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Research Article

Neuroprotective Effects of *Citrullus colocynthis* Ethanolic Extract in a 6-OHDA-Induced Rat Model of Parkinson's disease

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Abstract:

Parkinson's disease (PD), a prevalent neurological disorder, is marked by the degeneration of dopamine-producing neurons. In this study, a Wistar rat model of PD induced by 6-hydroxydopamine (6-OHDA) was used to investigate the potential neuroprotective effects of an ethanolic extract of Citrullus colocynthis. The rats were injected with 6-OHDA using a Hamilton syringe and stereotaxic equipment to mimic Parkinsonism. After a 30-day post-surgery survival period, the rats received an oral dose of 200 mg/kg of the *Citrullus colocynthis* extract, which was prepared using a Soxhlet device with a 70:30 ethanol-to-water solvent ratio. The rats also underwent step-testing exercises. For comparison, a group received conventional therapy with 5 mg/kg of L-dopa+carbidopa. The efficacy of the treatment was evaluated based on tremor, salivation, and performance in pole tests, open field tests, and rotarod tests. The findings suggest that *Citrullus colocynthis* may possess significant neuroprotective properties, making it a promising adjunctive therapy for Parkinson's disease.

Keywords: Parkinson’s disease, *Citrullus Colocynthis*, 6-OHDA, Dopamine

Introduction:

Globally, idiopathic Parkinson's disease is common. Every racial and socioeconomic group exhibits it. As one ages, the incidence and prevalence of Parkinson's disease (PD) rise, impacting 1% of those over 65. By 2040, Parkinson's disease (PD) and other neurodegenerative illnesses including motor neuron disease and Alzheimer's disease are predicted to overtake cancer as the second leading cause of death (Singhal B, Lalkaka J et al,.2003).

1 Parkinson’s disease

Parkinson's disease (PD) is a long-term, degenerative neurological disorder that mostly affects motor function. In his 1817 publication "An Essay on the Shaking Palsy," Dr. James Parkinson first described the symptoms; the memorial is in his honour. Parkinson's disease is characterised by the degeneration of nerve cells

in the brain region known as the substantia nigra. These cells create the neurotransmitter dopamine, which is necessary for coordinating smooth, purposeful movements **(**Parkinson J. et al,.1817).

Clinical symptoms: The most identified motor symptoms associated with Parkinson's disease (PD) include loss of postural reflexes, stiffness, bradykinesia, and rest tremor. However, when the illness worsens, other symptoms might be seen, including respiratory problems, neuro-ophthalmological anomalies, and bulbar dysfunction.

1.1 Bradykinesia

Bradykinesia is the most common main motor symptom of Parkinson's disease (PD). It is caused by a decrease in neuronal density in the SN and is characterized by slow movement, amplitude drop, and problems with fine motor control. Patients with bradykinesia have trouble using their executive muscles rapidly because their energy supply is insufficient. Initial symptoms include difficulty multitasking and slower reaction times; later symptoms include difficulty swallowing, loss of gesture, and sluggish blinking **(**Berardelli A, Rothwell JC et al,.2001).

1.2 Tremor

One of the most obvious symptoms of Parkinson's disease (PD) is rest tremor, which is characterized by rhythmic muscle contraction and relaxation mostly on the limbs but also affecting the lips, chin, and jaw. The two primary characteristics of rest tremors in the hands are supination and pronation, which can be decreased by movement or while you sleep. PD patients have also been shown to have a postural tremor, which is an extended horizontal posture against gravity. Postural tremor is more visible than rest tremor and might be one of the first signs of Parkinson's disease (Jankovic J, Schwartz KS, Ondo W. et al.,1999). The two kinds of tremors, in contrast to essential tremor, occur in the same frequency range (4–6 Hz) and are treatable with dopaminergic drugs. Alcohol, beta-blockers, and botulin toxin may be helpful in treating essential tremor, which more frequently affects the head and voice (5–10). Early-life essential tremor has been implicated in studies of a possible risk factor for Parkinson's disease (Shahed J, Jankovic J.et al., 2007).

1.3 Rigidity

Rigidity is defined by increased resistance during passive mobilization of an extremity, independent of direction and velocity of movement and is one of the cardinal diagnostic features of Parkinson’s disease (PD), along with tremor, bradykinesia, and postural instability (Fung V. S. C., Thompson P. D. et al,.2007). It is challenging to explain stiffness in Parkinson's disease (PD) using the traditional paradigm of basal ganglia pathophysiology as the underlying mechanism is poorly known and there is no clear correlation between rigidity and dopamine deficit (Rodriguez-Oroz M. C., Jahanshahi M. et al,.2009). In contrast to what is observed, the classic description of basal ganglia activity in Parkinson's disease (PD) predicts that increased neuronal activity in the internal globus pallidus (GPi) and subthalamic nucleus (STN), and its subsequent inhibition of thalamocortical projections, should result in decreased muscle activation and a reduced response to stretching (Bezard E., Przedborski S. et al,.2011).

1.4 Postural Deformities

One prevalent and incapacitating characteristic as Parkinson's disease (PD) progresses is postural abnormalities. But the best course of treatment and its underlying pathophysiology remain unknown. We wanted to find key areas of study, noteworthy accomplishments, and new directions in postural abnormalities in

Parkinson's disease (PD). The onset of postural abnormalities poses a danger to one's independence, self-esteem, and quality of life and is a typical consequence of severe Parkinson's disease. Like kyphosis, scoliosis, antecollis, retrocollis, Pisa syndrome, and camptocormia. When a person has camptocormia, their thoracolumbar spine is severely anteriorly flexed for more than 45°. In this work, we utilized the bibliometric analysis approach to review previous research findings by extracting bibliography data on postural abnormalities in Parkinson's disease (PD) from the Web of Science Core Collection (WoSCC) database between 2002 and 2021 (Liu W, Wu J, Zhang N.et al,.2023).

1.5 Postural Instability:

PD patients' most frequent cause of emergency room (E.R) visits and the main motor-related cause of health care expenses is instability, which frequently results in falls. Reduce the increasing financial and psychological burden of PI in patients with Parkinson's disease, early detection of instability, on-going monitoring, and prompt, efficient treatments are required. The rise in falls reflects suboptimal diagnostics, which are brought on by insufficient pathogenic factor characterisation and quantification (Ozinga S.J., Machado.et al,.2015).

1.6 Etioglogy

The complicated condition known as Parkinson's disease (PD) is influenced by both genetic and environmental factors. Aging represents the biggest risk factor for Parkinson's disease (PD), with a median age of onset of 60 years (Lees AJ, Hardy J et al,.2009).

The disease's incidence rises with age in the 70–79 age range, reaching 93.1 (per 100,000 person-years) In addition, there are cross-cultural differences; Europe, North America, and South America have reported greater incidence rates than African, Asian, and Arabic nations (Kalia LV, Lang AE. el al,.2009).

1.6.1 Cigarette smoking

The link between cigarette smoking and Parkinson's disease (PD) has been thoroughly investigated, with generally positive findings. Many epidemiological findings are case-control studies that demonstrate a lower risk of Parkinson's disease development; bigger cohort studies also support this finding. Smoking and Parkinson's disease (PD) were shown to be inversely correlated in a major meta-analysis that included 44 case-control studies and 8 cohort studies from 20 different countries. The pooled relative risk for current smokers was 0.39 Smoking and Parkinson's disease (PD) were shown to be inversely correlated in two further meta-analyses, with pooled odds ratios ranging from 0.23 to 0.70, suggesting a protective mechanism against PD (Nicholl DJ, Appleton JP. Et al,.2015).

1.7 Pathology

When PD patients have minor frontal cortical degeneration and ventricular dilatation, their brains are frequently unremarkable macroscopically. The transverse slice of the brainstem exhibits the distinctive morphological alteration in Parkinson's disease (PD) brain, with the loss of darkly pigmented patches in the SNpc and locus coeruleus (see Figure 1 for a brain schematic). The loss of noradrenergic neurons in the locus coeruleus and dopamine (DA) neuromelanin-containing neurons in the SNpc is linked with this loss. Dorsal and medial neuronal cells are less vulnerable to neuronal loss in the substantia nigra (SN) than is the ventrolateral tier of pars compacta (A9) neurons (Dickson, D.W. et al,.2012).

This approach is criticized for not accounting for neuronal loss, instead focusing solely on the spread of Lewy pathology. The severity of motor

characteristics and the length of the disease are highly correlated with the nigral DA neuron loss, which rises to 60% or more after the onset of motor symptoms. The nigrostriatal pathway's denervation is the cause of this amazing cell loss, which lowers dopamine levels in the striatum. The emergence of the cardinal motor symptoms in Parkinson's disease (PD) is thought to be caused by a decrease in dopaminergic signalling. The locus coeruleus, the nucleus basalis of Meynert, the dorsal motor nucleus of the vagus nerve, the pedunculopontine nucleus, the raphe nuclei, the hypothalamus, and the olfactory bulb are among the subcortical nuclei that exhibit extensive cell loss in addition to the SNpc (Giguère, N.; Nanni, S.B.; Trudeau et al,.2018).

There are several different mechanisms that may be implicated in the beginning of Parkinson's disease. Apoptosis, or programmed cell death, is a possibility for SN neurons based on chromatin condensation and nuclear TUNEL staining observed in PD brains. After more investigation, PD brains showed altered iron deposition, inflammation, glial activation, elevated oxidative stress, lysosomal dysfunction, protein aggregation, and poor degradation (Miller, D.B.; O'Callaghan, J.P. et al,.2014).

1.7.1. Oxidative Stress

The brain uses around 20% of the oxygen that is supplied to the body in its whole.

In neurons and glia, a substantial percentage of t his oxygen transforms into reactive oxygen spec ies (ROS) from several sources.

The primary source of ROS at the mitochondria l level is the electron transport chain; other sour ces include nitric oxide (NO), monoamine oxida se, NADPH oxidase, and other flavoenzyme.

The respiratory chain is hampered by the oxidative damage of mitochondrial DNA, which inhibits complex. Furthermore, SN and striatum of PD patients have been shown to exhibit ROS-mediated DNA damage, protein oxidation, and increased levels of malondialdehyde, thiobarbituric acid, and 4-hydroxynonenal (HNE) (Umeno, A.; Biju, V.; Yoshida, Y. et *al*,.2017).

Similarly, different biological components are affected by reactive nitrogen species (RNS) such nitroxyl radicals, peroxynitrite ($ONOO^-$), and NO, which can lead to lipid peroxidation, protein oxidation, and DNA damage, eventually compromising cellular function. Proteins oxidized in this way produce thiols, carbon dioxide, and nitrotyrosine, which are detrimental to the protein's function. There are three distinct isoforms of nitric oxide synthase (NOS), and they all produce NO Neuronal NOS is found in astrocytes, neutrophils, and neurons. Endothelial NOS is found in astrocytes and vascular endothelium. Neuronal cultures, glial cells, hepatocytes, and macrophages all express inducible NOS. Three mechanisms exist for how NO damages DNA: direct interaction between RNS and DNA; inhibition of repair mechanisms; and generation of genotoxic substances such H2O2 and alkylating chemicals. Dinitrogen trioxide (N2O3) nitrosates amines to produce nitrosamines. N2O3 metabolizes to produce a variety of mutagenic alkylating species that damage DNA and prevent it from repairing itself (Kavya, R.; Saluja, R.; Singh, S.; Dikshit, M, Weiss, B et al,.2006).

1.7.2 Dopamine

In the presynaptic terminals of DA neurons, DA is biosynthesised. Through both high- and low-affinity amino acid transporters, tyrosine enters the neuron and, in the presence of the rate-limiting enzyme tyrosine hydroxylase, is transformed into dihydroxyphenylalanine (L-DOPA). Further decarboxylation of L-DOPA results in DA, which is then transported by vesicle monoamine transporter 2 into vesicles where a low pH environment inhibits

autoxidation (Maguire-Zeiss, K.A.; Short, D.W.; Federoff, H.J. et al,.2005).

Two isoforms of monoamine oxidase (MAO), MAO-A and MAO-B, and catechol-O-methyl transferase (COMT) are responsible for the enzymatic metabolism of DA. Glial cells are the main source of COMT, which is responsible for converting DA into 3-methoxytyramine (3-MT). After that, 3-MT is reduced by MAO to homovanillic acid (HVA), which is then excreted in the urine. However, MAO-B is in astrocytes, while MAO-A is mostly found in catecholaminergic neurons in SN. DA is broken down by MAO into 3,4-dihydroxyphenylacetaldehyde (DOPAL), which is then broken down into 3,4-dihydroxyphenylacetic acid (DOPAC) and H2O2 by aldehyde dehydrogenase (Olguín, H.J.; Guzmán, D.C.; García, E.H.; Mejía, G.B. et al,.2016).

1.7.3 Iron

As a co-factor of tyrosine hydroxylase, which transforms tyrosine into DA and then norepinephrine, iron plays a crucial role in the production of neurotransmitters. Iron may be bound by the chemical molecule known as catechol. A high iron intake can induce a significant rise in free radical generation, which overwhelms the body's defences and damages cells on several levels. Iron levels were higher in the SN of PD patients in both the pars compacta and pars reticulate (Bjørklund, G.; Hofer, T.; Nurchi, V.; Aaseth, J. et al,.2019).

1.7.4 Neuromelanin

An oxidative metabolite of norepinephrine and dopamine produces the dark, brown insoluble material known as neuromelanin NM. It interacts with lipids, poisons such as paraquat, and metal ions such as iron as well as pesticides. NM has two distinct functions: inside neurons, it shields cells from the damaging effects of toxins, active redox metals, and an excess of cytosolic catecholamines; outside of neurons, however, it triggers neuroinflammation by activating neuroglia when a neuron dies. A recent study found that GSH was oxidized to GSSG with the generation of H2O2 by protein-linked DA produced melanin, indicating a strong prooxidant activity. The high expression of major histocompatibility complex class I (MHC-I) in NM-containing organelles implies that MHC-I presents antigenic peptides on the neuronal membrane of catecholaminergic neurons containing NM, which is another possible harmful mechanism involving NM. Thus, it seems that there are several ways in which iron, DA, and NM interact (Zucca, F.A.; Basso et al,.2013).

1.7.5 Lipids

The process of lipid peroxidation produces HNE. Immunocytochemistry in SN revealed elevated levels of HNE in dopaminergic cells and in PD's cerebrospinal fluid. Further evidence of elevated lipid peroxidation levels in SN comes from reduced polyunsaturated fatty acids and elevated malondialdehyde levels. Protein nucleophiles such as amines and thiols can form persistent adducts with HNE, a lipophilic α-beta alkenyl that is extremely reactive. Activation of caspases 8, -9, -3, and DNA fragmentation are other ways that HNE might induce apoptosis. In addition, it suppresses complexes I and II of the mitochondrial respiratory chain, diminishes GSH level owing to quick consumption by GSH peroxidase, and promotes PARP cleavage. It also inhibits the NF-kB signalling pathway (Perfeito, R.; Cunha-Oliveira, T.; Rego, A.C. et al,.2012).

1.7.6. Glutathione

Tripeptide glutathione, which is composed of glutamate, cysteine, and glycine, is a vital non-enzymatic antioxidant. It takes two stages to synthesize glutathione. First, in the presence of a rate-limiting enzyme called γ-glutamylcysteine ligase, glutamate and cysteine are converted to γ-

glutamylcysteine. Afterward, glycine is added to γ-glutamylcysteine in the presence of glutathione synthase. Reduced oxidized glutathione is what glutathione reductase does to keep GSH levels stable. But GSH levels are changed by oxidative stress. Lower levels of glutathione are the consequence of glutathione conjugating with oxidized products of DOPAC, L-DOPA, and DA. Decreases in SN might potentially hinder the removal of H2O2 produced by DA autoxidation, which could lead to an increase in iron-induced OH- production. By producing more ROS, the compromised complex I also lowers GSH levels. Reduced synthesis because of glutathione reductase inhibition or an increase in glutathione disulfide (GSSG) levels and a changed GSH: AGSSG. On the other hand, SN glutathione depletion inhibits mitochondrial complex I by thiol oxidation, which in turn lowers mitochondrial activity (Voshavar, C.; Shah, M.; Xu, L.; Dutta, A.K. et al,.2015).

1.7.7. Mitochondria Dysfunction

Reduction of energy and oxidative phosphorylation are two major functions of mitochondria. The five primary multi-subunit complexes that make up the oxidative phosphorylation system are complex I (NADH dehydrogenase-ubiquinone oxidoreductase), complex II (succinate dehydrogenase-ubiquinone oxidoreductase), complex III (ubiquinone-cytochrome c oxidoreductase), complex IV (cytochrome c oxidase), and complex V (ATP synthase) (Kaidery, N.A.; Thomas, B. et al,.2018).

1.7.8. α-Synuclein

Presynaptic neuronal protein α-synuclein is 140 amino acids (AA) long and highly expressed. Seven highly conserved 11 AA repeat sequences create an amphipathic α-helix at their N terminals (1–60), which enables them to connect with membranes. Protein aggregation is brought on by the amyloidogenic hydrophobic nonamyloid component (61–95). The polar C terminal (96–140) facilitates the interaction of α-synuclein with other proteins, ligands, and metal ions. It is composed of charged amino acid residues that are responsible for post-translational modification. The two forms of α-synuclein—its membrane-bounded α-helical structure and its soluble, naturally unstructured form—coexist in equilibrium [92]. Because of α-synuclein's distinct structure, it may easily interact with anionic lipids to modify their conformation and promote aggregation. Consequently, autophagy, vesicular homeostasis, microtubule transport, lysosomal and mitochondrial activity, and autophagy may all be hampered by these aggregate-prone soluble forms of α-synuclein. Anterograde transport will be hampered by disruption of tubulins, kinesin-, and dynein-containing complexes, respectively [93]. Misfolding and aggregation are brought about by filamentous aggregates of phosphorylated and ubiquitinated α-synuclein found in the widespread proteinaceous cytoplasmic inclusions, LB, and LNs. Oligomers are soluble transient pre-fibrillar intermediates that are formed along with a non-fibrillar off-pathway. Oligomers undergo conversion into distinct cross beta-sheet conformational insoluble fibrillar aggregates. Numerous animal and cellular models demonstrate that overexpression of α-synuclein cause's cytotoxicity. The aggregation pathway is comprised of three stages: (1) a lag phase that limits the rate at which an aggregation-competent nucleus forms; (2) an elongation phase in which the nucleus transforms into protofibrils and higher-order aggregating species; and (3) a stationary phase where most of the soluble protein transforms into amyloid fibrils and a dynamic equilibrium between fibrils and monomers occurs. Failure in any of these pathways leads to the build-up of α-synuclein.

The pace of α-synuclein production and clearance, which happens by direct proteolysis, chaperones, autophagy, and proteasome-mediated destruction, maintains the amount of protein in the CNS (Rocha, E.M.; De Miranda, B.; et al,.2018).

1.8 Risk Factors

1.8.1 Genetic Risk

There are two types of Mendelian genetic transmission: autosomal dominant and recessive. Enhanced tendency of α-synuclein to form oligomers or fibrils is associated with the six-point mutations in the SNCA gene (53T, E46P, A30P, H50Q, G51D, and A53E) in autosomal transmission. Likewise, autophagy, mitochondrial function, and vesicle trafficking are implicated by LRRKS. α-synuclein breakdown is linked to cathepsin D trafficking and retrograde vesicle transport, which is caused by a mutation in the VPS35 gene. The mitochondrial respiratory complex is also connected to the CHCHD2 gene. In recessive transmission, PTEN-induced putative kinase 1 (PINK1) and FBXO7 interact with Parkin to degrade defective mitochondria. Additionally, to recruit to mitochondria, PINK1 interacts with Parkin. These genes are implicated in processes related to mitophagy, and stress-induced apoptosis can be prevented by overexpressing wild-type PINK1. A chaperone protein called DJ1 controls the translocation of PINK1-Parkin to mitochondria and stops α-synuclein from aggregating. Mutation results in changes to the morphology of the mitochondria and an increase in ROS. A juvenile-onset condition with Parkinsonism, dystonia, and supranuclear palsy is caused by ATP13A2. PLA2G6 is linked to iron buildup in the brain and neurodegeneration. FBXO7 is directly involved in mitophagy through interactions with Parkin and PINK1, and when mutant, it mislocalizes to the cytosol. Auxilian's NAJC6 coding is involved in the production and recycling of synaptic vesicles as well as clathrin uncoating. Mutations in the glucocerebrosidase (GCase) gene (GBA) cause Gaucher disease, a lysosomal storage disease. Carriers with heterozygous GBA mutations are also more likely to develop Parkinson's disease (PD). N370S, L444P, and E326K are GBA mutations. They disrupt the lysosomal-autophagy pathway, which is followed by a reduction in α-synuclein degradation. Mutations in GBA1 disrupt the normal functioning of mitochondria by producing more reactive oxygen species (ROS) and reducing the synthesis of ATP, oxygen consumption, and membrane potential. Additionally, the buildup of damaged and fragmented mitochondria is caused by GBA1 mutations (Hernandez, D.G.; Reed, X.; Singleton, A.B. et al,.2016).

1.8.2. Environmental Risk Factors

Oxidative stress and disruption of neurotransmission caused by neurotoxins in the brain have deleterious effects on the basal ganglia. Iron and copper are two chemical elements that contribute to oxidative stress; copper operates through the Fenton-Haber-Weiss reaction and the 6-OHDA redox cycle. Producing the α-synuclein aggregation dopamine decrease, and superoxide dismutase 1 reduction. Exposure to manganese (Mn) can alter oxidative parameters, including elevated lipid peroxidation, reduced glutathione levels, and increased protein oxidation. Lead (Pb), which mimics calcium and enters the brain through calcium channels, causes a decrease in voluntary muscular movements and swelling as well as the death of neurons in the central nervous system and peripheral nervous system. Pb may also aggravate neuronal dysfunction associated with Parkinson's disease (PD), impairing cognition. Mercury (Hg) can cause tremors and loss of voluntary muscular action by reducing the number of neurons in the brain. Moreover, pesticides can target the SN. Dieldrin,

an organochlorine pesticide, damages the dopaminergic system neurotoxically. The organophosphate rotenone inhibits the mitochondrial complex and enhances α-synuclein aggregation. Methamphetamine reduces the integrity of DA neuron terminals in basal ganglia and decreases DA and dopamine transporter expression. High doses of amphetamine may cause damage to dopaminergic neurons and axon terminals within the human brain. Cocaine binds to DA transporters causing short-term DA inhibition. Cocaine addiction can result in iron dysregulation. A variety of illicit substances can elevate ROS leading to neurotoxicity (Ball, N.; Teo, W.-P.; Chandra, S.; Chapman, J. et al,.2019).

1.9 Diagnosis

The neurological examination and medical history are usually the foundation of a physician's first evaluation. They use clinical diagnostic criteria to evaluate motor symptoms, such as bradykinesia and rest tremors. The conclusive evidence of Parkinson's disease (PD) is typically seen in the autopsy report when Lewy bodies are discovered in the midbrain. Over time, the illness's clinical history could deviate from Parkinson's disease (PD), thus it is important to routinely assess the presentation to ensure the diagnosis is accurate (Jankovic J. et al,.2008).

1.9.1 Medical history

The information that doctors learn via medical interviews makes up a patient's medical history. Get trustworthy and impartial data for managing the medical diagnostic and suggesting effective medical treatments, it involves the patient and eventually, those who are close to them. Symptoms are medically relevant concerns that are reported by the patient or those who know the patient; clinical signs, on the other hand, are determined by direct examination by medical experts. A history will usually be collected during a medical appointment. The breadth and focus of medical histories differ. For instance, an ambulance paramedic would normally restrict their history to pertinent information such as identity, past complaints, allergies, etc. A psychiatric history, on the other hand, is usually long and detailed since a lot of information about the patient's past is important to include when creating a treatment plan for a mental health condition. The doctor and other healthcare providers can develop a diagnosis and treatment plan based on the information gathered in this manner in addition to the physical examination. If a diagnosis cannot be made, a provisional diagnosis may be made, and additional options (known as differential diagnoses) may be added. These diagnoses are conventionally given in order of likelihood. The next step in the treatment strategy could be to do more research to confirm the diagnosis (Jankovic J. et al,.2008).

1.9.2 Neurological examination:

A neurological examination evaluates motor responses, particularly reflexes, and sensory neurons to see if the nervous system is compromised. A physical examination and a review of the patient's medical history are usually part of this. But not deeper investigation such as neuroimaging. It can be used both as a screening tool and as an investigative tool, the former of which is when examining the patient when there is no expected neurological deficit and the latter when examining a patient where you do expect to find abnormalities. If a problem is found either in an investigative or screening process, then further tests can be conducted to focus on a particular aspect of the nervous system (such as lumbar punctures and blood tests). In general, a neurological examination is focused on finding out whether there are lesions in the central and peripheral nervous systems or is another diffuse process that is troubling the

patient. Once the patient has been thoroughly evaluated, it is then the role of the physician to determine whether these findings combine to form a recognizable medical syndrome or neurological disorder such as Parkinson's disease or motor neuron disease. Finally, it is the role of the physician to find the cause for why such a problem has occurred, for example finding whether the problem is due to inflammation or is congenital (Nicholl DJ, Appleton JP. et al,.2015).

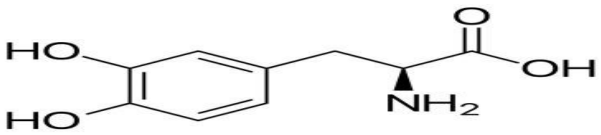
1.10 Management of Parkinson's Disease

Due to the chronic nature of Parkinson's disease (PD), a broad-based program is needed that includes patient and family education, support-group services, general wellness maintenance, exercise, and nutrition. At present, no cure for the disease is known, but medications or surgery can provide relief from the symptoms. While many medications treat Parkinson's, non-reverses the effects of the disease. Furthermore, the gold-standard treatment varies with the disease state. People with Parkinson's, therefore, often must take a variety of medications to manage the disease's symptoms ("Medications & Treatments et al,.2016).

1.10.1 Medication- The three primary medication families that help treat motor symptoms are MAO-B inhibitors, dopamine agonists, and levodopa. Depending on the stage of the disease, several treatment approaches are most frequently utilized. Typically, PD patients are divided into two phases: an early stage during which they have already experienced some degree of impairment that calls for medication, and a later stage during which they have motor problems associated with levodopa use. The goal of treatment in the early stage is to achieve the best possible balance between effective symptom management and negative effects that arise from increased dopaminergic activity. Postpone the development of dyskinesias, additional drugs including dopamine agonists and MAO-B inhibitors may be used before starting L-DOPA therapy reducing symptoms while managing drug response variations is the goal of the second stage. Overuse by certain individuals and abrupt drug withdrawals must also be managed (National Collaborating Centre for Chronic Conditions et al,.2006).

1.10.2 Levodopa

The most popular medication for more than 30 years has been levodopa, often known as L-DOPA. Dopa-decarboxylase converts L-DOPA into dopamine in dopaminergic neurons. L-DOPA treatment momentarily reduces motor symptoms since they are caused by a deficiency of dopamine in the substantia nigra.



Structure of 3, 4-dihydroxy-L-phenylalanin (levodopa)

L-DOPA only passes across the blood-brain barrier in 5–10% of cases. The remainder frequently undergoes further metabolism to produce dopamine, which can result in a broad range of adverse consequences, including as stiffness, nausea, and dyskinesia. Benserazide and carbidopa are examples of peripheral L-dopa decarboxylase inhibitors. By impeding the peripheral metabolism of L-DOPA, they enhance the supply of levodopa to the central nervous system. Usually, they are used in combination formulations with levodopa. Benserazide/levodopa (co-beneldopa, commercial name Madopar) and carbidopa/levodopa (co-careldopa, trade names Sinemet, Pharmacopa, Atamet) are the currently available formulations. Punding and dopamine

dysregulation syndrome, which is characterized by compulsive abuse of the drug, have also been linked to levodopa. Benserazide/levodopa (co-beneldopa, commercial name Madopar) and carbidopa/levodopa (co-careldopa, trade names Sinemet, Pharmacopa, Atamet) are the currently available formulations. Punding and dopamine dysregulation syndrome, which is characterized by compulsive abuse of the drug, have also been linked to levodopa (Samii A, Nutt JG, Ransom BR. et al,.2004). Madopar and Sinemet come in controlled, slow-release forms that prolong the effects of levodopa. Levodopa and carbidopa are combined to form duodopa. Compared to immediate-release formulations, slow-release levodopa has not demonstrated improved control over motor symptoms or consequences. Levodopa ultimately loses its beneficial effects and decreases the body's natural production of L-DOPA. Long-term use of levodopa preparations might result in the development of motor problems, such as dyskinesia's, or involuntary movements, and variations in the response to medicine. A former strategy to reduce motor complications was to withdraw patients from L-DOPA for a period; this is discouraged now because it can bring dangerous side effects such as neuroleptic malignant syndrome. Most people eventually need levodopa and later develop motor complications. When this occurs, PD patients change rapidly from stages with good response to medication and few symptoms ("on" state) to phases with no response to medication and important motor symptoms ("off" state). For this reason, levodopa doses are kept as low as possible while maintaining functionality (National Collaborating Centre for Chronic Conditions et al,.2006).

1.10.3 Dopamine agonists

Dopamine agonists function similarly to levodopa in the brain because they attach to dopaminergic postsynaptic receptors. Nowadays, most motor symptoms are treated with dopamine agonists alone to delay the development of motor issues. They were initially used as an adjunctive therapy for patients experiencing dyskinesias and intermittent hyperactivity in response to levodopa. Using them to reduce off-key moments in late-stage Parkinson's disease may be beneficial. Cabergoline, apomorphine, lisuride, ropinirole, pramipexole, pergolide, and piribedil are a few examples of dopamine agonists. Agonists cause notable, but minor, adverse effects such as sleeplessness, nausea, constipation, hallucinations, and insomnia. Occasionally, adverse effects might manifest at the lowest clinically effective dosage, prompting the doctor to look for another agonist or medication class. In contrast to levodopa, they treat severe symptoms while delaying motor problems. However, they often work well enough to control symptoms throughout the first several years. In addition, they cost more. Along with other adverse effects, dyskinesias with dopamine agonists are more prevalent in elderly individuals but rare in younger ones. Because of all of this, agonists are now the preferred first therapy for the former rather than levodopa for the latter. Reduce off periods and dyskinesia in late Parkinson's disease (PD) with the use of apomorphine, a dopamine agonist that is not taken orally. Patients using apomorphine should be continuously observed since side effects including disorientation and hallucinations are very uncommon. A little pump that the patient carries can be used to inject apomorphine subcutaneously. By continuously delivering a low amount of dopaminergic stimulation throughout the day, this low dose automatically reduces the swings in motor symptoms. During a first "apomorphine challenge" in the hospital to assess its efficacy, the primary caregiver—typically a spouse or partner—takes over pump maintenance. Prevent nodules from forming, the injection site needs to be switched every day and cycled across the body. For emergency dosages,

such as those required following a fall or first thing in the morning, apomorphine is also offered in a more acute dose as an auto injector pen. Vomiting and nausea are frequent, and domperidone, an antiemetic, may be necessary. Patients who took dopamine agonists were less likely to experience dyskinesia, dystonia, and motor fluctuations, according to a study comparing the effectiveness of the drug to levodopa. However, they were also more likely to stop their therapy because of adverse side effects like nausea, oedema, constipation, etc (National Collaborating Centre for Chronic Conditions et al,.2006).

1.10.4 MAO-B inhibitors

Drugs known as monoamine oxidase inhibitors (MAOIs) block the actions of either or both monoamine oxidase enzymes, monoamine oxidase B (MAO-B) and monoamine oxidase A (MAO-A). Their most well-known use is as powerful antidepressants, particularly for atypical and treatment-resistant depression. Along with many other illnesses, they are also used to treat Parkinson's disease, panic disorder, and social anxiety disorder (Cristancho, Mario A. (20 November 2012). prevent the metabolization of dopamine to raise its level in the basal ganglia. They prevent the breakdown of dopamine released by dopaminergic neurons by blocking monoamine oxidase-B (MAO-B). As a result, the striatum has more L-DOPA when MAO-B is reduced. When taken as monotherapy in the preliminary stages of the illness, MAO-B inhibitors relieve motor symptoms and postpone the need for levodopa, much like dopamine agonists do. However, they are less effective and have greater side effects than levodopa. While there is no evidence to support their effectiveness at the advanced stage, it does suggest that they may be helpful in lessening the oscillations between times of on and off. While a preliminary study suggested that taking selegiline together with levodopa increased the chance of mortality, this has now been refuted (National Collaborating Centre for Chronic Conditions et al,.2006).

1.11 Other

While there is evidence to support the usefulness of amantadine and anticholinergic in treating motor symptoms in both early and late stages of Parkinson's disease (PD), these drugs are not first-choice treatments. Multiple symptoms are present in addition to motor symptoms in Parkinson's disease (PD). Solve certain of these issues, different substances are employed. The use of atomoxetine for executive dysfunction, modafinil for daytime somnolence, clozapine for psychosis, and cholinesterase inhibitors for dementia are a few examples. According to a pilot trial, donepezil (Aricept) may assist Parkinson's patients avoid falling. Donepezil is a presently authorized treatment for the cognitive symptoms of Alzheimer's disease. It works by increasing the neurotransmitter acetylcholine levels. Participants in the trial who were taking donepezil fell half as frequently as those who were receiving a placebo, and those who had fallen most frequently before improved the most. A significant advance in the management of Parkinson's disease psychotic symptoms is the availability of clozapine, often known as Clozaril. Before it was developed, the only options for treating psychotic symptoms were first-generation antipsychotic medication or reducing dopamine therapy, both of which harmed motor function. Aripiprazole (Abilify), paliperidone (Invega), ziprasidone (Geodon), and quetiapine (Seroquel) are further atypical antipsychotics that are helpful in therapy. The medication clozapine is thought to be the most effective and least likely to cause extrapyramidal adverse effects (Hasnain M, Vieweg WV, Baron MS, Beatty-Brooks M, Fernandez A, et al,.2009)

1.11.1 Diet

It is common to develop gastroparesis (food sitting in the stomach for longer than normal) and constipation due to Parkinson's disease (PD) affecting the muscles and nerves that govern the digestive process. It is advised to have a balanced diet to aid with digestion. High-fiber meals and lots of water should be a part of every diet. Levodopa and proteins compete with one another for access to the blood-brain barrier and the intestine's similar transport system. When combined, these rivalries result in a decrease in the medication's efficacy. Thus, it is advised to avoid consuming too much protein when levodopa is first introduced. In later stages, it is also advised to consume more low-protein foods like bread or pasta for the same reasons. It is advised to take levodopa half an hour before meals to reduce protein interactions. In addition, PD regimens limit protein intake at breakfast and lunch, except supper. As the illness progresses, dysphagia might manifest. Specific actions in these situations include the use of thickening agents for liquid intake, unique feeding positions, and in the worst situations, a gastrostomy (Barichella M, Cereda E, Pezzoli G et al,.2009).

1.11.2 Surgery

Surgery was originally a prominent method of treating Parkinson's disease (PD), but it was limited to a small number of cases when levodopa was discovered. Due to advancements in surgical procedures brought about by research over the last several decades, patients with severe Parkinson's disease (PD) for whom medication treatment is no longer effective are once again undergoing surgery. Fewer than 10% of people with Parkinson's disease are good candidates for surgery. Ablative surgery, which involves the irreversible burning or freezing of brain tissue, stimulation surgery, often known as deep brain stimulation (DBS), and transplanting or restorative surgery are the three different modes of surgical therapy for Parkinson's disease (PD). The thalamus, the globus pallidus (the lesion method known as pallidotomy), or the subthalamic nucleus are the target locations for DBS or lesions (The National Collaborating Centre for Chronic Conditions, ed. (2006).

1.11.3 Neuroablative lesion surgery

During neuroablative lesion surgery, the brain regions linked to Parkinsonian neurological symptoms are identified and thermally destroyed. Typically, the operations entail a pallidotomy or thalamotomy. In 80–90% of cases, a thalamotomy involves destroying a portion of the thalamus, specifically the ventralis intermedius, to reduce tremor. The location of ablation is the subthalamis nucleus if stiffness and akinesia are evident. Pallidotomies are performed on Parkinson's patients who exhibit stiffness and akinesia. This procedure includes destroying the globus pallidus, specifically the globus pallidus interna. Multiple sessions of surgery are sometimes required to resolve tremors since it is challenging to precisely calculate the quantity of tissue to be removed. Because examining smaller sections of tissue is safer and prevents more catastrophic effects like paralysis or stroke, tissue is irreparably damaged and removed. Deep brain surgery has mostly taken the place of this technique (The National Collaborating Centre for Chronic Conditions, ed. (2006).

1.11.4 Deep brain stimulation

During surgery, a neurostimulator and electrodes are implanted to provide electrical impulses to specific brain locations that regulate movement. This technique is known as deep brain stimulation, or DBS. The medication is intended for a variety of movement disorders, including dystonia, essential tremor, and Parkinson's disease, as well as several neuropsychiatric

illnesses, such as epilepsy and obsessive-compulsive disorder (OCD). It is recognized that DBS modifies brain activity in a systematic manner, however its precise processes remain unclear and complicated. Since 1997, the Food and Drug Administration has authorized DBS as a therapy for Parkinson's disease (PD) and essential tremor. Because deep brain stimulation (DBS) is reversible, does not damage brain tissue, and can be customized to each patient at their specific illness stage, it is now the most widely utilized surgical therapy technique. Three pieces of hardware are used in DBS: an implanted pulse generator (IPG), also known as a neurostimulator, which produces electrical impulses that are used to modulate neural activity; a lead wire that guides the impulses to several metallic electrodes in the vicinity of the stimulation target; and an extension wire that joins the lead to the IPG. Typically, the titanium-encased, battery-operated IPG is implanted beneath the collarbone. and is linked to the lead by the subcutaneous extension, which runs from the exterior of the skull beneath the scalp all the way down into the brain to the stimulation target. Due to the popularity and historical precedent of cardiac pacemakers, as well as the structural similarities between the IPG and the three components of the system, the complete three-component system is frequently referred to as a brain pacemaker. Both the indirect and direct approaches can be used to pinpoint appropriate implantation locations preoperatively. The indirect approach locates the anterior and posterior commissures using computer tomography, magnetic resonance imaging, or ventriculography, and then defines the target region using predefined coordinates and distances from the anteromesial line. Verification of the target region can also be done using later histologically determined atlas maps. Using stereotactic preoperative MRI, the direct technique targets and visualizes deep nuclei. Unlike the indirect method, this method considers the anatomic variance in the size, location, and functional segregation of the nuclei among people. Patients with Parkinson's disease (PD) who do not have significant neuropsychiatric contraindications and whose medication-induced tremor and motor fluctuations are poorly managed, or who are drug intolerant, are advised to consider DBS. DBS is useful in reducing Parkinson's disease symptoms, particularly tremors. Recommendations on which Parkinson's patients are most likely to benefit from DBS were derived from recent clinical research (García MR, Pearlmutter BA, Wellstead PE, Middleton RH. et al,.2013).

1.12 Rehabilitation

There are few, poorly-quality studies on rehabilitation in Parkinson's disease. There is some evidence to suggest that rehabilitation can help with speech or movement issues. Preserve and enhance mobility, flexibility, strength, gait speed, and quality of life, regular physical activity and/or treatment might be helpful. Constipation may also be relieved by exercise. Research has demonstrated that exercise therapies can improve physical performance, health-related quality of life, balance, and fall risk in people with Parkinson's disease. No negative events or side effects followed any of the exercise interventions in an analysis of 14 trials looking at the benefits of exercise on people with Parkinson's disease; similar findings were reported in a more recent, broader evaluation conducted in 2023. There are five recognized potential processes by which exercise improves neuroplasticity. Exercise at an early stage of the disease can slow its progression. Intense activity maximizes synaptic plasticity. Complex activities promote greater structural adaptation. Rewarding activities raise dopamine levels, which in turn promote learning and relearning. Dopaminergic neurones are highly responsive to exercise and inactivity.

The Lee Silverman voice therapy (LSVT), which focuses on raising vocal volume and includes an intense one-month strategy, is one of the most often used therapies for speech impairments linked to Parkinson's disease. Improved voice and speech function may result from speech treatment, particularly LSVT. By assisting those who are afflicted with a disease in engaging in as many activities of daily living as they can, occupational therapy (OT) seeks to improve health and quality of life. While there is a dearth of high-quality research on the efficacy of occupational therapy, certain indications suggest that it may enhance motor skills and quality of life during therapy. Research teams are looking at whether virtual house calls might take the role of in-person visits to healthcare institutions for Parkinson's disease patient monitoring. After a year of such video encounters, participants in the experiment preferred the distant specialist. Although it involves access to and knowledge of Internet-enabled devices, home care was deemed convenient (Dorsey ER, Glidden AM, Holloway MR, Birbeck GL et al,.2018).

1.12.1Exercise

It can be helpful to maintain and enhance mobility, flexibility, strength, gait speed, and quality of life with regular physical activity, whether physiotherapy is administered. Parkinson's disease frequently results in sedentary behaviors that ultimately diminish the quality of life. Generalized relaxation methods, such as soft rocking, have been shown to reduce excessive muscular tension, which can help individuals suffering from stiffness become more flexible and range of motion. Slow rotations of the torso and limbs, rhythmic initiating, diaphragmatic breathing, and meditation practices are also helpful methods for fostering relaxation. Numerous techniques are used to enhance functional mobility and safety in response to common gait abnormalities linked to the condition, such as hypokinesia (slowness of movement), shuffling, and reduced arm swing. During rehabilitation programs, enhancing gait speed, stride length, base of support, trunk movement, and arm swing movement are all goals. Using pole walking and treadmill walking as assistive technology, verbal cueing (manual, visual, and auditory), workouts (marching and PNF patterns), and changing surroundings (surfaces, inputs, open vs. closed) are some strategies. Patients with primary muscular weakness and weakness attributable to inactivity in cases of mild to severe Parkinson's disease have shown gains in strength and motor skills after engaging in strengthening activities (O'Sullivan SB, Schmitz TJ et al,.2007).

1.13 Ayurvedic treatment

The age-old Indian medical system known as Ayurveda takes a comprehensive approach to treating illness, emphasizing the harmony of the body, mind, and soul. Although Ayurveda offers a unique viewpoint on neurological conditions, it is crucial to remember that Parkinson's disease is a complicated illness, and modern studies have not conclusively shown how effectively Ayurvedic remedies work in controlling it. When providing therapy, ayurvedic practitioners may consider a patient's lifestyle, food habits, and constitution (dosha). Ayurvedic treatments for Parkinson's disease patients may include the following.

1.13.1 Panchakarma Treatment: This is a series of cleansing techniques meant to help the body become more detoxified. Usually, it entails treatments like massage, herbal steam, and the use of herbal oils topically. Herbal Remedies: In Ayurveda, herbal formulations are frequently used to promote general health. Herbs like Mucuna pruriens and ashwagandha (Withania somnifera) may be taken into consideration due to their possible neuroprotective qualities. Ayurvedic medicine places a strong emphasis on nutrition to preserve health. A diet that balances

the doshas and places a focus on readily digested and nutritious meals is one of the recommended approaches. Ayurvedic doctors may suggest lifestyle adjustments to lower stress, get better sleep, and increase general well-being. Exercises like yoga and meditation could be recommended. It is critical to proceed cautiously with Ayurvedic remedies for Parkinson's disease and to consult medical experts before beginning any new regimen. Ayurveda may provide supporting measures for Parkinson's disease, but it is not a replacement for standard medical therapy. Parkinson's disease is a degenerative neurological illness. It is important to let your healthcare provider know about any complementary or alternative therapies you are thinking about, as certain herbs or treatments can cause unexpected adverse effects or interact negatively with pharmaceuticals. It is crucial to adhere to your neurologist's or other healthcare provider's recommendations and to keep up with routine check-ups. Because there is little data on the efficacy and safety of Ayurvedic remedies for Parkinson's disease, people should proceed with caution and consult with their healthcare practitioners to make educated decisions (Dwivedi PC. et al,.2017).

MATERIALS AND METHODS

2.1 Materials

S. No	List of Chemicals and Equipments
1	*Citrullus colocynthis* root (200g)
2	Ethanol (70% v/v)
3	H_2O
4	Soxhlet Apparatus
5	Water Bath
6	Whatman Filter Paper
7	Desiccator
8	Mortar and Pestle
9	Muffle Furnace
10	6-OHDA (6-Hydroxydopamine)
11	Ketamine
12	Xylazine
13	Imipramine
14	Ascorbic Acid (0.1%)
15	Lidocaine HCL (2%)
16	Meloxicam (1mg/kg)
17	Levodopa (5mg/kg)
18	Stereotaxic Apparatus
19	Hamilton Syringe (10 µl)

20	Scalpel and blades
21	Scissors
22	Scraper
23	Tweezers
24	Infusion pump
25	Amphetamine (2.5mg/kg)
26	Ascorbic Acid (0.1%)
27	LidocaineHCL (2%)
28	Meloxicam
29	Povidone-iodine
30	Levodopa

Chemical reagents: - All the chemicals used in this study were obtained from Labex corporation Pvt Ltd. (New Delhi, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), Mahesh medical agency (Sagar MP), Suresh medical store (Sagar MP) All the chemicals and solvent used in this study were of analytical grade.

2.2 Preparation of Plant Material

Collection of plant material

Root of *Citrullus colocynthis* were collected from Chambal ravines Morena Madhya Pradesh and dholpur Rajasthan region in separate sterile bags from Morena, Madhya Pradesh, and month of August 2023 the plant is authenticated from botany department of Dr Hari Singh Gour University Sagar authentication BOT/H/03/74/106 Plant material (root part) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water, they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder Powdered plant material was observed for their colour, odour, taste, and texture Dried plant material was packed in airtight container and stored for phytochemical and biological studies. The ground material is filtered through a sieve with mesh number 22 to ensure uniform particle size.

Shade Drying and Powdering:

The powdered root of *Citrullus colocynthis* was shade dried at room temperature to preserve its bioactive compounds.

The shade dried plant material was coarsely powdered to increase its surface area, facilitating the extraction process.

Defatting of plant material

The provided process involves the extraction of bioactive compounds from the powdered root of *Citrullus colocynthis* using a combination of Soxhlet and maceration methods. Here is a detailed breakdown of the process.

Extraction Process

Soxhlet Extraction - Setup the Soxhlet apparatus. Use a solvent mixture of 70% ethanol and 30%

Water (H_2O.). Run the Soxhlet extraction cycle to extract the bioactive compounds from the ground root material.

Soxhlet Extraction: The powdered plant material was subjected to extraction using a Soxhlet apparatus with a solvent mixture of Ethanol 70% and H_2O. 30%

The extraction was continued until the defatting of the material had taken place, indicating the removal of non-polar compounds and the enrichment of polar compounds.

Maceration Method: The powdered plant material was extracted with ethanol using the maceration method, which involves soaking the material in the solvent for an extended period. This method allows for the extraction of a broader range of compounds, including those that might not be extracted using the Soxhlet method alone.

Filtering and Evaporation: The resultant extract was filtered using Whatman filter paper no. 1 to remove any impurities.

The filtered extract was then evaporated to obtain a dry, concentrated extract.

Post-Extraction Processing Evaporation: After the extraction process, the solvent mixture is evaporated using water bath. Continue evaporation until the extract is completely dry.

Drying: - Further drying is done using a desiccator to ensure all moisture is removed.

Powder Form: - The dried extract is then converted into a fine powder form for further analysis and use.

Yield Calculation and Storage: The dried crude concentrated extract was weighed to calculate the extractive yield, which represents the percentage of bioactive compounds extracted from the original material. The extract was then transferred to glass vials (6 x 2 cm) and stored in a refrigerator at 4°C until used for analysis. This process is a common method for extracting bioactive compounds from plant materials, particularly those with a high content of polar compounds. The combination of Soxhlet and maceration methods allows for a comprehensive extraction of various compounds, which can be useful for identifying potential bioactive molecules and understanding their properties and activities.

2.3 Phytochemical screening: -Phytochemical screening is a critical process in the identification of bioactive compounds in plant extracts. Here is a detailed breakdown of the standard procedures for some common phytochemical tests, focusing on the visual observations expected:

- **Alkaloids:**
  - Mayer's Test: Add Mayer's reagent (potassium mercuric iodide) to the extract. A creamy white precipitate indicates the presence of alkaloids.
  - Wagner's Test: Add Wagner's reagent (iodine in potassium iodide) to the extract. A reddish-brown precipitate suggests the presence of alkaloids.
- **Flavonoids:**
  - Shinoda Test: Add a few pieces of magnesium ribbon and concentrated hydrochloric acid to the extract. A pink, red, or magenta colour indicates the presence of flavonoids.
  - Alkaline Reagent Test: Add a few drops of sodium hydroxide solution to the extract. An intense yellow colour, which becomes colourless upon addition of dilute acid, indicates the presence of flavonoids.
- **Saponins:**
  - Foam Test: Shake the extract with water in a test tube. Persistent frothing or foam formation indicates the presence of saponins.

- **Tannins:**
  - Ferric Chloride Test: Add ferric chloride solution to the extract. A blue-green or dark green colour indicates the presence of tannins.
- **Phenols:**
  - Ferric Chloride Test: Add a few drops of ferric chloride solution to the extract. A deep blue or black colour indicates the presence of phenolic compounds.
- **Terpenoids:**
  - Salkowski Test: Add a few drops of concentrated sulphuric acid to the extract along with chloroform. A reddish-brown interface indicates the presence of terpenoids.
- **Glycosides:**
  - Keller-Killiani Test: Add glacial acetic acid, one drop of ferric chloride solution, and concentrated sulphuric acid to the extract. A blue-green colour in the upper layer and a reddish-brown colour at the junction indicate the presence of glycosides.
- **Steroids:**
  - Liebermann-Burchard Test: Add acetic anhydride and concentrated sulphuric acid to the extract. A blue-green colour indicates the presence of steroids.

Each test relies on a specific reagent that reacts with the compound of interest, leading to a colour change or precipitate formation that is visible to the naked eye. This method allows for a preliminary identification of the types of bioactive compounds present in the plant extracts.

Experimental animal: In the present study, Albino rats weighing around 200 to 250 g were chosen. The rats were fed food, water, and ad libtium during the experiments. This study was conducted by the ethical standards outlined by the Institutional Animal Ethical Review Committee of Adina institute of pharmaceutical sciences, Sagar (Madhya Pradesh) The committee, comprised of experts in the field of ethics and research, reviewed and approved the study protocol (1546/PO/RE/S/11/CCSEA)

2.4 In-vivo antiparkinsonian activity

Induction of Parkinson's disease by 6-OHDA administration

The primary purpose of the 6-OHDA model is to reproduce the motor symptoms of PD, including tremors, ataxia, spasticity, salivation, lacrimation, and hypothermia. These symptoms are induced by the selective destruction of dopaminergic neurons, which results in an imbalance between the direct and indirect striatal pathways. The 6-OHDA model allows researchers to investigate the underlying mechanisms of these motor symptoms and to test potential therapeutic strategies.

6-Hydroxydopamine is the first toxin-based animal model of PD consisted of rats sustaining intracerebral injections of 6-hydroxydopamine (6-OHDA) (Ungerstedt, 1968). This chemical is a hydroxylated analog of DA that also occurs in the brain (Jellinger et al., 1995). 6-OHDA is a catecholamine-selective neurotoxin because it enters neurons via the dopamine or noradrenaline transporter. Once inside the neuron, 6-OHDA undergoes auto-oxidation and conversion to reactive oxygen species (ROS) Study the neuroprotective effect of *Citrullus colocynthis* ethanolic extract, the unilateral 6-OHDA lesion model was employed in our study (Rotman and Creveling, et,.1976).

Albino rats were used for the experimental study. The animals were maintained under standard husbandry conditions in polypropylene cages and provided with food and water ad-libitum. They were fasted overnight prior to the experimentation. Throughout the experiment, the animals were kept at room temperature under suitable nutritional and environmental

conditions. All experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by the Ministry of Environment and Forest, Government of India, New Delhi, India.

Concerning the study performed with *Citrullus colocynthis* extract for PD elicited by 6-OHDA, the accurate delivery of neurotoxin to rat brains was made by employing a stereotaxic instrument. For surgery, we used a computerized stereotaxic frame (Stoelting Co., USA) which came with an incisor bar and non-puncture ear bars. The process was conducted as follows:

- ➔ Anaesthesia: Rats were anaesthetized with isoflurane in oxygen: 3% for induction, and 1%. 5–2% for maintenance.
- ➔ Positioning: The positioning of the incisor bar at - 3 was done. Below the interaural line was 3 mm, and the rat was then anesthetized and secured in the stereotaxic frame.
- ➔ Preparation for Surgery: A povidone-iodine solution was applied to the patient's scalp following shaving to disinfect it.
- ➔ Incision and Exposure: The skull was flapped after making an incision on the midline of the scalp.
- ➔ Identification of the Bregma: The Bregma was measured, calculated, and then used as the standard stereotaxic coordinate.
- ➔ Coordinates: The atlas employed in this study was the rat brain atlas as described by to determine the coordinates of MFB, which are ML: -1. 2 mm from the midline, DV: Therefore, X has a negative balance of -7 in the current period. 8 mm from the dura, and AP: On the other hand, some had a severity level of 4 we implanted the electrode 4 mm from the bregma.
- ➔ Burr Hole: At the said coordinates, a small hole was drilled.
- ➔ Injection: A Hamilton syringe with a 33-gauge needle and a 10-l capacity was loaded with 8 g of 6-OHDA solution in 8 l of 0.
- ➔ Infusion: A micro infusion pump was used to infuse the 6-OHDA solution at a rate of 0.5 µL/min.
- ➔ The 4-5 cm needle was left in place for another 5 minutes after the injection to enhance diffusion and prevent backflow.
- ➔ Closure of the Wound: They used absorbable sutures that were used to close the incision on the scalp area.
- ➔ Post-operative Care: During their healing, the animals were attentively determined and given Meloxicam (0.05 mg/kg, S.C.) for analgesia.

2.5 Treatment schedule of *Citrullus colocynthis* extract

Following treatment schedule of *Citrullus colocynthis* extract was adopted to assess antiparkinsonian activity.

Group	Treatment	Dose & Route	No of Animals	Administration of Toxicant	Administration of Drugs	Behavioural assessment: Post Treatment
Control	Saline	1 mL, oral	6	-	Saline treatment	On 30th day Behavioural assessment was done

Disease Control	6-OHDA	0.4 µL, ICV	6	Single administration of 6-OHDA on day 0	-	On 30th day Behavioural assessment was done
Standard	6-OHDA + L-DOPA + Carbidopa	5.0 mg/kg + 1.250 mg/kg, oral	6	Single administration of 6-OHDA on day 0	15 days consecutive administration from day 15 of surgery	On 30th day Behavioural assessment was done
Test 1	6-OHDA + *Citrullus colocynthis* extract	0.4 µL ICV + 100 mg/kg oral	6	Single administration of 6-OHDA on day 0	15 days consecutive administration from day 15 of surgery	On 30th day Behavioural assessment was done
Test 2	6-OHDA + *Citrullus colocynthis* extract	0.4 µL ICV + 200 mg/kg oral	6	Single administration of 6-OHDA on day 0	15 days consecutive administration from day 15 of surgery	On 30th day Behavioural assessment was done

Animal groups were treated as below:

- ➔ Group I: control animals, which received saline treatment throughout the study period.
- ➔ Group II: Disease control animals, which were administered with a single dose of 6OHDA.
- ➔ Group III: Standard drug treatment animals, which received standard dose of L-Dopa + Carbidopa along with 6OHDA.
- ➔ Group IV: animals received 100 mg/kg *Citrullus colocynthis* extract + 6-OHDA.
- ➔ Group V: animals received 200 mg/kg *Citrullus colocynthis* extract + 6-OHDA.

6-OHDA was administered to all the animals from Group II to V, through stereotaxis surgery. Post surgery animals were left for 15 days. Animals in Group III, IV and V received their respective treatment from the 15th day of surgery. This treatment was given for the next 15 days. On the 30th day of surgery all animals were subjected to motor and behavioural assessment.

A battery of tests used in the *in vivo* pharmacological screening was intended to assess several extraneous aspects of an animal's behaviour and motor.

- ➔ **2.5.1 Open Field Test:** Frequently used behavioral tests are the open field test, which estimates the mice's motor activity, playing activity, and anxiety-like response. This test is relevant to research on Parkinson's disease (PD) because it can measure motor impairment as well as non-motor phenomena such as anxiety and a reduction in exploratory activity (Seibenhener & Wooten, 2015). The apparatus in each learned

and conditioned test was a 100 cm x 100 cm x 40 cm square arena with black walls, and the floor was divided into 25 equal squares. Five minutes of behaviour were recorded.

Figure 5.1 Open Field Test

Following parameters were measured: Total distance covered in centimetres, mean speed (in cm/s), duration of stay in the main area (s), number of incidents raised and quantity of grooming incidents.

➔ **2.5.2 Rota-Rod Test:** This test is used in this study to establish the impact of *Citrullus colocynthis* ethanolic extract of rats with 6-OHDA lesions on the motor coordination of rats**.**

Figure 5.1 Rotarod apparatus

The assembly of the rotarod was confirmed by 5 equal parts divided and supported by discs with a diameter of 25 cm while the rod used to rotate on was with a diameter of 3 cm. They positioned the pole slightly more than fifty centimetres from the ground so that the animals could not jump over it. From 5 rpm to 40 rpm, with increments of 5 rpm within every five minutes, the speed was gradually increased. Of this, a maximum of 300 seconds was used to record the latency to fall (Deumens et al., 2013)

➔ **2.5.3 Tail Suspension Test:** The specific procedure that has been used involves modification of the TEST for PD models to capture non-motor function and assess depressive-like behaviour in mice (Can et al., 2012). In our study, we applied the TEST to investigate if *C. colocynthis* extract is likely to produce an antidepressant-like effect in rats with 6-OHDA lesions. Adhesive tape was placed approximately one centimetre from the extremity of rats' tails, and then the rats were hanged by the tails.

Figure 5.3 Tail Suspension Test

The suspension point was hanging 50 centimetres off the ground. This variable was assessed in the last four minutes of the test, and each animal was suspended for a total of six minutes. The meaning of immobility was that a

patient could not move any of his limbs or any other part of his body except for those movements that were caused by breathing (Can et al., 2012).

Results

3 Phytochemical Screening

Phytochemical screening of the different extracts of *C. colocynthis* fruit showed the presence of the following phytochemical compounds (Table 1).

Table 2: Phytochemical compounds present in *Citrullus colocynthis.*

Phytochemical Compounds	Hydroethanolic Extract
Tannins	+
Phenols	++
Alkaloids	+++
Saponins	++
Terpenoids	+
Flavonoids	+++
Steroids	++
Carbohydrates	+
Glycosides	+
Saponin	++
Protein	+

(+) = good; (++) = moderate; (+++) = excellent

The study establishes the neuroprotective effects of Citrullus colocynthis ethanolic extract in a 6-OHDA-induced rat model of Parkinson's disease through various statistical analyses.

3.2 Acute toxicity study

In acute toxicity study we did not observe any mortality during 14 days of observation period.

3.3 Analysis of Results from Open Field Tests

- ➔ Total distance travelled and average speed: Stereotaxing with 6-OHDA causes a significant reduction in motor movements. The results also revealed that lesions with 6-OHDA reduced motor activity. The L-DOPA therapy returned locomotion to a level comparable to the control group. Enhancement profiles of the extract from *Citrullus colocynthis* were also found to vary with the escalation of the doses near the 200 mg/kg level, which was as effective as L-DOPA.
- ➔ 6-OHDA-lesioned rats spend less time in the middle zone; this indicates increased anxiety-like behavior. The depression of the central zone time was significantly increased by both L-DOPA and *Citrullus colocynthis* (200 mg/kg), which suggests anxiolytic effects.
- ➔ Rearing episodes: The lesioning of the rats with 6-OHDA led to a considerable decrease in what is known as rising or the episodes of vertical exploration. Rearing behaviour was highly increased, suggesting better motor function and/or inquisitive drive by L-DOPA and *Citrullus colocynthis* (200 mg/kg).
- ➔ Grooming episodes: 6-OHDA lesions lessened grooming behaviour as a manifestation of DAD. The control group, the L-DOPA, and the C. Col extract at a dosage of 200 mg/kg normalized the grooming degree, which means the patients improved their self-care practices and overall well-being.

These outcomes revealed that the chosen dosage of *Citrullus colocynthis* extract, namely 200 mg/kg, possesses significant neuroprotective effects in the model of PD induced by 6-OHDA. Reduced anxiety-like behaviour and motor function (locomotor activity and rearing) were improved by the extract.

The extract from *Citrullus colocynthis* confirmed comparable effectiveness to L-DOPA in several parameters when used at a larger

dosage, indicating that it might be used as an extra or alternative remedy for PD. The results are indicated in table 3.3.

Table 3.3: Open Field Test

Parameter	Control	6-OHDA	L-DOPA+ Carbidopa	6-OHDA +*Citrullus colocynthis* (100 mg/kg)	6-OHDA + *Citrullus colocynthis* (200 mg/kg)
Total distance (cm)	2485 ± 152	1187 ± 98*	2312 ± 145#	1785 ± 123*#	2098 ± 138#
Average velocity (cm/s)	8.28 ± 0.51	3.96 ± 0.33*	7.71 ± 0.48#	5.95 ± 0.41*#	6.99 ± 0.46*#
Time in central zone (s)	42.5 ± 4.8	15.3 ± 2.7*	38.7 ± 4.5#	27.8 ± 3.6*#	34.6 ± 4.2#
Rearing episodes	18.3 ± 2.1	7.5 ± 1.4*	16.8 ± 2.0#	12.4 ± 1.7*#	15.2 ± 1.9#
Grooming episodes	4.2 ± 0.6	2.1 ± 0.4*	3.9 ± 0.5#	3.0 ± 0.5*	3.6 ± 0.5#

*p < 0.05 vs. Control; #p < 0.05 vs. 6-OHDA

3.4 Analysis of Results from tail suspension test

Based on the study, the immobility period to tail suspension was significantly (p<0. 001) prolonged in the 6-OHDA-lesioned animals compared to the control animals, which characterized the depressive-like behavior. L-DOPA treatment of the animals significantly reduced the immobility period (p<0.01), suggesting an antidepressant-like effect. Compared to the 6-OHDA, both dosages of *Citrullus colocynthis* extract caused a significant reduction in immobility time (p < 0.01), like the effect of L-DOPA treatment with a higher dose of *Citrullus colocynthis* extract (200 mg/kg). Therefore, from the results of this experiment, it may be inferred that the extract of *Citrullus colocynthis* could possess motor-improving properties along with antidepressant-like activity. This might be attributed to changes that take place in the neurotransmitter systems like norepinephrine or serotonin that are proved to influence mood [189]. As a result, *Citrullus colocynthis* can be used as a therapeutic agent due to the scaled-up improvements that were evidenced in the PD's motor and non-motor symptomatology. The results are depicted in Table 3.4

Table 3.4: Immobility time in the tail suspension test for experimental groups

Group	Treatment	Immobility time (s)
1	Control (Saline)	98.5 ± 7.2
2	6-OHDA	186.3 ± 12.4*
3	6-OHDA + L-DOPA	124.7 ± 9.5#
4	6-OHDA + *Citrullus colocynthis* (100 mg/kg)	147.2 ± 10.8#
5	6-OHDA + *Citrullus colocynthis* (200 mg/kg)	132.6 ± 9.7#

mean ±SEM (n=6). "*p<0.001 vs. Control; #p<0.01 vs. 6-OHDA group"

3.5 Analysis of Results from Rota-road: The results demonstrate that lesioning with 6-OHDA significantly impaired motor activity, as indicated by decrease in latency. In the current study, it was verified that the administration of L-DOPA enhanced motor function, being almost equivalent to the sham-treated group. Notably, the rotarod result proved to be ameliorated by both dosages of *Citrullus colocynthis* extract compared to the 6-OHDA group.

Table 3.5: Rota rod performance of experimental groups

Group	Treatment	Latency to fall (s) at 20 rpm	Latency to fall (s) at 40 rpm
1	Control (Saline)	285.3 ± 10.2	198.7 ± 8.5
2	6-OHDA	92.6 ± 7.8*	45.3 ± 5.2*
3	6-OHDA + L-DOPA	246.8 ± 11.5#	162.4 ± 9.7#
4	6-OHDA + *Citrullus colocynthis* (100 mg/kg)	198.5 ± 9.3#	124.6 ± 7.8#
5	6-OHDA + *Citrullus colocynthis* (200 mg/kg)	237.2 ± 10.7#	153.9 ± 8.9#

Mean ±SEM (n=6). "*p<0.001 vs. Control;" "#p<0.01 vs. 6-OHDA group"

Discussion

The protective effects have been established by using the 6-OHDA-induced rat model of PD in the study findings recorded in this work to affirm *Citrullus colocynthis* ethanolic extract as neuroprotective. The statistical analyses demonstrate, in several motor and behavioral indicators, that there is a difference between the treatment groups.

Motor Function: These results of the Rotarod test ($p < 0.0001$) indicated that motor coordination in the 6-OHDA group was significantly reduced as compared to the control group. Thus, concerning

the 6-OHDA group, the depicted doses of *C. colocynthis* extract significantly improved motor activity ($p < 0.001$), leveling up to L-DOPA in the case of the 200 mg/kg dose. The two-way ANOVA confirms that treatment and rotarod speed have significant effects on motor performance.

These findings are consistent with the stepping test and cylinder test, which clearly show an improvement in motor activity and limb utilization after administering *C. colocynthis* treatment. As depicted in the analysis, the stepping test also correlated significantly with the cylinder test, another evaluation procedure ($r = 0.791$, $p < 0.0001$), indicating that the stepping test conforms to the cylinder test's consistent positive progressive pattern in evaluating motor function improvement.

Behavioral Assessments: In the apomorphine-induced rotation test, both concentrations of *C. colocynthis* were able to reduce the rotation behavior in comparison to the 6-OHDA group ($p <$). Qualified group differences were confirmed by the Kruskal-Walli's test, whose results are presented as $p < 0.0001$. The results from the elevated plus maze and forced swim tests reflect a decrease in anxiety- and depression-like behavior while on the *C. colocynthis* medication. The forced swim test ANOVA result shows a significant difference between the groups ($p < 0.0001$), and the post-hoc test also supports the efficacy of the 200 mg/kg concentration.

Cognitive Function: It also results in significant improvements in the new object recognition test results. As a result, treatment with *C. colocynthis* improves cognitive function. Though the 200 mg/kg dosage was effective as compared with the 6-OHDA group, it was statistically at par with the L-DOPA group ($p = 0.1679$), suggesting similar efficacy in enhancing cognitive ability.

Open Field Test: Therefore, in the context of the open field test, it was discovered that *C. colocynthis* therapy enhanced locomotor activity, inquisitive behaviour, and anxiety-related activities. There has been a continuous increase in general motor activity and exploratory behavior, as evidenced by the strong positive correlation ($r. = 0.724$, $p<0.0001$) between the total distance moved and the rearing episodes.

Physiological Parameters: This study concludes that time and therapy had both highly positive effects on the patients, with repeated measures ANOVA giving $p < 0.0001$ and $p = 0.0412$ for time and therapy, respectively. Based on this, it can be concluded that body weight is affected by 6-OHDA-lesioning and subsequent therapies; the change may not be constant and could be different over time.

As for the survival rate, it can be seen in the survival rate analysis of patients that the survival rate is higher in the *C. colocynthis* treatment group but failed to reach a significant difference ($p = 0.0726$). This may have happened due to the small sample of participants; therefore, such studies should be conducted on large populations in the future. Effects Dependent on Dose: At the dosage of 200 mg/kg, the effect of *C. colocynthis* extract was significantly higher than at the 100 mg/kg dose in most of the analyzed indicators, often stabilizing at the level of L-DOPA influence. This extract's dose-dependent action implies that there may be a therapeutic window for employing *C. colocynthis* extract in the treatment of Parkinson's disease, depending on the doses administered. Mechanism of Action: Even though the mode of action was not explored in detail in this work, the observed changes in behaviour, motor activity, and cognition that have been reported suggest that the extract of *C. colocynthis* might be neuroprotective in nature. A potential restoration

of striatal dopamine balance is indicated with the aid of lower apomorphine-induced rotations. This reduction may be attributable to the antioxidant, anti-inflammatory, or neurotrophic traits of the extract's components.

Conclusion

As a result, the findings of this extensive study, which support the neuroprotective effects of *C. colocynthis* ethanolic extract in a 6-OHDA-induced rat model of Parkinson's disease, highlight the need for more research. The approach reveals the extract's multiple positive impacts on the disease, including assessments of motor, behavioural and cognitive changes. All the tests, including the Rotarod, stepping, and cylinder tests, demonstrated a marked enhancement in the functionalities, which might mean that the use of *C. colocynthis* extract may help lower the general motor manifestations related to PD. This is why such results are especially encouraging, especially since in this case, the efficacy is directly proportional to the dosage, and the 200 mg/kg dose is known to reach the effectiveness of the conventional therapy, L-DOPA. This discovery not only confirms *C. colocynthis* as a valuable therapeutic cure, but it also provides more specific information about the next research on dosage optimization. The extract demonstrated a significant improvement in overall non-motor symptoms, which are associated with Parkinson's disease, as well as motor spectra. The FST and EMP findings reveal a decrease in anxiety- and depression-like behaviors that confirm the presence of the extensive array of symptoms' treatment based on *C. colocynthis* in the present study. This is especially important because non-motor manifestations have a significant impact on Parkinson's disease patients' quality of life. This is complemented by the cognition enhancements observed in the new object recognition test and the versatility of the list of benefits that *C. colocynthis* extract might offer. Students with Parkinson's disease exhibit cognitive dysfunction, which can be quite disturbing. Therefore, on a therapeutic note, therapies intended to slow down this negativity are always highly valuable. In this test, the high-dose *C. colocynthis* group gave an equivalent result to the L-DOPA group; this suggests that the extract could have similar cognitive impacts to the standard treatment. The open field test results further support the extract's effects regarding motor and non-motor symptoms; increases in locomotor activity, exploratory behaviour, and decreases in anxiety-like behaviour might be suggested. These results indicate the stimuli for the potential of *C. colocynthis* extract in Parkinson's disease therapy.

It clearly suggested an improvement to a certain extent in the balance of dopamine within the striatal area of the brain, although the exact way apomorphine works is still so far from precise apprehension. Such effects might exist due to the extract's anti-inflammatory properties, benefits in promoting the growth of new neurons, and antioxidant effects. Therefore, for future investigations to be more focused, understanding the effects of *C. colocynthis* is important.

Nonetheless, the tendency that has been observed regarding the groups that received *C. colocynthis* to have a higher survival rate outcome is something that should be investigated in the future. Thus, conducting the research more comprehensively and with longer follow-up times could provide stronger evidence of any possible survival benefits associated with the administration of *C. colocynthis* therapy. Still, referring to such results, it is important to emphasize that *C. colocynthis* extract is being developed only as a potential source of a new parkinsonism treatment. Though the mechanism of action, efficacy, and safety profiles were

highlighted, additional research is needed to decipher its safety profile for habitual consumption, optimal schedule of administration, and chronic effects. To enhance the use of the extract in the clinical treatment of the disorder, more studies should be conducted on the efficacious compounds and their exclusive means of operation.

As a result, the present study provides convincing evidence to support the claim *that Citrullus colocynthis* ethanolic extract has neuroprotective potential using an in vitro and in vivo rat model of Parkinsonism. The extract demonstrated a range of improvements in the motor control, behaviour, and cognition of tested subjects and even equaled or, in most cases, nearly placed the performance at par with the use of the medication L-DOPA. These findings provide more justification for using *C. colocynthis* extract as an innovative approach to Parkinson's disease treatment and mitigation, a supplement, or a potential replacement for the current agents. The possibility of using *C. colocynthis* for two cardinal symptoms of PD is seen, with the non-motor and motor symptoms having been shown in this study to improve. This could significantly improve patients' health and quality of life. These findings promise to guide efforts to translate these results into clinical trials as this field of study progresses and aims at determining the efficiency and efficacy of *C. colocynthis* extract on patients.

Though promising, these findings should be viewed with caution and optimism. More thorough research is required because of the complexity of Parkinson's disease and the difficulties in extrapolating its consequences from animal models to human patients. However, this study lays a solid foundation for further research into the medicinal potential of *Citrullus colocynthis* in the treatment of Parkinson's disease and possibly other neurodegenerative conditions.

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