Neuroprotective Effect of Ethanolic Extract of *Portulaca quadrifida* L. in Rotenone-Induced Locomotor Impairment in *Drosophila* Model and Haloperidol-Induced Catalepsy Rat Model

SWATI DHANDE* AND V. PATIL[1]

Department of Pharmacology, Bharati Vidyapeeth College of Pharmacy, Belapur, Navi Mumbai 400614, [1]Department of Pharmacognosy, Tapi Valley Education Society's Hon'ble Loksevak Madhukarrao Chaudhari College of Pharmacy, Faizpur, Jalgaon, Maharashtra 425503, India

The neurodegenerative diseases are characterized by the depletion of neuronal physiology and death as a virtue of potential oxidative stress. Parkinson's disease is a one of the debilitating neurodegenerative disease wherein the depletion of dopaminergic neurons results mainly in motor dysfunction. The present study was focused to study the neuroprotective effect of ethanolic extract of *Portulaca quadrifida* L. (Portulacaceae) against Parkinson's like symptoms. The experimental designs included were rotenone-induced locomotor impairment in *Drosophila* model and haloperidol-induced catalepsy in rat model; both resembling the motor dysfunction observed clinically in Parkinson's disease. The behavioral parameters were evaluated. Additionally the biochemical parameters of oxidative stress markers were evaluated in haloperidol-induced catalepsy in rat model. The locomotor behavior of rotenone-exposed *Drosophila* was improved significantly ($p<0.05$) when treated with 1 and 2 mg/ml doses of ethanolic extract of *Portulaca quadrifida*. The behavioral response of the catalepsy induced experimental rats (disease control group) was significantly ($p<0.05$) lower than that of vehicle control and standard control group. The co-exposed rats with haloperidol showed marked improvement at doses 100 and 200 mg/kg body weight of ethanolic extract of *Portulaca quadrifida*. The significant improvement in biochemical parameters further confirmed the antioxidant efficacy of ethanolic extract of *Portulaca quadrifida*. The standard drug used was Zandu Zandopa powder. Thus the study reveals that the ethanolic extract of *Portulaca quadrifida* imparts neuroprotective effect against motor dysfunction and hence has the potential to be used in the treatment of Parkinson's disease.

Key words: Parkinson disease, *Portulaca quadrifida*, haloperidol, catalepsy, *Drosophila*, rotenone

Neurodegenerative Disorders (NDDs) are characterized by a progressive loss of neuron structure or function which is often associated with neuron death. The selective loss of a particular subset of neurons is a common feature of NDDs. The cause of NDDs has not yet fully understood; however increased oxidative stress has been suggested as one of the potential common etiology in various neurodegenerative diseases. Cumulative oxidative stress may induce oxidative Deoxyribonucleic Acid (DNA) damage, mitochondrial dysfunction, excitotoxicity and alteration in antioxidant enzymes activities. These are the known key factors in acceleration of aging process and the development of NDDs like Parkinson's and Alzheimer's disease[1-3].

Neuroprotection is the preservation of the structure and function of neurons from insults arising from cellular injuries induced by a variety of agents or neurodegenerative diseases. The several study models are implemented with the aim of understanding their underlying mechanisms and identifying new therapeutic strategies. The paramountcy of the plants as medicine has gained popular therapeutic diversity. These plants

***Address for correspondence**
E-mail: dswatir@gmail.com

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms

Accepted 11 May 2022
Revised 22 November 2021
Received 24 March 2021
Indian J Pharm Sci 2022;84(3):791-796

constitute the backbone of many traditional medicine systems. They are enriched with many different types of phytoconstituents which have a wide array of pharmacological activities[4]. The herbal medicines are considered as time tested and has relatively safer for both human use and environment friendly and hence are used as sources of many lead compounds. They are also economic, easily available and affordable[1]. Therefore, there is need to look inwards to search for herbal medicinal plants with the aim of validating the ethno medicinal use and subsequently an isolation and characterization of compounds which will be added to the potential lists of drugs. One such plant selected for the study is *Portulaca quadrifida* L. (Portulacaceae). It is a small, succulent annual, mat-forming species[5]. The authors have reported the antioxidant effect of this plant in healthy rats[6]. Thus the ethanolic extract of whole plant will be explored, studied and evaluated for neuroprotective efficacy in the management of neurodegenerative disease in *Drosophila* and rat model. Rotenone and 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) were procured from Sigma-Aldrich (USA). Haloperidol (Inj. Serenace) manufactured by RPG Life Sciences and Zandu Zandopa powder manufactured by Zandu Pharmaceutical Works Ltd. Dimethyl sulfoxide, disodium hydrogen phosphate, methyl hydroxybenzoate, orthophosphoric acid and propionic acid were obtained from S.D. Fine Chem Ltd. *Drosophila melanogaster* wild-type, Canton special strain was procured from the Indian Institute of Science and Education Research, Pune, India. The flies were housed and propagated in glass bottles under 12:12 light-dark cycle at room temperature. The flies were fed with growth medium containing maize flour, glucose, orthophosphoric acid, methyl hydroxybenzoate, propionic acid and yeast granules. Albino Sprague Dawley male rats were procured from Mumbai Veterinary College, Mumbai. The rats were housed in the animal house under 12:12 light-dark cycle at 23°±2°. The animals were fed with standard rat pellets and water was provided *ad libitum*. The study was approved by the Institutional animal ethics committee (protocol number BVCP/IAEC/03/2019) and carried out as per the guidelines set by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The whole plant of *Portulaca quadrifida* L. family Portulacaceae was collected from the local market of Jalgaon. The plant material was authenticated by Dr. Rajendra Shinde, Blatter Herbarium, St. Xavier's College, Mumbai 400001 with herbarium specimen number 16926 dated 6/8/2018. The shade dried plant material was reduced to coarse powder manually. The dried powder was extracted with 95 % ethanol by the Soxhlet extraction method under reflux at 50° for 24 h. After completion of the extraction process the filtrate was concentrated using a Rotavac evaporator to obtain a dry mass of Ethanolic Extract of *Portulaca quadrifida* L (EEPQ). The percent yield obtained was 7.63 % w/w. The 80 adult *Drosophila* of 7 d old were divided into 5 groups of 16 flies per group. All the experiments were performed during the light cycle. Group I served as a control, group II as disease control, group III as low dose EEPQ (1 mg/ml); group IV as high dose EEPQ (2 mg/ml) and group V as a standard drug (2 mg/ml). The 500 µmol of rotenone was added into the culture media of all groups except group I. Similarly, the treatment was given by adding the respective drugs to each group culture media. The locomotor behavior of flies was evaluated after 1 w of treatment by negative geotaxis assay. Briefly, the flies were transferred into 2 cm wide and 12 cm long flat bottom test tube. The test tube was gently tapped so that flies settle down at the base of the test tube. The climbing activity of flies was monitored for 60 s. The locomotor behavior was expressed as a percentage number of flies escaping 10 cm distance within 60 s[7,8]. The 40 rats weighing between 120-150 g were segregated into 5 groups with 8 rats per group. Group I was vehicle control; group II disease control; group III low dose EEPQ (100 mg/kg of body weight); group IV high dose EEPQ (200 mg/kg of body weight) and group V standard Zandopa (775 mg/kg of body weight). The treatment drugs were suspended in 1 % Carboxymethyl Cellulose (CMC) solution and administered daily by per oral route for 15 d. Group I received 1 % CMC solution. Animals from all groups except group I were challenged with haloperidol (1 mg/kg of body weight) by intraperitonial route after 30 m of treatment for 15 d. Similarly, the group I animals were administered with sterile water for injection by intraperitonial route[9]. The behavioral parameters were evaluated at different time interval on d 1, 7, 13 using Dolphin's rotarod apparatus[10] and on d 3, 9, 15

using Dolphin's digital actophotometer (activity cage)[11]. The time intervals were 30 m, 60 m, 120 m and 180 m after haloperidol challenge. On d 15 after behavioral parameter evaluation, the rats were sacrificed by exposing them to carbon dioxide overdose to isolate the brain. The brain homogenate was further subjected to biochemical parameter evaluation i.e. reduced Glutathione (GSH), Catalase (CAT) and Malondialdehyde (MDA) as oxidative stress markers[10]. The obtained data was subjected to one-way Analysis of Variance (ANOVA) statistical analysis followed by Tukey's multiple comparisons test with $p<0.05$ as level of significance. The ROT-induced locomotor deficits in *Drosophila melanogaster* simulate the dopaminergic neurodegeneration caused specifically by the oxidative stress[12] while in haloperidol-induced catalepsy in rat resembles the deficiency of dopamine in nigrostriatal pathway that give rise to catalepsy[13]. *Drosophila melanogaster* shares homology with the human gene hence commonly used as study model in medicine and biomedical sciences. In recent years the fruit fly model has been developed for behavior studies[14,15]. The rotenone is a pesticide and herbicide commonly used to induce locomotor impairment mimicking Parkinson's disease symptom[16,17]. Rotenone being lipid soluble rapidly crosses the blood brain barrier and accumulates in subcellular organelles like mitochondria. It leads to inhibition of the mitochondrial enzyme Complex I *viz*. nicotinamide adenine dinucleotide dehydrogenase and hence increases the oxidative stress[12]. This oxidative stress causes damage of the dopaminergic neurons resulting in locomotor impairment. In flies the locomotor deficit is reflected by impairment of flying ability[18]. Approximately 60 % of the flies from the disease group i.e., rotenone exposed group were unable to climb and escape the 10 cm distance. Thus, the flies remained at the bottom depicting induction of locomotor impairment as compared to the control group flies. The flies from group treated with low and high dose of EEPQ showed significant ($p<0.05$) dose dependent improvement in the locomotor activity as shown in Table 1. The standard Zandopa treated group showed significant ($p<0.05$) improvement in locomotor activity which was similar to the normal locomotor activity in the control group flies. This model signifies that EEPQ exerts neuroprotective activity against rotenone-induced locomotor deficit. The intraperitoneal administrations of haloperidol in rats manifest the catalepsy i.e., Parkinson's like symptom. These symptoms include mainly the muscle rigidity and reduced locomotor activity[13]; which was evaluated as behavioral parameters using rotarod and actophotometer. The vehicle group values are depicting the basal values of muscle tone and locomotor activity in rats. The disease group i.e. haloperidol challenged animals showed significant ($p<0.05$) reduction in rotarod and locomotor activity when compared to control group animals and depicting the successful induction of catalepsy in rats after haloperidol challenge. The rotarod activity and locomotor activity of animals receiving only haloperidol challenge showed significant reduction ($p<0.05$) in activity when compared with the animals in vehicle group. The rotarod activity was performed to assess the muscle coordination and balancing ability in rats while on rotating rod and was evaluated in terms of latency of fall[19]. The latency of fall for animals treated with EEPQ at different doses and standard Zandopa in comparison to disease group was represented in Table 2. The rotarod activity of both EEPQ treated on d 1 and d 7 of treatment while Zandopa on d 1 was found to be non-significant. The spontaneous locomotor activity was assessed using actophotometer[20]. The motor activity in EEPQ treated and standard zandopa treated was represented in Table 3. The effect of EEPQ and Zandopa was not significant on locomotor activity on d 1 and on d 7 up to 1 h of treatment. The effectiveness of EEPQ at doses 100 and 200 mg/kg was found to be same and comparable to standard zandopa on d 13 of treatment in both rotarod and locomotor activity. In living organisms, the Reactive Oxidative Stress (ROS) can form in different ways, including normal aerobic respiration, stimulated polymorphonuclear leucocytes, macrophages and peroxisomes. These appear to be the main endogenous sources of most of the oxidants produced by the cells[21,22]. The neurons are vulnerable to oxidative damage as a result of ROS. The ROS formation manifests the neuronal death which is the major concern in neurodegenerative diseases. The antioxidant ability of particular bioactive can serve as a useful tool in providing the neuroprotection in ROS induced neuronal damage[23,24]. The endogenous anti-oxidants like GSH and CAT are beneficial

components that fight with free radicals and neutralize them before they can attack the cells and hence prevent damage to cell proteins, lipids and carbohydrates[25]. The GSH and CAT levels were found to be decreased in brain homogenate of disease group animal in catalepsy model as shown in Table 4. The unsaturated lipids present in cells interact with ROS which leads to toxic peroxide ions formation[26]. Lipid peroxidation is known to occur in a variety of pathological conditions including neurodegenerative disease. The MDA levels are indicative of lipid peroxidation; which was found to be increased in brain homogenate of disease group animal in haloperidol induced catalepsy model (Table 4). It is revealed that the group treated with both doses of EEPQ showed good antioxidant activity which was comparable to that of Zandopa group. Thus, from the behavioral observations made in *Drosophila* fly model and muscle coordination, spontaneous locomotor activity and biochemical GSH and CAT levels in rat brain homogenate catalepsy model; it can be concluded that the neuroprotective effect is exerted by the EEPQ though not dose dependent at doses 100 mg/kg and 200 mg/kg but equivalent to the standard marketed formulation i.e., Zandopa. The EEPQ can also be studied at higher dose to verify if the neuroprotective effect is dose dependent. The huge scope lies in exploring the bioactive present in EEPQ which can be responsible for the neuroprotective activity.

TABLE 1: EFFECT OF EEPQ ON LOCOMOTOR ACTIVITY USING NEGATIVE GEOTAXIS ASSAY IN ROTENONE-INDUCED LOCOMOTOR DEFICIT FRUITFLY MODEL

Treatment groups	Total number of flies escaped 10 cm distance	Percent locomotor behavior (%)
Group I (Vehicle control)	15.17±0.40*	94.79±2.5*
Group II (Disease control)	6.33±0.33	39.58±2.08
Group III (Low dose EEPQ)	10.17±0.48*	63.54±2.98*
Group IV (High dose EEPQ)	12.67±0.49*	79.17±3.09*
Group V (Standard Zandopa)	14.00±0.26*	87.50±1.61*

Note: Zandopa is marketed churna of Mucuna pruriens. All values are expressed as mean±SEM of experiment performed in sextuplate, (*) indicates significant difference ($p<0.05$) when compared with group II i.e. disease control by one-way ANOVA followed by Tukey's multiple comparisons test

TABLE 2: EFFECT OF EEPQ ON MUSCLE COORDINATION USING THE ROTAROD TEST IN HALOPERIDOL-INDUCED CATALEPSY RAT MODEL

Time (m)	Treatment groups				
	Group I Vehicle control	Group II Disease control	Group III Low dose EEPQ	Group IV High dose EEPQ	Group V Standard Zandopa
Activity time (s) d 7					
30	48.25±4.93*	16.75±1.80	20.63±3.35	22.50±2.36	21.88±2.42
60	57.13±6.56*	22.13±5.31	27.75±4.03	23.38±1.63	43.88±5.86*
120	58.63±8.22*	25.00±3.99	29.25±3.76	27.13±1.88	39.00±4.26*
180	60.00±8.33*	27.75±5.63	29.63±5.72	40.50±7.25*	44.87±7.18*
Activity time (s) d 13					
30	54.63±6.47*	14.75±3.62	25.12±5.49*	26.75±2.30*	22.75±2.45*
60	55.87±5.31*	19.63±3.92	32.37±4.33*	34.62±2.54*	35.75±7.05*
120	65.25±8.52*	18.12±3.57	33.25±5.25*	37.88±3.75*	31.75±4.01*
180	66.50±8.48*	18.25±3.64	34.75±7.00*	31.87±4.37*	32.50±4.48*

Note: Zandopa is marketed churna of Mucuna pruriens. All values are expressed as mean±SEM with n=8, where n is the number of animals per group, (*) indicates significant difference in activity time ($p<0.05$) when compared with disease control by one-way ANOVA followed by Tukey's multiple comparisons test. Time indicates recording interval after haloperidol challenge

TABLE 3: EFFECT OF EEPQ ON SPONTANEOUS LOCOMOTOR ACTIVITY USING ACTOPHOTOMETER IN HALOPERIDOL-INDUCED CATALEPSY RAT MODEL

Time (m)	Treatment groups				
	Group I Vehicle control	Group II Disease control	Group III Low dose EEPQ	Group IV High dose EEPQ	Group V Standard Zandopa
Activity time (s) day 9					
30	203.50±8.72*	26.50±6.14	28.13±5.02	19.00±1.72	17.88±2.26
60	204.25±10.72*	24.13±4.82	31.38±5.27	22.00±3.14	31.63±3.27
120	210.75±12.06*	26.50±2.85	48.00±4.53*	32.25±5.86	41.38±6.56*
180	184.88±11.61*	27.75±3.30	55.50±6.30*	47.13±8.04*	71.25±8.17*
Activity time (s) day 15					
30	215.00±13.53*	20.13±2.75	47.75±6.49*	35.75±4.57	60.25±6.31*
60	239.63±14.39*	20.13±1.68	55.25±6.84*	57.63±5.01*	67.75±5.45*
120	211.75±16.61*	29.00±4.73	86.25±5.48*	86.63±3.60*	85.38±9.01*
180	221.38±12.61*	24.62±4.23	99.25±8.95*	100.50±8.02*	85.13±8.35*

Note: Zandopa is marketed churna of Mucuna pruriens. All values are expressed as mean±SEM with n=8, where n is the number of animals per group, (*) indicates significant difference in activity time ($p<0.05$) when compared with disease control by one-way ANOVA followed by Tukey's multiple comparisons test. Time indicates recording interval after haloperidol challenge

TABLE 4: PROTECTIVE EFFECT OF EEPQ AGAINST OXIDATIVE STRESS IN HALOPERIDOL-INDUCED CATALEPSY RAT MODEL

Treatment groups	GSH (ng/mg of protein)	CAT (µmol of peroxide used/ min/mg of protein)	MDA (ng/mg of protein)
Group I (Vehicle control)	3.10±0.17*	3.04±0.34*	2.37±0.18*
Group II (Disease control)	2.02±0.10	1.17±0.09	4.04±0.59
Group III (Low dose EEPQ)	2.97±0.05*	3.32±0.32*	2.24±0.13*
Group IV (High dose EEPQ)	3.13±0.09*	3.08±0.33*	0.84±0.01*
Group V (Standard Zandopa)	3.68±0.13*	2.89±0.32*	0.74±0.02*

Note: Zandopa is marketed churna of Mucuna pruriens, All values are expressed as mean±SEM with n=8, where n is the number of animals per group, (*) indicates significant difference in activity time ($p<0.05$) when compared with disease control by one-way ANOVA followed by Tukey's multiple comparisons test

Acknowledgments:

Authors would like to acknowledge to Indian Institute of Science and Education Research, Pune, for providing *Drosophila* culture. We would like to extend our thanks to Dr. Vilasrao Kadam, for his support in completion of this project.

Conflict of interests:

The authors declared no conflicts of interest.

REFERENCES

1. Neurological disorders: Public health challenges. Switzerland: World Health Organization; 2006.
2. Ayala A, Venero JL, Cano J, Machado A. Mitochondrial toxins and neurodegenerative diseases. Front Biosci 2007;12(3):986-1007.
3. Salim S. Oxidative stress and the central nervous system. J Pharmacol Exp Ther 2017;360(1):201-5.
4. Shakya AK. Medicinal plants: Future source of new drugs. Int J Herb Med 2016;4(4):59-64.
5. Dhande SR, Patil VR. An overview of Protulaca quadrifida. Int J Veg Sci 2020;26(5):450-6.
6. Shahwan M, Gacem AS. Neuroprotective antioxidant effect of *Portulaca quadrifida* Linn. leaves extract on immobilization stress-induced changes in rat's brain. Asian J Pharm Clin Res 2018;11(10):378-81.
7. Khatri DK, Juvekar AR. Abrogation of locomotor impairment in a rotenone-induced *Drosophila melanogaster* and zebrafish model of Parkinson's disease by ellagic acid and curcumin. Int J Nutr Pharmacol Neurol Dis 2016;6(2):90-6.
8. Nichols CD, Becnel J, Pandey UB. Methods to assay *Drosophila* behavior. J Vis Exp 2012;7(61):e3795.
9. Bhangale JO, Acharya SR. Anti-Parkinson activity of petroleum ether extract of *Ficus religiosa* (L.) leaves. Adv Pharmacol Sci 2016;2016:1-9.
10. Adedeji HA, Ishola IO, Adeyemi OO. Novel action of metformin in the prevention of haloperidol-induced catalepsy in mice: Potential in the treatment of Parkinson's disease? Prog Neuropsychopharmacol Biol Psychiatry 2014;48:245-51.

11. Pany SU, Pal AB, Sahu PK. Neuroprotective effect of quercetin in neurotoxicity induced rats: Role of neuroinflammation in neurodegeneration. Asian J Pharm Clin Res 2014;7(4):152-6.
12. Perier C, Vila M. Mitochondrial biology and Parkinson's disease. Cold Spring Harb Perspect Med 2012;2(2):a009332.
13. Banasikowski TJ, Beninger RJ. Haloperidol conditioned catalepsy in rats: A possible role for D1-like receptors. Int J Neuropsychopharmacol 2012;15(10):1525-34.
14. Tolwinski NS. Introduction: *Drosophila*-A model system for developmental biology. J Dev Biol 2017;5(3):9.
15. van der Voet M, Nijhof B, Oortveld MA, Schenck A. *Drosophila* models of early onset cognitive disorders and their clinical applications. Neurosci Biobehav Rev 2014;46:326-42.
16. Zeng XS, Geng WS, Jia JJ. Neurotoxin-induced animal models of Parkinson disease: Pathogenic mechanism and assessment. ASN Neuro 2018;10:1-15.
17. Nisticò R, Mehdawy B, Piccirilli S, Mercuri N. Paraquat- and rotenone-induced models of Parkinson's disease. Int J Immunopathol Pharmacol 2011;24(2):313-22.
18. Aggarwal A, Reichert H, VijayRaghavan K. A locomotor assay reveals deficits in heterozygous Parkinson's disease model and proprioceptive mutants in adult *Drosophila*. Proc Natl Acad Sci USA 2019;116(49):24830-9.
19. Pritchett K, Mulder GB. The rotarod. J Am Assoc Lab Anim Sci 2003;42(6):49.
20. Pandian P. A study on analgesic, CNS depressant, muscle relaxant activity of marine gastropode species of. Int J Pharm Sci Res 2015;6(11):1397-400.
21. Gülçin İ, Büyükokuroğlu ME, Oktay M, Küfrevioğlu Öİ. Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn. subsp. *pallsiana* (Lamb.) Holmboe. J Ethnopharmacol 2003;86(1):51-8.
22. Yildirim A, Oktay M, Bilaloğlu V. The antioxidant activity of the leaves of *Cydonia vulgaris*. Turk J Med Sci 2001;31(1):23-7.
23. Salim S. Oxidative stress and the central nervous system. J Pharmacol Exp Ther 2017;360(1):201-5.
24. Elufioye TO, Berida TI, Habtemariam S. Plants-derived neuroprotective agents: Cutting the cycle of cell death through multiple mechanisms. Evid Based Complement Alternat Med 2017;2017:1-27.
25. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J Med 2018;54(4):287-93.
26. Yin H, Xu L, Porter NA. Free radical lipid peroxidation: Mechanisms and analysis. Chem Rev 2011;111(10):5944-72.