"EVALUATION OF PHYTOCONSTITUENT FOR ANTI-PARKINSON ACTIVITY USING ZEBRAFISH AS A MODEL ORGANISM"

Submitted in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy

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CERTIFICATE

This is to certify that the project entitled **EVALUATION OF PHYTOCONSTITUENT FOR ANTI-PARKINSON ACTIVITY USING ZEBRAFISH AS A MODEL ORGANISM**, is a bonafide work of SHAIKH MOHAMMED IKRAM IKHLAQUE (16PH52), ANSARI TAMANNA VAHAJUDDIN KASIMA (16PH02), SHAIKH AFZAL SHARFUDDIN (16PH48) & SAIWANKAR NEEHA NOORUDDIN NAZNEEN (16PH40) submitted for the appreciation of the degree of Bachelor of Pharmacy in Department of Pharmaceutical Chemistry.

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Declaration

I declare that this written submission represents my ideas in my own words and where others ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and Integrity and have not misrepresented or fabricated or falsified any idea / data / fact/ source in my submission. I understand that any violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

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ABSTRACT

EVALUATION OF PHYTOCONSTITUENT FOR ANTI-PARKINSON ACTIVITY USING ZEBRAFISH AS A MODEL ORGANISM

Parkinson disease (PD) is the second most common neurodegenerative disorder, after Alzheimer's disease. The incidence of PD increases after the age of 50 and rapidly increases after the age of 75. PD is characterized by motor and non-motor features. The most common motor symptoms are tremor, rigidity, akinesia (lack of movement), bradykinesia (slow movements), hypokinesia (reduced movement), that occur mainly due to the degeneration of dopaminergic neurons, involved in the movement coordination and located in the substantia nigra pars compacta. However, in later stages of the disease, it affects other brain regions.

Since the available medication of the drugs for the treatment of Parkinson's disease have seen to have many extrapyrimidal side effects and lesser drugs are present of herbal origin. The purpose of this study is to check and evaluate the efficacy of the phytoconstituent (curcumin) in treatment of parkinson's disease using zebrafish as a model organism .

Curcumin, a yellow pigment present in turmeric has been shown to exhibit numerous activities such as antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, anticarcinogenic, thrombosuppressive and cardiovascular as well have potential to show anti Parkinson activity, is extracted from one of the approved methods and characterized using thin layer chromatography technique. Curcumin related to neuroprotection is its anti-oxidant function that can protects substantia nigra neurons, increases striatal dopamine level , which is necessary for the treatment of parkinson's disease.

Keywords: Parkinson's disease, Zebrafish, Curcumin.

List of figures

List of tables

Table of contents

INTRODUCTION

Parkinson's disease (PD) was first described in 1817 by James Parkinson and is the second most common neurodegenerative disorder, after Alzheimer's disease. The incidence of PD increases after the age of 50 and rapidly increases after the age of 75. Onset of PD is clearly age-related, and its prevalence will increase as the population ages. PD is characterized by motor and non-motor features. The most common motor symptoms are tremor, rigidity, akinesia (lack of movement), bradykinesia (slow movements), hypokinesia (reduced movement), that occur mainly due to the degeneration of dopaminergic neurons, involved in the movement coordination and located in the substantia nigra pars compacta (Figure 1). However, in later stages of the disease, it affects other brain regions [3,4] . The degeneration of these wider circuits in the brain is responsible for the non-motor features of the disease, such as cognitive decline, depression and, in some cases, hallucination episodes.

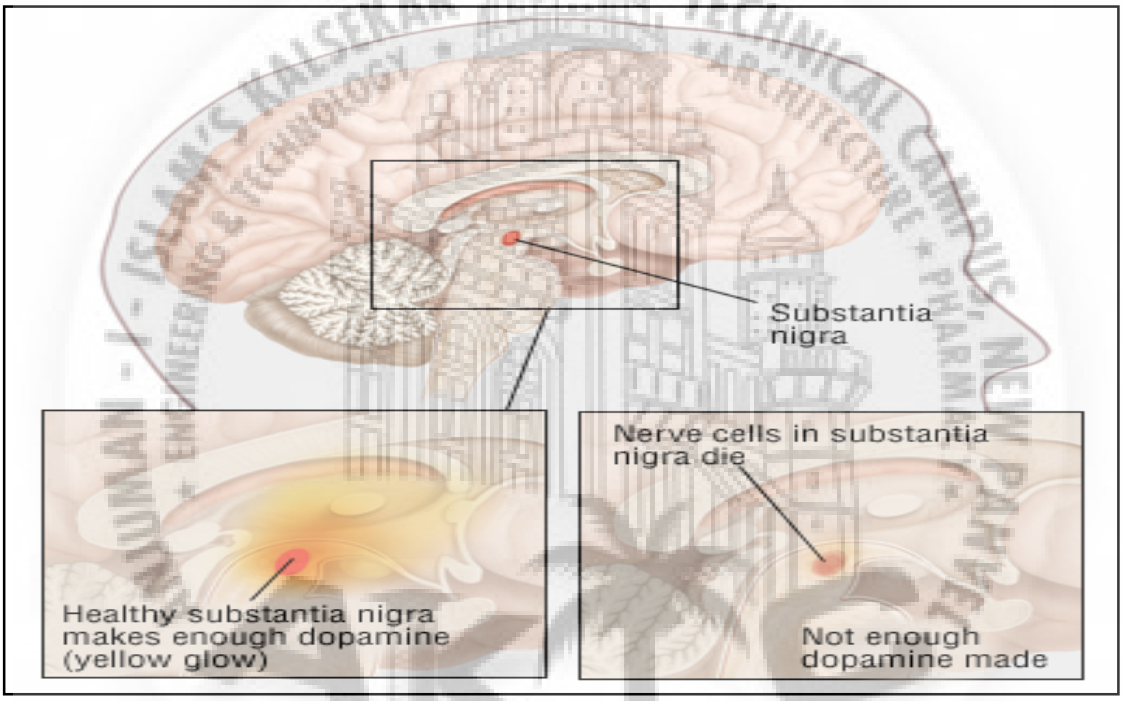

Fig. 1 – Affected region of brain

Some of the toxins such as rotenone, paraquat, and MPTP which are known to produce PD such as symptoms in mammals also cause dopaminergic loss in zebrafish. Antipsychotics such as haloperidol which act by temporary blockade of dopaminergic neurons are known to produce cataleptic movements in zebrafish, which leads to aberrant swimming patterns (upside down, circular, and arrow-like swimming toward bottom).

In PD patients it is visually possible to detect a depletion of dopaminergic neurons in the substantia nigra which is responsible for the majority of the motor symptoms found in the disease. The majority of the PD cases are sporadic and only 2% of the cases are familiar, being associated to specific gene mutations.

Phenotypically, both PD forms are very similar regarding motor symptoms, suggesting that the insult responsible for the disease development and progression may be identical in both cases

The major pathologic hallmark of PD, besides the degeneration of dopaminergic neurons, is the presence of cytoplasmic protein inclusions named Lewy Bodies (LBs) that can be found in the remaining surviving neurons. These inclusions are mainly constituted by alphasynuclein (a-syn) which was the first protein to be associated to the disease. The role of these bodies is still unknown, however, it is believed that they may present a protective effect in the disease, by sequestering dysfunctional and toxic protein species responsible for neurodegeneration [7]. The neuropathological hallmarks of PD are loss of nigrostriatal dopaminergic neurons and presence of intracellular alpha-synuclein, parkin, and ubiquitin contained in Lewy body (LB) inclusions.

Etiology:

The etiology of PD is currently unknown. It is believed to be caused by interplay of genetic and environmental factors. 1-methyl-4-phenyl-1,2,3,6-tetrapyridine (MPTP) was first produced as a side-product of MPPP (a type of synthetic heroin) synthesis and identified as a cause for parkinsonism in a college student in 1976 and in several heroin addicts in 1982[9]. Pesticides have been suspected of causing PD because of their mechanism of action – causing dysfunction in the respiratory chain of mitochondria. Meta-analyses of epidemiological data suggest that a positive association exists between pesticide exposure and PD [10,11]. In addition, the pesticide rotenone is used to produce experimental parkinsonism. Somewhat surprisingly, cigarette smoking is inversely associated with the risk of PD [12], which may be due to the inhibition of MAO.

Neuropathology of PD

Parkinsonism is a progressive motor disease that affects 1.5 million Americans. It is the second most common neurodegenerative disease after Alzheimer's and PD affects close to 5% of the population that are over 65 years old. The degeneration of dopaminergic neurons in the substantia nigra lead to PD. Due to abnormal protein folding and ER stress, a toxic protein named Lewy bodies are formed that are commonly observed in PD patients. This toxic protein, Lewy bodies, is made of different proteins such as α-synuclein, synphilin-1, and ubiquitin. Lewy bodies have also been observed in other areas of the brain such as hind brain, spinal cord and enteric nervous system. Lewy bodies first appear in the periphery, subsequently it travels to brain stem and eventually in the cortex. Although there is some controversy as to whether all PD patients do have α-synucleinopathy, all α-synucleinopathies have one thing common and that is pathology found in discrete location and clinical manifestations may be due prionopathy that arose from different regions of the brain. Thus, a misfolded synuclein can trigger a downstream cascade of events that may lead to clinical symptoms. There are other forms of PD that involve abnormally phosphorylated tau such as progressive supra nuclear palsy, cortico-basal degeneration, parkinsonism-dementia complex, and frontotemporal dementia with Parkinsonism.

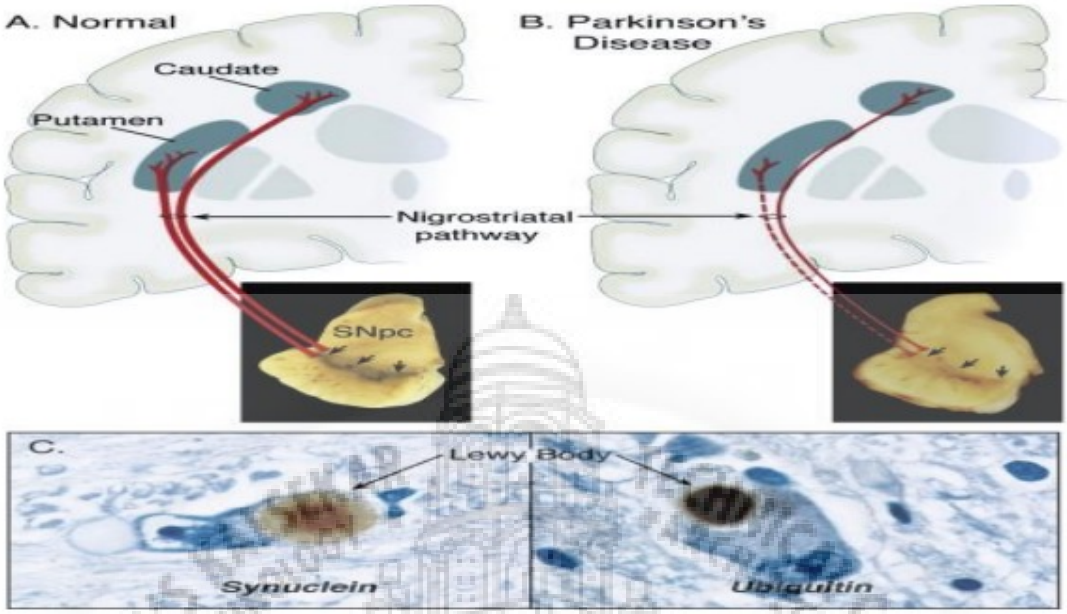

Fig.-2 Neuropathology of PD

Treatment:

The treatment of PD is currently symptomatic, as no preventive, curative, or even disease modifying treatment is available. L-DOPA, a precursor of dopamine, is the oldest and most efficacious drug for treating PD. However, long-term use of L-DOPA produces motor fluctuations and dyskinesia's and thus, treatment is usually started with other medications. These include amantadine, monoamine oxidase B (MAO-B) inhibitors, anticholinergics, and dopamine receptor, agonists. Neurosurgical options exist, such as deep brain stimulation of the subthalamic nucleus, which may be used after symptoms can no longer be managed with medication. There is an urgent need for new, disease-modifying treatment options for PD.

Efforts over the last 20 years to develop new therapies for PD can be divided in two categories: (1) improving symptomatic therapy of (1a) motor and (1b) non-motor symptoms and (2) addressing potential causes of PD, with a focus on the protein alpha-synuclein, its chemistry, synthesis, aggregation, degradation, and interaction with other proteins in order to develop a disease modifying treatment.

Dopamine replacement therapy	L-dopa + peripherally acting decarboxylase inhibitor (carbidopa, benserazide)
Direct dopamine agonists	Ropinirole, rotigotine, bromocriptine, pergolide, cabergoline, pramipexole, apomorphine
MAO-B inhibitors	Rasagiline, selegiline
COMT inhibitors	Entacapone*, tolcapone†
Antimuscarinic, DAT inhibitor	Benztropine‡, trihexiphenidyl
NMDA receptor antagonists§	Amantadine, memantine

MAO denotes monoamine oxidase, DA dopamine, DAT plasma membrane dopamine transporter.NET plasma membrane noradrenaline transporter, COMT catechol O-methyl transferase, and L-dopa, 3,4-dihydroxyphenylalanine.
* Peripherally acting inhibitor.
† Peripherally + centrally acting inhibitor.
‡ Also inhibits DA uptake via plasma membrane DA transporter.
§ Glutamate antagonists, moderate efficacy in Parkinsonism and L-dopa-induced dyskinesias.

Fig. 3- Drugs available for parkinson's disease in market

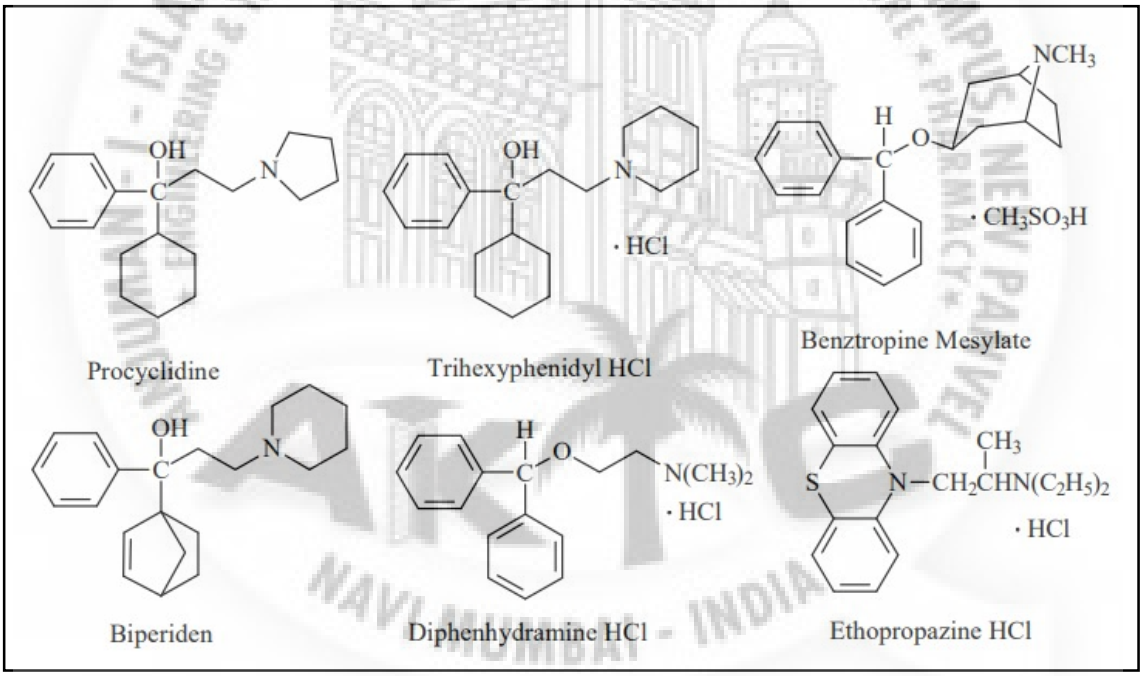

Fig. 4- Chemical structure of drugs used for parkinson's disease.

Management of PD

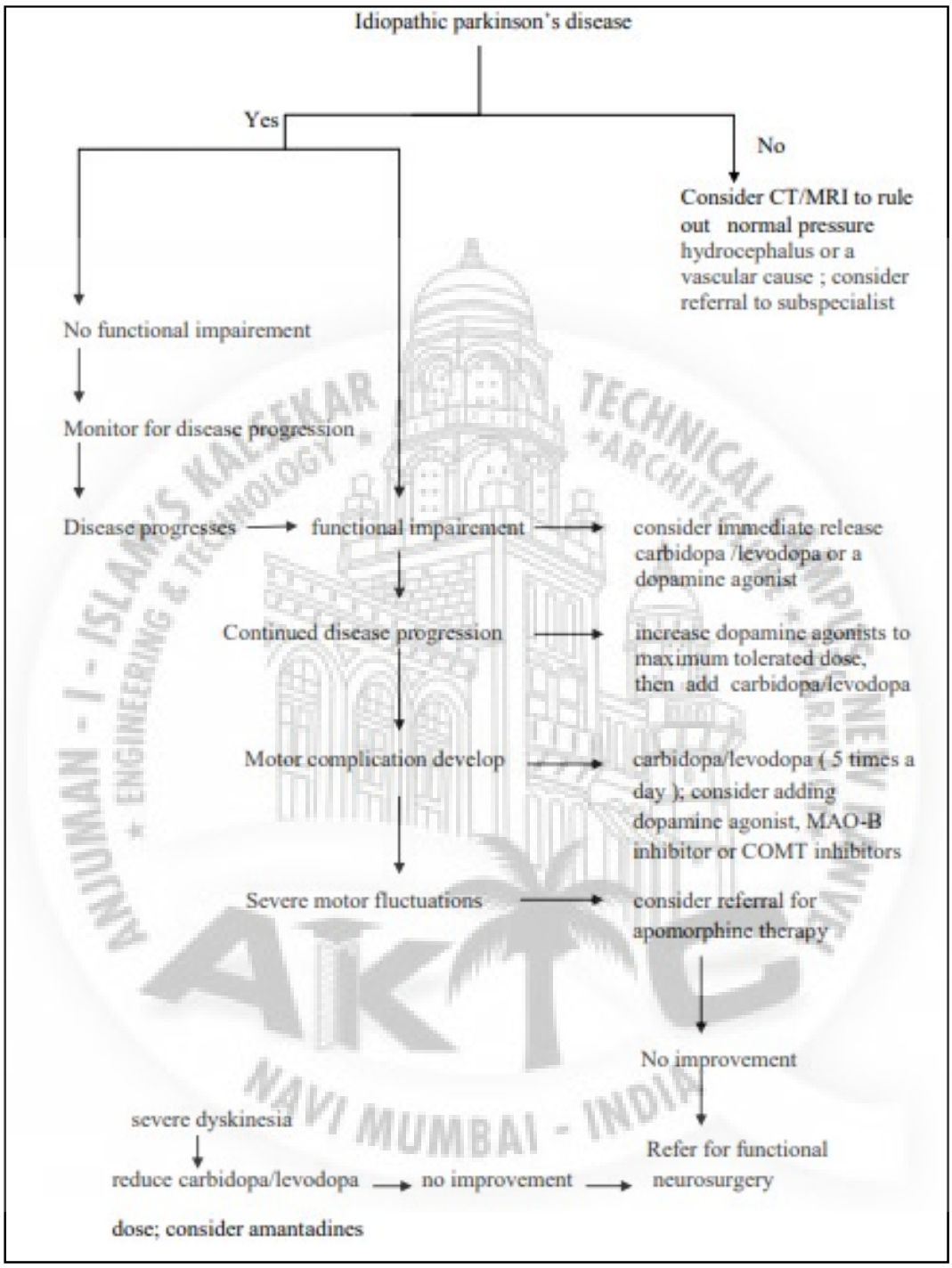

Fig- 5 Management of PD

Limitation of current therapies

Levodopa

The gold standard of PD therapy; acts as aprecursor of dopamine. When levodopa is administered orally it is rapidly decarboxylated; thus high dose is required for desired effect which induces nausea and vomiting in patients. Longterm treatment of levodopa results in adverse motor effects which limits its use including on-off phenomenon, wearing off, dose failure, akinesia and dyskinesias.

Selegiline

This MAO-B inhibitor prevents the in vivo metabolism of dopamine. Its main use is as adjuntive therapy with levodopa which may lead to potentiated side effects. Studies suggest that selegiline may retard disease progression and delays the need for levodopa. However, therapeutic effect is mild when used alone.

Amantadine

Amantadine, an antiviral agent was found by chance to be effective in PD. It is effective in reducing diskinesias. Its CNS effects include restlessness, depression, confusion and hallucinations.

Anticholinergics

Trihexyphenidyl or benztropine are specifically effective against tremor. Side effects such as confusion, drowsiness, agitation and hallucination are common. Drug withdrawal leads to precipitation of acute parkinsonian symptoms.

Dopamine receptor agonists

They may be used alone to delay the need for levodopa or used with levodopa to increase their effectiveness. The ergot derivatives causes psychiatric disturbances and cardiovascular problems that can progress to myocardial infractions. Even at lower doses patients experience orthostatic hypotension, constipation, dyskinesias, confusion and insomnia.

COMT inhibitors

COMT inhibitors are used mainly in combination with levodopa. The incidence of sleep disturbances, orthostatic hypotension, dyskinesias, confusion and insomnia. Tolcapone produce heparotoxicity and has been restricted in many countries.

Introduction to Zebrafish:

Zebrafish are a small fish usually less than 5 cm in length with stripes on the body. Geographically we can find them from Pakistan to Myanmar and from Nepal to Karnataka in India. They live in slow moving water like ponds, lakes, rice paddies etc. They are commonly kept as aquarium fish, as they are easy to maintain. Zebrafish were first introduced for

biomedical research purposes by George Streisinger in 1981. Streisinger chose zebrafish, among other species, due to their ideal combination of properties. Zebrafish have a tendency to live in shoals. They are active during the day and have a rest at night. They are omnivorous. Naturally, it feeds with zooplankton and insects [13].

Zebrafish as a model animal:

Zebrafish as a model animal progressed to be used during the 1960s and when the zebrafish genome started to be sequenced, their use rapidly increased (post-1996)[13]. Because their genetic sequence is quite similar to humans they became popular as a model animal for human diseases [14]. Now they are used mostly in molecular biology, developmental biology, neurobiology and genetics research [13]. Advantages of zebrafish as an animal model are its small size, short generation time and easy and cheap maintenance [15]. Another advantage of zebrafish larvae is that larvae are transparent so it is possible to observe the development of organs and tissues [16]. Their development is fast. Larvae one week old are already able to hide from predators, catch small preys or stabilize position in moving water.

Fish gender identification:

Zebrafish are mature when they are 3 months old [18]. Zebrafish male and female look very similar but it is important to recognize them. Zebrafish male (Fig. 3B) are more straight and narrow shaped with darker blue stripes. They are more golden, especially on the ventral fins. They have not gotas big belly as zebrafish female. They tend to be more active than zebrafishfemale. In contrast, zebrafish female (Fig. 3A) are more pale with bluish-white stripes and have bigger white belly than male zebrafish. Also, they have more visible typical oviduct in the caudal belly region.

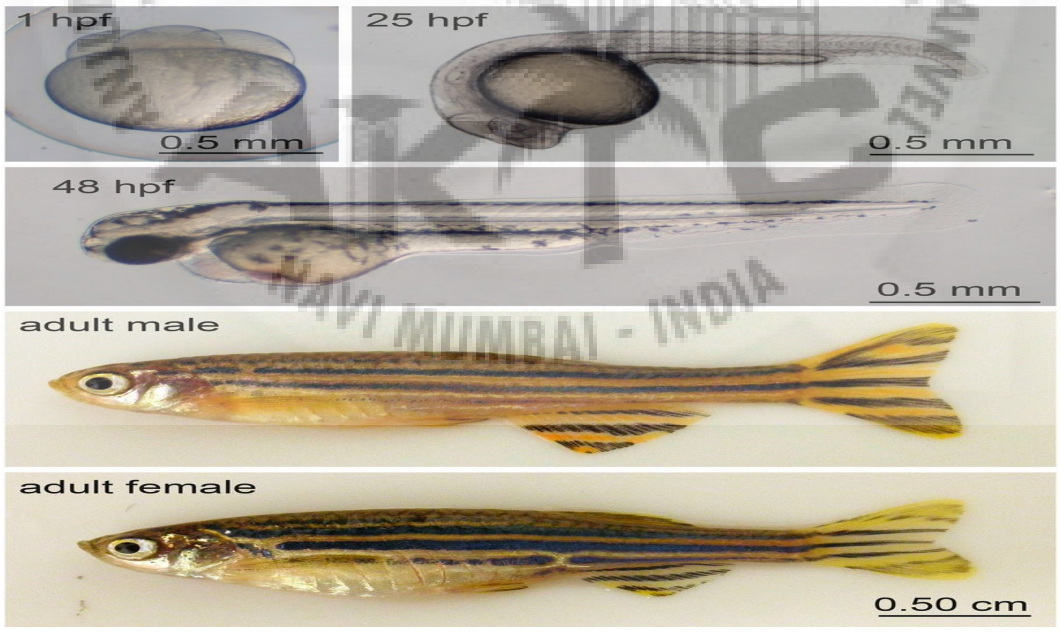

Fig 6 - Zebrafish gender difference

Zebrafish as a model organism in PD:

The zebrafish, Danio rerio, a vertebrate model organism for genetics that has emerged in the past decade or so, is a relatively uncharted system for modeling Parkinson's disease (PD). It has become a widely used model system for the study of development and gene function. Zebrafish are vertebrates and therefore more closely related to humans than other genetic model organisms such as Drosophila or Caenorhabditis elegans. They are relatively small fish (3–4 cm long as an adult) that can be easily managed in large numbers in specialized facilities. Zebrafish have a short generation time (3 months) and breed prodigiously (hundreds of offspring per female per week). Embryos develop externally, can be readily manipulated genetically and are transparent. Many factors suggest that the zebrafish is a powerful tool for the study of human diseases: patterning, pathfinding and connectivity in the CNS have all been deciphered and correlate with the human CNS; transparency of embryonic zebrafish facilitates analysis of single neuron activity during the execution of normal and pathological behavior; touch and behavioral responses such as movement patterns can be monitored; and cardiovascular, anti-angiogenic and anti-cancer drugs elicit compatible responses in zebrafish embryos to those in mammalian systems. Zebrafish mutations phenocopy many human disorders and the genome sequence of zebrafish is near completion.

Induction of Parkinson's disease in Zebrafish:

Some of the toxins known to induce DA cell loss in other animal models have now also been tested in adult zebrafish[22] . Four toxic substances are commonly used to produce experimental parkinsonism, they are MPTP, 6-hydroxydopamine, rotenone, paraquat.

Systemic injection of MPTP or 6-hydroxydopamine did not alter the number of DA neurons, but DA and noradrenaline concentrations in brain tissue were significantly decreased without a concomitant change of tyrosine hydroxylase (TH) or caspase 3 protein levels. The swimming velocity and total distance moved decreased after exposure to both neurotoxins. Apoptosis was not significantly increased in toxin-exposed fish. The lack of a clear decrease in the DA cell population may be due to the toxin exposure protocol; perhaps the single acute exposure (intramuscular injection) of neurotoxin, while enough to alter swimming behaviour and global brain levels of catecholamines, was an insufficient insult to result in neuronal cell loss.

Given the ease of delivering chemical compounds to zebrafish (by simply adding them to the tank water, which enables access to the CNS), the potential PD-inducing effects of MPTP, its metabolite MPP, and the pesticides including rotenone and paraquat, have been evaluated in both larval and adult zebrafish[22-25]. Motor behaviour (e.g. Swimming) can also be altered by administration of the pesticides rotenone and paraquat in both larval and adult zebrafish [23].

Adult fish were exposed to rotenone and paraquat via immersion (pesticides diluted in tank water) and exposed to MPTP and MPP+ via intraperitoneal injections. In adult zebrafish, only the highest single dose of MPTP resulted in a measurable effect on locomotor activity, and no effect was seen with rotenone or paraquat at sublethal doses.

In addition to neurotoxin-induced loss of DA neurons, it has been well established

that both antipsychotics and antidepressants can have extrapyramidal side effects (EPS) leading to movement disorders in individuals who are treated with these medications. This so-called drug-induced Parkinson is usually developing within 1 month of the initiation of the offending medication in approximately 60% patients and in approximately 90% within 3 months. The likely risk factors include prior history of movement defects, age, gender, and genetically determined differences in drug metabolism and possibly drug action. The conditions are generally reversible, once the medications are removed, suggesting that the drugs produce an interference with neuronal function rather than killing the neurons.

Behavioral parameters in zebrafish for testing catalepsy:

Catalepsy is the major symptom of Parkinson's disease. It can be induced in zebrafish using standardized dose of haloperidol (9 µg) by giving direct exposure to fishes. During induction of catalepsy, fish will start showing aberrant swimming patterns like upside down, arrow like swimming, circular swimming, and finally state of complete catalepsy can be achieved.

REVIEW OF LITERATURE

Goyal et al reported the various herbs antiparkinsonian activity for Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, affecting 1% of the population over the age of 65 years and 4–5% of the population over the age of 85 years. Current treatments are mainly symptomatic and can temporarily slow down disease progression, but can not halt this. To date there is a lack of effective preventive strategies for PD. Therefore, safe and effective treatment strategies are urgently required for management of PD.

Plant extracts have a wide range of medicinal properties and has been used to treat many types of diseases. Lycium Chinensis Miller, plant has been used as an anti aging therapy and a treatment of neurodegenerative diseases and recent research has confirmed neuroprotective effect of the fruits of plant in a rat model. Herbal plants and their phytochemicals might potentially offer a novel neuroprotective approach in a neurodegenerative diseases and might be developed for therapeutic use.

Pathan et al reported the zebrafish as model antiparkinsonian activity. The human brain is responsible for carrying out many complex tasks that are essential for the survival, successful and healthy functioning of an individual. These functions result from the cooperation of roughly 100 billion neurons. Neurons communicate with each other by secreting neurotransmitters. Dopamine is major neurotransmitter in brain whose absence hurts motor control.

Parkinson's disease (PD) is the second most common progressive neurodegenerative disease after Alzheimer's disease. It results from the slow degeneration of dopaminergic neurons in the substantia nigra. Zebrafish has been widely used as a model organism for various diseases. Zebrafish have many favorable properties as experimental animals. The embryos are small and transparent, and as adults, they are inexpensive and easy to maintain, develop rapidly, and breed in large quantities.

Victor et al had reported biological importance of Quercetin. Antioxidants are substances that may protect cells from the damage caused by unstable molecules such as free radicals. Flavonoids are phenolic substances widely found in fruits and vegetables. The previous studies showed that the ingestion of flavonoids reduces the risk of cardiovascular diseases, metabolic disorders, and certain types of cancer. These effects are due to the physiological activity of flavonoids in the reduction of oxidative stress, inhibiting low-density lipoproteins oxidation and platelet aggregation, and acting as vasodilators in blood vessels. Free radicals are constantly generated resulting in extensive damage to tissues leading to various disease conditions such as cancer, Alzheimer's, renal diseases, cardiac abnormalities, etc., Medicinal plants with antioxidant properties play a vital functions in exhibiting beneficial effects and employed as an alternative source of medicine to mitigate the disease associated with oxidative stress.

Flavonoids have existed over one billion years and possess wide spectrum of biological activities that might be able to influence processes which are dys regulated in a disease. Quercetin, a plant pigment is a potent antioxidant flavonoid and more specifically a

flavonol, found mostly in onions, grapes, berries, cherries, broccoli, and citrus fruits. It is a versatile antioxidant known to possess protective abilities against tissue injury induced by various drug toxicities.

Harsh et al reported flavnoids for Parkinson's disease. Oxidative stress is implicated in mitochondrial dysfunction associated with neurodegeneration in Parkinson's disease (PD). Depletion of the cellular antioxidant glutathione (GSH) resulting in oxidative stress is considered as an early event in neurodegeneration. We previously showed that curcumin, a dietary polyphenol from turmeric induced GSH synthesis in experimental models and protected against oxidative stress. Here we tested the effect of three bioconjugates of curcumin (involving diesters of demethylenated piperic acid, valine and glutamic acid) against GSH depletion mediated oxidative stress in dopaminergic neuronal cells and found that the glutamic acid derivative displayed improved neuroprotection compared to curcumin.

Hajialyani et al reported Hesperidin as a Neuroprotective Agent. Neuroprotection is the preservation of function and networks of neural tissues from damages caused by various agents, as well as neurodegenerative diseases such as Parkinson's, Alzheimer's, Huntington's diseases, and multiple sclerosis. Hesperidin, a flavanone glycoside, is a natural phenolic compound with a wide range of biological effects. Mounting evidence has demonstrated that hesperidin possesses inhibitory effect against development of neurodegenerative diseases. Our review discusses neuropharmacological mechanisms for preventive and therapeutic effects of hesperidin in neurodegenerative diseases. In addition, the review examines clinical evidence confirming its neuroprotective function.

Various cellular and animal models specific to neurodegenerative diseases have been conducted to evaluate the underlying neuropharmacological mechanisms of hesperidin. Neuroprotective potential of this flavonoid is mediated by improvement of neural growth factors and endogenous antioxidant defense functions, diminishing neuro-inflammatory and apoptotic pathways. Despite the various preclinical studies on the role of hesperidin in the neurodegenerative diseases, less is known about its definite effect on humans. A limited number of clinical trials showed that hesperidin-enriched dietary supplements can significantly improve cerebral blood flow, cognition, and memory performance. Further clinical trials are also required for confirming neuroprotective efficacy of this natural flavonoid and evaluating its safety profile.

Pitchai et al reported Zebrafish as an Emerging Model for Bioassay in Neurological Disorders. Most neurodegenerative diseases are currently incurable, with large social and economic impacts. Recently, there has been renewed interest in investigating natural products in the modern drug discovery paradigm as novel, bioactive small molecules. Moreover, the discovery of potential therapies for neurological disorders is challenging and involves developing optimized animal models for drug screening. In contemporary biomedicine, the growing need to develop experimental models to obtain a detailed understanding of malady conditions and to portray pioneering treatments has resulted in the application of zebrafish to close the gap between in vitro and in vivo assays.

Zebrafish in pharmacogenetics and neuropharmacology are rapidly becoming a widely used organism. Brain function, dysfunction, genetic, and pharmacological modulation considerations are enhanced by both larval and adult zebrafish. Bioassay-guided identification of natural products using zebrafish presents as an attractive strategy for generating new lead compounds. Here, we see evidence that the zebrafish's central nervous

system is suitable for modeling human neurological disease and we review and evaluate natural product research using zebrafish as a vertebrate model platform to systematically identify bioactive natural products.

Zhang et al reported recent advances in discovery and development of natural products as source for anti-Parkinson's disease lead compounds. Parkinson's disease (PD) is a common chronic degenerative disease of the central nervous system. Although the cause remains unknown, several pathological processes and central factors such as oxidative stress, mitochondrial injury, inflammatory reactions, abnormal deposition of a-synuclein, and cell apoptosis have been reported. Currently, anti-PD drugs are classified into two major groups: drugs that affect dopaminergic neurons and anti-cholinergic drugs.

The existing conventional strategies against PD are with numerous side effects, and cannot fundamentally improve the degenerative process of dopaminergic neurons. Therefore, novel therapeutic approaches which have a novel structure, high efficiency, and fewer side effects are needed. For many years, natural products have provided an efficient resource for the discovery of potential therapeutic agents. Among them, many natural products possess anti-PD properties as a result of not only their well recognized anti-oxidative and anti-inflammatory activities but also their inhibitory roles regarding protein misfolding and the regulatory effects of PD related pathways. Indeed, with the steady improvement in the technologies for the isolation and purification of natural products and the in-depth studies on the pathogenic mechanisms of PD, many monomer components of natural products that have anti-PD effects have been gradually discovered.

1. Haloperidol induced catalepsy

Zebrafish models have significantly contributed to our understanding of vertebrate development and more recently human disease. The growing number of genetic tools available in research on zebrafish has resulted in the identification of many more genes involved in developmental and disease processes.

Zebrafish is becoming an attractive model organism for understanding biology and developing therapeutics, because as a vertebrate, it shares considerable similarity with mammals in both genetic compositions and tissue/organ structures and yet remains accessible to high throughput phenotype-based genetic and small-molecule compound screening.

Parkinson's disease (PD) is a neurodegenerative disease of the central nervous system which mainly affects the motor system. It is also known as slowly progressive neurodegenerative disease where there is a loss of dopaminergic neurons projecting from *substantia nigra* pars compacta toward neostriatum leading to the imbalance between dopamine and acetylcholine.

In behavioral studies – The haloperidol induced catalepsy was about to be performed as per this above mentioned on model zebrafish and their behavioral studies should be observed.

2. Catalepsy in zebrafish

Latency to travel from one fixed point to another

Haloperidol significantly decreased the latency to travel from one fixed point to another as compared to vehicle control group. As shown in Fig. 1. It can be observed from Table 1 that groups treated with bromocriptine and HECG significantly recovered latency to travel from one point to another, as compared to haloperidol control group.

Time spent near the bottom of the tank

Haloperidol control group was significantly different from vehicle control. It can be observed that Bromocriptine and HECG exhibited significant reversal of the anxious behavior as compared to haloperidol control group.

Complete cataleptic time

Haloperidol control group significantly increased the complete cataleptic time in zebrafish. As shown in Fig. 2. It can be observed from Table 2 that Bromocriptine and HECG produced drastic and statistically significant reduction in complete cataleptic time as compared to haloperidol control group.

3. Extraction procedure of phytoconstituent

The extraction procedure of phytoconstituent was choosen and taken, Curcumin is an orange yellow crystalline powder with melting point 183 degree Celsius. It is insoluble in cold water, ether, and soluble in alcohol and glacial acetic acid. It dissolves in conc.sulphuric acid and gives yellow red coloration. In 0.1 N sodium hydrochloride it gives deep brown colour.

Under the established conditions, the content of soluble solids and curcumin in the extracts ranged from 0.8 to 3.4%, and from 0.1 to 1.8%, respectively. The most influential variable observed for the extraction was the ethanolic strength of the solvent.

The optimized condition involves an extraction time of 12 h, agitation speed of 30 rpm, drug to solvent ratio of 1/6, extraction temperature of 80 °C and the solvent with ethanolic strength of 70%. And extracts and to determine the optimum set of parameters for the extraction of curcumin using a 25 full factorial design and the response surface methodology. We were able to extract the curcumin compound and utilize it with the various purposes and further characterization is done.

4. Characterization of phytoconstituent (curcumin)

Validated TLC Method for Determination of Curcumin

Thin layer chromatography (TLC) has been widely used particularly in phytochemistry. Compared to HPLC, this method is relatively efficient and cheaper. TLC provides an opportunity to simultaneously analyse a large number of samples.

A satisfactory separation of curcuminoids has been performed by chromatography methods. High performance liquid chromatography methods (HPLC) based on a reverse phase (RP) system were reported as selective, sensitive, precise, and accurate for quantification of curcumin concentrations in the formulation studies
Curcumin [1.7-bis(4-hydroxy-3- methoxyphenyl)-1. 6-heptadiene-3.5-dione] as presented in Figure 1 is a naturally occurring phytoconstituent of C. longa and other curcuma species. In the plant of C.longa, curcumin(1) exists together with the two curcumin derivatives, demethoxycurcumin(2) and bis-demethoxycurcumin.

AIM AND OBJECTIVE

- An estimated seven to 10 million people worldwide have parkinson disease.

- The available drugs in treatment of parkinson disease have more side effect and very high cost.

- Since the drug available in the treatment of parkinson disease are mostly of synthetic origin and henceforth, we are trying to formulate drug which is more oriented towards natural source.

- Main reason for selecting the herbal origin to decrease the side effects, hope to increase the efficacy.

EXPERIMENTAL WORK

1) Extraction of phytoconstituent (drug)

Table 1: Materials used for extraction of drug.

Ingredients	Quantity required
Turmeric powder	**50g**
Alcohol (95%)	**q.s**
Benzene	**50ml**
Sodium hydroxide(0.1%)	**50ml**
Soxhlet apparatus	**1 Set**

Procedure

1) Extract about 50g of turmeric powder with 95% alcohol in a Soxhlet Apparatus until all the colouring matter is extracted.

2) Distil of alcoholic extract to a semi solid brown coloured mass (about 4.5%).

3) Dissolve the crude extract in 50ml of benzene, and extract twice with the equal volume of 0.1%sodium hydroxide solution.

4) Combine the alkaline extracts and acidify with dilute hydrochloric acid. A yellow colour precipitate is formed. Allow it to settle for about 15 minutes.

5) After setting of precipitate, concentrate the extract by boiling on water bath and at the same time dissolving precipitate in boiling water.

6) During this process, the resinous material would agglumerate and form lumpy mass.

7) Filter the solution in hot condition and concentrate filtrate to very small volume and finally cool to get curcumin (1.5%).

Curcumin is an orange yellow crystalline powder with melting point 183˚C. It is insoluble in cold water, ether, and soluble in alcohol and glacial acetic acid. It dissolves in conc.sulphuric acid and gives yellow red coloration. In 0.1 N sodium hydrochloride it gives deep brown colour.

2) Characterization of phytoconstituent (drug)

1) Dissolve 1 mg of curcumin in 1 ml methanol.

2) Apply the spots on silica gel-G plate and elute the plate in the solvent system chloroform-ethanol-glacial acetic acid (94:5:1).

3) Dry the eluted plate and visualize under 366 nm light.

4) Curcumin exhibits a bright yellow flurosencent spot at Rf value 0.79.

5) The other spot appering at Rf values 0.60 and 0.43 correspond to desmethoxycurcumin and bidesmethoxycurcumin.

6) Hence it proves the presence of phytoconstituent Curcumin is extracted from the method or process above.

7) The melting point of curcumin was also observed that should be around 183° C.

8) The yield expected to be obtain is about 1.5% of the taken quantity of the raw product that is turmeric.

3) Pharmacological study

1) Fish should be divided into eight groups (n=5),viz.,vehicle control, haloperidol control , bromocriptine and pramipexole treated group. Behaviour testing was done during day phase, i.e., between 10:00 am and 5:00 pm.

2) Each fish from bromocriptine and pramipexole-treated groups should be individually exposed to the solution of respective drugs at the concentrations of 2,5, and 10 micro/ml in a 300-ml beaker for 30 min.

3) Once this exposure is given , then fish should be transferred to another beaker containing fresh tank water, where they need to be kept for 15 min, then fish from all treatment groups should be individually transferred to fresh 300-ml beaker containing 9-micro haloperidol solution, where they will be kept for another 30 min. after haloperidol exposure, fish will be shifted to examination tank to evaluate various cataleptic parameters, where they are going to be habiturated for Examination tank should be filled with fresh aerated tank water .

4) It should consist of 5-L tank with number of vertical lines drawn on one of the faces of the tank at the spacing of 5cm and with one horizontal line which should divides the water filled portion of the tank in two equal halves.

5) This vertical lines are used to calculate the speed of fish by measuring the time taken by the fish to travel from first vertical line to the last and horizontal line gave idea about the time speed in the upper and lower half of the tank by the fish.

RESULTS

EXPERIMENTAL

- Extract about 660g of turmeric powder with 95% alcohol in a Soxhlet Apparatus until all the colouring matter is extracted.
- Distil of alcoholic extract to a semi solid brown coloured mass (about 4.5%).
- Dissolve the crude extract in 50ml of benzene , and extract twice with the equal volume of 0.1%sodium hydroxide solution.
- Combine the alkaline extracts and acidify with dilute hydrochloric acid. A yellow colour precipitate is formed. Allow it to settle for about 15 minutes.
- After setting of precipitate, concentrate the extract by boiling on water bath and at the same time dissolving precipitate in boiling water.
- During this process, the resinous material would agglumerate and form lumpy mass.
- Filter the solution in hot condition and concentrate filtrate to very small volume and finally cool to get curcumin (1.5%).

The quantity of phytoconstituent curcumin obtained from 660g of turmeric powder is about 9.9 grams of curcumin.

It is insoluble in cold water, ether, and soluble in alcohol and glacial acetic acid. It dissolves in conc.sulphuric acid and gives yellow red coloration. In 0.1 N sodium hydrochloride it gives deep brown colour.

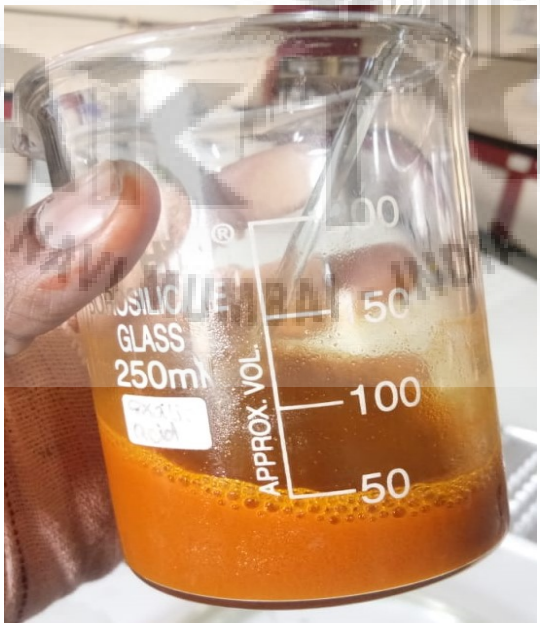

Fig 7 – Curcumin obtained after extraction.

ANALYTICAL

Thin layer Chromatography

- Dissolve 1 mg of curcumin in 1 ml methanol.
- Apply the spots on silica gel-G plate and elute the plate in the solvent system chloroform-ethanol-glacial acetic acid (94:5:1).
- Dry the eluted plate and visualize under 366nm light.
- Curcumin exhibits a bright yellow flurosencent spot at R value 0.79.
- Hence it proves the presence of phytoconstituent Curcumin is extracted from the method or process above.

The result of thin layer chromatography performed has given and proved the presence of Rf value of 0.78 was found , which proved the phytoconstituent curcumin compound.

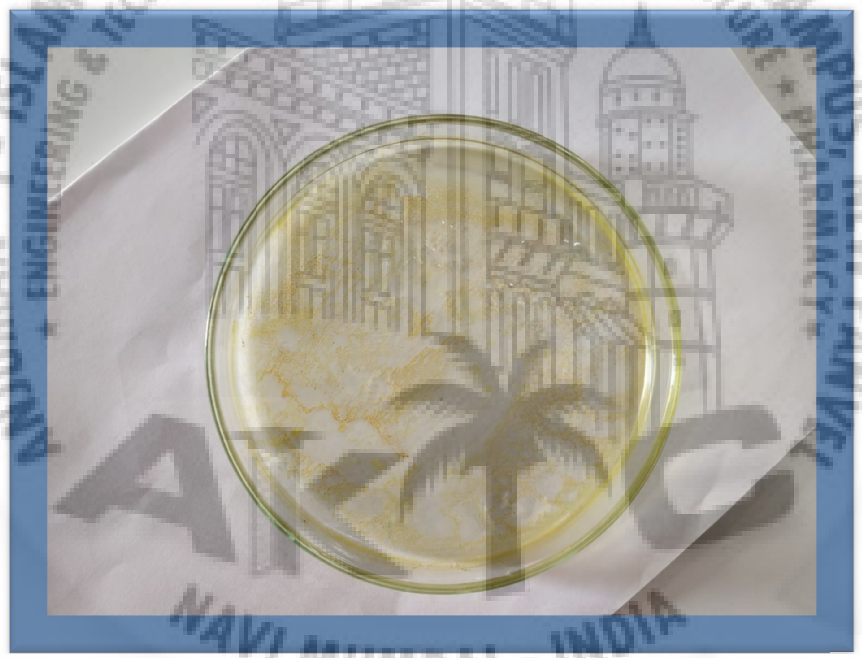

Fig 8 – Dried Curcumin obtained after extraction.

Determination of Melting point

1. Fill a capillary tube with crystals about 3 mm high.
2. Put the capillary tube (open end down) into the crystals and tap it on the bottom of the crystallization dish to get the crystals into the tube.
3. Force the crystals to slide to the bottom of the tube using one of the following methods: tap the tube (open end up) on the lab bench; drop the capillary tube through a 2-3 foot piece of glass tubing; or rub the capillary tube along a piece of wire gauze.

The obtained melting point was 184° C which is almost same as that of standard melting point given in Indian Pharmacopoeia.

PHARMACOLOGY

We planned to evaluate the anti-parkinson activity of curcumin using Zebra fish animal model. Due to unprecedented lockdown caused by COVID-19, we are not able to perform the pharmacological activity.

DISCUSSION

PD is a neurodegenerative disorder caused by environmental and genetic factors. However, the onset mechanisms of PD currently remain unclear, and this represents an obstacle to the development of effective treatments.

Previous studies demonstrated that oxidative stress is one of the main causes of PD. The formation of Lewy bodies and oxidative stress have been found in the pars compacta of the substantia nigra of PD patients. Therefore, antioxidants are considered to be a promising approach to decelerate the progression of PD. Curcumin is regarded as a powerful antioxidant .Previous studies reported the neuroprotective properties of curcumin in *in vitro* and *in vivo* PD models induced by several different environmental factors such as neurotoxins and genetic factors including *α-synuclein*,

The major pathologic hallmark of PD, besides the degeneration of dopaminergic neurons, is the presence of cytoplasmic protein inclusions named Lewy Bodies (LBs) that can be found in the remaining surviving neurons. These inclusions are mainly constituted by alphasynuclein (a-syn) which was the first protein to be associated to the disease. The role of these bodies is still unknown, however, it is believed that they may present a protective effect in the disease, by sequestering dysfunctional and toxic protein species responsible for neurodegeneration [7] . The neuropathological hallmarks of PD are loss of nigrostriatal dopaminergic neurons and presence of intracellular alpha-synuclein, parkin, and ubiquitin contained in Lewy body (LB) inclusions.

CONCLUSION

PD is a neurodegenerative disorder caused by environmental and genetic factors. However, the onset mechanisms of PD currently remain unclear, and this represents an obstacle to the development of effective treatments. Previous studies demonstrated that oxidative stress is one of the main causes of PD. The formation of Lewy bodies and oxidative stress have been found in the pars compacta of the substantia nigra of PD patients [41–44]. Therefore, antioxidants are considered to be a promising approach to decelerate the progression of PD. Curcumin is regarded as a powerful antioxidant [45]. Previous studies reported the neuroprotective properties of curcumin in *in vitro* and *in vivo* PD models induced by several different environmental factors such as neurotoxins and genetic factors including *α-synuclein*,

In conclusion, we can say that zebrafish may become efficacious tool for high throughput screening for many diseases. They can be used with ease and effectiveness for initial screening of various drugs before subjecting them to rodent testing. Thus, saving number of rodents and also it assures 3R‘s of pharmacological testing.

Canavalia gladiata exhibited significant antiparkinsonian activity in haloperidol mouse model and zebrafish. It appears to be the most promising plant due to its L-DOPA content and potential antioxidant activity. The predictable mode of action of this plant may be due to increased synthesis of dopamine from L-DOPA and decreased lipid peroxidation due to the presence of flavonoids and polyphenols. These findings provide evidence for its use as antiparkinsonian medication, including prevention of PD, improvement of PD symptoms. Further studies are required to investigate the phytoconstituents responsible for the activity and also to establish the exact mode of action.

FUTURE SCOPE

Curcumin, a compound found in the spice turmeric, is proving effective at preventing clumping of a protein involved in Parkinson's disease, says a Michigan State University researcher.

A team of researchers led by Basir Ahmad, an MSU postdoctoral researcher, demonstrated earlier this year that slow-wriggling alpha-synuclein proteins are the cause of clumping, or aggregation, which is the first step of diseases such as Parkinson's. A new study led by Ahmad, which appears in the current issue of the Journal of Biological Chemistry, shows that curcumin can help prevent clumping.

"Our research shows that curcumin can rescue proteins from aggregation, the first steps of many debilitating diseases," said Lisa Lapidus, MSU associate professor of physics and astronomy who co-authored the paper with Ahmad. "More specifically, curcumin binds strongly to alpha-synuclein and prevents aggregation at body temperatures."

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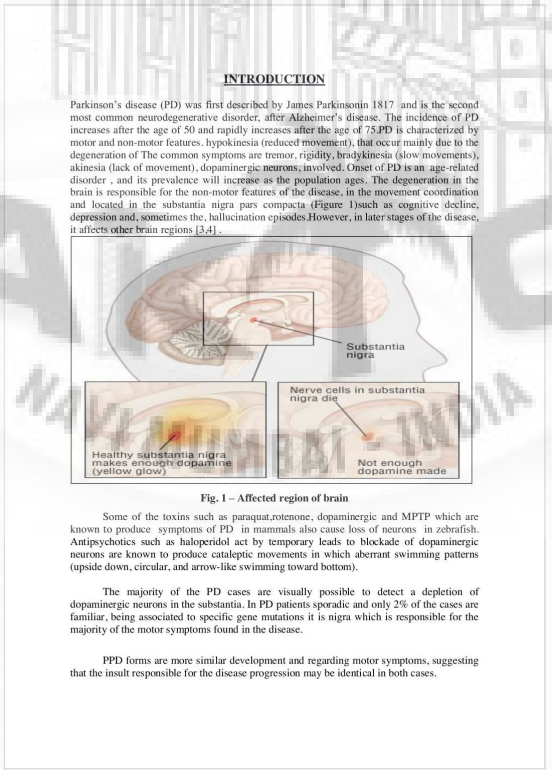

INTRODUCTION

Parkinson's disease (PD) was first described by James Parkinsonin 1817 and is the second most common neurodegenerative disorder, after Alzheimer's disease. The incidence of PD increases after the age of 50 and rapidly increases after the age of 75.PD is characterized by motor and non-motor features. hypokinesia (reduced movement), that occur mainly due to the degeneration of The common symptoms are tremor, rigidity, bradykinesia (slow movements), akinesia (lack of movement), dopaminergic neurons, involved. Onset of PD is an age-related disorder , and its prevalence will increase as the population ages. The degeneration in the brain is responsible for the non-motor features of the disease, in the movement coordination and located in the substantia nigra pars compacta (Figure 1)such as cognitive decline, depression and, sometimes the, hallucination episodes.However, in later stages of the disease, it affects other brain regions [3,4] .

Fig. 1 – Affected region of brain

Some of the toxins such as paraquat,rotenone, dopaminergic and MPTP which are known to produce symptoms of PD in mammals also cause loss of neurons in zebrafish. Antipsychotics such as haloperidol act by temporary leads to blockade of dopaminergic neurons are known to produce cataleptic movements in which aberrant swimming patterns (upside down, circular, and arrow-like swimming toward bottom).

The majority of the PD cases are visually possible to detect a depletion of dopaminergic neurons in the substantia. In PD patients sporadic and only 2% of the cases are familiar, being associated to specific gene mutations it is nigra which is responsible for the majority of the motor symptoms found in the disease.

PPD forms are more similar development and regarding motor symptoms, suggesting that the insult responsible for the disease progression may be identical in both cases.

EVALUATION OF PHYTOCONSTITUENT FOR ANTI-PARKINSON ACTIVITY USING ZEBRAFISH AS A MODEL ORGANISM

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