Methanolic Root Extract of Citrullus Colocynthis Ameliorate Parkinson's Disease in Experimental Animals

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Abstract: **Parkinson's disease (PD) is made, and a significant loss of dopaminergic neurons has already occurred. Identifying patients in the period between the presumed onset of dopaminergic cell loss and the appearance of clinical parkinsonism may be of major importance in the development of effective neuroprotective treatment strategies. In an effort to develop a feasible strategy to detect preclinical PD, a combination of olfactory processing tasks, including odor detection, odor identification, and odor discrimination was used to select groups of hyposmic and normosmic individuals from a total of 250 relatives (parents, siblings, or children) of subjects with PD.α-Synuclein (α-syn) aggregation is central to neuropathological changes in Parkinson's disease. The aggregates spread within the central nervous system according to a very predictable pattern. A prion-like transmission of α-syn aggregates has been recently proposed to explain this propagation pattern.**

Keywords: Citrullus Colocynthis Ameliorate, Natural Product, **α-Synuclein, Methanolic Root Extract**

1.INTRODUCTION

The second most prevalent neurodegenerative condition, Parkinson's disease is characterized by a gradual loss of dopaminergic neurons in the SN pars compacta. Between the initial cell damage in nervous system regions at risk and the appearance of clinical symptoms, there is a significant lag time in Parkinson's disease (PD). Typically, symptoms and indicators of Parkinson's disease (PD) do not appear until 70–80% of dopaminergic neurons have been destroyed (El-Agnaf et al., 2006). Therefore, locating patients in the window of time between the alleged start of dopaminergic cell loss and the emergence of clinical parkinsonism may be crucial for the creation of efficient neuroprotective treatment plans (Berendse et al., 2001). Based on the localization of the damaged brain regions, six neuropathological stages of this disease were identified by staining of LBs, the pathological characteristic of Parkinson's disease, to identify impaired neurons across the nervous system (Braak et al., 2003). Brain samples from hundreds of PD patients were examined, and the results showed that the degenerative process was largely homogeneous. The dorsal motor nucleus of the vagal nerve and the anterior olfactory structures in the lower medulla oblongata are where the pathology in the first stage starts. Stage 2 is characterized by worsening dorsal motor nucleus lesions, lower raphe nuclei inclusions, and locus ceruleus Lewy neuritis. The SN is impacted in stage three. In stage four, the temporal neocortex, in particular, begins to show lesions. Stages five and six both show cortical involvement, with the pathology first manifesting in the nearby temporal neocortical areas. Importantly, cognitive state and neuropathological stage are correlated (Braak et al., 2003). Finding trustworthy molecular biomarkers that can differentiate PD from other illnesses, track its development, or indicate a favorable response to therapeutic intervention is also crucial (Siderowf et al., 2018). Dopaminergic neurons in the SN are thought to be adversely impacted by a-synuclein (a-Syn) aggregates, and their creation may cause the spread of toxic a-syn from damaged cells to other nearby cells, leading to the formation of a cascade of LBs and, ultimately, cell death (Angot and Brundin, 2009; Steiner et al., 2018). By spreading pathogenic forms of a-syn to nearby cells, encourages the further death of dopaminergic cells (Luk et al., 2012). A natural channel for a-syn transfer between cells is possible. However, under stressful conditions, a-syn may begin to aggregate within the recipient cells, where pre-aggregated a-syn functions as a "seed," causing additional soluble a-syn to aggregate in a "prion-like" manner (Bernis et al., 2015). Furthermore, any flaw in the clearance systems could contribute to the progression of PD pathology as toxic a-syn spreads to other cells because a-syn aggregates are typically eliminated by the proteasome system or the lysosomes. According to this theory, lysosomal inhibition increases the amount of insoluble asn, which causes an increased release of exosomes that carry poisonous asn (Luk et al., 2012). Unwanted proteins are eliminated from healthy neurons through an exosome-mediated pathway, which explains why a-syn can be released from neurons under normal circumstances. However, any cellular or environmental issue that results in higher a-syn secretion can be harmful to neurons and can spread to other nearby cells. According to one theory, the enteric nervous system, which is comprised of nerves in the upper gastrointestinal tract, is where a-syn buildup in PD patients starts (GI). The migration of a-Syn from the stomach to the brain via the vagus suggests a critical function for the gut-brain axis in the development of Parkinson's disease (PD) (Liddle, 2018). In order to track the occurrence of PD, diagnose it in its early stages, be able to distinguish it from other parkinsonian syndromes, track its response to treatment, and track its course, it is necessary to identify reliable biomarkers. PDD can develop from PD after a long period of the disease. A recent thorough study describes the diagnostic techniques and differences between PD, PDD, and DLB (McKeith et al., 2017)

1.1 NEUROCHEMICAL BIOMARKERS

- **Orexin:** A tiny number of neurons in the dorsolateral hypothalamus express orexin also referred to as hypocretin, a neuropeptide hormone. The lateral and posterior hypothalamic neurons release orexin. Many physiological processes, including the sleep-wake cycle, cardiovascular reactions, heart rate, and hypertension are regulated by the hormone (Hagan et al., 1999).
- **Peripheral Proteasomes and Caspase Activity:** Large protein complexes called proteasomes are crucial for cell viability because they degrade and get rid of undesired and improperly folded proteins. The ubiquitin ligase attaches ubiquitin

molecules to damaged proteins, which causes the proteasome to begin its ATP-dependent proteolytic action (Lodish et al., 2004). Protein buildup within the neurons of people with PD results in the development of abnormal intracellular inclusions known as LBs. Protein aggregate formation and LBs may both be caused by protease dysfunction (Bentea et al., 2017)

- **Dopamine, Dopamine Receptor, and Dopamine Transporter Activity:** The SN, hypothalamus, and various other areas of the brain secrete the catecholamine neurotransmitter dopamine. L-aromatic amino acid decarboxylase converts the dopamine precursor (L-DOPA) that is produced by TH into dopamine (AADC). Dopamine serves as a precursor to noradrenaline (norepinephrine) and adrenaline in the brain (epinephrine). Dopamine levels drop in PD brains due to the loss of dopaminergic neurons in the midbrain and SN (Obeso et al., 2008). The reuptake of dopamine back into the cytosol is facilitated by the dopamine transporter (DAT), which regulates dopamine levels. However, since its oxidation results in deadly reactive quinones, unbound dopamine is toxic to neurons. Therefore, extra dopamine is stored in vesicles by the vesicular monoamine transporter 2 (VMAT2).
- **α-Synuclein:** As a potential molecular biomarker of Parkinson's disease, a-synuclein, which is found in an aggregated and fibrillar state, has received a lot of interest. (2017) Emamzadeh Human a-syn is primarily expressed in the neocortex, hippocampus, SN, thalamus, and cerebellum of the brain, as well as in LBs (Surguchov., 2015). It is encoded by the SNCA gene, which has six exons with sizes varying from 42 to 1,110 bp (McLean et al., 2000; Yu et al., 2007).
- **Apolipoprotein A1 (ApoA1):** The primary component of HDL particles is apolipoprotein ApoA1, which has a molecular weight of 28 kDa and contains 28 amino acids (Brewer et al., 1983). The liver and small intestine produce the majority of this apolipoprotein, which is in charge of removing excess cholesterol from cells. Together with apoE, apoA1 takes involved in lipid transport in the brain (Emamzadeh, 2017).

1.2 RNA-Based PD Biomarkers

MicroRNAs (miRNAs) are emerging as prospective PD biomarkers, according to recent research on this subject. This is particularly true given that exosomes and free microRNAs are both present in the peripheral circulation and CSF. Small non-coding RNAs called miRNAs, which have 21–24 nucleotides, control the post-transcriptional regulation of gene expression. MiRNAs can be used as practical PD biomarkers because they can cross the BBB. In a current investigation, Dos Santos et al (2018).

1.3 METABOLITE PROFILING

The intricate interaction of a person's genes, proteins, and environment is reflected in the metabolic profile of human tissues and/or bodily fluids. ATP, lactate, creatine, and other low molecular weight metabolites have all been studied using non-invasive imaging techniques such as proton (1H) and phosphorus (31P) magnetic resonance spectroscopy (MRS) (Havelund et al., 2017).

1.4 NEUROIMAGING BIOMARKERS

Using imaging methods like positron emission tomography (PET) scans, single-photon emission computed tomography (SPECT) scans, susceptibility-weighted imaging (SWI), diffusion-weighted imaging (DWI), and transcranial B-mode sonography (TCS), modern technology can now identify abnormalities in the brains of PD patients. (Chung et al., 2009)

- **Transcranial B-Mode Sonography (TCS)**Transcranial B-mode sonography measures the frequency of ultrasound waves and their reflections to track the blood flow velocity of the brain's blood arteries. This low-cost and trustworthy method demonstrates the higher echogenicity of the SN in PD brains compared to the normal group, which may be caused by increased iron and gliosis levels in the SN of PD patients (Skoloudk et al., 2014).
- **Magnetic Resonance Imaging (MRI)**
  A type of MRI called diffusion-weighted imaging evaluates the speed at which water diffuses through a tissue to identify its anatomical characteristics. Higher observed diffusivity indicates more water molecule mobility, which may result from cell death and a reduction in the volume of the region. While the early clinical symptoms of these illnesses are similar, this approach can distinguish PD from multiple system atrophy (MSA). DWI has specifically been used to report that MSA patients' middle cerebellar peduncles have more water diffusivity than PD patients do (Chung et al., 2009)
- **Single-Photon Emission Computed Tomography (SPECT) Scan**
  In order to create 3D pictures, radiotracers are used in both PET and SPECT scans to identify the loss of dopaminergic neurons and early PD onset. The majority of radiotracers are non-invasive radiopharmaceuticals with a limited lifespan, which typically decay shortly after the imaging process is over. Furthermore, although MRI can only monitor the morphology and structure of an organ, 3D pictures from PET and SPECT scans may show how the organ functions (Histed et al., 2012).
- **Positron Emission Tomography (PET) Scan**
  Electron anti-particles (positrons), which are positively charged and have the same mass as an electron, are released by the radiotracers used in PET scans. With the use of 18F and/or 11C radiolabeled dopamine analogs, it is possible to detect the existence of presynaptic DAT in dopaminergic neurons of the striatum and SN. 18F-dopamine (18F-dopa) (Ibrahim et al., 2016), 18F-FE-PE2Ib (Fazio et al., 2015), 18F-b-CFT (Rinne et al., 1999).

1.5 PARKINSON'S DISEASE TREATMENT

Since PD gradually reduces patients' functional abilities and quality of life, the discovery of neuroprotective medicines is a crucial unmet medical need. Determining new therapeutic targets is crucial, for this reason. There is presently no known cure for PD, despite the fact that several drugs and therapies are available to control its symptoms. PD therapies can range from different drugs to rehabilitation or even surgery, depending on the symptoms and demands of the patient. PD encompasses many clinical entities that have been identified in various research looking into the possibility of PD subtypes. Cluster analysis enables the identification of different PD subtypes based on the importance of both motor and non-motor symptoms, and the choice of a therapeutic strategy based on the presentation of the clustered symptoms (Lauretani et al., 2014).

MEDICATIONS

The most popular form of treatment for PD is using various commercially available drugs to address the dopamine deficiency in the SN. By increasing dopamine levels, simulating dopamine's actions, or blocking dopamine's oxidative metabolism, which produces reactive oxygen species (ROS), these drugs can temporarily relieve the symptoms of Parkinson's disease (PD) (Goldenberg, 2008). Another crucial area for PD treatment is the formation of protein aggregates that cause neuronal cell death. Levodopa (L-dopa, L-3,4-dihydroxyphenylalanine) is a potent medicine used to treat Parkinson's disease (PD). The immediate metabolic precursor of dopamine, which is made from L-tyrosine by TH, is levodopa. Levodopa is converted to dopamine in the dopaminergic neurons by the enzyme dopa decarboxylase. Before entering the central nervous system, levodopa given orally may be decarboxylated in auxiliary locations. Levodopa is therefore available in combination with carbidopa or benserazide, which are peripheral Dopa decarboxylase inhibitors but do not cross the BBB. In the presence of peripheral decarboxylase inhibitors, unchanged levodopa can enter the central nervous system and be employed as a precursor to dopamine (Goldenberg, 2008). Levodopa is converted into DA more effectively by serotonergic neurons of the nigrostriatal system than by dopaminergic ones after being stored in vesicles. The well-known adverse effects of L-DOPA therapy are brought on by the fact that the serotonergic distribution across the brain is considerably different from the dopaminergic one, which lowers the drug's effectiveness (De Deurwaerdère et al., 2017).

SURGERY

Deep brain stimulation therapy is rarely used for certain types of brain-related disorders including PD, dystonia, obsessive-compulsive disorder, and treatment-resistant depression (Herrington et al., 2016). When PD symptoms are very severe and medications cannot moderate them, surgery and DBS can be considered as the final options for treatment. It involves sending electrical impulses to certain parts of the brain (usually SN or globus pallidus, which communicate with the SN) by a neurostimulator device that is a brain implant known as a 'brain pacemaker.' The target area of DBS is usually the subthalamic nucleus (STN).

GENE THERAPY

The development of gene therapy for PD has made major progress in the recent decade. Advanced PD does not give a good response to levodopa therapy. Broaden loss of dopaminergic neurons is accompanied by a reduction in aromatic amino acid decarboxylase (AADC) levels that convert L-DOPA to dopamine. After successful preclinical studies, adeno-associated viral vectors carrying the human AADC gene are recently delivered into put aminal neurons and subthalamic nuclei of PD patients. In this method, a sufficient amount of dopamine production can be controlled by taking an adequate levodopa dose. Orally taken levodopa can be converted into dopamine by AADC and soothe PD symptoms (Muramatsu et al., 2010). The safety and efficiency of the method have been proven over 4 years by annual PET imaging from patients who received specific dosages of AAV2-hAADC (Mittermeyer et al., 2012).

NON-GENETIC RISK FACTORS OF PARKINSON DISEASE

Only 10–15% of PD cases are early onset familial PD, while the remaining cases are idiopathically pointing to an important role of non-genetic and environmental factors in PD pathogenesis. Exposure to environmental toxins can cause dopaminergic cell death. The accumulation of heavy metals in the SN enhances the risk of developing PD. The effect of exposure to heavy metals could increase oxidative stress in dopaminergic cells, leading to PD. MAO in the presence of oxygen can mediate dopamine oxidation in vitro into 3,4-dihydroxylphenylacetaldehyde (DOPAL).

GENETIC FORMS AND GENETIC RISK FACTORS OF PD

Although most cases of PD are idiopathic forms of the disease, about 15% of PD patients are recognized as having a first-degree family member with this disease. Recently, the genetic factors and gene loci involving in autosomal dominant and autosomal recessive forms of PD have been discovered due to advanced molecular genetics (Samii et al., 2004; Karimi-Moghadam et al., 2018) (**Tables 1**, **2**).
for PD (**Table 3**).

EPIGENETIC RISK FACTORS OF PD

Epigenetics refers to chromatin alternations, including DNA methylation and histone post-translational modifications that can alter gene expression without changes in DNA sequence. These modifications can be inherited, but environmental factors including nutritional, chemical, and physical factors can also affect epigenetics (Surguchov et al., 2017). In the sporadic form of PD involvement of environmental factors in the initiation and progression of the disease emerges an idea that epigenetics plays an important role in PD (Feng et al., 2015).

2.DRUG PROFILE

Plants have supplied many essential human needs, including a variety of therapeutic medications (Alagawany et al., 2020, 2021a, b; Dhama et al., 2021). Therefore, deliberate efforts towards cultivation are crucial for the continuous availability of those plant species. Medicinal plants have been used in healthcare for a long time, and their use to prevent and treat illness is expanding worldwide (Dhama et al., 2018; Bilal et al., 2021; Reda et al., 2021; Saeed et al., 2021). The medicinal properties of plants are due to the natural chemicals/compounds they contain (Saeed et al., 2019; Alagawany et al., 2021c; Garg et al., 2021; Zhang et al., 2021). Plants are a source of food and act as raw materials from which a variety of drugs are synthesized (Hassan, 2012). Citrullus colocynthis is a desert plant and a source of several bioactive compounds such as essential oils, glycosides, flavonoids, alkaloids, and fatty acids. Medicinal plants improve

the immune system. The dried fruit pulp of C. colocynthis has been used to treat gastrointestinal disorders like indigestion, gastroenteritis, and intestinal parasites

3. MATERIALS AND METHODS

3.1 Animals

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI), Bhopal. Wistar rats (150-200 g) were group housed (n = 6) under a standard 12 h light / dark cycle and controlled conditions of temperature and humidity (25 + 2 ° C, 55-65 %), Rats received standard rodent chow and water ad libitum. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried out in a noise-free room between 08.00 to 15.00 h. A separate group (n - 6) of rats was used for each set of experiments.

3.2 Test Herb

The roots of Citrullus colocynthis (2kg) were collected from district Morena (M.P.) and Dholpur (RJ .) in the month of November 2015. The roots were washed with water and dried under shade at room temperature. The roots were powdered to a moderately coarse powder and stored in an air-tight container. Extraction was done by soxhlet apparatus, In this method, the finely ground sample is placed in a porous bag or " thimble " made from a strong filter paper or cellulose, which is a place, is in thimble chamber of the Soxhlet apparatus. Extraction solvents are heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser, and drip back. When the liquid content reaches the siphon arm, the liquid contents are emptied into the bottom flask again and the process is continued (Amid et al., 2010). The solvent was then evaporated under reduced pressure till a resinous extract was obtained. The extract was golden brown in color and the total quantitative yield was 15.22 % (w / w). The methanolic extract was further subjected to pharmacognostical standardization for the detection of secondary plant metabolites and was found to be containing Alkaloids, flavonoids, saponins, and glycosides.

3.3 ABTS assay

This assay was carried out according to the procedure described by (Re et al., 1999) 10 mg ABTS was diluted in 2.6 ml of potassium persulfate solution (2.45 mM) and the final concentration of ABTS was 7 mM. The mixture was kept in the dark at room temperature for 12-16 h before use. The ABTS was diluted to an absorbance of 0.70 0.02 and stocked for offline and online assays. One milliliter of diluted PSR extract was added with 3 ml ABTS solution and kept in the dark at room temperature for 60 min. The absorbance was measured at 734 nm.

3.4 Tacrine-Induced Vacuous Jaw Movement

The anticholinesterase inhibitor tacrine is used therapeutically to improve memory function in patients with early and late-onset Alzheimer's disease. But it can lead to the production of parkinsonian side - effects such as bradykinesia, rigidity, and tremor. Ott and Lannon demonstrated that tacrine-induced Parkinsonism could be ameliorated by L - 3, 4 dihydroxyphenylalanines (L - DOPA). One of the motor effects produced by cholinomimetics is tremulous jaw movements (also known as VCM ' or ' purposeless ' chewing). These are characterized as rapid, vertical deflections of the lower jaw that resemble chewing but are not directed at any stimulus. They share some characteristics with human parkinsonian symptoms. Tacrine-induced tremulous jaw movements can be suppressed by antiparkinsonian agents (Cousins MS, Carriero, et al., 1997). Rats were divided into 4 groups, each containing three animals. The rats received orally, vehicle, and extract (200 mg/kg) 1 h prior to tacrine (2.5 mg/kg. i.p). Vitamin E (10 mg/kg. p.o) was used as a reference. Immediately after the injection of tacrine, rats were placed in a Plexiglas observation box (22x22x22 cm3) for a 10 min habituation period. An observer blind to treatment recorded the number of vacuous chewing movements (VCM) and the number of orofacial bursts (OB).

3.5 Assessment of Locomotor activity

The effect of Methanolic root extract on locomotion was determined using open - field apparatus. The total no. of squares traversed and the no. of rearing were counted for 5 minutes. The effect on catalepsy was determined for 3 hours at 30 - minute's intervals using the bas test (Gould, Todd D. & David T. Dao, 2009).

3.6 Biochemical Estimation

Immediately after the measurement of catalepsy on the 5th day, the animals were sacrificed. The brains were removed; the forebrain was dissected, rinsed with isotonic saline, and weighed. It was homogenized with 0.IN HCL. A 10 % (w / v) tissue homogenate was prepared in a 0.IM phosphate buffer (pH 7.4) was then centrifuged for 60 minutes at 4 ° C (Patil, Rupali A., Yogesh A. Hiray, and Sanjay B. Kasture . , 2012).

3.6.1 Measurement of Superoxide Dismutase Activity

The assay of SOD was based on the ability of SOD to inhibit the spontaneous oxidation of adrenaline to adrenochrome. To 0.05 mL supernatant, 2.0 mL of carbonate buffer and 0.5 ml. of EDTA were added The reaction was initiated by the addition of 0.5 mL of epinephrine and the auto-oxidation of adrenaline (3x10 M) to adrenochrome at pH 10.2 was measured by following changes in optical density at 480 nm. The changes in optical density every minute were measured at 480 nm against a reagent blank. The results are expressed as units of SOD activity (milligrams per protein). One unit of SOD activity induced approximately 50 % inhibition of adrenaline. The results were expressed as nmol SOD U per mg wet tissue (Flohe, L., 1984).

3.6.2 Measurement of Catalase Activity

The CAT activity assay was carried out as described by Beers and Sizer. The reaction mixture consisted of 2 mL phosphate buffer (pH 7.0), 0.95 mL of hydrogen peroxide (0.019 M), and 0.05 mL supernatant in a final volume of 3 mL. Absorbance was recorded at 240 nm every 10 seconds for 1 minute. One unit of CAT was defined as the amount of enzyme required to decompose I mmol of peroxide per minute, at 25 ° C and pH 7.0. The results were expressed as units of CAT activity (milligrams per protein). Units of activity were determined from the standard graph of H202. The results were expressed as catalase U per mg wet tissue (Beers R., Sizer I., 1952).

3.6.3 Estimation of Reduced Glutathione

GSH was determined by the method of Ellman. To the homogenate 10 % trichloroacetic acid was added and centrifuged, followed by the addition of 1.0 mL Ellman's reagent [19.8 mg of 5. 5-0 dithiobisnitro benzoic acid in 100 mL of 1.0 % sodium citrate and 3 mL of phosphate buffer (pH 8.0)]. The color that developed was measured at 412 nm. The results were expressed as nanomole GSH per milligram wet tissue (Ellman GL . , 1959).

3.6.4 Estimation of Lipid peroxidation

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was measured by the method of Niehaus and Samuelsson. In brief, 0.1 mL of homogenate (Tris - HCI buffer, pH 7.5) was treated with 2 mL of (1 : 1: 1 ratio) TBA - TCA - HCI reagent (thiobarbituric acid 0.37 %, 0.25N HCI, and 15 % TCA) and placed in a water bath for 15 minutes, cooled and centrifuged at room temperature for 10 minutes at 1000 g. The absorbance of clear supernatant was measured against a reference blank at 535 nm. The results were expressed as LPO nanomole per milligram wet tissue (Niehaus WG, Samuelsson B., 1968).

3.7 Membrane stabilizing Effect

Whole human blood was obtained from a healthy human volunteer and transferred to heparinized centrifuge tubes, centrifuged at 3000 rpm for 5 min, and washed three times with an equal volume of normal saline. The volume of the blood was measured and reconstituted as a 40 % (v / v) suspension with isotonic buffer solution (10 mM sodium phosphate buffer pH 7.4, the composition of the buffer solution (g / l) was NaH2PO4 (0.2), Na2HPO4 (1.15) and NaCl (9.0) (Chopade AR Some PM, Sayyad FJ 2012)

3.7.1 Heat-induced Haemolysis

The reaction mixture (2ml) consisted of a 1 ml test sample of different concentrations (100-500 µg / ml) and I ml of 10 % RBCs suspension, instead of the test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing the reaction mixture were incubated in a water bath at 56 ° C for 30min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min, and the supernatants' absorbance was taken at 560 nm. The experiment was performed in triplicates for all the test samples (Chopade AR, Some PM, Sayyad FJ. 2012). The Percentage inhibition of Haemolysis was calculated as follows: Percentage inhibition = (Abs control - Abs sample) X 100 / Abs control

3.8 Toxicity studies of the Methanolic extract

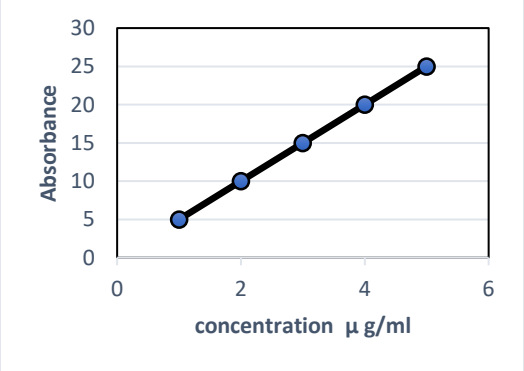
Acute oral toxicity was conducted according to the method of the Organisation for Economic Co-operation and Development (OECD). Animals were kept fasting providing only water, Methanolic extract of whole plant extract (50,100,150,200,300,400 mg/kg/day) was administered orally for 4 days in six groups of rats (n - 6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation (OECD, 2001).

3.9 Statistical Analysis

All the data represented as MEAN ± SD at n - 3 (3 Animals per group), One-way ANOVA followed by Bonferroni test, $P < 0.001$ compared to the tacrine group.

4. RESULTS

4.1 Total flavonoids content estimation I ml of extract Methanolic was mixed with 5 ml of Folin - Ciocalteu reagent (previously diluted with distilled water 1:10 v / v) and 4 ml (75g / l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min at 40 ° C for color development. The absorbance was measured at 765 nm using spectrophotometer



S.No.	Concentration	Absorbance
1.	5	0.216
2.	10	0.425
3.	15	0.625
4.	20	0.815
5.	25	1.021

Figure 1. Graph of Estimation of Total Flavonoids content

S.No.	Time (min.)	Concentration (mg / ml)					
		0.03	0.06	0.12	0.25	0.50	1.00
1	5	22.181±1.54	31.945±1.84	39.633±2.83	41.551±2.47	43.801±2.85	49.954±2.28
2	10	26.480±1.21	40.504±1.65	42.699±1.55	45.362±1.93	51.652±2.03	53.489±2.19

3	15	27.995±1.16	43.102±2.14	44.123±1.96	46.923±2.28	52.984±2.17	55.545±2.45
4	20	29.328±1.37	44.768±2.17	46.143±1.26	48.668±2.18	54.132±2.75	58.532±2.24

Total flavonoids content was calculated as quercetin equivalent (mg / g) using the equation based on the calibration curve: Y - 0.040X + 0.009, R^2 - 0.999, where X is the absorbance and Y is the quercetin equivalent (QE). Total flavonoid content was found to be 2.21 Equivalent to Quercetin mg / 100 mg of dried extract.

4.2 ABTS Assay Results

ABTS assay was carried out according to the procedure described by Re et al. , Percentage of Radical scavenging capacity was increasing with time and concentration the results were shown in Table 2. Radical scavenging capacity found to be 58.53 % at 20 minute and 1 mg / ml.

The scavenging capacity was calculated (1-Ab/A0) 100 % (where Ab is the absorbance at the scavenging capacity was calculated as (1 - Ab / A0) 734 nm; A0 refers to the absorbance of ABTS + without sample added at 734 nm). Results were expressed as Trolox equivalent antioxidant capacity (TEAC) only at 20 min and for a concentration of 1 mg/ml.

4.3 Effect on Tacrine-induced Orofacial dyskinesia

An increase in Bursts and jaw movements was seen in all groups except the control group throughout the whole observation. Although the extract-treated group (Group IV) showed a significant decrease in bursts and jaw movement compared to the tacrine control group (Group II)

S.No.	Treatment	Bursts	Jaw movement
1	Vehicle treated group	38.67±7.024	77.00±7.550
2	Tacrine group	175.33±14.978	593.67±18.610
3	Tacrine + Vit E	85.67±12.662	165.33±11.676
4	Tacrine + Extract 200	154.00±9.16	381.67±16.042

All the data represented as MEAN ± SD at n - 3 (3 Animals per group), One-way ANOVA followed by Bonferroni test, $P < 0.001$ compared to the tacrine group.

Tacrine administration on alternate days for a period of five days significantly ($P < 0.05$) decreased locomotor activity. Pretreatment with Methanolic extract of roots of Citrullus colocynthis (200 mg/kg) and Vitamin E (10 mg/kg) prevented attenuation of Tacrine-induced hyperlocomotion significantly [Table 4].

4.4 Effect on Locomotor Activities

S.No.	Locomotor activities	Vehicle	Vehicle Tacrine indu	Vit. E + T.I.	Extract 200 + T.I.
1	Squares traversed No. 42.01+ episodes)	42.01±0.03528	13.33±0.1693	19.89±1.111	15.72±1.042
2	Rearing effect	11.72±0.7748	1.887±0.4390	10.23±0.6934	7.207±0.5477

All the data represented as MEAN ± SD at n - 3 (3 Animals per group), One-way ANOVA followed by Bonferroni test, * $P < 0.05$ compared to the tacrine group.

Tacrine induced (T.L.) 13.33 0.1693 Tacrine induced time-dependent catalepsy in rats. Pretreatment with Methanolic extract of roots of Citrullus colocynthis (200 mg/kg), and Vitamin E (10 mg/kg) significantly inhibited Tacrine-induced catalepsy [Table 5].

4.5 Effect on Tacrine-Induced Catalepsy

S.No.	Treatment (mg/kg) time post-treatment	Duration of catalepsy (seconds)						
		0 min	30 min	60 min	90 min	120 min	150 min	180 min
1	Vehicle	2.067±0.08	2.050±0.07	3.617±0.16	2.917±0.13	2.917±0.13	3.133±0.12	2.867±0.09
2	Tacrine-induced (T.I.)	3.317±0.22	125.3±2.78	179.7±2.44	139.6±1.62	123.1±3.74	93.05±1.78	78.12±1.15
3	Vit. E + T.I.	2.587±0.21	128.0±1.56	128.0±1.56	112.5±0.46	99.70±0.93	89.58±1.12	77.77±1.51
4	Extract 200+T.I.	2.955±0.05	114.8±1.07	130.1±2.23	124.4±1.59	112.0±2.17	98.42±1.65	80.86±0.70

All the data represented as MEAN ± SD at n - 3 (3 Animals per group), One-way ANOVA followed by Bonferroni test, $P < 0.002$ compared to the tacrine group.

1. Vehicle S. no Biochemicals estimation Superoxide dismutase activity (u / mg of wet tissue) Catalase activity (u / mg of wet tissue) Reduced Glutathione 2.3. Tacrine induced (T.1)

4.6 Biochemical Estimation

In the present study. Tacrine-treated animals exhibited an increase in the levels of lipid peroxidation, and decreased levels of GSH and protective antioxidant enzymes such as SOD and CAT, suggesting a possible free radicals generation. Previous studies have also demonstrated that Tacrine-induced oral dyskinesia is closely associated with oxidative stress. Treatment with Methanolic root extract of Citrullus colocynthis (200 mg/kg) and vitamin E (10 mg/kg) attenuated these increased levels of lipid peroxidation. It also increased levels of GSH and protective enzymes such as SOD, and CAT, suggesting its possible antioxidant action is useful in the treatment of Parkinsonism.

S.No.	Biochemical Estimation	Vehicle	Tacrine (T.I.) induced	Vit E+ TI.	Extract 200+ T.I.
1	Superoxide dismutase activity (µ/mg of wet tissue)	2.837±0.09708	1.247±0.1608	1.768±0.1540	1.450±0.2124
2	Catalase activity (µ/mg of wet tissue)	7.908±0.1175	3.233±0.1215	4.182±0.1776	3.283±0.1295
3	Reduced Glutathione (nmol/mg of wet tissue)	14.36±0.1564	8.138±0.1423	13.28±0.1313	9.522±0.1531
4	Lipid peroxidase (uM / mg of wet tissue)	1.217±0.06059	14.13±0.04542	5.625±0.1459	7.202±0.1809

The observations are mean ± SEM (n = 6), #P < 0.05 compared to vehicle, P < 0.05 compared to tacrine (T.I.) treated group (one-way ANOVA followed by Dunnett's test)

15 10 Vehicle Tacrine induce E VILE + Vehicle Vehicle Tacrine to induce Tacrine Induce 0.1564 1.217 0.06059 Vehicle Tacrine induced Extract200 + + 21₂ + 7 % € hand Extractz Exit E + T. 0808 0.1531 7.202 0.1809 SOD CAT GSH LPO SOD = superoxide dismutase, CAT - catalase, GSH: reduced glutathione, LPO - lipid peroxidation**.**

4.7 Membrane stabilizing Effect

In the study of membrane stabilization activity the Methanolic extract of roots of Citrullus colocynthis at a concentration range of 100-200 µg / ml protected significantly the erythrocyte membrane against lysis induced by heat. Aspirin (200 µg / ml) also offered a significant (p < 0.01) protection of the RBCs against the damaging effect induced by heat and hypotonic solution. At a concentration of 100 µg / ml, the Methanolic extract showed 67.70 ± 1.049 % inhibition of heat-induced hemolysis when compared with blank (Table 7).

S. No.	Negative control	Positive control (Aspirin 100 µg / ml)	Test (100 µg / ml)	Test (200 µg / ml)
1	2.1281	1.7460	2.0672	1.9581
2	2.0129	1.7389	2.0129	1.9444
3	2.1441	1.7800	2.0603	1.9577
Mean	2.095±0.04133	1.755±0.01268	2.080±0.0.650	1.953±0.004502

All the data represented as MEANE SD at n - 3 (3 Absorbance per sample), One-way ANOVA followed by Unpaired t-test, * P < 0.0002 compared to the Negative control group.

4.8 Toxicity profile of the plant extract

Administration of Extract in a dose of 400 mg/kg did not produce any behavioral abnormalities in any of the test animals. No mortality was found throughout the test period.

5. Discussion and conclusion

The main phytochemical principles of Citrullus colocynthis are Cucurbitacins - cucurbitacin E. Colocynthoside A and colocynthoside B, cucurbitacin I 2-0 - B - D glucopyranoside , cucurbitacin L 2-0 - B - D glucopyranoside , cucurbitacin J 2-0 - B - D glucopyranoside . Phenolic Acids and Flavonoids- isosaponarin, isovitexin, and isoorientin 30 - O - methyl ether, p - hydroxybenzoic acid. catechin, myricetin, quercetin and kaempferol. Tocopherols- α-tocopherol, y - tocopherol and B carotene and Alkaloids- choline and two unidentified alkaloids (Al - Snafi AE., 2016). The plant has various previously reported medicinal properties like Anti-inflammatory, Anticandidal & antibacterial, Antioxidant, Hypoglycemic, Hypolipidemic, and Anti alopecia. Anticancer etc. In the present study, we have evaluated the Preventive Action of Methanolic root extract of Citrullus colocynthis on Motor Manifestation in Parkinson's disease by using tacrine-induced orofacial dyskinesia. In our study, there was a consistent decrease in jaw movements and bursts due to the presence of various anti-oxidant principles like quercetin and other flavonoids and tocopherols. The free radical scavenging properties of the extract may significantly prevent motor manifestation in the early stages of Parkinson's disease. The herb has many other active principles like glycosides and alkaloids which may also produce a synergic effect in preventing this root extract. Previous studies have also demonstrated the Tacrine-induced oral action dyskinesia to be closely associated with oxidative stress. Treatment with Methanolic root extract of Citrullus colocynthis increased levels of lipid peroxidation. It also increased levels of GSH and protective enzymes such as SOD, and CAT, suggesting its possible antioxidant action is useful in the treatment of Parkinsonism. However, the sole purpose of this study was to identify the preventive potential

of the herb in motor manifestation. The Bioactivity-guided holistic approach of the study of the plant can be able to provide the exact mechanism of action of this preventive effect against the motor manifestation of Parkinson's disease.

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