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Therapeutic Potential of Clitoria ternatea Leaf Extract in reducing Parkinson's Disease Symptoms in Drosophila

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ABSTRACT

Phytochemicals, bioactive non-nutrient compounds found in plants, have been linked to reduced risks of chronic diseases, particularly those associated with oxidative stress. Parkinson's disease (PD), a neurodegenerative disorder characterized by dopaminergic neuron loss and motor dysfunction, is influenced by oxidative stress and protein aggregation. In this study, we investigated the neuroprotective potential of ***Clitoria ternatea*** (CT) leaf extract in a Drosophila model of PD. The ethanolic extract of ***C. ternatea*** leaves was prepared using soxhlet extraction, and PD flies expressing human α-synuclein were generated through genetic crosses. Different concentrations (10, 50, and 100 µl/ml) of ***C. ternatea*** extract were administered to PD flies for 21 days, followed by behavioral assays to assess locomotion (climbing assay), seizure susceptibility (vortex assay), olfactory function (olfactory assay), and phototactic response (phototaxis assay). The results demonstrated that ***C. ternatea*** extract significantly improved locomotor function, reduced seizure recovery time, restored olfactory responses, and enhanced phototactic behavior in PD flies, with the highest improvement observed at 100 µl/ml concentration. These findings suggest that ***C. ternatea*** extract exerts neuroprotective effects, likely through its antioxidant and radical-scavenging properties, offering a potential therapeutic strategy for managing neurodegenerative disorders like Parkinson's disease. Further studies are warranted to explore the underlying molecular mechanisms and its translational potential.

Keywords: *Phytochemicals, Parkinson's disease, Oxidative stress, Neuroprotection*

1. INTRODUCTION

Major chronic disease risk reduction has been associated with phytochemicals which are the bioactive non-nutrient plant substances found in fruits, vegetables, leaves, grains or any other part of plant. Over 5000 phytochemicals are thought to have been found, but many more are currently undiscovered and must be discovered before their full health advantages are known (1). Numerous chronic illnesses is due to the generation of free radicals leading to oxidative stress and there is significant evidence that these phytochemicals present in the plants can act against these free radicals (2). The free radicals are generated by the oxidizing agents and some of these agents are found in substances like food, water or air and they can be created by cellular metabolism. Oxidative stress is caused when there is excess production of these oxidants leading to imbalance. To maintain body's ideal physiological balance, it is important to maintain oxidants and antioxidants balance (3).

Oxidants that evade the body's antioxidant defenses can harm DNA and other macromolecules within cells, which can result in mutations. It has been argued that oxidative DNA damage plays a significant role in ageing and the degenerative diseases that follow it. Micronutrients are part of the body's defenses against oxidants and other mutagens that lead to degenerative diseases. Since the severity of endogenous oxidative DNA damage is more, there are good scientific reasons for believing that antioxidants ought to be just as significant as they are now (4). Consuming adequate amounts of antioxidants is necessary to avoid or reduce the oxidative stress caused by free radicals. Plant products contain many antioxidant substances (phytochemicals) that may protect cellular system from oxidative damage and reduce the risk of chronic illnesses (5-9).

One such common illness which affects the aged people are neurodegenerative disorders and fighting this chronic disease by phytochemicals or herbal medicine has gained popularity and several studies have been published that phytochemicals are used to treat neurodegenertaive disorders. Parkinson's disease is one such neurodegenerative disease seen more prevalent in the old age population. The development of neurodegenerative illnesses is also significantly influenced by environmental and genetic conditions. These neurodegenerative disorders frequently exhibit oxidative stress, the buildup of certain aggregated proteins and neuroinflammation as clinical characteristic. Phytochemicals improve these condition by their anti-oxidative and radical scavenging capacity (10). The progressive loss of dopaminergic neurons of the substantia nigra that project to the striatum is the primary characteristic of Parkinson's disease (11). The deficiency of dopamine in the striatum leads to motor and non-motor symptoms which greatly impact the quality of life of patients (12). To combat all these progressive symptoms natural phytochemicals present in plant can be explored. Research on humans is difficult, therefore researchers are looking into animal models that could mimic some aspects of Parkinson's disease. One of the animal models that can replicate the symptoms of Parkinson's disease in order to look into the underlying causes of the condition and identify possible therapeutic drugs is the Drosophila model. The transgenic fly that expresses α-syn gene

Clitoria ternatea (CT), belongs to the Fabaceae family is a famous ayurvedic remedy and has been extensively studied scientifically and is used to treat a variety of illnesses. In Indian traditional machine, CT is referred to as shankapushpi, butterfly pea or counch flower. The roots, seeds and leaves of CT are widely utilised as brain tonic in Asian Indian medicinal systems notably in ayurveda where it is thought to enhance memory and intelligence (13). More recent studies have discovered that CT exhibits antioxidant qualities (14). Further research is still needed to determine the role of phytochemicals found in CT and their role in neuroprotection.

2. MATERIALS AND METHODS

Preparation of Leaf Extract

Fresh leaves samples of *Clitoria ternatea* were gathered from the Tumkur region. In GKVK, the plant samples were identified and confirmed (Accession no: UASB 5719). Before being allowed to air dry, the obtained plant sample was cleansed with tap water and then briefly rinsed with distilled water. The samples were air-dried and then processed into a fine powder. Using ethanol as a solvent for each weighed plant powder (20g IN 250ml), soxhlet extraction was carried out to extract the bioactive compounds.

Drosophila Stock

Under UAS control, transgenic fly lines express the wild type human synuclein gene in neurons. The fly lines w [1118]; P {w[+mC]=UAS-SNCA.J}1/CyO and TH-GAL4 were bought from the Indian Institute of Science ,Bangalore. By crossing TH-GAL4 females with UAS (Upstream Activation Sequence)-SNCA.J strain males, human α synuclein is expressed in the dopamergic cells of neurons. The resulting progeny expresses the human α synuclein gene (15).

Drosophila Culture and Crosses

The flies were bred at 25°C on standard Drosophila media, which included corn meal, agar, sucrose, and yeast. UAS-SNCA males were mated with virgin females of TH-GAL4 to create crosses and the resulting progeny flies were Parkinson's disease (PD) flies as they express human α synuclein (16). Different concentrations of *Clitoria ternatea* extract mixed with the culture media were administered to the PD flies. At final concentrations of 10µl/ml, 50µl/ml, and 100µl/ml, the plant extract was added to the medium. The control was the UAS-SNCA-CyO. Additionally, the PD flies were treated to 10^{-3}M Ldopamine.

Climbing Assay

A horizontal line was marked 10 cm from the bottom of an empty glass vial in order to conduct the climbing test. Using a small funnel, twenty flies were added to the vial, and they were allowed to adapt for ten minutes at room temperature. The vial was then placed upright after being safely sealed and gently tapped with a foam pad. The number of flies that ascended to the 10-cm mark in 10 seconds was counted using a timer that was started right away. In relation to the total number of flies, the percentage of flies that were able to reach the designated height was measured. After completing this process three times, the average climbing percentage (17).

Vortex Assay

The adult flies were subjected to a cold anesthesia method to induce temporary immobilization. Ten flies were transferred to a fresh, food-free vial and allowed to recover from the effects of anesthesia for one hour at a controlled temperature of 24–25°C. To induce seizures, the vial containing the flies was secured onto a benchtop vortex and shaken at maximum speed for 10 seconds. The flies were then observed for characteristic seizure symptoms, including paralysis, wing flapping, and body stiffness. Seizure recovery time is recorded which is the time taken for each fly to return to its normal (18).

Phototaxis Assay

Flies of the desired genotype were anesthetized and 20 flies were collected per food vial, preparing at least five vials for each genotype. The vials were maintained at 24°C with a 12-hour light/dark cycle for 24 hours before the assay. A clean Y-maze was prepared by plugging all three open ends with cotton. The Y-maze was placed flat on a table with a lamp positioned near the "light" arm, initially turned off.

To begin the assay, the Y-maze was held upright with the base arm facing upward. The cotton plug was removed, and a funnel was inserted into the base arm. Flies were gently transferred from the vial into the base arm. After ensuring all flies entered, the funnel was removed, and the base arm was replugged. The Y-maze was then laid horizontally, with one arm covered to create a dark zone. The light source was turned on, and after 30 seconds, flies in the light arm were counted as positively phototactic, while those in the base and dark arms were considered non-phototactic. The experiment was repeated for at least five replicates per genotype to ensure reliability (19).

Olfactory Assay

To obtain early third instar larvae, approximately 200 well-fed flies (3-5 days old) of the desired genotype were placed in food bottles and allowed to lay eggs for 12 hours at 25°C. After egg collection, the flies were transferred to a fresh food bottle, and the eggs were incubated at 25°C for 4-5 days until larvae reached the early third instar stage. The larvae were collected and washed in water at 25°C to remove food residue. The testing plate was placed on a black surface for better contrast, and two filter discs were positioned in the odorant zones (O1 and O2). Approximately 50 larvae were placed on filter paper to remove excess solution before being transferred to the start zone of the plate. A test odorant (e.g., ethyl acetate) and distilled water was applied to each filter disc, and the dish was sealed. The response was recorded after two minutes by counting larvae in the O1, O2, and control (C) zones. The experiment was repeated three times using fresh larvae and test plates (18).

3. RESULTS AND DISCUSSION

Using ethanol as a solvent, the phytochemicals found in *C. ternatea* leaves were extracted using the soxhlet extraction method. PD flies were obtained by crossing UAS-SNCA males with TH- GAL4 females and the resultant progeny which expresses the α- synuclein gene is referred as PD flies. The α- synuclein gene encodes α-Syn, a protein that is widely expressed in the central nervous system's presynaptic terminals. Although there is increasing evidence that α-Syn has a role in synaptic plasticity and neurotransmitter release, its precise function is still mostly to be understood (20). The PD flies with the expression of α-Syn were treated with different concentrations of *C. ternatea* leaf extract such as 10,50,100μl/ml for 21 days and were referred as treated group and the UAS-SNCA flies without plant extract treatment were referred as control flies. PD flies without plant extract and PD flies treated with commercially available drug L-Dopa was also used in the experiment. All the group of flies were subjected to various behavior assays. After 21 days of exposure of the flies to plant extract, the flies were subjected to climbing assay. One of the most common symptoms in PD is the effect on the locomotor functions and climbing assay is an efficient test to detect the locomotor capabilities. The flies treated with the leaf extract showed improved locomotor ability when compared to that of the untreated flies. Flies treated with 100μg/ml of plant extract showed more improvement in climbing ability (Fig 1). Epilepsy is more prone in people suffering from PD. Epilepsy is characterized by unprovoked recurring seizures. Various symptoms may arise from this increased electrical activity in the brain, depending on the specific region affected (19). The vortex assay was performed to imitate seizures in Drosophila and the time taken by the flies to recover from the seizure was recorded. The quickest recovery rate was seen in flies treated with plant extract at a concentration of 100μg/ml (Fig 2). One of the top five most common motor and nonmotor symptoms that have influenced the standard of life for people with early-stage Parkinson's disease is olfactory loss (20). The fruit fly, *Drosophila melanogaster*, has a highly sensitive and specific olfactory system that allows it to detect and differentiate a broad spectrum of odors. It relies on its sense of smell to find food, identify mates, and select suitable egg-laying sites. Due to its anatomically and genetically simple yet remarkably similar olfactory system to that of humans, *Drosophila* has become a preferred model for studying olfaction, learning, and memory (18). Hence PD model of Drosophila is used to study the olfactory response. The flies treated with the plant extract could smell ethyl acetate and move towards it when compared to that of the untreated flies (Fig 3). Parkinson's disease patients experience a variety of distinct visual abnormalities. These include challenges with complex visual activities and alterations in colour vision and contrast sensitivity (21). The basis of the phototaxis test is an organism's migration either in the direction of or away from the light source. Drosophila flies, like the majority of insects, respond favorably to light and hence migrate in the direction of the light source. The fly's phototaxis is impacted by defects that impact the way photoreceptors in the eyes operate. The flies with PD are unable to recognize between light and dark source and the flies treated with plant extract are able to migrate towards light (Fig 4). Hence plant extract supplementation improves the flies' resistance to oxidative stress damage, which is a mainly seen in neurodegenerative disease.

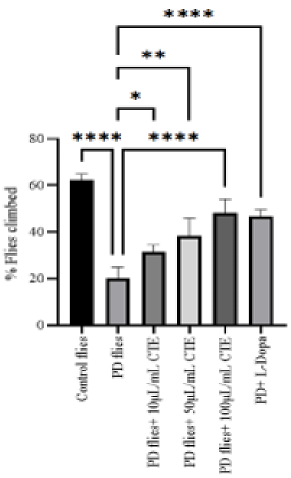

Fig 1: Effects of *Clitoria ternatea* leaf extract (CTE) on the climbing ability. Data are presented as the mean ±SE (n= 3). * Significant at *p* < 0.05; * Significant at *p* < 0.00**

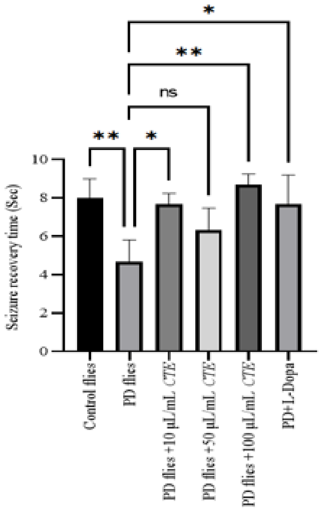

Fig 2: Effects of *Clitoria ternatea* leaf extract (CTE) on the Vortex assay. Data are presented as the mean ±SE (n= 3). * Significant at *p* < 0.05; * Significant at *p* < 0.00**

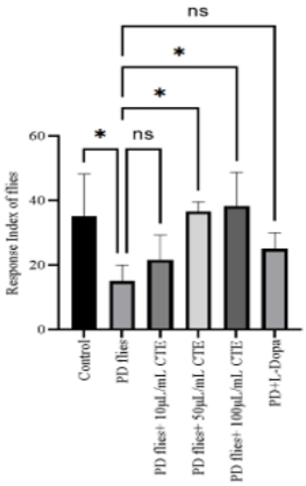

Fig 3: Effects of *Clitoria ternatea* leaf extract (CTE) on the Olfactory assay. Data are presented as the mean ±SE (n= 3). * Significant at *p* < 0.05; * Significant at *p* < 0.00**

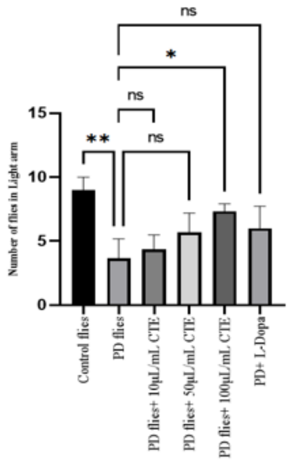

Fig 4: Effects of *Clitoria ternatea* leaf extract (CTE) on the Phototaxis assay. Data are presented as the mean ±SE (n= 3). * Significant at *p* < 0.05; * Significant at *p* < 0.00**

4. CONCLUSION

This study highlights the neuroprotective potential of *Clitoria ternatea* leaf extract in a Drosophila model of Parkinson's disease (PD). The phytochemicals present in the extract were tested for their ability to improve PD-associated impairments, including locomotor dysfunction, seizure susceptibility, olfactory deficits, and phototaxis abnormalities. Results from behavioral assays demonstrated that flies treated with *C. ternatea* extract showed significant improvements compared to untreated PD flies. The climbing assay revealed enhanced locomotor ability, indicating improved motor function. The vortex assay demonstrated a faster seizure recovery time, suggesting a potential anticonvulsant effect. Additionally, the olfactory assay showed better response to odor stimuli in treated flies, indicating the extract's role in preserving sensory functions. The phototaxis assay confirmed improvements in visual response, which is often impaired in PD patients.

These findings suggest that *C. ternatea* may exert neuroprotective effects through its antioxidant and free radical scavenging properties, potentially mitigating oxidative stress and neurodegeneration. The study supports the use of *C. ternatea* as a natural therapeutic agent for PD; however, further research is needed to isolate and characterize the active phytochemicals responsible for these effects. Future studies should also explore its efficacy in mammalian models and clinical applications for PD treatment.

REFERENCES

[1] Shahidi, F., & Naczk, M. (1995). Food Phenolics: Sources, Chemistry, Effects and Applications. Technomic Publishing Co., Lancaster, PA. ISBN: 978-1-56676-279-3

[2] Ames, B. N., & Gold, L. S. (1991). Endogenous mutagens and the causes of aging and cancer. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 250(1-2), 3-16.

[3] Liu, R. H., & Hotchkiss, J. H. (1995). Potential genotoxicity of chronically elevated nitric oxide: a review. Mutation Research/Reviews in Genetic Toxicology, 339(2), 73-89.

[4] Ames, B. N., Shigenaga, M. K., & Gold, L. S. (1993). DNA lesions, inducible DNA repair, and cell division: three key factors in mutagenesis and carcinogenesis. Environmental Health Perspectives, 101(suppl 5), 35-44.

[5] Doddamani, S., Kumar, R., Anchan, V., Sindhu, C. V., Athira, A. G., Bhat, S., Makhija, D., Kachare, K., Srikanth, N., Acharya, R. N., & Patil, S. J. (2024). Exploring the health status, nutritional deficiencies, dietary patterns, and laboratory investigation among tribal populations of villages in HD Kote, Karnataka: A cross-sectional study. Journal of Advanced Zoology, 45(3), 567-573.

[6] Rajeev Ramachandra Kolgi, Keshamma E. and Sharangouda J. Patil. Importance of Nutrition in Infectious Diseases. (ISBN: 978-1-63278-998-3, Innovationinfoebooks Publisher, United Kingdom, p: 209-217.

[7] Ekambaram, G., Sankaranarayanan, N., Nagarajan, P., Palanisamy, P., Mani, T., Raman, V., Kaliaperumal, R., Port Louis, L. R., & Patil, S. J. (2025). Integrative nutrition strategies and cardiovascular therapy from macronutrients to micronutrients. In A. Chatterjee, T. Sarkar, N. Bansal, & K. Adhikary (Eds.), Impact of yoga and proper diet on cardiopulmonary function (pp. 273-304). IGI Global Scientific Publishing. https://doi.org/10.4018/979-8-3693-8267-7.ch009.

[8] Eramma, N., Renuka Jyothi, S., Sadashiv, S. O., & Patil, S. J. (2024). Nutraceutical potential of selected vegetables. In Plant metabolites and vegetables as nutraceuticals (Vol. 1, pp. 33-58). Apple Academy Press – CRC Press. ISBN: 978-10-32680-125.

[9] D'mello, D., Renuka Jyothi, S., Sadashiv, S. O., & Patil, S. J. (2024). Plant metabolites and vegetables for liver disease prevention and treatment. In Plant metabolites and vegetables as nutraceuticals (Vol. 1, pp. 245-273). Apple Academy Press – CRC Press. ISBN: 978-10-32680-125.

[10] Velmurugan, B. K., Rathinasamy, B., Lohanathan, B. P., Thiyagarajan, V., & Weng, C. F. (2018). Neuroprotective role of phytochemicals. Molecules, 23(10), 2485.

[11] Xu, L., & Pu, J. (2016). Alpha-Synuclein in Parkinson's Disease: From Pathogenetic Dysfunction to Potential Clinical Application. Parkinson's disease, 2016(1), 1720621.

[12] Kalia, L. V., & Lang, A. E. (2015). Parkinson's disease. The Lancet, 386(9996), 896-912.

[13] Mukherjee, P. K., Kumar, V., Kumar, N. S., & Heinrich, M. (2008). The Ayurvedic medicine Clitoria ternatea - From traditional use to scientific assessment. Journal of Ethnopharmacology, 120(3), 291-301.

[14] Islam, M. A., Mondal, S. K., Islam, S., Akther Shorna, M. N., Biswas, S., Uddin, M. S., ... & Saleh, M. A. (2023). Antioxidant, cytotoxicity, antimicrobial activity, and in silico analysis of the methanolic leaf and flower extracts of Clitoria ternatea. Biochemistry Research International, 2023(1), 8847876.

[15] Beaulah Angel, P., & Manjula, K. R. (2025). Effect of Centella asiatica leaf extract on the transgenic Drosophila model of Parkinson's disease. Cuestiones de Fisioterapia, 54(4), 5233-5243.

[16] Siddique, Y. H., Ara, G., Jyoti, S., & Afzal, M. (2012). Protective effect of curcumin in transgenic Drosophila melanogaster model of Parkinson's disease. Alternative Medicine Studies, 2(1), e3-e3.

[17] Lakhotia, S. C., & Ranganath, H. A. (Eds.). (2022). Experiments with Drosophila for Biology Courses. Indian Academy of Sciences. ISBN: 978-81-950664-2-1

[18] Mituzaite, J., Petersen, R., Claridge-Chang, A., & Baines, R. A. (2021). Characterization of seizure induction

methods in Drosophila. Eneuro, 8(4).

[19] Gómez-Benito, M., Granado, N., García-Sanz, P., Michel, A., Dumoulin, M., & Moratalla, R. (2020). Modeling Parkinson’s disease with the alpha-synuclein protein. Frontiers in Pharmacology, 11, 356.

[20] Haehner, A., Hummel, T., & Reichmann, H. (2011). Olfactory loss in parkinson′ s disease. Parkinson’s disease, 2011(1), 450939.

[21] Weil, R. S., Schrag, A. E., Warren, J. D., Crutch, S. J., Lees, A. J., & Morris, H. R. (2016). Visual dysfunction in Parkinson’s disease. Brain, 139(11), 2827-2843.