Assessing the Ethanolic Impact of Bacopa Monnieri Leaf's Neuroprotective Potential in an Animal Model of Parkinson's disease Produced by Chlorpromazine

Hrishita A Singh V M, M. Meena Kumari, I Veena Rani
Department Of Pharmacology, SSJ College of Pharmacy, Vattinagulapally, Gandipet, Rangareddy District

ABSTRACT :
Background: Chlorpromazine, a commonly used antipsychotic, induces motor dysfunction and neurochemical imbalances, limiting its clinical use. This study investigates the neuroprotective effects of *BMLEE* (name of your extract) on chlorpromazine-induced motor and behavioral deficits, along with alterations in serotonin, GABA, and dopamine levels in rodents.
Methods: Animals were divided into five groups: Vehicle control, Chlorpromazine (25 mg/kg, i.p), Chlorpromazine + Standard treatment (Levodopa 12 mg/kg + Benserazide 3 mg/kg, i.p), and Chlorpromazine + *BMLEE* at 200 mg/kg and 400 mg/kg (p.o). Motor coordination (Rota Rod test), locomotor activity, depressive-like behavior (Forced Swim and Tail Suspension Tests), anxiety-like behavior (Hole Board Test), and neurotransmitter levels were evaluated.
Results: Chlorpromazine significantly impaired motor coordination, reduced locomotor and exploratory activity, increased immobility in depression-related tests, and decreased brain serotonin, GABA, and dopamine levels. Treatment with *BMLEE* dose-dependently improved motor and behavioral parameters and restored neurotransmitter levels, comparable to standard Levodopa + Benserazide treatment.
Conclusion: *BMLEE* demonstrates significant neuroprotective and neurorestorative effects against chlorpromazine-induced deficits, highlighting its potential as a therapeutic agent for neuropsychiatric and neurodegenerative disorders involving dopaminergic dysfunction.
Keywords: *BMLEE*,Chlorpromazine,antipsychotic,neuroprotective

I. INTRODUCTION

The human nervous system, comprising the brain, spinal cord, and peripheral nerves, is a complex and highly specialized network responsible for regulating vital physiological functions and cognitive processes. Neurons, the functional units of this system, are particularly sensitive to damage due to their high metabolic demands, complex connectivity, and limited regenerative capacity. The term “neuroprotection” refers to strategies, agents, or interventions that help preserve the structure and function of neurons and prevent their degeneration or death following injury or disease.[1-3]

In recent decades, the incidence of neurological disorders has risen sharply, largely due to aging populations and increased environmental stressors. Neurodegenerative diseases, such as Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, and amyotrophic lateral sclerosis (ALS), are characterized by progressive neuronal loss, leading to severe cognitive and motor impairments. Similarly, acute neurological conditions, such as ischemic stroke, traumatic brain injury, and spinal cord injury, result in sudden neuronal damage and long-term disability. These conditions not only compromise individual health and quality of life but also impose a substantial socio-economic burden on healthcare systems worldwide.[4-6]

The underlying mechanisms of neuronal damage are complex and multifactorial. Common pathological pathways include oxidative stress, glutamate excitotoxicity, inflammation, mitochondrial dysfunction, protein aggregation, and apoptosis. Therefore, neuroprotective strategies often aim to interrupt or modulate these pathways to delay disease progression or enhance neuronal survival. For example, antioxidants like vitamin E, coenzyme Q10, and flavonoids neutralize reactive oxygen species (ROS), preventing oxidative damage to neuronal membranes and DNA. Anti-inflammatory agents can inhibit microglial activation and cytokine release, thereby reducing inflammation-induced neurotoxicity.

Additionally, the role of natural compounds and herbal medicines in neuroprotection has gained increasing attention in recent years. Phytochemicals such as curcumin (from turmeric), resveratrol (from grapes), and bacosides (from Bacopa monnieri) have demonstrated neuroprotective effects through multi-target mechanisms,

including antioxidant activity, inhibition of acetylcholinesterase, and modulation of neurotransmitter systems. These compounds offer promising alternatives due to their low toxicity and biological versatility.[7-8]

The development and evaluation of neuroprotective therapies is a critical area of biomedical research. Animal models, in vitro studies, and clinical trials are essential for understanding the efficacy and safety of potential neuroprotective agents. Although many compounds have shown promise in preclinical studies, the translation of these findings into effective clinical treatments remains challenging due to the complexity of the central nervous system and variability in disease progression.

II. EXPERIMENTAL WORK

COLLECTION AND AUTHENTICATION OF PLANT

bacopa monnieri leafs were obtained collected from Thirupathi, Andhra Pradesh, India, andauthenticatedbyDrMadhava Chetty,ProfessorandHead,DepartmentofBotany, S.V. University, Thirupathi, Andhra Pradesh, India and Voucher specimens(PC/0220/06)weredeposited at S.V. University, Thirupathi, Andhra Pradesh.

EXTRACTION OF PLANT MATERIAL

Coarsely powdered leaves of *bacopa monnieri leafs*were extracted with ethanolic for 6-8 hours at room temperature. After extraction, the extracts were evaporated by using a rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4^{o}C for further analysis by adding few drops of chloroform as a preservative.

EVALUATION OF ANTI-PARKINSON STUDY OF ETHANOL EXTRACT OF *BACOPA MONNIERI LEAFS*

Selection of animal for chlorpromazine induced Parkinson evaluation

225-220 g body weight were offered by from our college animal house. All the rats were kept at room temperature and allowed to acclimate in standard conditions less than 12 hr light/ 12 hr dark cycle in the animal house. Animals are fed with commercial pellet diet and water ad libitum freely throughout the study. The experimental procedure was approved by IAEC (Institution of Animal Ethical Committee). The animals were divided into 5 groups each group of 6 animals

Group I: Vehicle control (normal saline i.p)
Group II: Only chlorpromazine. (25 mg/kg, i.p)
Group III: chlorpromazine. + Standard [Levodopa 12 mg/kg + Benzerazide 3mg/kg i.p]
Group IV: chlorpromazine. + BMLEE (200 mg/kg, p.o)
Group V: chlorpromazine. + BMLEE (400 mg/kg, p.o)

Preparation of Levodopa and Benzerazide

12mg/kg of levodopa and 3 mg/kg of Benzerazide was dissolved in distilled water. Levodopa and benzerazide was freshly prepared daily and given via i.p to the standard group.

Preparation of sample

200 mg/kg and 400 mg/kg were dissolved in distilled water and it was prepared freshly and given via oral route to group IV& V respectively for 7 days.

EVALUATION PARAMETERS

Motor Co-Ordination Test (Rota Rod Test)

Principle

The Rota rod performance test is carried out on a rotating rod that provides forced motor activity in animals. The animals were placed on a rotating rod which is placed horizontally, suspended above a cage floor, which is high enough to induce avoidance of fall. Animals naturally try to stay on the rotating rod avoid falling to the ground. The length of time (duration) the animal stay on the rod without falling, gives a measure of their coordination, balance, physical condition and motor-planning. [9]

Procedure

Motor Co-ordination test was conducted using rota rod apparatus. Animal was placed individually on the rotating rod and trained for 3 min trail at 25 rpm on the day before the first day of testing. A cut off time of 180s was fixed and each animal performed 3 separate trials at 5 min interval. After each trial, 5 min rest period was given to alleviate stress and fatigue. Motor coordination can be tested by comparing the latency to fall on the very first trial between treatment groups. The time taken by animals to fall from the rotating rod was noted.[10]

Locomotor Activity

Principle

The locomotor activity (horizontal activity) can be easily measured using an actophotometer which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded. An actophotometer could have either circular or square area in which the animal moves.[11]

Procedure

The spontaneous locomotor activity of each animal was recorded individually, using Actophotometer. The apparatus was placed in a sound attenuated and ventilated room during the testing period. All the animals were placed individually in the activity cage for 3 min to habituate them before starting actual locomotor activity task for the next 3 min. the basal activity score was noted. The units of the activity counts were arbitrary and based on the beam breaks by movement of the animal. Counts/3 min is used as an index of locomotor activity.[78]

Forced Swimming Test

Principle

Forced swimming test Is a Behavioural despair test. This test is most widely used to assess alterations in depression-like behaviour in animals. The time spent by the animal as immobile in water represents the depression-like behavior. [13]

Procedure

The test was performed according to the method described by Porsolt et al., 1977, with slight modifications. Animals were forced to swim in a glass cylinder (20 cm height, 14 cm diameter) containing 10 cm depth of water at 25^{o} c. After the initial 2 min acclimatization period, the total duration of immobility was measured during final 4 min of the 6 min test session. Animal were considered to be immobile, when they made no further attempts to escape except the movements necessary to kept their heads above the water. After 6 min, the animals were removed from water, allowed to dry, and returned back to their home cage.[14]

Hole Board Test

Principle

When a animal is placed on the hole board apparatus, which is elevated to 25 cm from the base, shows anxiety as it is exposed to a new environment, thus showing characteristics head poking behaviour. Decrease in anxiety shows increased exploration of the holes. Whereas increased anxiety shows lower number of head poking.[15]

Procedure

The hole board apparatus consist of a wooden board (40*40cm) placed 25 cm above the ground. It consists of 16 holes which is about 3 cm in diameter, spaced symmetrically in a diamond pattern. Animals were placed on the corner of the apparatus and were observed for the next 5 min for the number of head dipping. A head dipping is counted when the animal introduces its head into any hole of the box up to the level of the ears. The apparatus was thoroughly cleaned between each subject.

Tail Suspension Test

Principle

The tail-suspension test (TST) is a widely used assay for screening potential antidepressant drugs. The test is based on the principle that animal subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. [16]

Procedure

The tail suspension test is another well characterized test for assessing depression-like and anti-depressant like activity. In this test animal were individually suspended by the tail to a horizontal ring –stand bar (distance from floor = 30cm) using adhesive tape (distance from tip of tail = 2cm). Typically animal demonstrated several escape-orientated behaviours interspersed with temporally increasing bouts of immobility. A 6-mins test session was employed, which was videotaped. The parameter recorded was the number of seconds spent immobile. [17]

ESTIMATION OF BRAIN NEUROTRANSMITTER

Estimation of Serotonin, GABA and Dopamine

Preparation of tissue extracts

Reagents

- HCl – Butanol solution: (0.85 ml of 37% hydrochloric acid in one-litre *n*-butanol)
- Heptane
- M HCl: (0.85 ml conc. HCl up to 100 ml H_2O)

Procedure

At the end of experiment, rats were sacrificed and the whole brain was dissected out. 0.25 g of tissue was weighed and was homogenized in 5 mL HCl–butanol with motor driven Teflon coated homogenizer for about 1 min. The sample was then centrifuged for 10 min at 2000 rpm. An aliquot supernatant phase (1 mL) was removed and added to centrifuge tube containing heptane (2.5 mL) and 0.1 M HCl (0.31 mL). After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate the two phases, and the overlaying organic phase was discarded. The aqueous phase was then taken either for 5-HT or NA and DA assay.

Estimation of dopamine

Reagents

- 0.4 M HCl: 0.34 ml conc. HCl up to 10 mL H2O
- Sodium acetate buffer (pH 6.9): 0.72 mL of 1 M acetic acid (6 µL of glacial acetic acid up to 1000 µL with distilled water) + 6.84 mL of 0.3 M sodium acetate (0.408 g of sodium acetate in 10 mL distilled water) and volume were made up to 25 mL with distilled water. pH was adjusted with sodium hydroxide solution.
- 5 M sodium hydroxide: 5 g of NaOH pellets dissolved in distilled water and volume was made up to 25 mL with distilled water.
- M Iodine solution (in Ethanol): 1 g of potassium iodide + 0.65 g of iodine dissolved in ethanol and volume was made up to 25 mL.
- Sodium thiosulphate solution: 0.625 g Na2SO3 in 2.5 mL H_2O + 22.5 mL 5 M NaOH
- 10 M Acetic acid: 14.25 mL of glacial acetic acid dissolved in distilled water and made up to 25 mL.

Procedure

To 1 mL of aqueous phase, 0.25 mL 0.4 M HCl and 0.5 mL of Sodium acetate buffer (pH 6. 9) were added followed by 0.5 mL iodine solution (0.1 M in ethanol) for oxidation. The reaction was stopped after 2 min by the addition of 0.5 mL Na_2SO_3 solution. 0.5 mL Acetic acid was added after 1.5 min. The solution was then heated to 100°C for 6 min. When the sample reached room temperature, excitation and emission spectra were read from the spectrofluorimeter. The readings were taken at 330-375 nm for dopamine. Blanks for the assay were prepared by adding the reagents of the oxidation step in reversed order (sodium sulphite before iodine). Different concentration of dopamine and nor-adrenaline (1 mg/ml) was used as standard. [18]

Estimation of Serotonin

The serotonin content was estimated by the OPT method

Reagents

O-phthaldialdehyde (OPT) reagent: (20 mg in 100 ml conc. HCl)

Procedure

To 1.4 mL aqueous extract, 1.75 mL of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at 360-470 nm in the spectrofluorimeter. Concentrated HCI without OPT was taken as blank. Serotonin (1 mg/mL) at different concentration was used as standard. [19]

Estimation of brain GABA content

Preparation of tissue homogenate

Animals were sacrificed by decapitation and the whole brain was rapidly removed. 0.5 g tissue was weighed and placed in 5 mL of ice-cold TCA (10% w/v). The tissue was then homogenized and centrifuged at 10,000 rpm for 10 min at 0°C. The supernatant was used for estimation of GABA content.

Reagents:

- Carbonate-bicarbonate buffer, 0.5 M (pH 9.95): 1.0501 g sodium bicarbonate and 1.3249 g sodium carbonate dissolved in distilled water and made up to 25 ml. pH adjusted to 9.95 if necessary.
- 0.14 M ninhydrin solution: 499 mg ninhydrin dissolved in 0.5 M carbonate-bicarbonate buffer and made up to 20 ml.
- Copper tartarate reagent: 0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid.

Procedure:

0.1 mL of tissue homogenate was placed in 0.2 mL of 0.14 M ninhydrin solution in 0.5 M carbonate-bicarbonate buffer (pH 9.95), and kept in a water bath at 60°C for 30 min. It was then cooled and treated with 5 mL of copper tartarate reagent. After 10 min fluorescence at 377/455 nm in a spectofluorimeter was recorded. [20]

STATISTICAL ANALYSIS

The statistical analysis was carried out by using PRISM version 5 software. The data's of all parameters were analysed by means of one way ANOVA followed by Dunnett's test. The results were expressed as mean ±SEM.

REFERENCES

1. Mattson, M. P., & Magnus, T. (2006). Ageing and neuronal vulnerability. Nature Reviews Neuroscience, 7(4), 278-294.
2. Dirnagl, U., Iadecola, C., & Moskowitz, M. A. (1999). Pathobiology of ischaemic stroke: an integrated view. Trends in Neurosciences, 22(9), 391-397.
3. Lalkovičová, M., & Danielisová, V. (2016). Neuroprotection - Possibilities, Expectations and Limitations. Interdisciplinary Toxicology, 9(2), 90-99.
4. Sahu, R., & Saxena, J. (2014). Bacopa monnieri and neuroprotection: An overview. Journal of Natural Remedies, 14(1), 11–21.
5. Scuderi, C., & Steardo, L. (2013). Neuroglia in neurodegenerative diseases: From pathogenesis to therapy. Frontiers in Pharmacology, 4, 137.
6. Butterfield, D. A., & Halliwell, B. (2019). Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. Nature Reviews Neuroscience, 20(3), 148–160.
7. Singh, M., Arseneault, M., Sanderson, T., Murthy, V., & Ramassamy, C. (2008). Challenges for research on polyphenols from foods in Alzheimer's disease: Bioavailability, metabolism and cellular and molecular mechanisms. Journal of Agricultural and Food Chemistry, 56(13), 4855-4873.
8. Bisht, K., Wagner, K. H., & Bulmer, A. C. (2010). Curcumin, resveratrol and flavonoids as anti-inflammatory, cyto- and DNA-protective dietary compounds. Toxicology, 278(1), 88-100.
9. A.S. Rabelo, I.D. Oliveira, A.G. Guimaraes, J.S.S Quintans, A.P.N. Prata , D.P. Gelain, Antinociceptive, anti-inflammatory and antioxidant activities of aqueousextract from Remireamaritima (Cyperaceae), Journal of ethnopharmacology, 145 (2013): 11-17.
10. Wang C, Shang J.H, Xiao H, Dong F.W, Cycloartane triterpenoid saponins from water soluble of passiflora edulis sims and their antidepressant like effects, Journal of ethnopharmacology, 148 (2013): 812-817.
11. Rajon orantes J.C, Perdomo suarez D.P, Rejon-Rodringnez a, Hemandez SH, aqueous root extract from mimosa albidahumb and bonpl.ex willed display antinociceptive activity in mice, Journal of ethnopharmacology, 149 (2013): 522-526.
12. Annemieke Aartsma-Rus and Maaike van Putten, Assessing Functional Performance in the Mdx Mouse Model, J Vis Exp. 2014; (85): 51303.
13. Pabba Sindhu, Mukta N. Chowta, Nishith RS, Seema Rai, Akash Gadgade, Rajeshwari S, Evaluation of Antidepressant Activity of Bacopa Monnieri in Mice, Int J Pharm 2014; 4(3): 144-148.
14. Dauer, W., & Przedborski, S. (2003). Parkinson's disease: Mechanisms and models. *Neuron*, 39(6), 889-909.
15. Dilip Kumar Pal, Determination of Brain Biogenic Amines in CynodonDactylon Pers. and Cyperus Rotundus L. Treated Mice, International Journal Of Pharmacy And Pharmaceutical Sciences, 2009, 1(1):190-197.
16. Raju TR, Kutty BM, Sathyaprabha TN, Shankarnarayana Rao BS. Brain and behavior. National Institute of Mental Health and neurosciences, Bangalore. 2004. Pp. 134-138.
17. EllmanG.L, Fiches F.T. Quantitative determination of peptides by sulfhydryl groups. *BiochemBiophys*. 1959; 82: 70-72.
18. King J. The phosphohydrolases-acid and alkaline phosphatases, *PracticalClinical Enzymology*, 1st ed. London; 1965: 191-208.
19. Lowry O.H, Rosebrough N.J, Farr A.N. Protein measurement with the folin phenol reagent. J Bio Chem, 1951; 193:265-275.
20. Riachi, N.J., Harik, S.I., 1988. Strain differences in systemic 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity in mice correlate best with monoamine oxidase activity at the blood-brain barrier. Life Sci. 42, 2359–2363.