- Parihar M, Chaudhary M, Shetty R, Hemnani T. Susceptibility of hippocampus and cerebral cortex to oxidative damage in streptozotocin treated mice: prevention by extracts of Withania somnifera and Aloe vera. Journal of clinical neuroscience. 2004;11(4):397-402.
- . Dadkar VN, Ranadive N, Dhar H. Evaluation of antistress (adaptogen) activity of Withania somnifera (ashwagandha). 1987.
- Kaur P, Mathur S, Sharma M, Tiwari M, Srivastava K, Chandra R. A biologically active constituent of withania somnifera (ashwagandha) with antistress activity. Indian Journal of Clinical Biochemistry. 2001;16(2):195-198.
- Head K, Kelly G. Nutrients and botanicals for treatment of stress: adrenal fatigue, neurotransmitter imbalance, anxiety, and restless sleep. Alternative medicine review: a journal of clinical therapeutic. 2009;14(2):114-140.
- Bone K, Morgan M. Clinical applications of ayurvedic and Chinese herbs: monographs for the Western herbal practitioner. Phytotherapy Press; 1996.
- Mir BA, Khazir J, Mir NA, Hasan T-u, Koul S. Botanical, chemical and pharmacological review of Withania somnifera (Indian ginseng): An Ayurvedic Medicinal Plant. Indian Journal of Drugs and Diseases. 2012;1(6):147-160.
- Archana R, Namasivayam A. Antistressor effect of Withania somnifera. Journal of ethnopharmacology. 1998;64(1):91-93.
- Dhuley JN. Effect of ashwagandha on lipid peroxidation in stress-induced animals. Journal of ethnopharmacology. 1998;60(2):173-178.
- Elsakka M, Pavelescu M, Grigorescu E. Withania somnifera, a plant with a great therapeutical future. Revista medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi. 1988;93(2):349-350. The Canadian Journal of Clinical Nutrition, Volume 5, Issue 2, June 2017 ISSN 1927-8942 (Print Edition), ISSN 1927-8950 (Online Edition) The Canadian Journal of Clinical Nutrition is published by Global Science Heritage, (<http://www.globalscienceheritage.org>)

Registered publisher by the Library and Archives/Government of Canada, (www.collectionscanada.gc.ca) Page 50-59. Jain S, Shukla SD, Sharma K, Bhatnagar M. Neuroprotective Effects of Withania somnifera Dunn. in Hippocampal Sub-regions of Female Albino Rat. *Phytotherapy Research*. 2001;15(6):544-548. 60. Tohda C, Kuboyama T, Komatsu K. Search for natural products related to regeneration of the neuronal network. *Neurosignals*. 2005;14(1-2):34-45. 61

- . Sinha K, Chaudhary G, Gupta YK. Protective effect of resveratrol against oxidative stress in middle cerebral artery occlusion model of stroke in rats. *Life sciences*. 2002;71(6):655-665.
- Chaudhary G, Sinha K, Gupta Y. Protective effect of exogenous administration of α -tocopherol in middle cerebral artery occlusion model of cerebral ischemia in rats. *Fundamental & clinical pharmacology*. 2003;17(6):703-707.
- Sinha K, Degaonkar MN, Jagannathan NR, Gupta YK. Effect of melatonin on ischemia reperfusion injury induced by middle cerebral artery occlusion in rats. *European journal of pharmacology*. 2001;428(2):185-192.
- Chaudhary G, Sharma U, Jagannathan NR, Gupta YK. Evaluation of Withania somnifera in a middle cerebral artery occlusion model of stroke in rats. *Clinical and Experimental Pharmacology and Physiology*. 2003;30(5-6):399-404.
- . Nakayama N, Tohda C. Withanoside IV improves hindlimb function by facilitating axonal growth and increase in peripheral nervous system myelin level after spinal cord injury. *Neuroscience Research*. 2007;58(2):176-182. Ali S., Champagne D. L., Spaink H. P., Richardson M. K. (2011). Zebrafish embryos and larvae: A new generation of disease models and drug screens. *Birth Defect. Res.* 93 115–133. 10.1002/bdrc.20206
- Anichtchik O. V., Kaslin J., Peitsaro N., Scheinin M., Panula P. (2004). Neurochemical and behavioural changes in zebrafish *Danio rerio* after systemic administration of 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *J. Neurochem.* 88 443–453. 10.1111/j.1471-4159.2004.02190.x
- Anichtchik O., Diekmann H., Fleming A., Roach A., Goldsmith P., Rubinsztein D. C. (2008). Loss of PINK1 function affects development and results in neurodegeneration in zebrafish. *J. Neurosci.* 28 8199–8207. 10.1523/JNEUROSCI.0979-08.2008
- Ariga H., Takahashi-Niki K., Kato I., Maita H., Niki T., Iguchi-Ariga S. M. M. (2013). Neuroprotective function of dj-1 in Parkinson's disease. *Oxidat. Med. Cell. Longev.* 2013:683920. 10.1155/2013/683920

- Arslan B. K., Edmondson D. E. (2010). Expression of zebrafish (*Danio rerio*) monoamine oxidase (MAO) in *Pichia pastoris*: Purification and comparison with human MAO A and MAO B. *Protein Exp. Purific.* 70 290–297. 10.1016/j.pep.2010.01.005
- Ashrafi G., Schwarz T. L. (2013). The pathways of mitophagy for quality control and clearance of mitochondria. *Cell Death Diff.* 20 31–42. 10.1038/cdd.2012.81
- Audira G., Sampurna B. P., Juniardi S., Liang S. T., Lai Y. H., Hsiao C., et al. (2018). A versatile setup for measuring multiple behavior endpoints in zebrafish. *Inventions* 3:3040075. 10.3390/inventions3040075
- Audira G., Siregar P., Strungaru S. A., Huang J. C., Hsiao C. Der. (2020). Which zebrafish strains are more suitable to perform behavioral studies? A comprehensive comparison by phenomic approach. *Biology* 9 1–22. 10.3390/biology9080200
- Avdesh A., Chen M., Martin-Iverson M. T., Mondal A., Ong D., Rainey-Smith S., et al. (2012). Regular care and maintenance of a Zebrafish (*Danio rerio*) laboratory: An introduction. *J. Visualiz. Exp.* 69:4196. 10.3791/4196
- Bajpai P., Sangar M. C., Singh S., Tang W., Bansal S., Chowdhury G., et al. (2013). Metabolism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by mitochondrion-targeted cytochrome P450 2D6 implications in parkinson disease. *J. Biol. Chem.* 288 4436–4451. 10.1074/jbc.M112.402123
- Ball N., Teo W.-P., Chandra S., Chapman J. (2019). Parkinson's Disease and the Environment. *Front. Neurol.* 10:00218. 10.3389/fneur.2019.00218
- Barazzuol L., Giamogante F., Brini M., Calì T. (2020). PINK1/Parkin Mediated Mitophagy, Ca²⁺ Signalling, and ER-Mitochondria Contacts in Parkinson's Disease. *Int. J. Mole. Sci.* 21:21051772. 10.3390/ijms21051772
- Barbazuk W. B., Korf I., Kadavi C., Heyen J., Tate S., Wun E., et al. (2000). The syntenic relationship of the zebrafish and human genomes [1]. *Genome Res.* 10 1351–1358. 10.1101/gr.144700
- Barbeau A., Dallaire L., Buu N. T., Poirier J., Rucinska E. (1985). Comparative behavioral, biochemical and pigmentary effects of MPTP, MPP⁺ and paraquat in *rana pipiens*. *Life Sci.* 37 1529–1538. 10.1016/0024-3205(85)90185-7
- Barbosa J. S., Ninkovic J. (2016). Adult neural stem cell behavior underlying constitutive and restorative neurogenesis in zebrafish. *Neurogenesis* 3:1148101. 10.1080/23262133.2016.1148101
- Barnhill L. M., Murata H., Bronstein J. M. (2020). Studying the pathophysiology of Parkinson's disease using zebrafish. *Biomedicines* 8:8070197. 10.3390/BIOMEDICINES8070197

- Barodia S. K., Creed R. B., Goldberg M. S. (2017). Parkin and PINK1 functions in oxidative stress and neurodegeneration. *Brain Res. Bull.* 133 51–59. 10.1016/j.brainresbull.2016.12.004
- Barone P. (2010). Neurotransmission in Parkinson's disease: Beyond dopamine. *Eur. J. Neurol.* 17 364–376. 10.1111/j.1468-1331.2009.02900.x
- Barré-Sinoussi F., Montagutelli X. (2015). Animal models are essential to biological research: Issues and perspectives. *Future Sci. OA* 1:63. 10.4155/fso.15.63
- Barros T. P., Alderton W. K., Reynolds H. M., Roach A. G., Berghmans S. (2008). Zebrafish: An emerging technology for in vivo pharmacological assessment to identify potential safety liabilities in early drug discovery. *Br. J. Pharm.* 154 1400–1413. 10.1038/bjp.2008.249
- Bastías-Candia S., Zolezzi J. M., Inestrosa N. C. (2019). Revisiting the Paraquat-Induced Sporadic Parkinson's Disease-Like Model. *Mol. Neurobiol.* 56 1044–1055. 10.1007/s12035-018-1148-z [[PubMed](#)] [[CrossRef](#)] [[Google Scholar](#)]

-
-