Parkinson's disease in experimental animals is improved by methanolic root extract of *citrullus colocynthis*

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Abstract: *Citrullus colocynthis (CC)* has traditionally been used in Africa, Asia, and Europe for a number of reasons. Citrullus colocynthis has been utilised in a number of polyherbal and monoherbal formulations in the Ayurvedic medical system to treat Kamp-vaat (Parkinson's illness). The goal of the research was to evaluate how well Tacrine-induced catalepy, hypolocomotion, and other biochemical changes were protected against by *Citrullus colocynthis* root methanolic extracts. Materials and Procedures The percolation method was used to create a methanolic extract of *Citrullus colocynthis* root. Tacrine (2.5 mg/kg, i.p.) was administered to cause orofacial dyskinesia and hypolocomotion, and the quantity of vacuous chewing movements (VCM), orofacial bursts (OB), and locomotor activity were quantified. All of the rats were monitored for Tacrine-induced catalepsy over the course of 1 to 5 days.Free radical scavenging activity was measured using the ABTS + assay. Methanolic extract affected the forebrain's superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSH), and lipid peroxidation (LPO) inhibition. Results: During the investigation, root ethanolic extract significantly decreased vacuous chewing movements, other motor manifestations including hypolocomotion, and catalepsy. It also significantly enhanced locomotion and rearing in the open-field test. Additionally, the root extract's capacity to scavenge free radicals was dose-dependent. The antioxidant action of plant extract is shown by increases in GSH and antioxidant enzymes like SOD and CAT. Our findings are in line with the usage of *Citrullus colocynthis* root extract for Parkinson's disease treatment that has been used traditionally.

Key words: *Citrullus colocynthis*, Motor manifestation, Tacrine, Biochemical Estimation, Free radical scavenging, Membrane stabilizing

INTRODUCTION

Parkinson's disease (PD) is a progressive, debilitating neurodegenerative disease that often begins with the gradual loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). It is a common age-related movement disorder that often appears sporadically [1]. The pathogenesis of PD remains poorly understood, but emerging evidence implicates various genetic and environmental factors in the initiation and progression of PD. The multifactorial etiopathogensis of PD includes mitochondrial dysfunction, excitotoxicity, endoplasmic reticulum stress, oxidative/nitrosative stress, and inflammation, along with ubiquitin-proteasome system dysfunction. Altogether, these events lead to the accumulation of abnormal or misfolded α-synuclein (α-syn) protein [2, 3]. Numerous genetic, biochemical, cellular, pathological, and molecular studies indicate PD pathogenesis is associated with environments where α-syn is susceptible to polymerization, aggregation and fibril formation, and propagation [4]. The α-syn oligomers cause mitochondrial dysfunction and induce endoplasmic stress, oxidative stress, neuroinflammation, and inhibit proteasomal activity and autophagy. Current PD treatment options, such as dopamine agonists, cholinesterase, and monoamine oxidase inhibitors provide only symptomatic relief [5]. Dopamine-based drugs have reduced effectiveness in relieving symptoms with disease progression. The oligomerization and fibrillation of α-syn is linked with the onset and progression of PD, and is believed to be a unique and convincing disease-modification therapeutic strategy for PD, dementia with Lewy body (DLB), and related α-synucleinopathy [6]. Several molecules including antibodies, polyamines. heat shock proteins, chaperones and pharmaceuticals have been shown to affect different forms of α-syn (i.e., monomers, soluble oligomers, protofibrils, or fibrils) and oligomerization, fibrillation, and clearance. Therefore, targeting α-syn aggregation, oligomerization, fibrillation, and propagation to reduce α-syn toxicity emerged as an important therapeutic target for slowing or halting disease progression [7]. But the problem associated with the current approaches is having number of side effects. To minimize these potential side effect Scientist all over the searching for alternative natural origin drug which have minimum side effect and maximum therapeutic efficacy [8]. Many plant products including *Citrullus colocynthis* are used as folk medicine for the treatment of PD [9]. To the best of my knowledge no one any research so far done to explore the scientific evidence of their Anti PD activity.

Several recent reviews and research highlighted the neuroprotective potential of plant extracts and phytochemicals in PD through antioxidant and anti-inflammatory activities. However, despite the enormous success of antioxidants (whether of synthetic or natural origin) in preclinical studies, coenzyme Q10, creatine, and vitamin E either failed or showed marginal neuroprotection in patients. Recently, α-syn antibodies (PRX002) showed safety in phase 1 studies and were indicated for further phases of clinical studies [10]. Similarly, natural products (mainly plant extracts and phytochemicals) emerged to specifically target α-syn [11]. This research, therefore, focuses on the neuroprotective properties and mechanism of action of plant extracts, extract-based formulations, and plant-derived phytochemicals that target α-syn oligomerization, fibrillation, aggregation, and toxicity in various experimental PD models. The

present aim of the study was to evaluated anti Parkinson's potential of methanolic root extract of *Citrullus colocynthis* in Experimental animals.

MATERIALS AND METHODS

Animals

The Institutional Animal Ethics Committee (IAEC) of our institution gave its approval to the experimental protocol. Wistar rats (150-200 g) were housed in groups of six (n = 6) and kept in a controlled environment with a temperature and humidity range of 25+2°C and 55-65%. Rats were fed a regular rodent diet and were given access to unlimited amounts of water. Prior to the experiments, rats spent 7 days becoming used to the lab environment. Between 8:00 and 15:00 hours, all studies were conducted in a room with no background noise. Each series of tests used distinct groups (n-6) of rats.

Test Herb

In the months of November 2021, 2 kg of Citrullus colocynthis roots were procured from the districts of Haridawar (U.K) and Meerut (U.P). The roots were water-washed, then air-dried at room temperature in a shaded area. The roots were somewhat coarsely ground into a powder and placed in an airtight container for storage. The Soxhlet apparatus was used for the extraction. Heat from the bottom flask causes the extraction solvent to evaporate into the sample vial, condense in the condenser, and drip back. The process is then continued once the liquid content is drained into the bottom flask by the syphon arm (Amid et al., 2010). Following that, the solvent was expelled under lower pressure until a resinous extract was produced. The overall quantitative yield of the extract, which was 15.22% (w/w) in colour, was golden brown. Following pharmacognostical standardisation for the detection of secondary plant metabolites, methanolic extract was discovered to contain alkaloids, flavonoids, saponins, and glycosides.

ABTS assay

Procedures for this test were carried out as per (Re et al., 1999) After diluting 10 mg of ABTS into 2.6 ml of potassium persulfate solution, the final concentration was 7 mM. (2.45 mM). The mixture was left out in the dark and at room temperature for 12 to 16 hours before being used. For both off-line and online testing, ABTS was diluted to an absorbance of 0.70 0.02 and kept indefinitely. After diluting the PSR extract by a factor of three, three millilitres of the ABTS solution were added to one millilitre of the mixture and the mixture was kept at room temperature and out of the light for sixty minutes. The absorbance was checked at 734 nm [12].

Tacrine Induced Vacuous Jaw Movement

The anticholinesterase inhibitor tacrine is given to people with early and late onset Alzheimer's disease as a kind of therapy in order to improve memory function in these patients. Bradykinesia, stiffness, and tremor are only some of the parkinsonian side effects that might be caused by this. Ott and Lannon's research shown that L-3,4-dihydroxyphenylalanine might ameliorate some of the symptoms of Parkinson's disease caused by tacrine (L - DOPA). Cholinergic medications may cause tremors in the jaw, which is one of the motor effects they have (also known as VCM or purposeless chewing). These are characterised as brief, vertical deflections of the lower jaw that mimic chewing but are unconnected to any external stimuli. These movements may be seen in people who have TMJ (temporomandibular joint disorder). They have some of the characteristics that are associated with human Parkinson's disease symptoms. Medications that treat Parkinson's disease have the capacity to lessen the tremors in the jaw that are caused by tacrine (Cousins MS, Carriero et al., 1997). There were a total of

four groups of rats, and each group consisted of three rats. Orally administered vehicle and extract at a dose of 200 mg/kg were given to the rats one hour before to the intraperitoneal administration of tacrine at a dose of 2.5 mg/kg. Vitamin E at a dose of 10 milligrammes per kilogramme taken orally was utilised as a benchmark. After being injected with tacrine, rats were placed for observation in a Plexiglas cage measuring 22 by 22 by 22 centimetres for a period of ten minutes. An observer who was unaware of the therapy was tasked with the responsibility of tallying the amount of orofacial bursts and vacuous chewing motions (VCM) (OB) [13].

Assessment of Locomoter activity

The effect of Methanolic root extract on movement was measured using open-field equipment. The impact on catalepsy was measured over the course of three hours at 30-minute intervals using the bas test [14].

Biochemical Estimation

On the fifth day, once the catalepsy measurement was completed, the animals were murdered soon thereafter. After the brains were removed, the forebrain was dissected, then it was washed with isotonic saline, and then it was weighed. In order to homogenise the material, 0.IN HCL was utilised. After using a 0.IM phosphate buffer with a pH of 7.4 to make a tissue homogenate that was 10% (w/v), the mixture was centrifuged for an hour and a half at 4 degrees Celsius [15, 16]

Measurement of Superoxide Dismutase Activity

The SOD assay was based on the enzyme's ability to block the natural conversion of adrenaline to adrenochrome. We monitored minute-to-minute fluctuations in reagent optical density as measured at 480 nm and compared them to those seen in a blank. Findings are shown in terms of superoxide dismutase activity units (milligram per protein) [17]. One unit of SOD activity, or SOD activity, partly inhibited adrenaline. The data was presented as nmol SOD U per mg of fresh tissue (Flohe, L., 1984).

Measurement of Catalase Activity

Methods described by Beers and Sizer were followed for the CAT activity test. Two millilitres of phosphate buffer (pH 7.0), one millilitre and a half of hydrogen peroxide (0.019 M), and a half millilitre of supernatant made up the 3 millilitre final volume of the reaction mixture. Absorbance was monitored at 240 nm at 10-second intervals for a full minute. CAT is measured in units of how much of the enzyme is required to break down one millimoles of peroxide per minute at 25 degrees Celsius and pH 7.0 [18]. The data was shown in CAT activity units (milligram per protein). Based on the H2O2 standard curve, activity units were determined. The results were presented as catalase U per mg of fresh tissue (Beers R., Sizer I., 1952).

Estimation of Reduced Glutathione

Ellman's technique was used in order to get an accurate reading on GSH. The next stage included adding Ellman's reagent to the homogenate that had previously been centrifuged with 10% trichloroacetic acid. The formulation called for 19.8 mg of 5.5-0 dithiobisnitro benzoic acid to be dissolved in 100 mL of 1.0% sodium citrate and 3 mL of phosphate buffer with a pH of 8.0. The shadow that was produced had a wavelength that was 412 nm [19]. The findings were expressed as the number of nanomoles of GSH present per milligramme of water-containing tissue (Ellman GL., 1959).

Estimation of Lipid peroxidation

The Niehaus and Samuelsson method was used in order to ascertain the level of lipid peroxidation present, which was evaluated based on the production of thiobarbituric acid reactive compounds (TBARS). The gist of the situation is that the mixture of 0.1 millilitres of homogenate (in Tris-HCI buffer, pH 7.5) and 2 millilitres of the TBA-TCA-HCI reagent (1:1:1) (thiobarbituric acid 0.37%, 0.25N HCI, and 15% TCA) was heated in a water bath for 15 minutes. The reaction was carried out at a temperature of 7.5. The mixture was allowed to cool before being centrifuged at room temperature for ten minutes using a g-force of one thousand grammes. In order to estimate the absorbance of the clear supernatant at 535 nm, a comparison was carried out with a blank standard [20]. The results were expressed as the amount of LPO nanomoles contained in one millilitre of wet tissue(Niehaus WG, Samuelsson B., 1968).

Membrane stabilizing Effect

Blood was collected from healthy participants, spun at 3000 rpm for five minutes, and then washed three times with normal saline solution before being used. After measuring its volume, blood was reconstituted as a 40% (v/v) suspension in a sodium phosphate buffer (10 mM, pH 7.4) containing 0.2 grammes of NaH2PO4, 1.15 grammes of Na2HPO4, and 9.0 grammes of NaCl per litre [21].

Heat induced Haemolysis

One millilitre of test sample in various concentrations (100-500 g/ml) and one millilitre of a 10% RBC suspension made up the reaction mixture (2 ml); the control test tube's only addition was saline. Aspirin was a widely prescribed medication. The reaction mixture in each centrifuge tube was incubated for 30 minutes at 56 ° C in a water bath. The tubes were cooled under running water after the incubation period. Centrifuging the reaction mixture at 2500 rpm for 5 minutes allowed the supernatants' absorbance to be measured at 560 nm. For each test sample, the experiment was run in triplicate (Chopade AR, Somade PM, Sayyad FJ., 2012). Following is how the percentage inhibition of hemolysis was determined: Percentage inhibition = (Abs control - Abs sample) X 100/Abs control [22].

Toxicity studies of the Methanolic extract

The method recommended by the Organization for Economic Cooperation and Development was used to test acute oral toxicity (OECD). Six groups of rats (n-6) were kept on a fast with only water available, and a methanolic extract of the entire plant (50,100,150,200,300,400mg/kg/day) was given orally for four days. The rats were then monitored for mortality as well as any behavioural changes for evaluation (OECD, 2001) [23].

Histopathological examination

Following fixation, tissues were dehydrated in escalating alcohol grades and embedded in wax in accordance with standard procedure. After being cut into 57 um thick paraffin slices, they were stained with hematoxylin-eosin in accordance with Humanson's instructions [24, 30-34].

Statistical Analysis

With respect to the tacrine group, all results were reported as MEAN SD at n = 3 (3 animals per group), one-way ANOVA was followed by the Bonferroni test, and P 0.001 was calculated.

RESULTS

Total flavonoids content estimation

4 ml (75g/l) of sodium carbonate and 1 ml of extract in methanol were combined with 5 ml of the Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v). The mixture was

vortexed for 15 seconds before being left to stand for 30 minutes at 40°C to develop the colour. In order to determine the absorbance, a spectrophotometer was used at 765 nm.

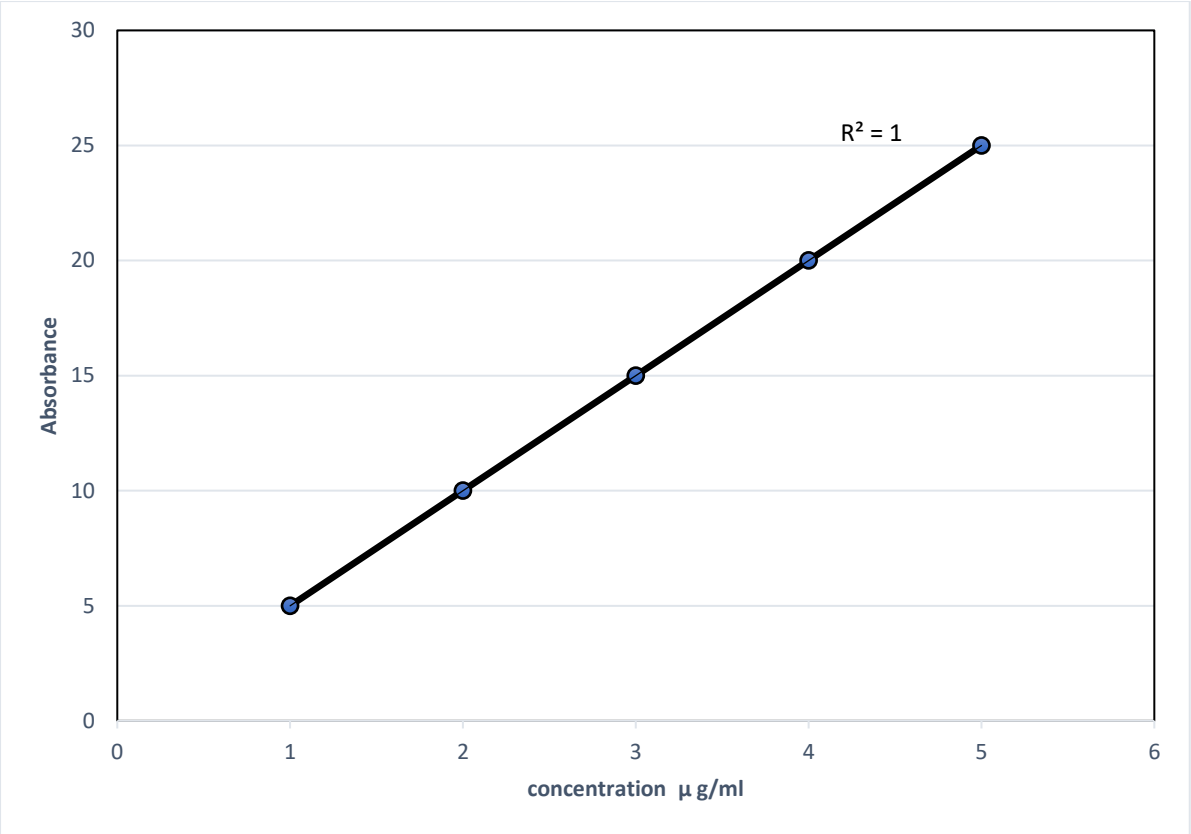

Figure 1: Estimated total flavonoid content graph

Using the equation based on the calibration curve: Y-0.040X + 0.009, R2-0.999, where X is the absorbance and Y is the quercetin equivalent, the total flavonoid content was determined as quercetin equivalent (mg/g) (QE). It was discovered that the total flavonoid content was 2.21 Eq. to quercetin mg/100 mg of dry extract.4.2 ABTS Assay Results

According to Re et alinstructions, .'s an ABTS assay was conducted. The results are shown in Figure 2.

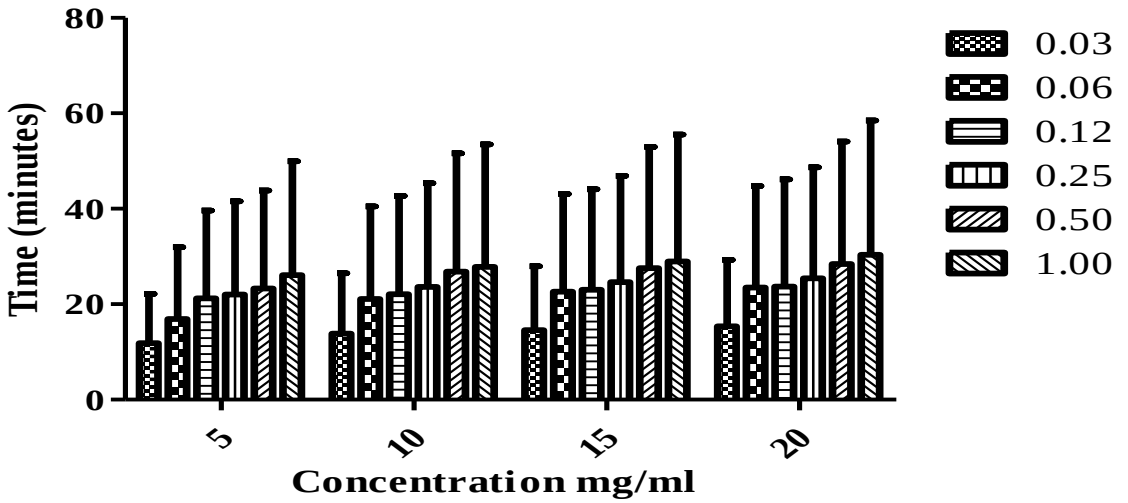

Figure 2: Plot of ABTS + Assay

The percentage of Radical Scavenging Capacity increased with time and concentration. At 20 minutes and 1 mg/ml, the radical scavenging capacity was found to be 58.53%.

Using the formula (1-Ab/A0) 100%, the scavenging capacity was determined. The calculation for the scavenging capacity was (1 - Ab / A0) 734 nm; A0 is the absorbance of ABTS + without sample added at 734 nm. Only at 20 minutes and for a concentration of 1 mg/ml were the results expressed as Trolox equivalent antioxidant capacity (TEAC).

Effect on Tacrine - induced Orofacial dyskinesia

Throughout the entire observation, all groups excluding the control group experienced an increase in bursts and jaw movements. Despite a considerable reduction in bursts and jaw movement compared to the tacrine control group in the extract-treated group (Group IV) given in Figure 3.

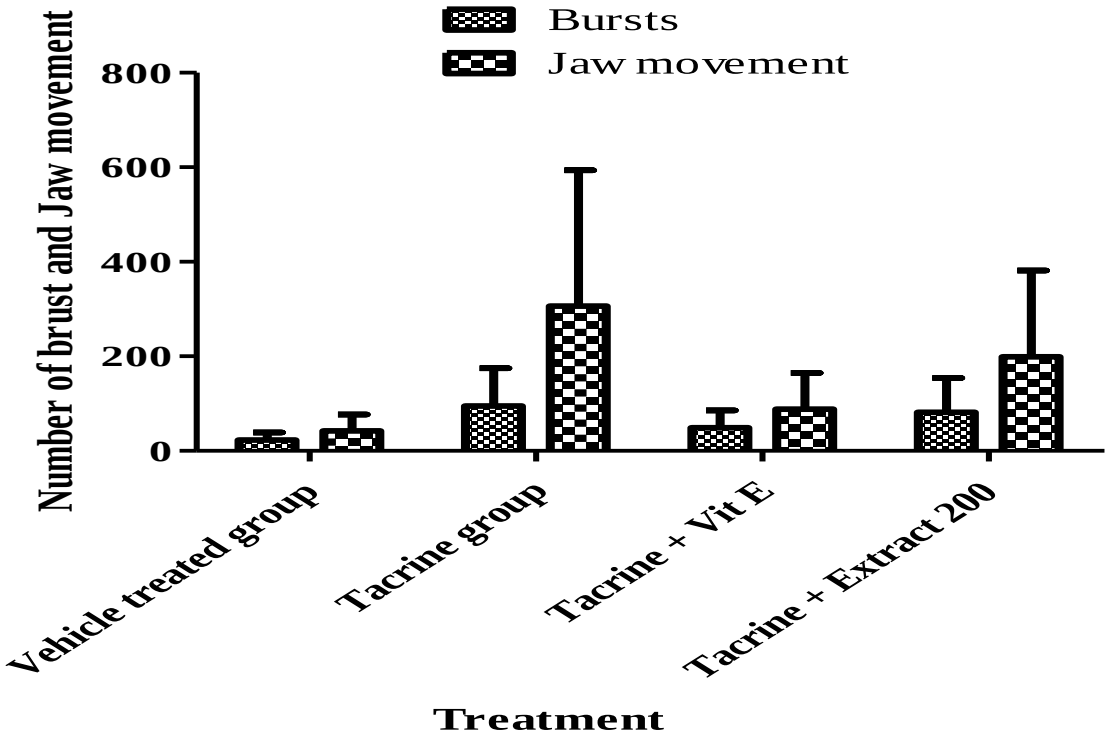

Figure 3: Effect on Tacrine - induced Orofacial dyskinesia

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.

Following a five-day course of alternate-day tacrine treatment, locomotor activity was considerably (P 0.05) reduced. Vitamin E (10 mg/kg) and a methanolic extract of Citrullus colocynthis roots (200 mg/kg) were used as a pretreatment to dramatically reduce the effects of tacrine-induced hypolocomotion [Table 1].

Table 1: Effect on Locomoter Activities

S.N	Locomoter activities	Vehicle	Tacrine induced (T.L.)	Vit . E + T.I.	Extract 200 +T.I.
1	Squares traversed	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 .

Rats exposed to tacrine developed a time-dependent catalepsy. Tacrine-induced catalepsy was greatly reduced when the roots of Citrullus colocynthis were extracted in methanol (200 mg/kg) and supplemented with vitamin E (10 mg/kg) [Table 2].

Table2: Effect on Tacrine Induced Catalepsy

S.N	Treatment	Duration of catalepsy (seconds)						
		0 min	30 min	60 min	90 min	120 min	150 min	180 min

1	Vehicle	2.067±0.08819	2.050±0.07638	3.617±0.1641	2.917±0.1352	2.917±0.1352	3.133±0.1202	2.867±0.09189
2	Tacrine induced (T.I.)	3.317±0.2242	125.3±2.781	179.7±2.447	139.6±1.626	123.1±3.742	93.05±1.789	78.12±1.159
3	Vit. E + T.I.	2.587±0.2136	128.0±1.565	128.0±1.565	112.5±0.4684	99.70±0.9363	89.58±1.123	77.77±1.516
4	Extract 200+T.I.	2.955±0.05667	114.8±1.079	130.1±2.232	124.4±1.598	112.0±2.170	98.42±1.653	80.86±0.7093

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 .

Biochemical Estimation

In the current study, Tacrine-treated animals showed an increase in lipid peroxidation levels and a decrease in GSH and protective antioxidant enzymes like SOD and CAT, which may indicate the generation of free radicals. Previous research has also shown that the oral dyskinesia brought on by tacrine and oxidative stress are closely related. These elevated levels of lipid peroxidation were reduced by treatment with vitamin E (10 mg/kg) and a methanolic root extract of Citrullus colocynthis (200 mg/kg). Additionally, it raised levels of GSH and defense-related enzymes like SOD and CAT, indicating that its potential antioxidant action may be helpful in the treatment of Parkinsonism mention in Table 3.

Table 3: Results of Biochemical Estimation

S.N	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)

Membrane stabilizing Effect

The erythrocyte membrane was effectively preserved against lysis brought on by heat when the Methanolic extract of Citrullus colocynthis roots was used in a study on membrane stabilisation activity. The RBCs were also significantly protected (p 0.01) by aspirin (200 mg/ml) from the

harmful effects of heat and hypotonic solution. When compared to a control sample, the Methanolic extract showed a 67.70 1.049% inhibition of heat-induced hemolysis at a concentration of 100 g/ml (Table 4).

Table 4: Membrane stabilizing activity, Data obtain from UV Absorbance at 560mm

S. No.	Negative control	Positive control (Aspirin 100µg/ml)	Test (100µg/ml)	Test (200µg/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±0.041	1.755±0.012	2.080±0.0.65	1.953±0.0045

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 .

Toxicity profile of the plant extract

None of the test animals exhibited any abnormal behaviour after receiving an extract dose of 400 mg/kg. Throughout the test period, no mortality was discovered.

Histopathology

Cell degeneration was calculated using hematoxylin and eosin staining. Neuronal cells stained with H&E were quantified in the surface of the substantia nigra. Each midbrain section was viewed at low power (×10 magnification) and the outlines of SNpc were determined. Histological assessment demonstrated that control group's rats showed normal SNpc neurons whereas tacrine treated rats showed marked neuronal degeneration. In comparison to the tacrine group, the control group had a higher mean number of SNpc neurons. When compared to the tacriine group, pharmacological treatment with a methanolic extract of Citrullus colocynthis (200 g/ml), vitamin E therapy, increased the number of SNpc dopaminergic neurons by a higher percentage and decreased neuronal degeneration.

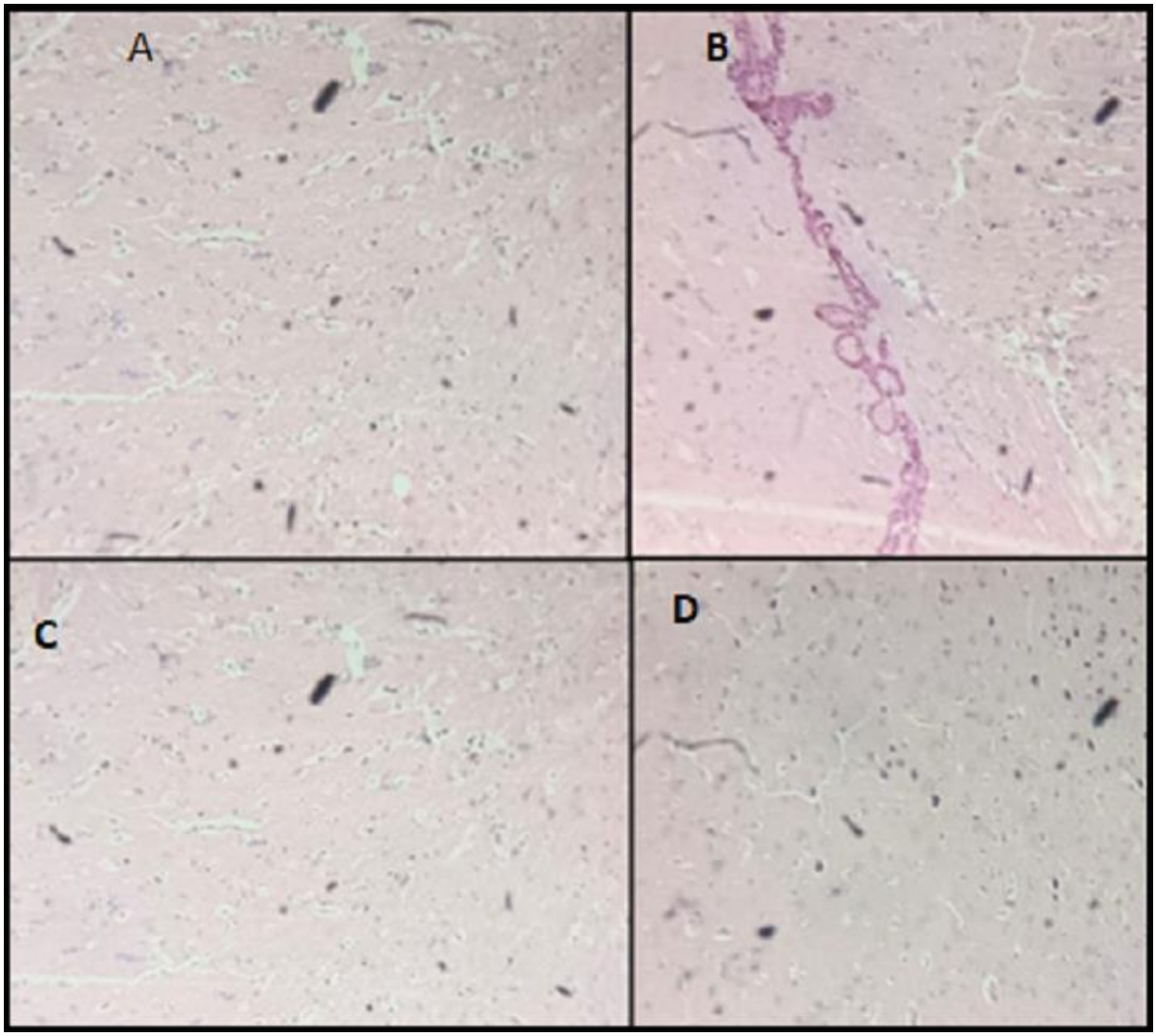

Figure 4: Histopathological changes in the mid brain of rats: (A) Control section showing normal histoarchitecture. (B) Rats treated with tacrine with prominent degeneration of neurons. (C) Rats treated with tacrine and Vitamin E. (D) Rats treated methanolic extract of *Citrullus colocynthis*. Sections stained with haematoxylin and eosin ×10.

DISCUSSION AND CONCLUSION

Cucurbitacins and cucurbitacin E are Citrullus colocynthis primary phytochemical components. Cucurbitacin I 2-0-B-D glucopyranoside, Cucurbitacin L 2-0-B-D glucopyranoside, and Cucurbitacin J 2-0-B-D glucopyranoside are all colocynthosides A and B. Isosaponarin, isovitexin, and isoorientin 30-O-methyl ether, p-hydroxy-benzoic acid, catechin, myricetin, quercetin, and kaempferol are phenolic acids and flavonoids. Tocopherols-a - tocopherol, y - tocopherol and B carotene and Alkaloids-choline and two unidentified alkaloids (Al - Snafi AE., 2016). In recent years, studies of CC are mainly focused on its therapeutic potential of other disease, for example, anticonvulsant activity [25], anti-diabetes activity [26, 27], anti-DNA damage [28], antiInflammatory and analgesic activities [29, 35-40]. In our study, we found CC had the protective effect against PD model in vitro and in vivo for the first time. 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 the motor manifestation in early stages of Parkinson's disease. The herb has many other active principles like glycosides and alkaloids which may also produce the synergic effect in preventive this root extract. Previous studies have also demonstrated the Tacrine-induced oral action dyskinesia to be closely associated with the oxidative stress. Treatment with Methanolic root extract of Citrullus colocynthis increased levels of lipid peroxidation. Additionally, it raised levels of GSH and defense-related enzymes like SOD and CAT, indicating that its potential antioxidant effect may be helpful in the treatment of Parkinsonism. In histopathological study, substantia nigra showed neuronal degenerationin tacrine group whereas showed better result with no swelling in treatment group, it was show significant effect when treated with methanolic extract. In summary, these findings demonstrated that CC may be utilized as a neuroprotective agent that inhibit autophagy-associated cell death, which would exploiting CC as a new candidate drug in the treatment of PD.

CONCLUSION

We think that the methanolic extract of Citrullus colocynthis roots' ability to prevent Parkinson's disease motor manifestations is a result of its capacity to scavenge free radicals and maintain membrane stability. Our findings support the conventional usage of Citrullus colocynthis root extract for the treatment of Parkinson's disease.

CONFLICT OF INTEREST

None

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REFERENCES

1. Dorsey ER, Sherer T, Okun MS, Bloem BR. The Emerging Evidence of the Parkinson Pandemic. *J Parkinsons Dis*. 2018;8(s1):S3-S8. doi:10.3233/JPD-181474.
2. Mao L, Nicolae A, Oliveira MA, He F, Hachi S, Fleming RM. A constraint-based modelling approach to metabolic dysfunction in Parkinson's disease. *Comput Struct Biotechnol J*. 2015;13:484-491. Published 2015 Sep 2. doi:10.1016/j.csbj.2015.08.002.
3. Mele B, Van S, Holroyd-Leduc J, Ismail Z, Pringsheim T, Goodarzi Z. Diagnosis, treatment and management of apathy in Parkinson's disease: a scoping review. *BMJ Open*. 2020;10(9):e037632. Published 2020 Sep 9. doi:10.1136/bmjopen-2020-037632.
4. Hickman S, Izzy S, Sen P, Morsett L, El Khoury J. Microglia in neurodegeneration. *Nat Neurosci*. 2018;21(10):1359-1369. doi:10.1038/s41593-018-0242-x.
5. Elsworth JD. Parkinson's disease treatment: past, present, and future. *J Neural Transm (Vienna)*. 2020;127(5):785-791. doi:10.1007/s00702-020-02167-1.
6. Hershey LA, Coleman-Jackson R. Pharmacological Management of Dementia with Lewy Bodies. *Drugs Aging*. 2019;36(4):309-319. doi:10.1007/s40266-018-00636-7.

7. Lehtonen Š, Sonninen TM, Wojciechowski S, Goldsteins G, Koistinaho J. Dysfunction of Cellular Proteostasis in Parkinson's Disease. *Front Neurosci*. 2019;13:457. Published 2019 May 10. doi:10.3389/fnins.2019.00457.
8. Elkouzi A, Vedam-Mai V, Eisinger RS, Okun MS. Emerging therapies in Parkinson disease - repurposed drugs and new approaches. *Nat Rev Neurol*. 2019;15(4):204-223. doi:10.1038/s41582-019-0155-7.
9. Yin R, Xue J, Tan Y, et al. The Positive Role and Mechanism of Herbal Medicine in Parkinson's Disease. *Oxid Med Cell Longev*. 2021;2021:9923331. Published 2021 Sep 3. doi:10.1155/2021/9923331.
10. Schenk DB, Koller M, Ness DK, et al. First-in-human assessment of PRX002, an anti-α-synuclein monoclonal antibody, in healthy volunteers. *Mov Disord*. 2017;32(2):211-218. doi:10.1002/mds.26878.
11. Kwon S, Iba M, Kim C, Masliah E. Immunotherapies for Aging-Related Neurodegenerative Diseases-Emerging Perspectives and New Targets. *Neurotherapeutics*. 2020;17(3):935-954. doi:10.1007/s13311-020-00853-2.
12. Mhaidat I, Alwedian F, Ababneh T, Shdefat A, Tashtoush H. Synthesis, characterization, computational, antioxidant and fluorescence properties of novel 1,3,5-trimesic hydrazones derivatives. *Heliyon*. 2021;7(9):e08074. Published 2021 Sep 25. doi:10.1016/j.heliyon.2021.e08074.
13. Mak S, Li W, Fu H, et al. Promising tacrine/huperzine A-based dimeric acetylcholinesterase inhibitors for neurodegenerative disorders: From relieving symptoms to modifying diseases through multitarget. *J Neurochem*. 2021;158(6):1381-1393. doi:10.1111/jnc.15379.
14. Opara J, Małecki A, Małecka E, Socha T. Motor assessment in Parkinson`s disease. *Ann Agric Environ Med*. 2017;24(3):411-415. doi:10.5604/12321966.1232774
15. Shahbaz H, Gupta M. Creatinine Clearance. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; July 25, 2022.
16. Abstracts of Presentations at the Association of Clinical Scientists 143rd Meeting Louisville, KY May 11-14,2022. *Ann Clin Lab Sci*. 2022;52(3):511-525.
17. Li DN, Lian TH, Zhang WJ, et al. Potential roles of oxidative distress on neurodegeneration in Parkinson's disease with neuropsychiatric symptoms. *Front Aging Neurosci*. 2022;14:875059. Published 2022 Dec 15. doi:10.3389/fnagi.2022.875059.
18. Antunes MS, Goes AT, Boeira SP, Prigol M, Jesse CR. Protective effect of hesperidin in a model of Parkinson's disease induced by 6-hydroxydopamine in aged mice. *Nutrition*. 2014;30(11-12):1415-1422. doi:10.1016/j.nut.2014.03.024.
19. Vora U, Vyas VK, Wal P, Saxena B. Effects of eugenol on the behavioral and pathological progression in the MPTP-induced Parkinson's disease mouse model. *Drug Discov Ther*. 2022;16(4):154-163. doi:10.5582/ddt.2022.01026.
20. Pal N, Mandal S, Shiva K, Kumar B. Pharmacognostical, Phytochemical and Pharmacological Evaluation of Mallotus philippensis. Journal of Drug Delivery and Therapeutics. 2022 Sep 20;12(5):175-81.

21. Singh A, Mandal S. Ajwain (Trachyspermum ammi Linn): A review on Tremendous Herbal Plant with Various Pharmacological Activity. International Journal of Recent Advances in Multidisciplinary Topics. 2021 Jun 9;2(6):36-8.
22. Mandal S, Jaiswal V, Sagar MK, Kumar S. Formulation and evaluation of carica papaya nanoemulsion for treatment of dengue and thrombocytopenia. Plant Arch. 2021;21:1345-54.
23. Mandal S, Shiva K, Kumar KP, Goel S, Patel RK, Sharma S, Chaudhary R, Bhati A, Pal N, Dixit AK. Ocular drug delivery system (ODDS): Exploration the challenges and approaches to improve ODDS. Journal of Pharmaceutical and Biological Sciences. 2021 Jul 1;9(2):88-94.
24. Shiva K, Mandal S, Kumar S. Formulation and evaluation of topical antifungal gel of fluconazole using aloe vera gel. Int J Sci Res Develop. 2021;1:187-93.
25. Ali S, Farooqui NA, Ahmad S, Salman M, Mandal S. Catharanthus roseus (sadabahar): a brief study on medicinal plant having different pharmacological activities. Plant Archives. 2021;21(2):556-9.
26. Mandal S, Jaiswal DV, Shiva K. A review on marketed Carica papaya leaf extract (CPLE) supplements for the treatment of dengue fever with thrombocytopenia and its drawback. International Journal of Pharmaceutical Research. 2020 Jul;12(3).
27. Mandal S, Vishvakarma P, Verma M, Alam MS, Agrawal A, Mishra A. Solanum Nigrum Linn: An Analysis Of The Medicinal Properties Of The Plant. Journal of Pharmaceutical Negative Results. 2023 Jan 1:1595-600.
28. Vishvakarma P, Mandal S, Pandey J, Bhatt AK, Banerjee VB, Gupta JK. An Analysis Of The Most Recent Trends In Flavoring Herbal Medicines In Today's Market. Journal of Pharmaceutical Negative Results. 2022 Dec 31:9189-98.
29. Mandal S, Vishvakarma P, Mandal S. Future Aspects And Applications Of Nanoemulgel Formulation For Topical Lipophilic Drug Delivery. European Journal of Molecular & Clinical Medicine.;10(01):2023.
30. Chawla A, Mandal S, Vishvakarma P, Nile NP, Lokhande VN, Kakad VK, Chawla A. Ultra-Performance Liquid Chromatography (Uplc).
31. Mandal S, Raju D, Namdeo P, Patel A, Bhatt AK, Gupta JK, Haneef M, Vishvakarma P, Sharma UK. Development, characterization, and evaluation of rosa alba l extract-loaded phytosomes.
32. Mandal S, Goel S, Saxena M, Gupta P, Kumari J, Kumar P, Kumar M, Kumar R, Shiva K. Screening of catharanthus roseus stem extract for anti-ulcer potential in wistar rat.
33. Shiva K, Kaushik A, Irshad M, Sharma G, Mandal S. Evaluation and preparation: herbal gel containing thuja occidentalis and curcuma longa extracts.
34. Javed H, Azimullah S, Meeran MFN, Ansari SA, Ojha S. Neuroprotective Effects of Thymol, a Dietary Monoterpene Against Dopaminergic Neurodegeneration in Rotenone-Induced Rat Model of Parkinson's Disease. *Int J Mol Sci*. 2019;20(7):1538. Published 2019 Mar 27. doi:10.3390/ijms20071538.
35. Xu J, Wang L, Zhang L, et al. Mono-2-ethylhexyl phthalate drives progression of PINK1-parkin-mediated mitophagy via increasing mitochondrial ROS to exacerbate cytotoxicity. *Redox Biol*. 2021;38:101776. doi:10.1016/j.redox.2020.101776.

36. Anwar S, Raut R, Alsahli MA, et al. Role of Ajwa Date Fruit Pulp and Seed in the Management of Diseases through In Vitro and In Silico Analysis. *Biology (Basel)*. 2022;11(1):78. Published 2022 Jan 5. doi:10.3390/biology11010078.
37. Harun NH, Rasdi NA, Salleh RM, Safuan S, Ahmad WANW, Fuad WEM. Subacute Toxicity Evaluation of Methanolic Extract of *Syzygium polyanthum* in Rats. *Trop Life Sci Res*. 2021;32(2):65-81. doi:10.21315/tlsr2021.32.2.5.
38. Safadi A, Carmel-Neiderman NN, Toledano R, et al. The Efficiency of Routine Histopathological Examination for Bilateral Nasal Polyposis. *Ear Nose Throat J*. 2021;100(5_suppl):670S-674S. doi:10.1177/0145561319872728.
39. Mehrzadi S, Shojaii A, Pur SA, Motevalian M. Anticonvulsant activity of hydroalcoholic extract of citrullus colocynthis fruit: involvement of benzodiazepine and opioid receptors. J Evid Based Compl Altern Med 2015;13(4):76–7.
40. Ostovan F, Gol A, Javadi A. Investigating the effects of Citrullus colocynthis pulp on oxidative stress in testes and epididymis in streptozotocin-induced diabetic male rats. Int J Reprod Biomed (Yazd) 2017;15(1):41–8.
41. Shi C, Karim S, Wang C, Zhao M, Murtaza G. A review on antidiabetic activity of Citrullus colocynthis Schrad. Acta Pol Pharm 2014;71(3):363.
42. Shokrzadeh M, Chabra A, Naghshvar F, Ahmadi A. The mitigating effect of citrullus colocynthis (L.) fruit extract against genotoxicity induced by cyclophosphamide in mice bone marrow cells. Sci World J 2013;2013(1):980480.
43. Pashmforosh M, Rajabi Vardanjani H, Rajabi Vardanjani H, Pashmforosh M, Khodayar MJ. Topical anti-inflammatory and analgesic activities of Citrullus colocynthis extract cream in rats. Medicina 2018;54(4):pii: E51.