The Review Of
DIABETIC
STUDIES OPEN ACCESS

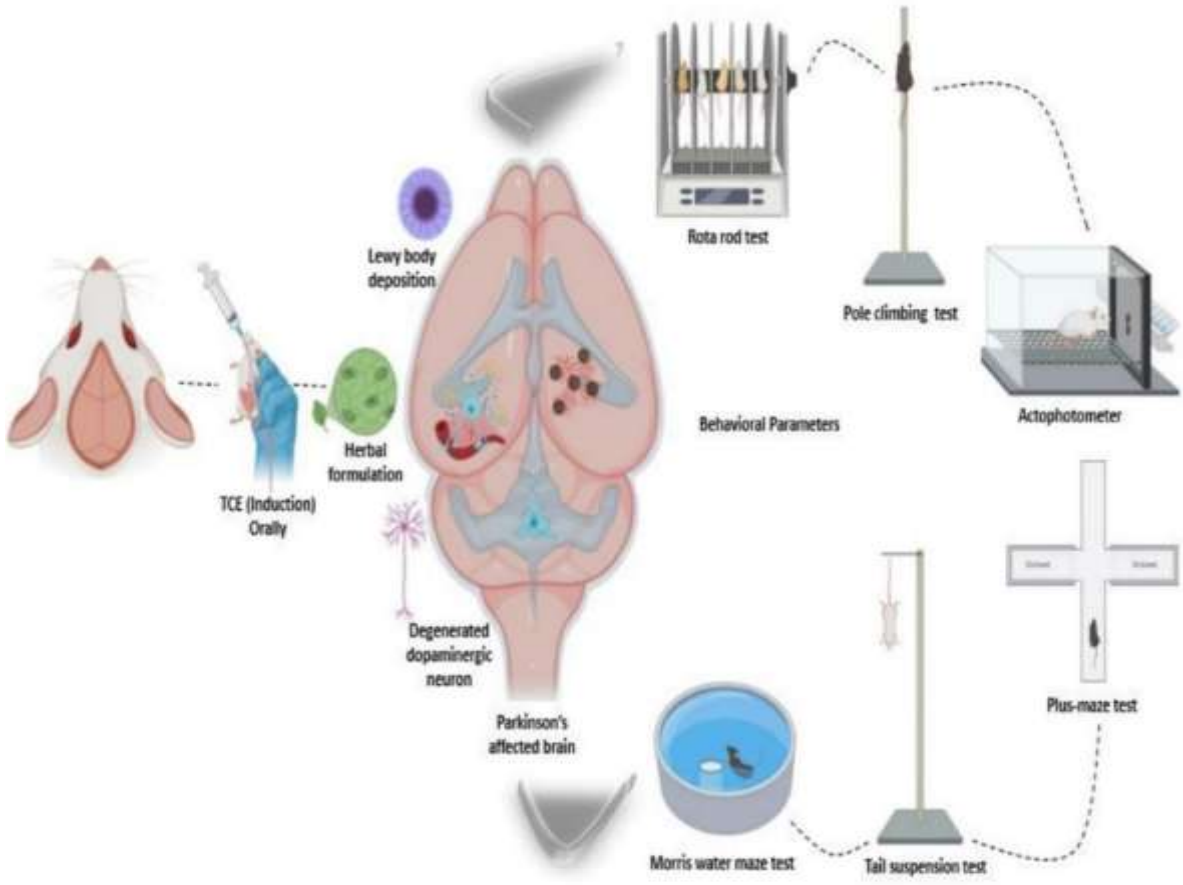
Behavioral And Biochemical Approaches On The Therapeutic Effectiveness Of Leucas Aspera In Managing Trichloroethylene-Induced Neurotoxicity

Varsha Rani[1], Jayalakshmi Muniramiah[2], Manjula Kannasandra Ramaiah[1*]

[1,2] Department of Biotechnology, School of Applied Sciences, REVA University, Bengaluru, Karnataka-560064
**Corresponding author: manjula.kr@reva.edu.in*

Key Contribution:
This study highlights the importance of Leucas aspera, which can be used therapeutically to restore motor and behavioral abilities that have been compromised by Parkinsonism brought on by trichloroethylene. In the mammalian model, the extract showed notable neuroprotective effects by lowering oxidative stress and increasing dopaminergic activity. Notably, the individual's coordination and mobility significantly improved after treatment. According to these results, Leucas aspera shows promise as a natural treatment for Parkinson's disease.

GRAPHICAL ABSTRACT



ABSTRACT

The widely used chlorinated solvent, trichloroethylene (TCE), has been closely linked to neurotoxicity and the emergence of pathologies resembling Parkinson's disease (PD). This is mostly because TCE can cause neuronal damage, oxidative stress, and mitochondrial dysfunction. TCE exposure is a crucial experimental paradigm for researching neurodegeneration because these changes not only upset the metabolic balance but also affect behavioral function in individuals, causing major imbalance and instability. Plant-based neuroprotective drugs have gained attention recently as safer substitutes for treating brain damage brought on by toxins. In this study, the potential of the Methanolic extract of Leucas aspera, a medicinal plant renowned for its antioxidant and neuroprotective properties, to mitigate TCE-induced neurotoxicity in Wistar rats was investigated. Both behavioral and biochemical parameters are beneficial in examining oxidative stress and evaluating the effectiveness of the treatment in managing PD. The Rotarod test for motor coordination, the tail suspension test for depressive-like behavior, and the raised plus maze for anxiety-related reactions were among the behavioral evaluations. Superoxide dismutase (SOD) and catalase (CAT) activity were evaluated as indicators of antioxidant defense, while lipid peroxidation (LPO) was examined as an indicator of oxidative damage in the hippocampus. The results showed that rats exposed to TCE exhibited increased anxiety-like behavior, extended immobility, and marked deterioration in motor coordination and enzyme estimation using Acetylcholinesterase (AChE). Elevated lipid peroxidation levels and a significant decrease in SOD and CAT activity coincided with these behavioral changes, indicating that oxidative stress had been induced in the hippocampus tissue. Through the restoration of motor function, the reduction of depressive-like characteristics, and the normalization of anxiety indicators, treatment with Leucas aspera extract markedly improved behavioral results. Leucas aspera extract also decreased oxidative damage by increasing SOD and CAT activity toward normal levels and decreasing lipid peroxidation. When combined, these findings suggest that Leucas aspera has high neuroprotective potential against TCE-induced neurotoxicity, most likely via its antioxidant mechanism, and could be a viable natural treatment option for Parkinson's disease.

Keywords: motor dysfunction, neuroprotection, oxidative stress, antioxidant enzyme, phytotherapy

INTRODUCTION

Parkinson’s disease (PD) is known as the most common progressive neurodegenerative disorder, affecting more than 9 million people around the world. In the year 2019, it was estimated that over the period of 2000, 8.5 million global population showed a 100% increase due to poor lifestyle changes in humans, and it will be doubled by the year 2040 **[1].** The only cure for such neurodegenerative disorders is the management of the disease by different modes of rehabilitation and various physiotherapies to strengthen muscles and bones.

Trichloroethylene (TCE) is one such volatile chemical compound that helps in the induction of Parkinson’s disease in animal models. These halogenated organic solvents are widely used in the chemical industry by factories, causing neuronal damage and dysfunction. As such, the past 3 major cases are well-proven examples of Parkinson's disorder in the year 2008, reported by Gash and colleagues **[1, 2].** TCE is known for its neurotoxic effects, which cause neuronal loss at the primary stage due to extreme exposure to airborne toxicity from the release of chemicals in the factories. Animal models exposed to TCE lead to neuronal damage in the basal ganglia pars compacta part of the midbrain, causing loss of dopaminergic neurons due to deposition of Lewy bodies. The impact of the chemical on the rat model is well-studied by doing behavioral parameter testing concerning many tests toward locomotor activity and muscle functioning, memory testing, and cognitive functions **[3] (Figure 1).**

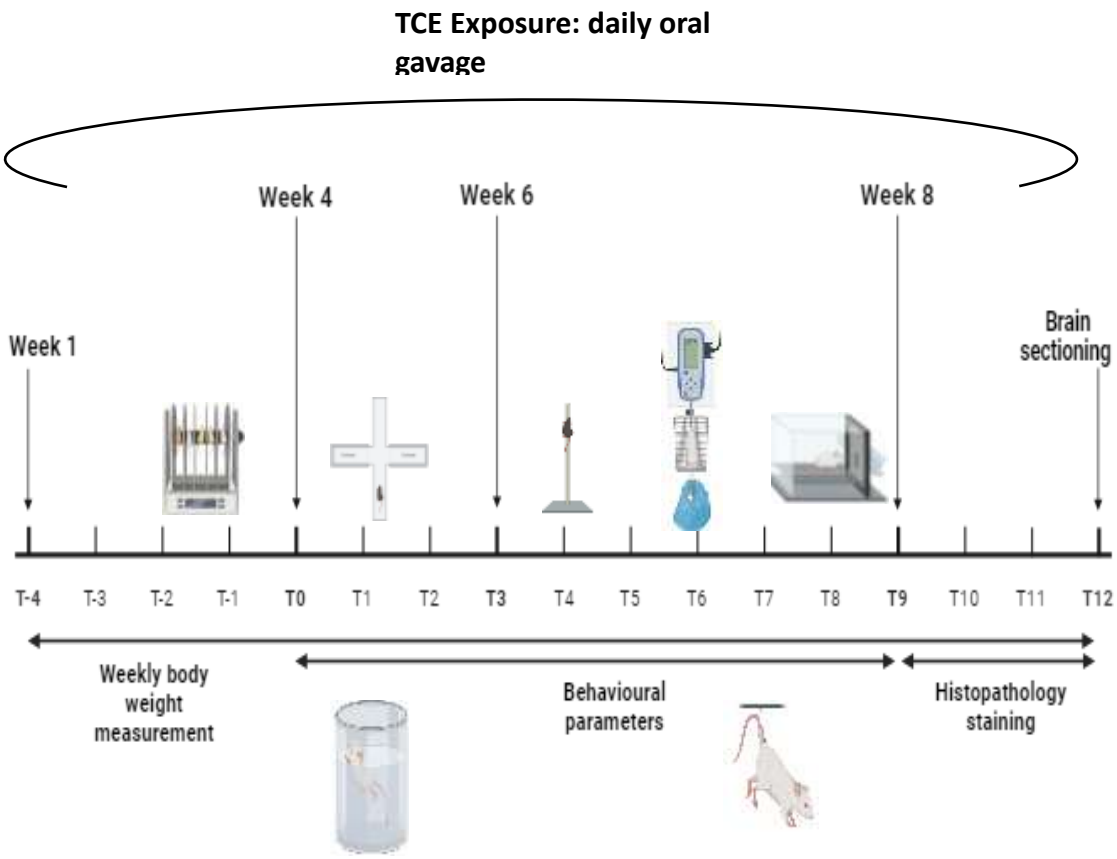


Figure 1. 3-4 months old rat model, exposed to 250 and 500 mg/kg TCE vehicle (olive oil) by oral gavage for 8 weeks.

This research aims to address the gaps in therapeutic drug management of neurodegenerative disorders by targeting the novel mechanism of secondary metabolites obtained from herbal formulations through several steps involving extraction and purification techniques. In the plant extract, the bioactive substances present act as an herbal formulation approach. One such plant extract used in our study is Leucas aspera, commonly known as Thumbai, belonging to the Lamiaceae family. All the parts of the plant sample are well known to have medicinal properties [4]. As Leucas aspera consists of various secondary metabolite compounds in the treatment of PD, our study focuses on the catechins and epicatechin derived from the Leucas aspera plant sample acting as a therapeutic approach against the induction of Parkinson's disease using TCE chemical [5, 6].

The study involves the evaluation of the biochemical and behavioral parameters, aiming to assess the therapeutic potential of Leucas aspera methanolic extract against neurotoxin-induced Parkinsonism. As the behavioural test involves the raised plus maze and Rotarod test for the assessment of motor coordination, oxidative stress markers were evaluated using biochemical parameters such as lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase (CAT), and enzyme estimation using Acetylcholinesterase (AChE). This study attempts to offer new insights into the neuroprotective potential of Leucas aspera and its potential therapeutic value in treating Parkinson's disease-related disorders brought on by environmental toxins like TCE by combining behavioral and biochemical evaluations.

2. MATERIALS AND METHODS

2.1 Animal model

Male albino Wistar rat models in the 2-3-month age group were taken for the experiment from the India Institute of Science, Bangalore, and well maintained till they reached near middle-aged age. Animals

were divided into different groups concerning body weight in polypropylene cages with stainless steel wire mesh, with a properly maintained temperature of 25-28±1°C, relative humidity of 76.5±1%. All the animals were fed daily with freely accessible food from Amruth Feeds, Bangalore, and mineral water **[7].**

2.2 Experimental design

All the rats concerning body weights were chosen and separated into different groups: Control (CON, n=6) and experimental groups: Trichloroethylene (TCE 250), Trichloroethylene (TCE 500), Leucas aspera (LA 250), Leucas aspera (LA 500). All the animals were supplemented with food and water according to their weekly body weight (BW) measurement for 8 weeks. During the supplementation and induction period, animals were daily subjected to behavioral experimental parameters concerning different groups, respectively **[7].**

2.3 Plant extraction

The whole leaf extraction procedure was carried out by air-drying the powdered plant leaf samples in the shade and powdering them. The Soxhlet apparatus was used for extraction using methanol as solvent. The resultant plant extract was reduced using a rotary vacuum evaporator, and for the dissolving of the plant extract, an aqueous solution was used as a vehicle **[4, 7].**

2.4 TCE induction

Male Wistar rats of 2-3 months of age were used for the study. Animals were supplemented with TCE (dissolved in olive oil) as a vehicle by oral gavage using OECD guidelines for dosage supplementation according to the body weight of animals once a day/week. Animals were administered TCE in two doses, 250 and 500 mg/kg, by oral administration using an oral gavage needle. All the experimental design was performed under the guidelines, and the animal care setup was maintained throughout the experimental duration **[8].**

2.9 Behavioral parameter

2.9.1 Body weight

Body weight changes in the individual rats were noted manually every 8 weeks in total before the drug administration **[9].**

2.9.2 Rotarod test

The test was carried out to assess the motor activity using an automated apparatus for Wistar rats after drug administration, which involved different groups compared with the control animals. To judge the memory, movement, and coordination of the TCE-induced Parkinson's disease rat model, the testing apparatus was maintained at a speed of 25 RPM for (5 min) with a computerized electronically controlled recorder for each animal separately, automated apparatus was used for the study consisting of a hallow rotating drum bottom for the animal movement testing (7.5 cm diameter) and 30 cm upgraded from the bottom grid providing rough grip for the animal to endorse. During the testing period, the speed of the apparatus was maintained at 25 RPM, not exceeding. The readings were recorded in seconds automatically by the apparatus, and later, the mean of three test trials was taken. All the rats were trained for a week before the experimental period **[10, 11].**

2.9.3 Elevated Plus Maze test

The ability of the study is derived from testing the anxiety level in the animal model after drug administration, compared with the control group. The setup consists of two open arms (16 × 5cm) and two closed arms (16 × 5 × 12 cm). Each group containing 6 rats was placed individually on the apparatus with the time duration recorder for 5 min for each rat. During the test, animals were placed at the open arm end and allowed to move towards the center and enter the enclosed arm. The time duration covered by the rat to reach the center and the time of the enclosed arm were noted for the test in seconds.

Similarly, for the other experimental groups, the duration was noted and compared with the control group. The study was carried out for 8 weeks, plus a maze test on a one-day/week basis **[12, 13].**

2.9.7 Tail suspension test

The tail suspension test (TST) helps in evaluating the depression-like behavior in rodent models after drug administration. Assessment of immobility was tested for a time duration of 6 min for individual rat models. Rats were suspended onto the metal rod with adhesive tape 30cm above the bench of the laboratory for 6 min. The behavioral changes were recorded and noted manually for each rat model compared with the control group animals' activity. The major aim of the study is to test the acute antidepressant and immobility score **[14, 15].**

2.10 Enzymatic assay

2.10.1 Determination of Acetylcholinesterase

The acetylcholinesterase activity is used to analyze the neuromuscular functioning ability of the animal model post-experimental design using the hypothalamus tissue sample collected post-sectioning of the Wistar rat model brain region by gently using a glass homogenizer with chilled phosphate buffered saline maintained pH, 2mM acetylcholine chloride was used an enzymatic substrate (Ellman's method) against the sample incubating for 2-3 min for the hydrolyzation process to take place and measured the acetylcholine esterase level in the brain region at 412nm using a spectrophotometer using indoxylacetate as standard against acetylcholinesterase **[16].**

2.11 Biochemical assay

2.11.1 Determination of Superoxide Dismutase

The superoxide dismutase activity was carried out to analyze the biological and pathogenic functioning of the oxygen molecules present in the reaction, which can lead to damage to the normal cellular mechanism of the enzyme present in the superoxide dismutase molecule, helping in the mitochondrial functioning and signalling mechanism pathway. The Hypothalamus region is taken and homogenized using a glass homogenizer for different groups. Pyrogallol is used as the autoxidizing agent with Tris-HCl buffer maintaining pH 8.5, and Phenazine Methosulfate to activate the superoxide radical ions. Later, the enzyme activity is measured at 420nm upon the color change reaction using Nitro blue tetrazolium (NBT) dye for detecting the enzymatic reaction. The superoxide dismutase activity is calculated by measuring the 50 %inhibition of the Pyrogallol against the enzymatic reaction **[17].**

2.11.2 Determination of Catalase Activity

Catalase activity was analyzed by detecting the reduction in the level of hydrogen peroxide. The tissue was isolated, and the mixture contained Tris-HCl buffer and 5mM EDTA solution with 10mM hydrogen peroxide. The enzyme activity was measured spectrophotometrically at 240nm, and the decomposed amount of hydrogen peroxide solution was measured and calculated **[18].**

2.11.3 Determination of Malondialdehyde level using lipid peroxidation assay

The malondialdehyde level in the enzymatic assay was measured using lipid peroxide in the presence of Thiobarbituric acid (TBA) and acetic acid, maintaining a pH of 3.5. The brain region was smoothly dissected, and the tissue was homogenized using freshly prepared KCl and refluxed for 60min at 95°C using the above mixture of acetic acid and TBA. After the reaction, the sample was measured at 532nm using a spectrophotometer and calculated using the standard formula **[18].**

2.12 Statistical analysis

Data was analyzed using two-way ANOVA, Bonferroni's multiple comparisons test for all the groups, and the values were given as the mean ± SEM. The GraphPad Prism 9 software was used to analyze the data. The probability values $P<0.05$, $P<0.01$, and $P<0.0001$ were considered significant.

3. RESULTS

3.1 Behavioral study

3.1.1 Body weight

Bonferroni's multiple comparison test was conducted to check the level of significance for the 8-week comparison with the control group. The plant extract effect was also tested on the body weight of the animal model. Not much significant difference was noted in the different groups during the study period. As the TCE induction of 250 and 500 mg/kg to the Wistar rat model, the graph shows that the TCE 250 mg/kg lower dosage shows induction of Parkinson's slowly compared to the control group. Similarly, concerning TCE 500 mg/kg, the rat model is closer to death rate as the graph represents. Plant extract induction using a vehicle has shown a protective effect, respectively, for LA 250 and LA 500 mg/kg. Also, a two-way ANOVA interaction test was conducted to evaluate the quantitative variable changes in the different groups, $p<0.05$

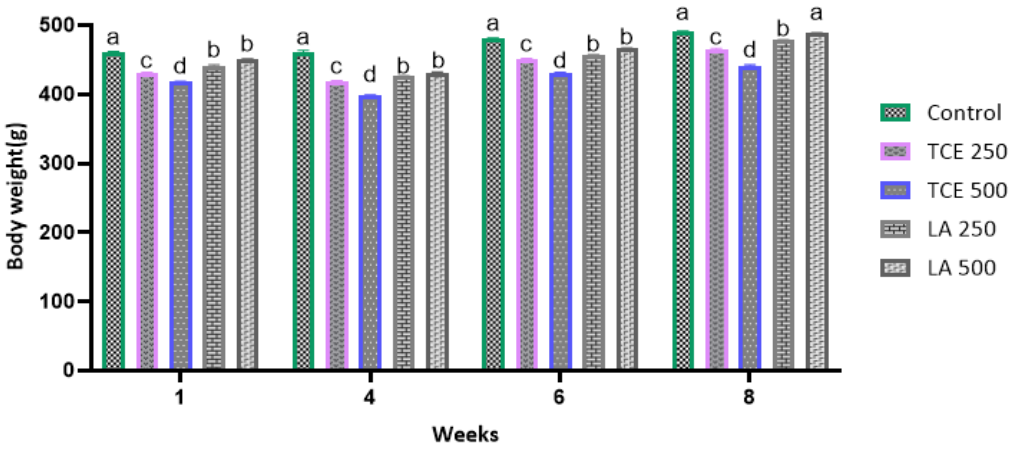
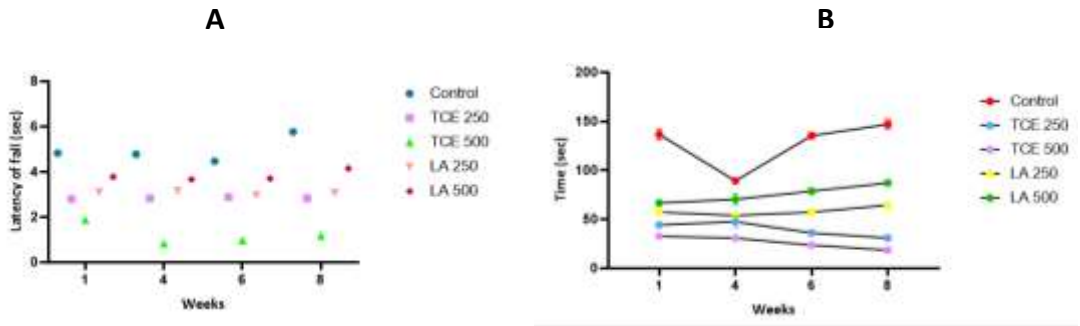


Figure 2. Body weight changes during the study period of 8 weeks

3.1.2 Rotarod test

The main approach of this test was to note the motor coordination of the drug and the TCE-induced rat model. Bonferroni's multiple comparisons test was conducted using two-way ANOVA for the latency of fall and wheeling time at a constant speed mode. The treatment approach of the plant extract-induced model showed better locomotor activity compared with TCE induction. The significance value was tested for the control and experimental group for the 8-week study duration; the level of significance was not significant **Figure 3. (A), (B).**

Figure 3. The result of the Rotarod test is compared with the control group, for 8 weeks: **(A)** Latency of fall (sec), **(B)** Wheeling time (sec), n=6 rats for each group; ****$p<0.0001$ ***$p<0.001$ when compared with normal and TCE induced groups.



3.1.3 Plus-Maze test

The plus-maze test was carried out to test the anxiety level in the Wistar rat model. The apparatus consists of two open arms and two closed arms, which are compared to the rat model of the experimental and control groups. Animals were more towards the closed-arm chamber for TCE 250 and 500mg/kg compared to the open-arm chamber. Comparatively, the plant extract group showed a better approach towards the anxiety level as more movement towards the open arm chamber *p<0.05, ****p<0.0001, **Figure 4, Table 1.**

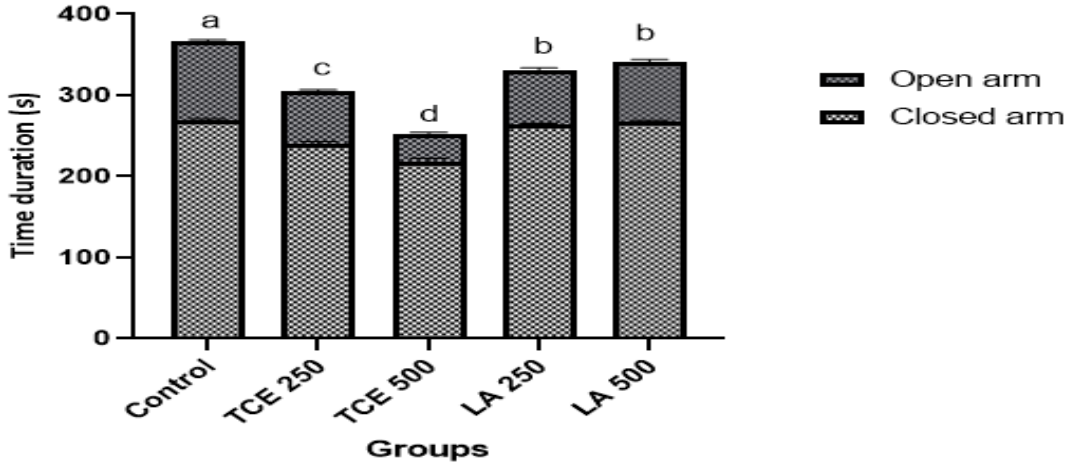


Figure 4. Elevated plus maze; for the open and closed arm to test for anxiety in different groups, the activity of the animals was more in the closed arm compared to the open arm ****p<0.0001 showing statistical significance, LA 250 and LA 500 displayed significantly better activity in the open arm chamber, as the treated group displayed better activity p<0.0001.

Table 1. Elevated plus maze test, the effect of Methanolic extract of Leucas aspera and locomotor changes in rat model using the latency test in the plus-maze.

Sl. No	Treatment (Dose & Route)	Measurement of transfer latency of Leucas aspera at different intervals of (min) mean (±SD)	
		Open arm	Closed arm
1	CONT	96.9 ± 1.09	269 ± 0.88
2	TCE (250 mg/kg)	63.5 ± 0.98**	241 ± 0.74**
3	LA (250 mg/kg)	33.3 ± 1.20**	218 ± 1.76*
4	TCE (500 mg/kg)	65.25 ± 1.46***	264.9 ± 0.35***
5	LA (500 mg/kg)	73.5 ± 1.26*	267.75 ± 0.39

Mean ± SEM, n = 6, ***p<0.001, **p<0.05, ****p<0.0001 are the values.

3.1.4 Tail Suspension test

The tail suspension test, also called an antidepressant test in animal models, is used to test the level of despair-like behavior by suspending the tail. The rat is subjected to stress to check the effect of the treated antidepressant drug against the TCE-induced Parkinson's disease in the rat model. Being suspended to tail the rat tends to escape until they reach the flat platform. The time of escape for different experimental groups compared to the control group was calculated. The testing result showed a higher average immobility episode activity by the experimental group animals than the control group due to

the TCE-induced dosage, and comparatively better improvement in the treated group animals. Showing Significance p<0.0001, immobility test, the resulting level of significance p<0.001, **Figure 5.**

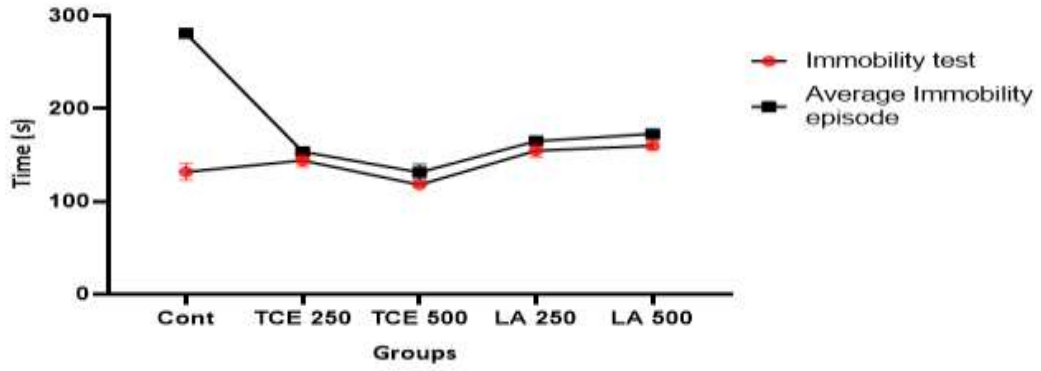


Figure 5. The tail suspension test was conducted by checking the immobility and average episodes on the swing without any disturbance. In the sec, the average immobility episode of the rat was comparatively higher than in the immobility test. The significance level was tested, showing P<0.05 in contrast to the control.

3.2 Enzymatic activity

3.2.1 Determination of Inhibition of Acetylcholinesterase (AChE)

Acetylcholinesterase activity of the brain region was analyzed and assessed, taking a standard reference to determine the difference between the activity of different groups and the impact of TCE on the methanolic extract of Leucas aspera. The control group had the highest AChE activity at all tested concentrations (50, 100, and 150 µg/ml), as seen in **Figure 6 (A).** AChE activity was significantly decreased in a dose-dependent manner ($p < 0.01$) by treatment with both extracts, with the reduction being more noticeable in the groups that received Leucas aspera. AChE was moderately inhibited at 50 µg/ml, but at 100 µg/ml, there was a noticeable decrease, with LA 500 exhibiting the least amount of activity of any group. Additional inhibition of enzyme activity was visible at 150 µg/ml, with LA 500 continuing to have a noticeably stronger inhibitory impact than TCE at comparable dosages. Significant differences between treatments were confirmed by the statistical grouping shown by various superscript letters. These results imply that Leucas aspera may have a role as a natural AChE inhibitor with neuroprotective significance, as its methanolic extract has higher inhibitory effects on hippocampus AChE than TCE **Figure 6 (A).**

3.3 Biochemical Assay

3.3.1 Determination of Superoxide Dismutase (SOD)

The superoxide dismutase (SOD) activity of the hippocampus brain tissue was assessed at different incubation time periods (6, 12, and 24 hours). The control group showed the highest percentage of inhibition at all-time points, indicating strong SOD activity at the base, as shown in **Figure 6 (B)**. Superoxide activity was significantly decreased, showing (***$p < 0.001$; ****$p < 0.0001$) in a dose-dependent manner with respect to the Leucas aspera methanolic extract. The activity of the treated group showed modest activity for the treated group, compared with the control at 6 hours of time duration. The activity continued to decrease at 12 hours, with the 150 µg/mL group exhibiting the greatest suppression. Significant variance among treatments was confirmed by unique superscript letters, and within 24 hours, SOD activity was still significantly lower in all treated groups when compared to the control. These results suggest that although Leucas aspera therapy reduces SOD activity, it may alter antioxidant defense by reorienting the defenses against oxidative stress to other enzymatic or non-enzymatic pathways.

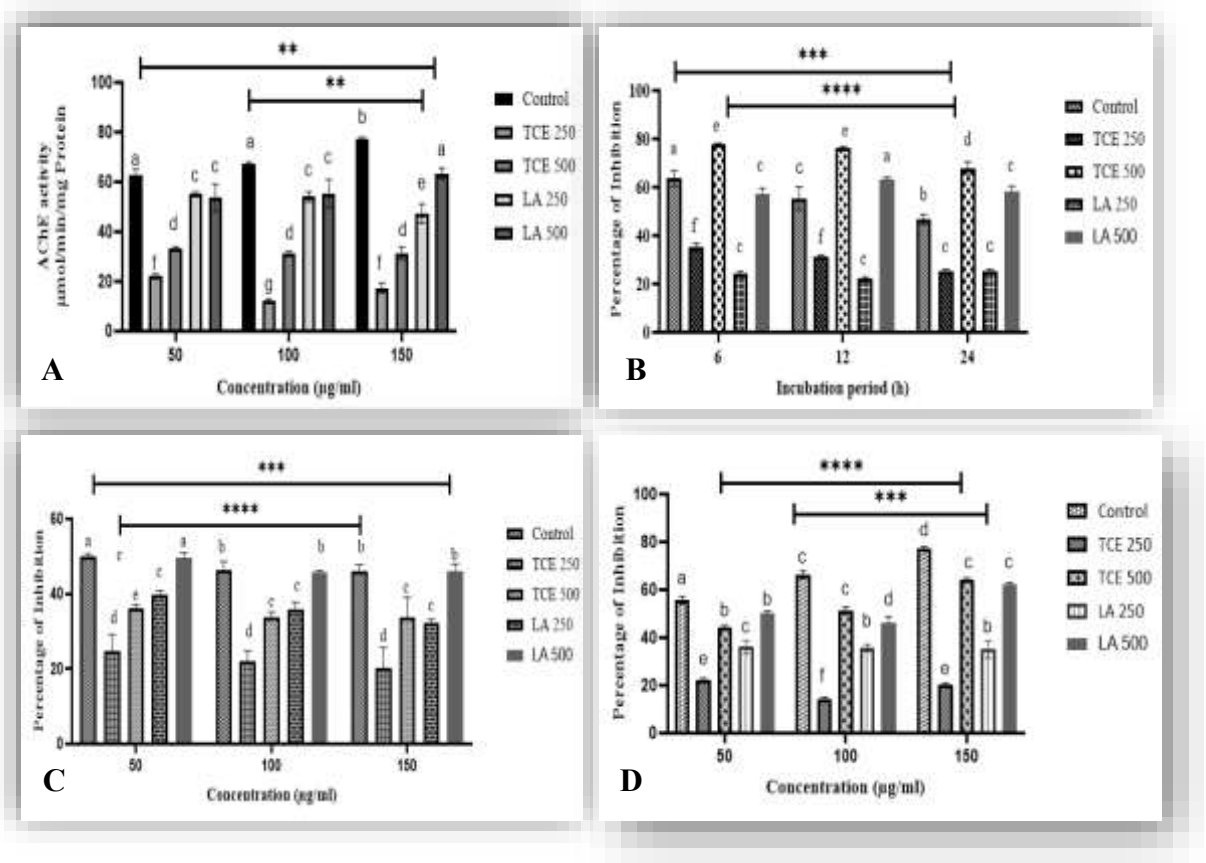


Figure 6: (A) The graph represents AChE activity at various concentrations shown in the bar graph, for the control and treatment groups. Activity noticeably greater at LA and TCE groups, with a reduction in a dose-dependent manner by both extracts at (250and 500 µg/ml), with the highest inhibition exhibited at higher doses **(B)** The bar graph represents the different percentages of inhibition with respect to incubation time periods of (6, 12, and 24 hours), showing high inhibition activity compared with the control and treatment groups. Both extracts (250 and 500 µg/ml) exhibited significantly lower inhibition, with variations across time points **(C).** The bar graph represents the different percentages of inhibition with respect to concentration of (50, 100, and 150 µg/ml), showing high inhibition activity compared with the control and treatment groups. Both extracts (250 and 500 µg/ml) exhibited significantly lower inhibition, with variations across time points **(D).** The Percentage of Inhibition at various concentrations of (50, 100, and 150 µg/ml) is compared with respect to the control and treatment groups, showing maximum inhibition in the control group and a considerable decrease in the TCE-induced group.

3.3.2 Determination of Catalase activity (CAT)

Catalase activity of the Leucas aspera methanolic extract was evaluated in contrast with the standard extract (TCE), although the incubation time showed a decrease in the percentage of inhibition, as shown in Figure 6 (C), displaying weekend antioxidant defense against free radicals causing oxidative stress. On the other hand, TCE and Leucas aspera therapy both markedly increased CAT activity in a time and dose-dependent manner (***$p < 0.0001$; ****$p < 0.0001$). All treatment groups showed a moderate increase in CAT activity after 50µg/mL, with TCE 500 and LA 500 exhibiting higher activity than their corresponding lower dosages. The activity continued to rise at 100 hours, and by 150 µg/mL, the highest CAT activity was observed, especially in the LA 500 and TCE 500 groups, both of which were above 80% inhibition, while the control group stayed noticeably lower. Significant variations between treatments and incubation periods were validated by statistical analysis, denoted by various superscript letters. These results show that the methanolic extract of Leucas aspera efficiently increases catalase

activity, strengthening antioxidant defense systems and shielding hippocampus tissue from oxidative stress-induced damage.

3.3.3 Determination of Malondialdehyde level using lipid peroxidation assay (LPO)

The Inhibition level of the hippocampus region was evaluated in the methanolic extract of the Leucas aspera sample to analyze the ability of lipid peroxidation in contrast with the standard reference group. High oxidative stress was indicated by the control group's lowest percentage of LPO inhibition over the course of all incubation periods (50, 100, and 150 µg/mL), as seen in Figure 6 (D). However, in a dose and time-dependent manner, both TCE and Leucas aspera therapy markedly increased LPO inhibition (***$p < 0.001$; ****$p < 0.0001$). All treatment groups showed moderate inhibition at 50 hours, with TCE 500 and LA 500 showing noticeably greater action than their lower dosages. The inhibitory impact became even more significant at 100 µg/mL, with LA 500 demonstrating comparable efficacy to TCE 500 and better protection than LA 250. The greatest percentage of inhibition was observed at 150 µg/mL, especially in the LA 500 and TCE 500 groups, which both approached 80–90%, whilst the control group stayed much lower. Significant differences between groups were validated by statistical analysis, denoted by separate superscript letters. These results show that the methanolic extract of Leucas aspera efficiently inhibits lipid peroxidation in a concentration- and time-dependent manner, indicating strong antioxidant activity and a possible function in reducing neuronal damage. Damage caused by oxidative stress.

4. DISCUSSION

The incorporation of Leucas aspera into Parkinson's disease management regimens is likely to represent a change and advancement toward the incorporation of more natural and herbal formulation approaches for the cure and management of neurodegenerative dysfunction, as described in the study by Kalpana et al. (2016) [19]. The existing evidence in the research points towards the usage of commercially available drugs in the market. Here, the study specifies the significant importance of therapeutic potential that could bring a huge change in medical practice. As enhancement of traditional herbal remedies has always proven to be a more promising strategy with minimal side effects [20]. The major cause of this disease is memory impairment and tremors in patients affected with PD. Oxidative stress in the cells is the major cause of the overproduction of free radicals, which causes non-enzymatic dopamine levels to increase. α-synuclein is one such protein molecule that gets deposited, causing neurogenesis [21]. Depletion of the dopamine level is a universal factor resulting in neuronal loss, which is responsible for motor dysfunction [22].

Results showed that body weight across different groups did not have significant variations. TCE-treated rats tended to lose weight, especially at higher doses, indicating systemic toxicity. These findings align with previous observations that TCE causes physiological stress and disrupts metabolic processes in animal models [23, 24]. The administration of LA extract mitigated these effects, suggesting a stabilizing influence on metabolic balance. The Rotarod test evaluated the motor coordination, showing a reduction in the activity of the group induced with TCE, which showed a reduction in the locomotor performance, indicating dopaminergic dysfunction seen in PD [25]. With respect to other research showing that plant-derived antioxidants can improve motor function and performance in neurotoxin-induced Parkinsonian models, LA-treated rats showed noticeably enhanced latency to fall and wheeling time [26]. The raised plus maze, which measures anxiety behavior, revealed that TCE-treated groups preferred closed arms, indicating increased anxiety-like reactions, which are indicative of hippocampal impairment [27].

On the other hand, rats given LA exhibited more open-arm exploration, suggesting anxiolytic potential that is probably mediated by flavonoids that alter neurotransmitter systems [28]. TCE increased immobility duration in the tail suspension test, which is consistent with toxin-induced neurobehavioral changes and depressive-like behavior [29]. In accordance with the other medicinal plants that have antioxidant and monoaminergic modulatory qualities, the methanolic extract of LA therapy decreased immobility, confirming its antidepressant-like action [30].

Neurotoxin-induced TCE showed oxidative and neurochemical dysfunction, which was further validated by biochemical tests. According to earlier research relating environmental pollutants to

cognitive impairments, TCE groups showed a significant decrease in acetylcholinesterase (AChE) activity, suggesting compromised cholinergic transmission **[31].** The dose-dependent inhibition of AChE activity by LA extract is in keeping with data that phytochemicals have neuroprotective properties as natural cholinesterase inhibitors **[32].** In accordance with earlier findings of free radical formation after neurotoxic exposure, antioxidant enzyme assays demonstrated significantly reduced superoxide dismutase (SOD) activity in TCE groups, indicating oxidative imbalance [33]. According to observations that plant polyphenols enhance antioxidant defense, treatment with LA partially restored SOD activity [34]. In line with earlier research showing that botanical extracts can regulate catalase activity under oxidative stress, the LA-treated groups showed increased catalase (CAT) activity, indicating better hydrogen peroxide detoxification and reinforcement of enzymatic antioxidant systems [27]. According to earlier research on TCE-related mitochondrial dysfunction, lipid peroxidation (LPO) was also higher in TCE-exposed groups, suggesting oxidative damage to hippocampus lipids [23]. Similar to prior research paper showing substantial antioxidant activity of plant extracts in attenuating neuronal oxidative damage, LA administration dramatically decreased LPO levels in a dose-dependent manner [35]. The protective effect of plant extract is due to the presence of flavonoid compounds present in the formulation, which act as antioxidant molecules to fight against reactive oxygen species molecules due to oxidative stress [36].

5. CONCLUSION

The study summarizes the significant role of the Wistar rat model in understanding the advancement in technology for Parkinson's disease (PD) and the utilization of novel therapeutic approaches in the treatment. Utilization of Trichloroethylene for the induction of Parkinson's disease showed regressive loss of dopaminergic neurons in the substantia nigra part of the brain, causing behavioral changes in the animal model such as motor dysfunction, tremors, bradykinesia, cognitive disability, and rigidity. All these parameters were tested using the Rotarod test, plus-maze for open and closed arms to test the anxiety level, for analyzing the depressive-like behavior, and the Tail suspension test to test the antidepressant activity in the rat model, and biochemical parameters for the confirmation of enzymatic activity in the treated and control region against neurotoxins causing PD. Therefore, the administration of Leucas aspera methanolic extract at a dosage level exhibited a relatively improving effect on motor and cognitive function, suggesting methanolic extract as a potential therapeutic approach.

Findings in the study indicated that Leucas aspera not only helps minimize the depletion of dopaminergic neurons but also plays a vital role in managing PD. It relates better motor coordination and cognitive function to activity. Therefore, implementing a therapeutic approach involving herbal formulations can be beneficial in treating symptoms associated with PD. As the bioactive compounds present in the flavonoid molecule of Leucas aspera methanolic extract help fight against the oxidative stress causing neuronal damage in the temporal region of the brain, future research could aim at elucidating the mechanism of action as a promising approach to effectively cure diseases related to the neurological disorder PD.

6. ABBREVIATIONS

TCE – trichloroethylene, PD - Parkinson's disease, SOD - Superoxide dismutase, CAT – catalase activity, LPO - lipid peroxidation, AChE – Acetylcholinesterase, CON – Control, LA – Leucas aspera, BW – Body Weight, OECD - Organisation for Economic Co-operation and Development, RPM - Revolutions Per Minute, TST - tail suspension test, NBT - Nitro blue tetrazolium, TBA - Thiobarbituric acid, MDA – Malondialdehyde, KCl – Potassium Chloride, ANOVA – Analysis of Variance

7. ACKNOWLEDGEMENTS

We would like to acknowledge REVA University for the Research facility and constant support in carrying out the research work.

8. AUTHOR CONTRIBUTIONS

Manjula K.R. conceptualized and designed the study, supervised the experimental work, and contributed to data interpretation. Varsha Rani performed the laboratory experiments, collected data,

and assisted in statistical analysis. Both authors collaborated on drafting and revising the manuscript. Manjula K.R verified final approval of the version.

9. CONFLICT OF INTEREST

The Authors declare no conflict of interest.

10. REFERENCES

1. Dorsey ER, Zafar M, Lettenberger SE, Pawlik ME, Kinel D, Frissen M, Schneider RB, Kieburtz K, Tanner CM, De Miranda BR, Goldman SM, Bloem BR. Trichloroethylene: An Invisible Cause of Parkinson's Disease? J Parkinson's Dis 2023. 13(2):203-218.
2. Greggio E. Role of LRRK2 kinase activity in the pathogenesis of Parkinson's disease. Biochem Soc Trans 2012. Oct;40(5):1058-62.
3. De Miranda BR, Greenamyre JT. Trichloroethylene, a ubiquitous environmental contaminant, is associated with the risk of Parkinson's disease. Environ Sci Process Impacts 2020. Mar 1;22(3):543-554.
4. Anisetti, Ravinder N & Ramani, Ramalingam & Madhavi, Bindu & K, Sujeevana & Rao, Raja & Nagulu, Malothu. Anti-Parkinson's Studies of Ethanolic Extract of Leucas aspera in Mice. Antiparkinson's studies of the ethanolic extract of Leucas aspera in mice. World Journal of Pharmacy and Pharmaceutical Sciences 2014. 3(7), 1226-1235.
5. Meghashri SH, Vijay Kumar, S Gopal. Antioxidant Properties of a Novel Flavonoid from Leaves of Leucas aspera." Food Chemistry 2010. 122(1): 105–10.
6. Priya R. & Mahendran, Nirmala & Shankar T. & Malarvizhi, Arthanari. Phytochemical compounds of Leucas aspera L. Pharmacological Benefits of Natural Products 2018. (2), 19-35.
7. Asha Devi S, Sagar Chandrasekar BK, Manjula KR, Ishii N. Grape seed proanthocyanidin lowers brain oxidative stress in adult and middle-aged rats. Exp Gerontol 2011. Nov;46(11):958-64.
8. Liu M, Choi DY, Hunter RL, Pandya JD, Cass WA, Sullivan PG, Kim HC, Gash DM, Bing G. Trichloroethylene induces dopaminergic neurodegeneration in Fisher 344 rats. J Neurochem 2010. Feb;112(3):773-83.
9. Potrebić MS, Pavković ŽZ, Srbovan MM, Dmura GM, Pešić VT. Changes in the Behavior and Body Weight of Mature, Adult Male Wistar Han Rats after Reduced Social Grouping and Social Isolation. J Am Assoc Lab Anim Sci 2022. Nov 1;61(6):615-623.
10. Bera I, Sabatini R, Auteri P, Flace P, Sisto G, Montagnani M, Potenza MA, Marasciulo FL, Carratu MR, Coluccia A, Borracci P, Tarullo A, Cagiano R. Neurofunctional effects of developmental sodium fluoride exposure in rats. Eur Rev Med Pharmacol Sci 2007. Jul-Aug;11(4):211-24.
11. Monir, Dina M, Omyma G Ahmed, Motamed E Mahmoud, and Amany A Abdel Hamid. Behavioral Evaluation of Rotenone Model of Parkinson's Disease in Male Wistar Rats. Sohag Medical Journal 2020.
12. Tamboli FA, Rangari VD, and More HN. The anxiolytic action of natural and micropropagated plant extracts of Bacopa monnieri (L.) In Swiss albino mice, by elevated plus maze and swim test were used. International Journal of Biology, Pharmacy and Allied Sciences 2021.10(11).
13. Johnson SA, Javurek AB, Painter MS, Murphy CR, Conard CM, Gant KL, Howald EC, Ellersieck MR, Wiedmeyer CE, Vieira-Potter VJ, Rosenfeld CS. Effects of a maternal high-fat diet on offspring behavioral and metabolic parameters in a rodent model. J Dev Orig Health Dis 2017. Feb;8(1):75-88.
14. Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neurosci Biobehav Rev 2005. 29(4-5):571-625.
15. Bonito-Oliva A, Masini D, Fisone G. A mouse model of non-motor symptoms in Parkinson's disease: focus on pharmacological interventions targeting affective dysfunctions. Front Behav Neurosci 2014. Aug 27;8:290.
16. Ellman Gl, Courtney Kd, Andres V Jr, Feather-Stone Rm. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961. Jul;7:88-95.

17. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974. Sep 16;47(3):469-74.
18. Ezekwesili, Chinwe & Ogbunugafor, Henrietta & Ezekwesili-Ofili, Josephine. Anti-diabetic Activity of Aqueous Extracts of Vitex doniana Leaves and Cinchona calisaya Bark in Alloxan–Induced Diabetic Rats. International Journal of Tropical Disease and Health 2012. (2): 290 - 300.
19. Kalpana VN, V Devi Rajeswari. Phytochemical and Pharmacological Investigation of an Indigenous Medicinal Plant, Leucas aspera. International Journal of Pharm Tech Research 2016. 9(8), 399-407.
20. Menezes, Ashton. Computational analysis of Phytocompounds present in Leucas aspera to target Parkinson's disease-causing alpha-synuclein. Innovare Journal of Medical Sciences 2022. 10 (6): 2321-4406.
21. Stefanis L. α-Synuclein in Parkinson's disease. Cold Spring Harb Perspect Med 2012. Feb;2(2):a009399.
22. Lo Bianco C, Ridet JL, Schneider BL, Deglon N, Aebischer P. alpha -Synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. Proc Natl Acad Sci U S A 2002. Aug 6;99(16):10813-8.
23. Lash LH, Chiu WA, Guyton KZ, Rusyn I. Trichloroethylene biotransformation and its role in mutagenicity, carcinogenicity and target organ toxicity. Mutat Res Rev Mutat Res 2014. Oct-Dec;762:22-36.
24. Cichocki JA, Guyton KZ, Guha N, Chiu WA, Rusyn I, Lash LH. Target Organ Metabolism, Toxicity, and Mechanisms of Trichloroethylene and Perchloroethylene: Key Similarities, Differences, and Data Gaps. J Pharmacol Exp Ther 2016. Oct;359(1):110-23.
25. Goldman JG, Postuma R. Premotor and nonmotor features of Parkinson's disease. Curr Opin Neurol 2014. Aug;27(4):434-41.
26. Jávega-Cometto M, Naranjo-Viteri AJ, Champarini LG, Hereñú CB, Crespo R. Plant-Derived Monoterpene Therapies in Parkinson's Disease Models: Systematic Review and Meta-Analysis. Plants (Basel) 2025. Mar (22):14(7):999.
27. Blossom SJ, Melnyk SB, Li M, Wessinger WD, Cooney CA. Inflammatory and oxidative stress-related effects associated with neurotoxicity are maintained after exclusively prenatal trichloroethylene exposure. Neurotoxicology 2017. Mar(59):164-174.
28. Bell R, Duke AA, Gilmore PE, Page D, Bègue L. Anxiolytic-like effects observed in rats exposed to the elevated zero-maze following treatment with 5-HT2/5-HT3/5-HT4 ligands. Sci Rep 2014. Jan 24;4:3881.
29. Lad HV, Liu L, Payá-Cano JL, Fernandes C, Schalkwyk LC. Quantitative traits for the tail suspension test: automation, optimization, and BXD RI mapping. Mamm Genome 2007. Jul;18(6-7):482-91.
30. Shah MS, Tayab MA, Rahman A, Hasan MN, Talukder MSH, Uddin AMK, Jabed M, Chy MNU, Paul A, Rahman MM, Bin Emran T, Seidel V. Anxiolytic, antidepressant and antioxidant activity of the methanol extract of Canarium resiniferum leaves. J Tradit Complement Med 2022. Aug 3;12(6):567-574.
31. Kalafatakis K, Gkanti V, Mackenzie-Gray Scott CA, Zarros A, Baillie GS, Tsakiris S. Acetylcholinesterase activity as a neurotoxicity marker within the context of experimentally-simulated hyperprolinaemia: An in vitro approach. J Nat Sci Biol Med 2015. Aug;6(Suppl 1):S98-S101.
32. Lopa SS, Al-Amin MY, Hasan MK, Ahammed MS, Islam KM, Alam AHMK, Tanaka T, Sadik MG. Phytochemical Analysis and Cholinesterase Inhibitory and Antioxidant Activities of Enhydra fluctuans Relevant in the Management of Alzheimer's Disease. Int J Food Sci 2021. Jan 11;2021:8862025.
33. Chidambaram SB, Anand N, Varma SR, Ramamurthy S, Vichitra C, Sharma A, Mahalakshmi AM, Essa MM. Superoxide dismutase and neurological disorders. IBRO Neurosci Rep 2024. Jan 23;16:373-394.

34. Rudrapal M, Rakshit G, Singh RP, Garse S, Khan J, Chakraborty S. Dietary Polyphenols: Review on Chemistry/Sources, Bioavailability/Metabolism, Antioxidant Effects, and Their Role in Disease Management. Antioxidants (Basel) 2024. Mar 30;13(4):429.
35. Subash S, Essa MM, Al-Adawi S, Memon MA, Manivasagam T, Akbar M. Neuroprotective effects of berry fruits on neurodegenerative diseases. Neural Regen Res 2014. Aug 15;9(16):1557-66.
36. Chouman A, El-lakany A, Aboul-ela M, Ali Hijazi M. Review on phenolic constituents and pharmacological activities of genus Ononis. J Appl Pharm Sci 2025.15(07):027–036.