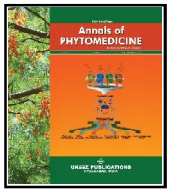
Annals of Phytomedicine 11(2): 318-325, 2022

DOI: http://dx.doi.org/10.54085/ap.2022.11.2.37

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Evaluation of anti-parkinsonian activity of *Pueraria tuberosa* (Roxb. ex Willd.) DC. on experimental animals

Nimisha Vasava, Vipulkumar Gajera◆, Vijay Lambole*, Tanvi Desai, Binal Patel and Ashok Akabari**
Department of Pharmacology, Shree N.L Patel College of Pharmacy, Umrakh-394601, Surat, Gujarat, India
**Department of Pharmacology, Datta Meghe College of Pharmacy, Wardha-442004, Maharashtra, India*
***Department of Quality Assurance, Shree N.L Patel College of Pharmacy, Umrakh-394601, Surat, Gujarat, India*

Article Info

Article history
Received 9 August 2022
Revised 26 September 2022
Accepted 27 September 2022
Published Online 30 December-2022

Keywords
Pueraria tuberosa (Roxb. ex Willd.) DC
Parkinson's disease
Antioxidant
Haloperidol
Neuroprotective

Abstract

Pueraria tuberosa (Roxb. ex Willd.) DC. possess neuroprotective and antioxidant property. The purpose of this study was to investigate anti-parkinsonian activity of *Pueraria tuberosa* (Roxb. ex Willd.) DC. on experimental animals. The haloperidol model was selected for screening anti-parkinsonian activity and haloperidol was administered at a dose of 1 mg/kg i.p., for 15 days. Ethanolic extract of *P. tuberosa* (EEPT) of 150 and 300 mg/kg and aqueous extract of *P. tuberosa* (AEPT) of 200 and 400 mg/kg and standard drug (levodopa 100 mg/kg + carbidopa 25 mg/kg) were orally administered for 15 days. In a haloperidol-induced model, behavioral and biochemical parameters like superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were evaluated. Locomotor activity, motor coordination and catalepsy were evaluated using the actophotometer, open field test, rotarod test, despair swim test, bar test and hang test.

In the haloperidol model, EEPT (150 and 300 mg/kg) and AEPT (200 and 400 mg/kg) increase the locomotor activity and motor coordination and decrease the cataleptic behaviour in the bar test and increase the hanging time in the hang test, which indicates the better cataleptic behaviour compared to the disease-induced group. The findings suggest that *P. tuberosa* exerted the anti-parkinsonian activity against haloperidol induced Parkinson's disease in rats *via* suppressing oxidative stress and apoptosis due to the presence of different bioactive compounds.

1. Introduction

Parkinsonism is portrayed by the degeneration of dopaminergic neurons in the substantia nigra and a deficiency of dopamine in the striatum. Rigidity, bradykinesia, tremors and postural instability can happen for various reasons; however, are generally idiopathic (Parkinson's illness or loss of motion agitans). Mental degradation happens in numerous patients as the disease advances (Dickson *et al*., 2012).

The degeneration of dopaminergic neurons in the substantia nigra standards compacta (SNcp) is a pathologic sign of PD (Sharma *et al*., 2021). Dopamine is a synapse liable for equilibrium, step and developments and degeneration of dopaminergic neurons from nigral projections prompts engine intricacies. A lack of dopamine can begin from unreasonable oxidative pressure, free extreme collection, natural poisons and hereditary transformations (Khatri *et al*., 2020).

In the flow situation, pharmacological and non-pharmacological medicines are utilized to treat Parkinson's disease. In pharmacological treatment, tranquilizes that influence the mind's dopaminergic framework and medications that influence the cerebrum's cholinergic framework are utilized to treat Parkinson's disease patients. Many people with Parkinson's disease take part in non-pharmacological treatment like activities or sports, either to forestall muscle firmness and development limitations or to work on their capacity to move (Sun *et al*., 2021; Mele *et al*., 2021).

P. tuberosa otherwise called Indian Kudzu (vidari kand), is a Fabaceae plant. It is a quickly developing, enormous enduring climber with huge tuberous roots and is dispersed all through India, Pakistan and Nepal. Lianas of *P. tuberosa* have likewise been found to develop at 4,000 feet in the Himalayan Mountain range. In Ayurveda, it is known as vidari (vidarikand), Indian kudzu in English, bhoikolu in Guajarati, vidarikanda in Hindi and bhumikusmanda in Sanskrit. Phytochemical investigation of the tuber separate uncovered the presence of various classes of phytoconstituents, including alkaloids, carbs, steroids, glycosides, tannins, terpenoids, flavonoids, coumarins and anthocyanidins (Sarmah *et al*., 2022). Late RPHPLC examination of the tuber separate was found to contain flavonoids; for example, puerarin, daidzein and genistein (Sekeroglu *et al*., 2019). The tuber likewise contains other flavonoid mixtures; for example, daidzin, genistin, puerarone, tuberosin, 4-methoxypuerarin, hydroxy-tuberosone, quercetin, biochanin A, biochanin B, irisolidon, tectoridin, robinin and glycoside. The tuber also contains, phytosterols (β-sitosterol, stigmasterol), natural acids (p-coumaric corrosive, arachidonic corrosive, eicosanoic corrosive, hexadecanoic

Corresponding author: Dr. Vipulkumar Gajera
Department of Pharmacology, Shree N.L Patel College of Pharmacy, Umrakh-394601, Surat, Gujarat, India.
E-mail: gajera15184@gmail.com
Tel.: +91-9909240815

corrosive, tetracosanoid corrosive). The tuber likewise contains lupinoside, anthocyanins and pterocarpintuberosin in a low amount (Bharti *et al*., 2021). *P. tuberosa* separates and its refined mixtures have different exercises; for example, antidiabetic, anti-inflammatory, antioxidant, antistress, cardioprotective, nephroprotective, nootropic, neuroprotective and wound healing (Bharti *et al*., 2021; Maji *et al*., 2014). Both extracts possess nootropic (Rao *et al.,* 2008), neuroprotective (Rani *et al.,* 2017) and antioxidant activity. Aqueous extract, has antioxidant and antiapoptotic effect (Shukla *et al.,* 2018) where has ethanolic extract has anti-inflammatory activity (Tripathi *et al*., 2013). Here, we have studied ethanolic and aqueous extracts of *P. tuberosa* for antiparkinsonian activity.

2. Materials and Methods

2.1 Plant material

P. tuberosa was locally procured from Samarghat, Dediyapada, Gujarat. And, it was authenticated by Dr. B. R. Patel, Associate Professor of Botany, The Patidar Gin Science College, Bardoli, Dist. Surat, Gujarat (Authen. 02/2022 Botany) on January 3rd, 2022.

2.2 Preparation of extract

2.2.1 Preparation of ethanolic extract

The fine powder of the dried tubers of *P. tuberosa* (50 g) was defatted by Soxhlet extraction using petroleum ether for 24 h and then the defatted mark was subjected to ethanolic extraction using 100 ml of 95% ethanol. The extract was concentrated by evaporating the solvent in a water bath at 60°C (Patel *et al*., 2016).

2.2.2 Preparation of aqueous extract

50 g of coarse powdered PT was boiled with five volumes of water. The volume was decreased to the 1/4 and separated. Sifted extricate was washed with hexane in a separating funnel. The watery part was gathered and concentrated by a rotatory evaporator. It is kept at 20°C until use (Shukla *et al*., 2018).

2.3 Drugs

Haloperidol (Inj. serenace; RPG Life Sciences Ltd, Ankleshwar, India), L-dopa plus carbidopa in 10:1 ratio (syndopa; Sun Pharmaceuticals, Mumbai, India) was obtained from respective sources. PT extract, l-dopa plus carbidopa was suspended in 0.5% w/v carboxy methyl cellulose (CMC) in distilled water and administered *via* oral route. The stock solution contained 100 mg/ml of PT. Haloperidol was obtained in an injectable form and diluted with water for injection I.P. Haloperidol was injected *via* i.p. route.

2.4 Experimental animals

Experiments were performed in accordance with the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). The experimental protocol in the study was approved by the Institutional Animal Ethical Committee (CPCSEA/SNLPCP/IAEC/22/01/130).

The Wistar albino rats, weighing 150-180 g, were obtained from the Jay Research Foundation, Vapi, Gujarat. The rats were housed at a temperature (25 ± 1°C) with 50 ± 55% relative humidity. Rats were fed a standard chow diet and water ad libitum. Animals were acclimatised in institutional animal houses and were exposed to a 12 h day and night cycle. LD_{50} of ethanolic and aqueous extract of *P. tuberosa* is 1500 mg/kg. and 2000 mg/kg, respectively. Here, the selected dose was 1/5 and 1/10 of LD_{50} (Rao *et al*., 2008).

2.5 Pharmacological evaluation for anti-parkinsonian activity

2.5.1 Experimental design

Male Wistar albino rats (150-180 g) were used. They were housed under standard conditions, maintained on a 12 h light/dark cycle, and had free access to food and water up to experimentation. The rats were acclimatised to the laboratory environment 1 h before the experiments. All experiments were conducted during the light period. The animals were divided into seven groups of six animals each.

Group 1: Normal Group (0.5% carboxy methyl cellulose)

Group 2: Model Control (Haloperidol 1 mg/kg) I.P.

Group 3: Standard Group (Levodopa 100mg/kg + Carbidopa 25mg/kg) Orally

Group 4: EEPT (150 mg/kg) Orally

Group 5: EEPT (300 mg/kg) Orally

Group 6: AEPT (200 mg/kg) Orally

Group 7: AEPT (400 mg/kg) Orally

The standard drug and test drug were given by oral route, followed by a haloperidol challenge (1 mg/kg; i.p.) to all groups except group I daily for 15 days of experimental period (Nagarjuna *et al.,* 2015).

2.6 Behavioural parameters

2.6.1 Locomotor activity

2.6.1.1 Actophotometer

This test estimates exploration and voluntary locomotion within an encased region. The goal an incentive for the spontaneous motor activity was gotten utilizing a photoactometer (INCO Ltd., India). The animals were set independently into a 30 cm × 30 cm dark metal chamber with a screen floor and a light close cover. Six light emissions light were engaged 2 cm over the floor into photocells on the contrary side. Each bar interference was enlisted as an occasion on the outside counter. The light bar breaks were counted for 5 min (Chaudhary *et al*., 2020).

2.6.1.2 Open field test

The open field device comprises of a major square region 76 × 76 with walls 42 cm high. The floor was separated into 25 equivalent squares. To decide action, a creature was set at the corner of a square in the open field and following the situation, the quantity of squares crossed was scored for 5 min.

2.6.2 Motor coordination

2.6.2.1 Rotarod test

The rotarod apparatus comprises of motor rod with a drum of 7.0 cm in diameter (Aarson, haryana, India). It was acclimated to a speed of 12 cycles each moment during the test meeting. The latency to fall in a test meeting of 180 s was taken as a proportion of engine coordination (Saleem *et al*., 2019).

2.6.2.2 Despair swim test

Every animal was brought into a pool (45 cm long; 22 cm wide, and 20 cm high) loaded up with 10 cm of water. The creatures were permitted to make rotations. The number of revolutions made per 3 min was recorded.

2.6.3 Cataleptic behavior

2.6.3.1 Bar test

Catalepsy, characterized as a diminished capacity to start development and an inability to address unusual stance, was estimated through the bar test. To test for catalepsy, animals were positioned so that their hindquarters were on the bench, and their forelimbs laid on a 1 cm width level bar, 6-9 cm over the seat. The period of time that an animal kept up with this position was recorded by stopwatch to a limit of 180 s (mean of three continuous preliminaries; interval: 1 min). Animals would be determined to be cataleptic if they maintained this position for 30 seconds or more (Prasad *et al*., 2020).

2.6.3.2 Hang test

This test is utilized to assess the skeleton's muscle strength, tone and perseverance as an element of time. The contraption is comprised of 1.5 mm in measurement treated steel wire that is 10 cm and 45 cm long or more the level surface of the device, separately the rodents are permitted to hang by the wire midway between the backings through their lower arms and the hanging time was recorded, which is corresponding to the rats' global muscular strength (Hegab *et al*., 2020).

2.7 Biochemical parameters

The brain tissue was homogenised with a 1:10 ratio in phosphate buffer (7.4 pH) and centrifuged at 600 rpm at 4°C for 10 min. The resultant clear supernatant was used for estimation of the following biochemical parameters of oxidative stress like superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH).

2.7.1 Assay of superoxide dismutase (SOD)

SOD activity was estimated spectrophotometrically by incubating 0.5 ml of brain homogenate with 0.1 ml of NADH for 90 s. In a similar arrangement, 0.5 ml, 10% acetic acid and 4 ml of butanol were added. The colour change was estimated at 520 nm by gathering the upper layer of butanol. SOD activity was expressed in µg per mg of tissue (Patel *et al*., 2020; Manju *et al*., 2020).

2.7.2 Assay of glutathione peroxidase (GSH)

The supernatant (1 ml) was precipitated with 10% of TCA (1 ml) and its aliquot was additionally blended in with phosphate solution (4 ml) and 0.5 ml of DTNB reagent. The solution was additionally utilized for spectroscopic investigation at 412 nm to determine the GSH content (Saleem *et al*., 2011).

2.7.3 Assay of catalase (CAT)

CAT activity was assessed using the method of Luck and Bergmeyer. Here, hydrogen peroxide breakdown was assessed. In 0.2 ml of brain homogenate, added 3 ml of H_2O_2 phosphate buffer, absorbance was quickly recorded at 240 nm. CAT was communicated in µg per mg of tissue (Patel *et al*., 2020).

2.7.4 Dopamine assay

2.7.4.1 Preparation of tissue extract

A weighed quantity of tissue was homogenized in 3 ml of HCl butanol in a cool climate. The sample was centrifuged for 10 min at 2000 rpm, then, at that point, 0.8 ml of supernatant phase was taken out and added to an Eppendorf reagent tube containing 2 ml of heptanes and 0.25 ml of 0.1M HCl. After 10 min, the tube was shaken and centrifuged under same circumstances to isolate the two phases. The upper organic phase was disposed of, and the watery phase was utilized for the dopamine assay (DA) (Shastry *et al*., 2020).

2.7.4.2 Assay procedure

To 0.02 ml of the HCl phase, 0.005 ml, 0.4 ml HCl and 0.01 ml of EDTA sodium acetate buffer (pH 6.9) were added, trailed by 0.01 ml of iodine solution for oxidation. The response was stopped after 2 min by the addition of 0.1 ml of sodium thiosulfate in 5 M sodium hydroxide. 1.5 min later, 10 M acetic acid was added. The solution was then heated to 100°C for 6 min. At the point when the samples again arrived at room temperature, excitation and emanation spectra were read (330-375 nm) in a spectrofluorimeter. Then, the tissue values (fluorescence of tissue extract-fluorescence of tissue blank) were compared and an inner reagent standard (fluorescence of inward standard-fluorescence of inside reagent clear). Tissue blanks for the assay were prepared by adding the reagents of the oxidation step backward request (sodium thiosulfate before iodine). Inner reagent guidelines were acquired by adding 0.005 ml of twofold distilled water and 0.1 ml of HCl butanol to 20 ng of dopamine standard. It is expressed in pg/mg protein (Nagarjuna *et al*., 2015).

2.8 Statistical analysis

The data were all expressed as mean standard error of the mean. Statistical significance between more than two groups was tested using one-way ANOVA, followed by Dunnett's multiple comparison tests. Using the computer-based fitting programme Prism Graph Pad version 8.0.2, compared all the column tests. Statistical significance was set accordingly.

3. Results

3.1 Locomotor activity

Table 1: Effect of EEPT and AEPT on locomotor activity using actophotometer

Groups	Cut off No. (5 min)	
	Day 7	Day 13
Normal	569.167 ± 9.826	562.333 ± 9.003
Haloperidol (1 mg/kg)	388.833 ± 13.151#	348.500 ± 4.847#
Syndopa 125 (Levodopa+Carbidopa)	509.500 ± 6.284**	518.167 ± 4.956**
EEPT (150) mg/kg	363.167 ± 6.400**	406.167 ± 4.996**
EEPT (300) mg/kg	397.667 ± 9.416**	414.833 ± 8.207**
AEPT (200) mg/kg	364.167 ± 6.735**	404.167 ± 5.076*
AEPT (400) mg/kg	363.667 ± 7.033**	411.833 ± 8.010**

Values are mean ± SEM; Statistical analysis by One-way ANOVA, followed by Dunnett's Multiple Comparison test. The signs (**) and (*) indicate values significantly different from control at $p<0.05$ (significant), respectively. # Indicates significant difference from normal control. * Indicates significant difference from model control.

Table 2: Effect of EEPT and AEPT on locomotor activity using open field test

Groups	Number of square crossed (5 min)	
	Day 7	Day 13
Normal	144.333 ± 4.226	145.667 ± 4.412
Haloperidol (1 mg/kg)	52.1667 ± 4.070#	50.333 ± 3.559#
Syndopa 125 (Levodopa+Carbidopa)	136.833 ± 6.524**	138.833 ± 7.111**
EEPT (150) mg/kg	63.000 ± 3.949**	64.833 ± 3.544**
EEPT (300) mg/kg	83.000 ± 4.857**	85.500 ± 5.991**
AEPT (200) mg/kg	59.833 ± 6.585*	61.666 ± 6.683*
AEPT (400) mg/kg	75.500 ± 6.284**	77.500 ± 6.655**

Values are mean ± SEM; Statistical analysis by One-way ANOVA, followed by Dunnett's Multiple Comparison test. The signs (**) and (*) indicate values significantly different from control at $p<0.05$ (significant), respectively. # Indicates significant difference from normal control. * Indicates significant difference from model control.

3.2 Motor coordination

Table 3: Effect of EEPT and AEPT on motor coordination using rotarod test

Groups	Fall of time (Sec)/3 min	
	Day 8	Day 14
Normal	67.000 ± 3.033	69.666 ± 4.589
Haloperidol (1mg/kg)	36.000 ± 2.190#	32.166 ±2.714#
Syndopa125 (Levodopa+Carbidopa)	63.000 ± 3.577**	64.833 ± 3.710**
EEPT (150) mg/kg	44.500 ± 3.728**	47.000 ± 4.427**
EEPT (300) mg/kg	53.500 ± 2.880**	55.833 ± 5.269**
AEPT (200) mg/kg	42.000 ± 2.449**	44.500 ± 3.391**
AEPT (400) mg/kg	47.166 ± 5.115**	50.500 ± 5.319**

Values are mean ± SEM; Statistical analysis by One-way ANOVA, followed by Dunnett's Multiple Comparison test. The signs (**) and (*) indicate values significantly different from control at $p<0.05$ (significant), respectively. # Indicates significant difference from normal control. * Indicates significant difference from model control.

Table 4: Effect of EEPT and AEPT on motor coordination using despair swim test

Groups	Number of rotations/3 min	
	Day 8	Day 14
Normal	7.000 ± 0.632	8.000 ± 0.632
Haloperidol (1mg/kg)	3.166 ± 0.408#	2.650 ± 0.547#
Syndopa 125 (Levodopa+Carbidopa)	6.666 ± 0.516**	7.500 ± 0.547**
EEPT (150) mg/kg	4.166 ± 0.408*	4.666 ± 0.516*
EEPT (300) mg/kg	6.000 ± 0.632**	6.500 ± 0.547**
AEPT (200) mg/kg	4.500 ± 0.547**	4.666 ± 0.516*
AEPT (400) mg/kg	5.000 ± 0.632**	5.500 ± 1.048**

Values are mean ± SEM; Statistical analysis by One-way ANOVA, followed by Dunnett's Multiple Comparison test. The signs (**) and (*) indicate values significantly different from control at $p<0.05$ (significant), respectively. # Indicates significant difference from normal control. * Indicates significant difference from model control.

3.3 Cataleptic behavior

Table 5: Effect of EEPT and AEPT on cataleptic behavior using bar test

Groups	Number of Sec/3 min	
	Day 9	Day 15
Normal	7.951 ± 0.672	8.281 ± 0.593
Haloperidol (1mg/kg)	134.003 ± 3.678#	138.785 ± 2.753#
Syndopa 125 (Levodopa+Carbidopa)	65.748 ± 3.319**	63.581 ± 2.812**
EEPT (150) mg/kg	85.168 ± 3.183**	82.213 ± 2.086**
EEPT (300) mg/kg	75.246 ± 3.091**	72.580 ± 3.125**
AEPT (200) mg/kg	123.342 ± 2.503**	120.860 ± 2.003**
AEPT (400) mg/kg	95.485 ± 2.882**	91.51 ± 3.475**

Values are mean ± SEM; Statistical analysis by One-way ANOVA, followed by Dunnett's Multiple Comparison test. The signs (**) and (*) indicate values significantly different from control at $p<0.05$ (significant), respectively. # Indicates significant difference from normal control. * Indicates significant difference from model control.

Table 6: Effect of EEPT and AEPT on cataleptic behavior (neuromuscular strength) using Hang test

Groups	Number of Sec/3 min	
	Day 9	Day 15
Normal	25.273 ± 1.556	26.516 ± 1.004
Haloperidol (1 mg/kg)	8.475 ± 0.564#	7.141 ± 0.613#
Syndopa 125 (Levodopa+Carbidopa)	22.885 ± 1.912**	23.551 ± 2.684**
EEPT (150) mg/kg	12.375 ± 1.437*	12.875 ± 1.741*
EEPT (300) mg/kg	14.733 ± 4.328**	15.863 ± 4.830**
AEPT (200) mg/kg	12.505 ±1.281*	12.838 ± 1.867*
AEPT (400) mg/kg	13.173 ± 3.400**	13.673 ± 3.806*

Values are mean ± SEM; Statistical analysis by One-way ANOVA, followed by Dunnett's Multiple Comparison test. The signs (**) and (*) indicate values significantly different from control at $p<0.05$ (significant), respectively. # Indicates significant difference from normal control. * Indicates significant difference from model control.

3.4 Antioxidant parameters

Brain tissue homogenate for the estimation of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH).

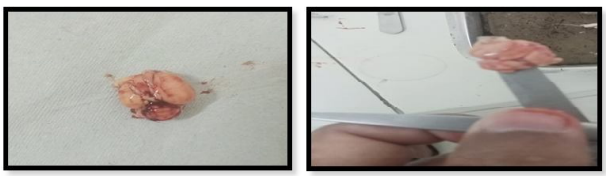


Figure 1: Brain isolated from wistar albino rats.

Table 7: Effect of EEPT and AEPT on SOD, CAT and GSH

Groups	SOD (µg/mgof brain tissue)	CAT (µg/mg of brain tissue)	GSH (µg/mg of brain tissue)
Normal	21.333 ± 0.276	15.435 ± 0.269	40.840 ± 0.399
Haloperidol (1 mg/kg)	14.260 ± 0.474#	4.256 ± 0.293#	20.863 ± 0.528#
Syndopa 125 (Levodopa+Carbidopa)	20.320 ± 0.494**	11.695 ± 0.464**	36.736 ± 0.324**
EEPT (150 mg/kg)	17.441 ± 0.185**	7.145 ± 0.219**	26.611 ± 0.213**
EEPT (300 mg/kg)	19.670 ± 0.242**	9.516 ± 0.464**	34.025 ± 0.647**
AEPT (200 mg/kg)	15.786 ± 0.971*	5.843 ± 0.298*	24.095 ± 0.322*
AEPT (400 mg/kg)	18.455 ± 0.176**	7.911 ± 0.123**	29.545 ± 0.303**

Values are mean ± SEM; Statistical analysis by One-way ANOVA, followed by Dunnett's Multiple Comparison test. The signs (**) and (*) indicate values significantly different from control at $p<0.05$ (significant), respectively. # Indicates significant difference from normal control. * Indicates significant difference from model control.

Table 8: Effect of EEPT and AEPT on DA levels in brain tissue homogenate

Groups	Day 15
Normal	613 ± 23
Haloperidol (1 mg/kg)	219.12 ± 18.2#
Syndopa 125 (Levodopa+Carbidopa)	523.55 ± 12.64**
EEPT (150) mg/kg	445.86 ± 14.30*
EEPT (300) mg/kg	452.87 ± 11.71**
AEPT (200) mg/kg	413.20 ±23.80*
AEPT (400) mg/kg	432.83 ± 18.67*

Values are mean ± SEM; Statistical analysis by One-way ANOVA, followed by Dunnett's Multiple Comparison test. The signs (**) and (*) indicate values significantly different from control at $p<0.05$ (significant), respectively. # Indicates significant difference from normal control. * Indicates significant difference from model control.

4. Discussion

Parkinson's disease (PD) is a constant age-related neurodegenerative problem and it is characterized by α-synuclein aggregation and death of dopaminergic neurons characterised chiefly by motor dysfunctions including resting tremors, muscular rigidity, bradykinesia and postural reflex impairments (Saleem *et al*., 2019; Olsen *et al*., 2021).

The degeneration of dopaminergic neurons in the substantia nigra standards compacta (SNcp) is a pathologic sign of PD. Dopamine is a synapse liable for equilibrium, step and developments and degeneration of dopaminergic neurons from nigral projections prompts engine confusions. A lack of dopamine can begin from exorbitant oxidative pressure, free extreme collection, natural poisons and hereditary changes. Transformations of the qualities like Parkin (PARK2), PINK1 (PARK6) and DJ-1 (PARK7) can cause Parkinson's disease. Responsive oxygen species (ROS) like hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxyl free extremist (Å"OH) and peroxynitrite (ONO_2^-) are delivered in the mitochondria as a result of cell breath. It has been imagined that expanded degrees of ROS add to the etiology of PD. Natural poisons like rotenone, 6-OHDA (6-hydroxydopamine) and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) follow up on the mitochondrial complex and hinder ATP age, which at last causes neurodegeneration. Drug-induced Parkinsonism (DIP) is the second-most-normal etiology of Parkinsonism in the older, after Parkinson's illness. Numerous patients with DIP might be misdiagnosed with PD on the grounds that the clinical highlights of these two circumstances are unclear (Khatri *et al*., 2020).

Over the years, different pharmacological models have been employed to evaluate the Parkinsonism and anti-parkinsonian activity of herbal plants. Animal models of Parkinson's disease (PD) have been proven to be highly effective in the discovery of novel treatments for motor symptoms of PD and in the search for clues to the underlying cause of the illness. Models based on specific pathogenic mechanisms may subsequently lead to the development of neuroprotective agents for PD that stop or slow disease progression. Various *in vivo* and *in vitro* models have been used in various research to date in PD (Salari *et al*., 2019). Haloperidol Induced Parkinson's Disease and Chlorpromazine Induced Parkinson's Disease, 6-OHDA model, Rotenone model, tremorine and oxotremorine antagonism, MPTP model, reserpine antagonism, circling behaviour in nigrostriatal lesioned rats, elevated body swing test, skilled paw reaching in rats, stepping test in rats, *etc*., are the animal models which have been used in various research till date (Duty *et al*., 2011)

In this research, haloperidol induced PD models are used to assess the behavioural parameters such as locomotor activity, muscle coordination and catalepsy. Brain homogenate is also carried out to investigate the antioxidant parameters such as SOD, CAT, and GSH.

It was used to induce Parkinsonism with haloperidol, which is an antipsychotic class of drug. Neuroleptics are widely utilized in the treatment of schizophrenia and other affective disorders. Sadly, their utilization is frequently connected with upsetting after effects including Parkinsonism and tardive dyskinesia (Nair *et al*., 2008). Neuroleptic-prompted catalepsy has for some time been utilized as a model for the extrapyramidal side effects (EPS); for example, parkinsonian like bradykinesia, related with antipsychotic use in people. Proof demonstrates that haloperidol prompts catalepsy in rats and this conduct reaction has for some time been utilized as a model for EPS impacts. Other than dopamine receptor bar and catecholamine consumption, other neurochemical speculations have been proposed for the advancement of catalepsy; for example, striatonigral GABAergic, cholinergic, glutamate and serotonergic exhaustion and so on. The current review utilizes three behavioural parameters like locomotor movement, motor coordination and

catalepsy to evaluate haloperidol induced Parkinson's disease in rats. Locomotor action was assessed by utilizing an actophotometer and an open field test. Engine coordination was evaluated utilizing the rotarod test and the misery swim test. Cataleptic way of behaving was assessed by utilizing the bar test and the hang test (Nagarjuna *et al*., 2015).

The locomotor activity of the experimental rats was investigated on days 7th and 13th of the experimental duration, employing an actophotometer and an open field test. In the actophotometer, the cut off number was measured. The disease control haloperidol (1 mg/kg) group revealed a significant decrease in locomotor activity compared to the normal group. Animals that received the standard drug syndopa 125 (levodopa 100 + carbidopa 25 mg/kg) showed a significant increase in locomotor activity compared to the disease control group. On treatment with test drugs (EEPT 150-300 mg/kg and AEPT 200-400 mg/kg), the locomotor activity was increased significantly compared to the disease control group. test drug, EEPT 300 mg/kg, had the most significant effect.

In an open field test, the number of squares crossed was measured on the 7th and 13th days. The haloperidol (1 mg/kg) treated animal showed a significant decrease in the number of squares crossed compared to the normal group, resulting in lower locomotor activity. The squares crossed were recovered in the treatment groups. Animals that received the standard drug syndopa 125 (levodopa 100 + carbidopa 25 mg/kg) showed a significant increase in the number of squares crossed compared to the disease control group. Animals that received test drugs (EEPT 150-300 mg/kg and AEPT 200-400 mg/kg) showed a significant increase in the number of squares crossed compared to the disease control group. Test drug, EEPT 300 mg/kg, had the most significant effect.

Motor coordination was assessed using a rotarod test and a despair swim test on the 8th and 14th days. In the rotarod test, the fall of time was measured. The rats treated with haloperidol (1 mg/kg) were found to exhibit motor incoordination and imbalance, typically expressing the symptoms of Parkinson's. Animals that received haloperidol (1 mg/kg) showed a significant decrease in fall time compared to the normal group. Animals that received the standard drug syndopa 125 (levodopa 100 + carbidopa 25 mg/kg) showed a significant increase in fall time compared to the disease control group. Animals that received test drugs (EEPT 150-300 mg/kg and AEPT 200-400 mg/kg) showed a significant increase in fall time compared to the disease control group. Test drug, EEPT 300 mg/kg, had the most significant effect.

The despair swim test is a rat motor-function test. Haloperidol-induced swim disability was monitored in rats at doses of 1 mg/kg. In the despair swim test, the number of rotations is measured on the 8th and 14thday. Animals that received haloperidol (1 mg/kg) showed a significant decrease in the number of rotations compared to the normal group. Animals that received the standard drug syndopa 125 (levodopa 100 + carbidopa 25 mg/kg) showed a significant increase in the number of rotations compared to the disease control group. Animals that received test drugs (EEPT 150-300 mg/kg and AEPT 200-400 mg/kg) showed a significant increase in the number of rotations compared to the disease control group.

In rodents, systemic administration of haloperidol can induce catalepsy, a behavioural state of bradykinesia and rigidity in which the animal can not right remotely forced stances. Haloperidol-prompted catalepsy results from the blockage of D2 dopaminergic receptors in the nigrostriatal pathway (Waku *et al*., 2021).

Cataleptic behaviour was assessed using a bar test and a hang test on the 9th and 15th days. In the bar test, the haloperidol (1 mg/kg) group significantly increased their cataleptic score as compared to the normal group. Animals that received the standard drug syndopa 125 (levodopa 100 mg/kg + carbidopa 25 mg/kg) showed a significant decrease in catalepsy as compared to the disease control group. Animals treated with extracts (EEPT 150 and 300 mg/kg and AEPT 200 and 400 mg/kg) showed a significant decrease in catalepsy as compared to the disease control group. In which the test drug EEPT 300 mg/kg showed a more significant reduction in catalepsy.

In the hang test, hanging time was measured on the 9th and 15th days. This test is used to evaluate the skeleton's muscle strength, tone and endurance as a function of time. The rats treated with haloperidol (1 mg/kg) exhibited a sharp decrease in the hang time latency as compared to the normal group. The treatment groups were found to increase the hang time latency in a dose-dependent manner. When compared to the control group, animals given the standard drug syndopa 125 (levodopa 1 mg/kg + carbidopa 25 mg/kg) had a significant increase in hanging time. Animals treated with extracts (EEPT 150-300 mg/kg and AEPT 200-400 mg/kg) showed a significant increase in hanging time as compared to the disease control group. Rest drug, EEPT 300 mg/kg, caused the greatest increase in hanging time.

Toward the finish of 15 days of the exploratory period, the animals were sacrificed utilizing anesthesia and their brains were taken out for assessment of oxidative stress. Oxidative stress to dopamergic neurons of SNpc is believed to be one of the leading causes of neurodegeneration in PD. Antioxidants may play an important role in the prevention of PD and combat against oxidative stress induced progressive neurodegeneration by reactive oxygen species (Chandrashekhar *et al*., 2012). Disturbances in the physiologic upkeep of the redox potential in neurons interfere with several biological processes,, eventually prompting cell death. Proof has been created for oxidative and nitrative harm to enter cell parts in the PD substantia nigra. Various sources and mechanisms for the age of receptive oxygen species (ROS) are perceived, including the digestion of dopamine itself, mitochondrial dysfunction, iron, neuroinflammatory cells, calcium and maturing (Chang *et al*., 2020).

It has been demonstrated that the cataleptic effects of haloperidol are mediated by dopamine receptors of the striatal neurons. It was also reported that the administration of haloperidol provokes an oxidative stress in the brain tissue. Under normal conditions, decrease in the activities of SOD, GPx and CAT enzymes in the brain leads to the accumulation of oxidative free radicals resulting in degenerative effects (Chitra *et al*., 2017).The animals that received haloperidol (1 mg/kg) alone for 15 days showed a significant decrease in superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) when compared to the normal group. Animals that received the standard drug syndopa 125 (levodopa 100 mg/kg + carbidopa 25 mg/kg) showed a significant increase in SOD, CAT and GSH when compared to the disease control group. Animals treated with extracts (EEPT 150 and 300 mg/kg and AEPT 200 and 400 mg/kg) showed a significant increase in SOD, CAT and GSH when compared to the control group. The test drug EEPT 300 mg/kg produced the greatest increase in SOD, CAT and GSH.

Depletion of dopamine levels was the major hallmarkas well as a biomarker in the diagnosis of PD. Loss of dopaminergic neurons leads to decrease inthe levels of dopamine and its metabolites (Kosaraju *et al*., 2014). The increased levels of dopamine by the treatment with standard drug syndopa 125 (levodopa 100 mg/kg + carbidopa 25 mg/kg) along with haloperidol caused a significant increase in the dopamine when compared to the negative control group. The animals treated with (EEPT 150 and 300 mg/kg and AEPT 200 and 400 mg/kg) along with haloperidol caused a significant increase in the dopamine when compared to negative control group. In which test, drug EEPT 300 mg/kg has showed the more significant increase in dopamine.

Effect of EEPT 150 and 300 mg/kg and AEPT 200 and 400 mg/kg are showing promising results in haloperidol model due to the presence of phenolic and flavonoids (puerarin, genistein, daidzein, daidzin, genistin, tuberosin) which gives the antioxidant activity (Tripathi *et al.*, 2013). Alkaloids, carbohydrates, proteins, saponins, flavonoids (puerarin, daidzein, genistein), isoflavonoids, tannins, tri-terpinoids and phenolic which gives the neuroprotective activity (Rani *et al*., 2017). Antioxidant and neuroprotective effect act by inhibiting the oxidative stress.

5. Conclusion

The results of the present study conclusively showed that among the both the extract ethanolic extract has more significant effect compare to aqueous extract of *P. tuberosa* and the higher dose of EEPT (300 mg/kg) give the best results among all the test drugs. The anti-parkinsonian activity of *P. tuberosa* may be due to its antioxidant and neuroprotective activity. Thus, the result of the present study conclusively shows the anti-parkinsonian activity of *P. tuberosa* in a haloperidol-induced Parkinson's disease model in rats.

Acknowledgments

The authors wish to thank Professor (Dr) Biren Shah, Department of Pharmacognosy, Shree N. L. Patel College of Pharmacy, Umrakh, Bardoli, Surat.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Bharti, R.; Chopra, B.; Raut, S. and Khatri, N. (2021). Pueraria tuberosa: A review on traditional uses,pharmacology, and phytochemistry. Frontiers in Pharmacology, **11**:1-20. doi: 10.3389/fphar. 2020. 582506.

Chandrashekhar, V.; Avinash, S.; Sowmya, C.; Ramkishan, A. and Shalavadi, M. (2012). Neuroprotective activity of *Stereospermum suaveolens* DC against 6-OHDA induced Parkinson's disease model. Indian Journal of Pharmacology, **44**(6):737-45. doi: 10.4103/0253-7613.103275.

Chang, K. and Chen, C. (2020). The role of oxidative stress in parkinson's disease. Antioxidants, **9**(7):597-605. doi: 10.3390/antiox9070597.

Chaudhary, P. and Dhande, S. (2020). Evaluation of anti-parkinson's activity of ethanolic extract of tridax procumbens (asteraceae). Indian Journal of Natural Products and Resources, **11**(1):9-17. doi: 10.56042/ijnpr. v11i1.21641.

Chitra, V.; Manasa, K.; Mythili, A; Tamilanban, T. and Gayathri, K. (2017). Effect of hydroalcoholic extract of achyranthes aspera on haloperidol induced parkinson's disease in wistar rats. Asian Journal of Pharmaceutical and Clinical Research, **10**(9):318-332. doi: 10.22159/ajpcr. 2017.v10i9.19285.

Dickson, D. (2012). Parkinson's disease and parkinsonism: Neuropathology. Cold Spring Harbor Perspectives in Medicine, **2**(8):a009258-a009258. doi: 10.1101/cshperspect. a009258.

Duty, S. and Jenner, P. (2011). Animal models of parkinson's disease: A source of novel treatments and clues to the cause of the disease. British Journal of Pharmacology, **164**(4): 1357-1391. doi: 10.1111/j.1476-5381.2011. 01426.x.

Hegab, d.; Ahmed, O.; Mahmoud, M. and Abdelhamid, A. (2020). Behavioral evaluation of rotenonemodel of parkinson's disease in male wistar rats. Sohag Medical Journal, **24**(2):8-14. doi: 10.21608/smj.2020. 21596.1089.

Khatri, D.; Choudhary, M.; Sood, A. and Singh, S. (2020). Anxiety: An ignored aspect of parkinson's disease lacking attention. Biomedicine and Amp. Pharmacotherapy, **131**:1-13. doi: 10.1016/j.biopha. 2020. 110776.

Kosaraju, J.; Chinni, S.; Roy, P.; Kannan, E.; Antony, A. and Kumar, M. (2014). Neuroprotective effect of *Tinospora cordifolia* ethanol extract on 6-hydroxy dopamine induced Parkinsonism. Indian Journal of Pharmacology, **46**(2):176-182. doi: 10.4103/0253-7613.129312.

Maji, A.; Pandit, S.; Banerji, P. and Banerjee, D. (2014). *Pueraria tuberosa*: A review on its phytochemical and therapeutic potential. Natural Product Research, **28**(23):1-19. doi.org/10.1080/14786419.2014.928291.

Manju, P. and Pushpa, A. (2020). Phytochemical analysis and *in vitro* free radical scavenging activity of rhizome of *Zingiber officinale* rosc. Ann. Phytomed., **9**(2):257-262. doi: 10.21276/ap.2020. 9.2.24.

Mele, B.; Ismail, Z.; Goodarzi, Z.; Pringsheim, T.; Lew, G. and Holroyd Leduc, J. (2021). Non-pharmacologic interventions to treat apathy in parkinson's disease: a realist review. Clinical Parkinsonism and Amp. Related Disorders, **4**:1-11. doi: 10.1016/j.prdoa.2021.100096.

Nagarjuna, S.; Reddy, Y.; Arifullah, M.; Kumar, A.; Srinath, B. and Reddy, K. (2015). Evaluation of antioxidant and antiparkinsonian activities of *Brassica oleraceain* haloperidol-induced tardive dyskinesia. International Journal of Green Pharmacy, **9**(3):143-149. doi: 10.4103/0973-8258.161230.

Nair, V.; Arjuman, A.; Gopalakrishna, H. and Nandini, M. (2008). Effect of *Withania somnifera* root extract on haloperidol-induced catalepsy in albino mice. Phytotherapy Research, **22**(2):243-246. doi: 10.1002/ptr.2299.

Olsen, A. and Feany, M. (2021). Parkinson's disease risk genes act in glia to control neuronal α-synuclein toxicity. Neurobiology of Disease, **159**:105-482. doi: 10.1016/j.nbd.2021.105482.

Patel, C.; Pande, S. and Acharya, S. (2020). Potentiation of anti-alzheimer activity of curcumin by probiotic *Lactobacillus rhamnosus* ublr-58 against scopolamine-induced memory impairment in mice. Naunyn-Schmiedeberg's Archives of Pharmacology, **393**(10):1955-1962. doi: 10.1007/s00210-020-01904-3.

Patel, J.; Doshi, N.; Bhalerao, A. and Bonagiri R. (2016). Immunomodulatory activity of ethanolic extract of *Pueraria tuberosa* DC. IJSER, **7**(11):708-712.

Prasad, E. and Hung, S. (2020). Behavioral tests in neurotoxin-induced animal models of parkinson's disease. Antioxidants, **9**(10):1007. doi: 10.3390/antiox9101007.

Rani, V.; Sudhakar, M. and Ramesh, A. (2017). Protective effect of *Pueraria tuberosa* Linn. in arsenic induced nephrotoxicity in rats. Asian Journal of Pharmaceutical Research, **7**(1):15. doi: 10.5958/2231-5691.2017. 00003.x.

Rao, N.; Basavaraj, pujar.; Nimbal, S.; Shantakumar, M. and Satyanarayana, S. (2008). Nootropic activity of tuber extract of *Pueraria tuberosa* (Roxb). Indian Journal of Experimental Biology, **46**:591-598.

Salari, S., and Bagheri, M. (2019). *In vivo, in vitro* and pharmacologic models of parkinson's disease. Physiological Research, **68**(1):17-24. doi: 10.33549/physiolres.933895.

Saleem, U.; Raza, Z.; Anwar, F.; Chaudary, Z. and Ahmad, B. (2019). Systems pharmacology based approach to investigate the *in vivo* therapeutic efficacy of *Albizia lebbeck* (L.). In experimental model of parkinson's disease. BMC Complementary and Alternative Medicine, **19**(1):1-16. doi: 10.1186/s12906-019-2772-5.

Sarmah, B.; Shukla, P.; Kumar, M.; Sarma, S. and Sharma, R. (2022). Quantification of puerarin and daidzein and *in vitro* pharmacological evaluation of *Pueraria tuberosa* (Willd.) DC.: An ethnopharmacological approach. Ann. Phytomed., **11**(1):505-516. doi: 10.54085/ap.2022.11.1.58.

Sekeroglu, N. and Gezici, S. (2019). Original article astragalus neurocarpus bioss. As a potential source of natural enzyme inhibitor associated with alzheimer's and parkinson diseases along with its rich polyphenolic content and antioxidant activities. Ann. Phytomed., **8**(1):82-87. doi: 10.21276/ap.2019.8.1.9

Sharma, S.; Kochhar, K.; Jain, S.; Jayasundar, R. and Divya, M. (2021). Therapeutic potential of Indian traditional medicines in parkinson's disease. Ann. Phytomed., **10**(2):252-263. doi: 10.21276/ap.2021.10.2.34

Shastry, R.; Habbu, R.; Smita, P.; Chetan, D. and Kulkarni, S.(2020). Isolation, characterization and evaluation of endophytic fractions of *Centella asiatica* (L.) Urb. (leaves) for the management of alzheimer's disease. Ann. Phytomed., **9**(1):171-180. doi: 10.21276/ap.2020.9.1.22

Shukla, R.; Banerjee, S. and Tripathi, Y. (2018). Antioxidant and antiapoptotic effect of aqueous extract of *Pueraria tuberosa* (Roxb. ex Willd.) DC. On streptozotocin-induced diabetic nephropathy in rats. BMC Complementary and Alternative Medicine, **18**(1).1-11. doi: 10.1186/s12906-018-2221-x.

Sun, C. and Armstrong, M. (2021). Treatment of parkinson's disease with cognitive impairment: Current approaches and future directions. Behavioral Sciences, **11**(54):1-21. doi: 10.3390/bs11040054.

Tripathi, Y.; Pandey, N.; Yadav, D. and Pandey, V. (2013). Anti-inflammatory effect of *Pueraria tuberosa* extracts through improvement in activity of red blood cell antioxidant enzymes. AYU (An International Quarterly Journal of Research in Ayurveda), **34**(3):297. doi: 10.4103/0974-8520.123131.

Waku, I.; Magalhães, M.; Alves, C. and de Oliveira, A. (2021). Haloperidol induced catalepsy as an animal model for parkinsonism: A systematic review of experimental studies. European Journal of Neuroscience, **53**(11): 3743-3767. doi: 10.1111/ejn.15222.

Citation **Nimisha Vasava, Vipulkumar Gajera, Vijay Lambole, Tanvi Desai, Binal Patel and Ashok Akabari (2022). Evaluation of anti-parkinsonian activity of *Pueraria tuberosa* (Roxb. ex Willd.) DC. on experimental animals. Ann. Phytomed., 11(2):318-325. http://dx.doi.org/10.54085/ap.2022.11.2.37.**