IJBPAS, September, 2023, 12(9): 4136-4151

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EVALUATION OF ANTI-PARKINSONIAN ACTIVITY OF *CONVOLVULUS PLURICAULIS* ON EXPERIMENTAL ANIMALS

JOSHI H[1], GAJERA V[1*], LAMBOLE V[2], KATARIYA A[1] AND SHAH D[3]

1: Department of Pharmacology, Shree N.L Patel College of Pharmacy, Umrakh-394601, Surat, Gujarat, India

2: Department of Pharmacology, Datta Meghe College of Pharmacy, Wardha -442004, Maharashtra., India

3: Department of Pharmaceutics, C.K. Pithawalla Institute of Pharmaceutical Science and Research, Surat-395007, Gujarat, India

[*]**Corresponding Author: Dr. Vipulkumar Gajera: E Mail:** gajera15184@gmail.com

Received 25th June 2022; Revised 18th Aug. 2022; Accepted 11th Dec. 2022; Available online 1st Sept. 2023

https://doi.org/10.31032/IJBPAS/2023/12.9.7404

ABSTRACT

Parkinson's disease (PD) is a well-known neurodegenerative disorder characterized by dopaminergic (DA) neuron loss in the substantia nigra pars compacta. In the present study, we evaluated Anti-parkinsonian activity of *Convolvulus pluricaulis* (CP) *in* Oxotremorine (OXO) and Haloperidol induced PD in mice. PD was induced by administering OXO (0.5mg/kg S.C.) and haloperidol (2.0 mg/kg i.p.). In OXO induced model tremor, hypothermia and salivation were evaluated and behavior parameters such as locomotor activity, motor coordination and catalepsy were evaluated using actophotometer, open field test, rotarod test, horizontal bar test, bar test and hang test in haloperidol induced mice model. In OXO model Ethanol extract (EE) and aqueous extract (AE) of CP at the dose of 125 and 250 mg/kg. were significant decreases the tremor and salivation score and increases the temperature compare to disease induce group. Effect of EECP and AECP are showing promising results in OXO model. In haloperidol model EECP and AECP at the dose of 125 and 250 mg/kg significantly increases the locomotor

activity, motor coordination and decreases the cataleptic behavior in bar test and increases the hanging time in hang test which indicates the better cataleptic behavior compare to disease induce group. Effect of EECP and AECP are showing better results in haloperidol model due to the presence of alkaloids, flavonoids, tropane alkaloids and scopoletin. The data show that CP extract has significant anti-parkinsonian activity and suggest that these effects may be mediated via antioxidant and neuroprotective and anticholinergic activity.

Keywords: ***Convolvulus pluricaulis,*** **Parkinson's disease, Oxotremorine, Haloperidol, Anticholinergic, Antioxidant**

INTRODUCTION

Parkinson's disease (PD) is a chronic, progressive, neurodegenerative disease characterized by bradykinesia, rigidity and tremor with postural instability developing at a later stage **[1]**. It is the second most common neurodegenerative disease, after Alzheimer's disease **[2]**. The clinical presentation of PD represents a nexus of three major components: motor symptoms, non-motor symptoms, and symptoms related to autonomic nervous system failures. Motor symptoms such as bradykinesia, Rigidity (Inability to bent), Tremor (Involuntary shaking or movement) & Postural instability are observed in patient with PD. In Non-motor symptoms patient suffers from Anxiety, Depression, Insomnia and Cognitive impairment or dementia. Autonomic dysfunction like Orthostatic hypotension, Impaired gastrointestinal motility, Sexual dysfunction and sweating is observed **[3, 4]**.

Even though the exact cause of PD is not yet known, it is considered to be potentiated by the interaction of environmental and genetic factors. Mutations of the gene like Parkin (PARK2), PINK1 (PARK6) and DJ-1 (PARK7) can cause Parkinson's disease. Environmental toxins such as Rotenone, 6-OHDA and MPTP acts on the mitochondrial complex and inhibit ATP generation which ultimately causes neurodegeneration **[4]**. Drug-induced parkinsonism (DIP) is the second-most-common etiology of parkinsonism in the elderly after Parkinson's disease. Many patients with DIP may be misdiagnosed with PD because the clinical features of these two conditions are indistinguishable **[5]**. Oxidative stress interferes with dopamine metabolism leading to PD. This oxidative damage leads to formation of reactive oxygen species (ROS) leading to neuronal death **[6, 7]**.

C. *pluricaulis* is a perennial herb that seems like morning glory. It belongs to the Convolvulaceae family. This medicinal herb has been reported to contain many bioactive phytoconstituents, such as, alkaloid (convolamine), flavonoid (kaempferol) and phenolics (scopoletin, b-sitosterol and ceryl alcohol), that have been ascribed to the observed medicinal properties. According to the ancient literature, this herb has been attributed with several therapeutic properties, such as anxiolytic, neuroprotective, antioxidant, analgesic, immunomodulatory, antimicrobial, antidiabetic and cardioprotective activities. It is also scientifically proven that due to its neuropharmacological properties, this herb has potential to be helpful in treatment of nervine disorders **[8-10]**.

PD mostly demonstrates oxidative stress, neurotoxicity, neuronal-inflammation and neuronal cell death, and CP has Anti-oxidant **[9]**, neuroprotective **[10]**, anti-inflammatory **[8]**, anticholinergic [11] and anti-apoptotic **[10]** effects. However, no report is available for its anti-parkinsonian activity. Hence, an effort is made to investigate the anti-parkinsonian activity in experimental animal

MATERIALS AND METHODS

Animals

Swiss albino mice (20-25g) 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. Mice was acclimatized to laboratory environment 1 h before the experiments. All experiments were conducted during the light period.

Ethical Approval

The study protocol was approved by Institutional Animal Ethical Committee, meeting held on 6th February, 2021 resolution No. **CPCSEA/SNLPCP/IAEC/21/01/120** at Shree Naranjibhai Lalbhai Patel College of Pharmacy, Umrakh, Gujarat, India.

Collection and Authentification of Plant

CP was procured from the Biotic Nature Products, Kelapur, Maharashtra, India. CP was authenticated by DR.B. R. Patel, Associate Professor of Botany, The Patidar Gin Science College, Bardoli, Dist. Surat, Gujarat. (Authen. /01/2021 Botany) on date 11th of January 2021.

Materials

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. Haloperidol was obtained in an injectable

form and diluted with water for injection I.P. Haloperidol was injected via i.p. route. OXO was procured from Sigma Aldrich Vadodara, Gujarat,

Extraction Procedure

Preparation of EECP: About 1 kg dried coarse powder of CP was imbibed in ethanol (95%) for 24 h. This moistened drug was extracted with ethanol (95%) at 70°C using a Soxhlet Apparatus for 48 h. The EE was concentrated and stored in a refrigerator below 4ºC until further use (EE:15.7% w/w). When needed, the extract was suspended/ dissolved in desired solvent and use **[12]**.

Preparation of AECP: CP was triturated in a blender until a finely granulated powder was obtained. AE was made from 100g of this powder by adding distilled water and soaking it overnight. After filtration the extract was stored at 4°C for experimental use (AE:13.7% w/w) **[13]**.

Phytochemical Investigations

In the present study, phytochemical screening was carried out using standard procedures.[14][15][16]

Pharmacological evaluation for Anti-parkinsonian activity

In this research, Oxotremorine (OXO) induced PD & Haloperidol induced PD models were used to evaluate the behavioural parameters such as tremor, hypothermia, salivation, locomotor activity, muscle coordination & catalepsy. Doses were selected based on LD_{50} **[8]**. here, the selected dose was 1/5 and 1/10 of LD_{50}.

OXO induced Parkinson Disease

OXO is a direct-acting cholinergic agonist and produces a variety of cholinergic responses. Administration of OXO, a centrally active, nonselective muscarinic receptor agonist, is known to induce hypothermia, tremor, salivation, bradycardia, hypotension and intestinal hypermotility. In this study tremor, hypothermia & salivation were assessed.

Experimental Design: Swiss albino mice (20-25g) was 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. Mice was acclimatized to laboratory environment 1h before the experiments. All experiments were conducted during the light period. Animals were divided into 7 group of six animal each.

- Group 1: Normal Group
- Group 2: Model Control (OXO 0.5 mg/kg) S.C
- Group 3: Standard Group (Atropine mg/kg) I.P
- Group 4: EECP (125 mg/kg) Orally
- Group 5: EECP (250 mg/kg) Orally
- Group 6: AECP (125 mg/kg) Orally

- Group 7: AECP (250 mg/kg) Orally

Tremor: Tremor were scored independently 30 min after injection of each does of OXO on a scale of 0 (no tremor), 1 (occasional isolated twitches) and 2(nearly continuous whole-body tremor), 3 (continuous tremor). The data was expressed as percent of the maximum possible score.

Hypothermia: The temperature was measured using a rectal probe inserted to a depth of 1.5 cm. Temperature was measured after the oxotremorine 0.5 mg/kg administration.

Salivation: Salivation were scored independently 30 min after injection of each does of OXO on a scale of 0 = No salivation, 1 = moderate salivation - moisture on face only, 2 = marked salivation - moisture on face & chest [17][18][19][20].

Haloperidol induced Parkinson's Disease

Haloperidol which is antipsychotic class of drug, was used to induce the Parkinson like symptoms.

Experimental Design: Swiss albino mice (20-25g) was 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. Mice was acclimatized to laboratory environment 1 h before the experiments. All experiments were conducted during the light period. Animal was divided into 7 group of six animal each.

- Group 1: Normal Group
- Group 2: Model Control (Haloperidol 1 mg/kg) I.P.
- Group 3: Standard Group (Levodopa + Carbidopa 10 mg/kg) I.P
- Group 4: EECP (125 mg/kg) Orally
- Group 5: EECP (250 mg/kg) Orally
- Group 6: AECP (125 mg/kg) Orally
- Group 7: AECP (250 mg/kg) Orally

Locomotor Activity:

Actophotometer: This test measures the exploration and the voluntary locomotion within an enclosed area. The objective value for the spontaneous motor activity was obtained using a photo actometer (INCO Ltd., India). The animal was placed individually into a 30 cm × 30 cm black metal chamber with a screen floor and a light-tight lid. Six beams of red light were focused 2 cm above the floor into photocells on the opposite side. Each beam interruption was registered as an event on the external counter. The light beam breaks were counted for 5 min**.**

Open Field Test: The apparatus was made of 50 cm in length, 50 cm in width, and 25 cm in height. The plain floor of the box was divided into 8 cm by 8 cm, with 16 squares on it. Sixteen squares were defined as the center and the others adjacent to the walls as

the periphery. Each mouse was gently placed at the center of the open field and number of squares crossed was counted for 5 min. The mouse was taken out of the apparatus after 5 min and the floor was cleaned with ethanol. A mouse was deemed to have crossed over from one square to another when all four paws had crossed. The number of squares crossed within the observation period of 5 min. was recorded **[21]**.

Motor coordination

Rotarod Test: The rotarod apparatus consists of a motor rod with a drum of 7.0 cm diameter. It was adjusted to a speed of 12 revolutions/min during the test session. The latency to fall in a test session of 180 s will take as a measure of motor coordination.

Horizontal Bar Test: Hold the mouse by the tail; place it on the bench in front of the apparatus. Slide it quickly backwards about 20 cms, rapidly raise it and let it grasp the horizontal bar at the central point with its fore paws only and release the tail simultaneously starting the stop clock. The criterion point is either a fall from the bar before the mouse reaches one of the end columns of the bar, or the time till one forepaw touches a column Maximum cut off time is 30 seconds **[22, 23]**.

Cataleptic Behaviour

Bar Test: Catalepsy, defined as a reduced ability to initiate movement and a failure to correct abnormal posture, was measured by means of the bar test. To test of catalepsy, animals was positioned so that their hindquarters were on the bench, and their forelimbs rested on a 1 cm diameter horizontal bar, 6–9 cm above the bench. The length of time that animal maintained this position was recorded by stopwatch to a maximum of 180 s (mean of three consecutive trials; interval: 1 min). Animals would determine judge to be cataleptic if they maintained this position for 30 s or more.

Hang Test: Neuromuscular strength was determined in the grid hang test. Mice were lifted by their tail and slowly placed on a horizontal grid and supported until they grabbed the gird with both their fore and hind paws. The grid was then be inverted so that the mice were allowed to hang upside down. The grid was mounted 20 cm above a hard surface, to discourage falling but not leading to injury in case of animal fall. Start a stop clock and note the time when the mice fall off or remove it. When the criterion time of 30 seconds is reached **[24]**.

Biochemical Parameters

After completing behavioural parameters, on 7^{th} day brain homogenate was carried out to

investigate the antioxidant parameters such as SOD, CAT & GSH.

Statistical analysis: All the data were expressed as mean ± standard error of the mean. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Dunnett's multiple comparison test. Compare all columns test using the computer-based fitting program Prism Graph Pad version 5.0 Statistical significance was set accordingly.

RESULTS

On the basis of literature among different extracts, ethanolic and aqueous extracts of CP showed promising results in general behaviour models **[25]**.

Table 1: Effect of EECP &AECP on Tremor induced by OXO

Groups	10 Min	20 Min	30 Min
Normal	0	0	0
Oxotremorine (0.5 mg/kg)	3±0#	3±0#	3±0#
Atropine (1 mg/kg)	0.6667±0.3333**	0.3333±0.3333**	0.3333±0.3333**
EECP (125 mg/kg)	1.667±0.3333*	1.667±0.3333*	1.333±0.3333**
EECP (250 mg/kg)	0.6667±0.3333**	0.6667±0.3333**	0.3333±0.3333**
AECP (125 mg/kg)	1.667±0.3333*	1.667±0.3333*	1.333±0.3333**
AECP (250 mg/kg)	1.333±0.3333**	1±0**	0.6667±0.3333**

Scale of 0 = (no tremor), 1 = (occasional isolated twitches) and 2(nearly continuous whole-body tremor) and 3 = (continuous tremor).
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<0.01 (extremely significant) and P<0.05 (significant) respectively.
 # Indicates significant difference from normal control.
*** Indicates significant difference from model control.**

Table 2: Effcet of EECP & AECP on Temprature induced by OXO

Groups	Temperature (°c)
Normal	37.67±0.3333
Oxotremorine (0.5 mg/kg)	31.33±0.3333#
Atropine (1 mg/kg)	36.67±0.3333**
EECP (125 mg/kg)	33.33±0.3333**
EECP (250 mg/kg)	35.67±0.3333**
AECP (125 mg/kg)	33.33±0.3333**
AECP (250 mg/kg)	34.33±0.3333**

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<0.01 (extremely significant) and P<0.05 (significant) respectively.
 # Indicates significant difference from normal control.
*** Indicates significant difference from model control.**

Table 3: Effect of EECP & AECP on Salivation induced by OXO]

Groups	Salivation
Normal	0.000±0.000
Oxotremorine (0.5 mg/kg)	2.000±0#
Atropine (1 mg/kg)	0.000±0.0000**
EECP (125 mg/kg)	0.6667±0.3333**
EECP (250 mg/kg)	0.3333±0.3333**
AECP (125 mg/kg)	1.000±0.0000*
AECP (250 mg/kg)	0.6667±0.3333**

Scale of 0 = No salivation, 1 = moderate salivation - moisture on face only and 2 = marked salivation - moisture on face & chest.
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<0.01 (extremely significant) and P<0.05 (significant) respectively.
 # Indicates significant difference from normal control.
*** Indicates significant difference from model control.**

Table 4: Effect of EECP & AECP on Locomotor activity using Actophotometer

Groups	Cut off No. (5 min)		
	Day 1	Day 4	Day 7
Normal	583.3±6.009	573.3±2.333	573.3±2.333
Haloperidol (2 mg/kg)	345±18.93#	341±16.86#	339.7±15.81#
Syndopa 110 (Levodopa+Carbidopa)	525.7±15.72**	524±17.79**	513.7±16.95**
EECP (125 mg/kg)	425±11.55**	411.3±6.936**	405.3±10.65**
EECP (250 mg/kg)	495±4.163**	481.3±5.239**	476.3±3.712**
AECP (125 mg/kg)	405.7±5.207*	396±5.033*	385.7± 4.372*
AECP (250 mg/kg)	446.7±16.95**	442.7±9.387**	436.7±6.119**

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<0.01 (extremely significant) and P<0.05 (significant) respectively.
# Indicates significant difference from normal control.
* Indicates significant difference from model control.

Table 5: Effect of EECP & AECP on Locomotor activity using Open Field Test

Groups	Number of Square Crossed (5 min)		
	Day 1	Day 4	Day 7
Normal	304.3±14.33	308.3±13.64	318±11.72
Haloperidol (2 mg/kg)	147.7±4.3333#	143.3±4.91#	136±5.859#
Syndopa 110 (Levodopa+Carbidopa)	269±10.69**	265.3±5.548**	261.3±4.667**
EECP (125 mg/kg)	190.7±3.48**	187±3.055**	183.7±2.404**
EECP (250 mg/kg)	241±13.58**	237.3±11.02**	230.3±9.062**
AECP (125 mg/kg)	199±2.646*	180±2.517*	173.3±2.333**
AECP (250 mg/kg)	211±2.309**	206±2.646**	200±3.606**

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<0.01 (extremely significant) and P<0.05 (significant) respectively.
# Indicates significant difference from normal control.
* Indicates significant difference from model control.

Table 6: Effect of EECP & AECP on Motor Coordination using Rotarod Test

Groups	Fall of Time (Sec.)		
	Day 1	Day 4	Day 7
Normal	67±3.606	71.33±4.807	73.33±4.177
Haloperidol (2 mg/kg)	33.33±0.8819#	29±0.5774#	26±1.528#
Syndopa 110 (Levodopa+Carbidopa)	59.67±2.728**	56±3.055**	55.33±2.404**
EECP (125 mg/kg)	46±0.5774**	42.67±1.202**	39.67±0.8819**
EECP (250 mg/kg)	54±1.528**	52.33±1.453**	49.33±0.8819**
AECP (125 mg/kg)	43±1.528*	40.33±0.3333*	37.67±0.3333**
AECP (250 mg/kg)	47.67±1.453**	46.67±0.8819**	43.33±0.8819**

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<0.01 (extremely significant) and P<0.05 (significant) respectively.
# Indicates significant difference from normal control.
* Indicates significant difference from model control.

Table 7: Effect of EECP & AECP on Motor Coordination using Horizontal Bar Test

Groups	Fall of Time (Sec.)		
	Day 1	Day 4	Day 7
Normal	25.33±0.8819	25.67±0.8819	27±0.5774
Haloperidol (2 mg/kg)	7.333±1.202#	7±1.155#	5.667±0.6667#
Syndopa 110 (Levodopa+Carbidopa)	22.33±0.8819**	19.67±0.8819	18.33±0.8819**
EECP (125 mg/kg)	13.67±1.453**	12.33±0.8819	12.33±0.6667**
EECP (250 mg/kg)	18±0.5774**	17.33±0.3333	16.33±0.6667**
AECP (125 mg/kg)	11.67±0.6667*	10.33±0.3333*	9.667±0.3333**
AECP (250 mg/kg)	13.33±0.8819**	12.67±0.6667	11±0.5774**

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<0.01 (extremely significant) and P<0.05 (significant) respectively.
# Indicates significant difference from normal control; * Indicates significant difference from model control.

Table 8: Effect of EECP & AECP on Cataleptic Behavior using Bar Test

Groups	Number of Seconds / 3 Min.		
	Day 1	Day 4	Day 7
Normal	8.333±0.3333	8.667±0.3333	8±0.5774
Haloperidol (2 mg/kg)	138.7±2.404#	143.7±2.963#	147.7±1.453#
Syndopa 110 (Levodopa+Carbidopa)	69±1.155**	66.33±1.856**	63.67±0.6667**
EECP (125 mg/kg)	89.33±3.844**	86.67±3.383**	82.67±3.383**
EECP (250 mg/kg)	73.33±1.202**	70±1.528**	59±1.155**
AECP (125 mg/kg)	130±0.5774**	127±1.528**	117.7±1.453**
AECP (250 mg/kg)	84.33±0.3333**	81.67±1.453**	80±3.215**

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<0.01 (extremely significant) and P<0.05 (significant) respectively.
 # Indicates significant difference from normal control.
*** Indicates significant difference from model control.**

Table 9: Effect of EECP & AECP on Cataleptic Behavior using Hang Test

Groups	Hanging Time (Sec.)		
	Day 1	Day 4	Day 7
Normal	24.67±1.202	26.67±0.8819	28.33±1.202
Haloperidol (2 mg/kg)	9.667±0.3333#	8.333±0.6667#	6.667±0.8819#
Syndopa 110 (Levodopa+Carbidopa)	23.33±0.8819**	21.67±1.202**	19.67±0.8819**
EECP (125 mg/kg)	14.67±0.3333**	12.67±0.3333*	12.33±0.3333**
EECP (250 mg/kg)	19.67±1.202**	17.67±1.202**	15.67±0.8819**
AECP (125 mg/kg)	13.67±0.3333*	12±0.5774*	11±0.5774**
AECP (250 mg/kg)	15.33±0.8819**	13.33±0.8819**	12.67±0.3333**

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<0.01 (extremely significant) and P<0.05 (significant) respectively.
 # Indicates significant difference from normal control.
*** Indicates significant difference from model control.**

Biochemical Parameters

Antioxidant Parameters: Brain tissue homogenate for the estimation of Superoxide dismutase (SOD), Catalase (CAT) and Reduced glutathione (GSH).

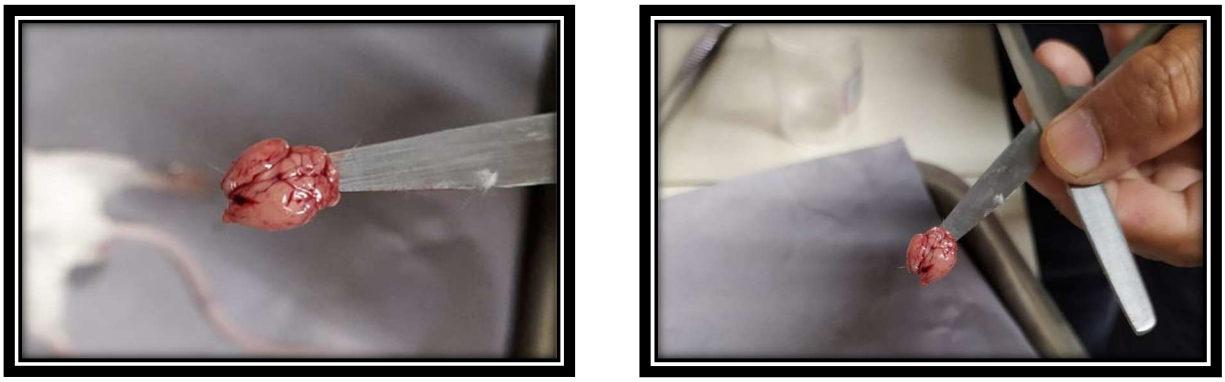

Figure 1: Brain isolated from Swiss Albino Mice

Table 10: Effect of EECP & AECP on SOD, CAT & GSH

Groups	SOD (U/mg of Protein)	CAT (U/mg of Protein)	GSH (µmol/mg of protein)
Normal	7.133±0.1856	3.833±0.08819	32±1.155
Haloperidol (2 mg/kg)	3.6±0.1#	1.3±0.1#	9.333±0.8819#
Syndopa 110 (Levodopa+Carbidopa)	6.7±0.1155**	3.433±0.1202**	29±0.5774**
EECP (125 mg/kg)	4.6±0.1**	2.633±0.1202**	21.67±0.3333**
EECP (250 mg/kg)	5.533±0.2028**	3.067±0.06667**	25.33±0.8819**
AECP (125 mg/kg)	4.167±0.03333*	1.667±0.03333*	13.67±0.8819*
AECP (250 mg/kg)	4.967±0.03333**	2.333±0.06667**	19.67±0.8819**

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<0.01 (extremely significant) and P<0.05 (significant) respectively.
 # Indicates significant difference from normal control; * Indicates significant difference from model control.

DISCUSSION

PD is the second most common progressive neurodegenerative disorder. It is characterized by degeneration of dopaminergic neurons in the substantia nigra and loss of dopamine in the striatum resulting in resting tremor, rigidity, bradykinesia and postural instability **[26]**. Various in vivo and in vitro models such as Tremorine and oxotremorine antagonism, MPTP model, Reserpine antagonism, circling behavior in nigrostriatal lesioned rats, Elevated body swing test, Skilled paw reaching in rats, stepping test in rats, Rotenone model, 6-OHDA model, Haloperidol Parkinson's Disease and Chlorpromazine Induced Parkinson's Disease etc. are used in various research till date **[27]**.

In this research, OXO induced PD & Haloperidol induced PD models are used to assess the behavioral parameters and biochemical parameters such as tremor, hypothermia, salivation, locomotor activity, muscle coordination & catalepsy and brain homogenate is also carried out to investigate the antioxidant parameters such as SOD, CAT & GSH.

The muscarinic agonist, oxotremorine, induces parkinsonism like signs such as tremor, salivation and hypothermia **[28]**. In which increased in the tremors & salivation was observed after the dose of OXO (0.5 mg/kg S.C.), to suppress it standard drug Atropine (1 mg/kg) S.C. is given, which succeed to suppress the tremors as well as salivation. EECP 125 mg/kg & 250 mg/kg and AECP 125 mg/kg & 250 mg/kg doses are given orally respectively. In which all extracts succeed to supress the tremors and salivation. While, EECP 250 mg/kg shows best result in both the parameters.

The temperature was measured using a rectal probe inserted to a depth of 1.5 cm. OXO was observed to produce hypothermia at the dose of 0.5 mg/kg S.C. To reverse the action of OXO, Atropine (1 mg/kg) S.C is given, which help to increase the temperature. EECP & AECP 125-250 mg/kg are given

orally to measure the effect on temperature. In which all extracts succeed to antagonize the action of OXO. While, EECP 250 mg/kg gives more promising result.

Effect of EECP and AECP 125-250 mg/kg are showing best results in OXO model due to the presence of flavonoids which gives anticholinergic activity **[11]**. Anticholinergic effect act by reducing the unbalanced cholinergic activity in the striatum.

Haloperidol which is antipsychotic class of drug, was used to induce parkinsonism. It was recognized that all typical antipsychotics drugs had the potential to cause parkinsonism. The present study uses three behavioural parameter such as locomotor activity, motor coordination and catalepsy to assess haloperidol induced PD in mice **[29]**.

Locomotor activity was assessed using actophotometer and open field test. In actophotometer cut off number was measured and number of squares crossed were measured in open field test. Animals received haloperidol (2 mg/kg) showed a significant decrease in cut off number and number of squares crossed on 1^{st},4^{th} and 7^{th} day compared to normal group. Animals received standard drug Syndopa 110 (Levodopa + Carbidopa 10 mg/kg) showed a significant increase in cut of number and number of squares crossed on 1^{st},4^{th} and 7^{th} day compared to disease control group. Animals received test drug (EECP & AECP 125-250 mg/kg) showed a significant increase in cut of number and number of squares crossed on 1^{st}, 4^{th} and 7^{th} day compared to disease control group. In which test drug EECP 250 mg/kg has showed the more significant increase in cut off number and number of squares crossed.

Motor Coordination was assessed using rotarod test and horizontal bar test, fall of time is measured in both. Animals received haloperidol (2 mg/kg) showed a significant decrease in fall of time on 1^{st},4^{th} and 7^{th} day compared to normal group. Animals received standard drug Syndopa 110 (Levodopa + Carbidopa 10 mg/kg) showed a significant increase in fall of time on 1^{st},4^{th} and 7^{th} day compared to disease control group. Animals received test drug (EECP & AECP 125-250 mg/kg) showed a significant increase in fall of time on 1^{st},4^{th} and 7^{th} day compared to disease control group. In which test drug EECP 250 mg/kg has showed the more significant increase in fall of time.

Cataleptic behavior was assessed using bar test and hang test, in bar test animals received haloperidol (2 mg/kg) significantly increases catalepsy which was seen on 1^{st}, 4^{th} and 7^{th} day as compared to the normal group. Animals received standard drug Syndopa 110

(Levodopa + Carbidopa 10 mg/kg) showed a significant decrease in catalepsy on 1st, 4th and 7th day as compared to the disease control group. Animals treated with extracts (EECP & AECP 125-250 mg/kg) showed a significant decrease in catalepsy on 1st, 4th and 7th day as compared to the disease control group. In which test drug EECP 250 mg/kg has showed the more significant decrease in catalepsy. In hang test hanging time was measured. Animals received haloperidol (2 mg/kg) significantly decreases hanging time which was seen on 1st, 4th and 7th day as compared to the normal group. In Animals received standard drug Syndopa 110 (Levodopa + Carbidopa 10 mg/kg) showed a significant increase in hanging time on 1st, 4th and 7th day as compared to the normal group. Animals treated with extracts (EECP & AECP 125-250 mg/kg) showed a significant increase in hanging time on 1st, 4th and 7th day as compared to the disease control group. In which test drug EECP 250 mg/kg has showed the more significant increase in hanging time.

At the end of 7 days of the experimental period, the animals were sacrificed, brains were taken out for assessment of oxidative stress. The animals received haloperidol (2 mg/kg) alone for 7 days showed a significant decrease in the Superoxide Dismutase (SOD), Catalase (CAT) and Reduced Glutathione (GSH) when compared to the normal group. Animals received standard drug Syndopa 110 (Levodopa + Carbidopa 10 mg/kg) showed a significant increase in the SOD, CAT and GSH when compared to the disease control group. Animals treated with extracts (EECP & AECP 125-250 mg/kg) showed a significant increase in the SOD, CAT and GSH when compared to normal group.

Effect of EECP and AECP 125-250 mg/kg are showing promising results in haloperidol model due to the presence of alkaloids (sankhpushpine, convolvine), flavonoids (Kaempferol, quercetin), tropane alkaloids (convolvine, convolaine) and scopoletin which gives antioxidant and neuroprotective activity. Antioxidant and neuroprotective effect act by inhibiting the oxidative stress.

This research revealed that EECP and AECP (125 and 250 mg/kg) possess anticholinergic, antioxidant and neuroprotective effect. In which higher dose of EECP gives the best result in both models. From which anticholinergic effect act by reducing the unbalanced cholinergic activity in the striatum. Antioxidant and neuroprotective effect act by inhibiting the oxidative stress. All these results thus predict that CP provide pharmacological rationale for the traditional

use of the plant against PD. Thus, the result of the present study conclusively shows the anti-parkinsonian activity of CP in OXO and haloperidol induced PD model in mice. However, further studies are necessary to evaluate the contribution of other substances that are isolated for the activity observed, because it still remains to be determined which components exactly were responsible for these effects.

CONCLUSION

From this study, aerial parts of C*onvolvulus pluricaulis possess* anti-parkinsonian activity in mice model. However, further investigations are required to isolate the phytoconstituents responsible for behavioral activity and to find their mechanism of action.

ACKNOWLEDGMENTS

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

Conflict of interest

There are no conflicts of interest.

REFERENCES

[1] Kumar A., Mishra K. Pharmacological evaluation of polyherbal suspension for anti-parkinsons activity in albino rat. IJPSR,11(8), (2020), 3848-3853

[2] Dag A., Lucia B., Glenda M., Gert J., Clive B., Parkinson disease-associated cognitive impairment. Nature Reviews Disease Primers, 7(1), (2021),1-21

[3] Váradi, C. Clinical Features of Parkinson's Disease: The Evolution of Critical Symptoms. Biology, 9(5), (2020), 103.

[4] Khatri D, Choudhary M, Sood A, Singh S. Anxiety: an ignored aspect of parkinson's disease lacking attention. Biomed Pharmacother. 131, (2020),1-13

[5] Blanchet, P., Kivenko, V. Drug-induced parkinsonism: diagnosis and management. Journal of parkinsonism and restless legs syndrome, 6, (2016), 83-91.

[6] Kizhakke P., Olakkaran S., Anet A., Tilagul K., Hunasanahally P. Convolvulus pluricaulis (shankhapushpi) ameliorates human microtubule-associated protein tau (hmap) induced neurotoxicity in alzheimer's disease drosophila model. Journal of Chemical Neuroanatomy. 95, (2019), 115-122.

[7] Rachitha, P., Krupashree, K., Jayashree, G., Kandikattu, H., Amruta, N., & Gopalan, N. Chemical

composition, antioxidant potential, macromolecule damage and neuroprotective activity of Convolvulus pluricaulis. Journal of Traditional and Complementary Medicine. 4(8), (2018), 483-496.

[8] Agarwa P., Sharma B., Fatima A., Jain, Sanjay. An update on ayurvedic herb convolvulus pluricaulis choisy. Asian Pac J Trop Biomed. 4(3), (2014), 245-252

[9] Kharoliwal S. A review on traditional indian herbs convolvulus pluricaulis linn and its medicinal importance. Int. J. Pure App. Biosci. 2 (6), (2014), 326-329

[10] Balkrishna A, Thakur P, Varshney A. Phytochemical profile, pharmacological attributes and medicinal properties of convolvulus prostratus - a cognitive enhancer herb for the management of neurodegenerative etiologies. Front Pharmacol. 11, (2020), 1-12

[11] Sharath S., Preethy J., Surendra kumar G., Revannaswamy A., Muralidhar P., Sujith R., Rohini K. Screening for anti-ulcer activity of convolvulus pluricaulis using pyloric ligation method in wister rats. International Journal of Pharma Sciences and Research. 6(1), (2015), 89-99.

[12] Bihaqi, S., Sharma, M., Singh, A., & Tiwari, M. Neuroprotective role of convolvulus pluricaulis on aluminium induced neurotoxicity in rat brain. Journal of Ethnopharmacology. 124, (2009), 409-415.

[13] Susan Duty, Peter Jenner. 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), (2011),1357-1391.

[14] Khandelwal K. Practical Pharmacognosy: Techniques and Experiments. Nirali Prakashan, 8th edition, (2001).

[15] Kokate C., Gokhale P.,. Text Book of Pharmacognosy. 4th ed, Pune: Nirali Prakashan; (1996). pp 8-32.

[16] Shah B., Nayak B. Experimental Pharmacognosy. S. Vikas & Co. Publishing House, Jalandhar, pp190-200.

[17] Vogel, H., Drug Discovery and Evaluation. 2nd ed., Springer-Verlag Berlin Heidelberg, New York, (2002) pp.577 to 585.

[18] Wang N., Orr-Urtreger A., Chapman J., Rabinowitz R., Korczyn A. Nicotinic acetylcholine receptor alpha5 subunits modulate oxotremorine-induced salivation and tremor. J. Neurol Sci. 222, (2004), 87-91.

[19] Fukuzaki, K., Kamenosono, T., Nagata, R. (2000). Effects of ropinirole on various parkinsonian models in mice, rats, and cynomolgus monkeys. Pharmacology, biochemistry, and behavior. 65, (2000), 503-508.

[20] Gerald, M., Skau, K., Maickel, R. Effects of various antihistamines on oxotremorine-induced hypothermia in mice. Eur J. Pharmacol. 17(1), (1972),189-193

[21] Zeenath B., Syeda J., Azra F., Sarwar F., Syeda F. Zohra, T. Phytochemical evaluation and pharmacological screening of antiparkinson's activity of allium sativum in swiss/albino mice. IOSR Journal of Pharmacy. 6(6), (2016), 1-12

[22] S. Nagarjuna, M. Arifullah, A. Sanjeeva Kumar, B. Srinath, K. Somasekhar Reddy, Y. Padmanabha Reddy. Evaluation of antioxidant and antiparkinsonian activities of Brassica oleracea in haloperidol-induced tardive dyskinesia. International Journal of Green Pharmacy. (2015), 143-149.

[23] Akindele, A., Sanni, H., & Edeh, P. Anxiolytic activity of aerial part hydroethanolic extract of Allium ascalonicum Linn. (Liliaceae) in mice. Functional foods in health and disease, 2(11), (2012), 448-459.

[24] Krishna K., Suma B., Shetty A., Nandini H., Prashanth B. evaluation of anti-parkinson's activity of novel glitazones on rats. International journal of pharmaceutical sciences review and research. 55(2), (2019), 64-69

[25] Nasir A. Siddiqui1, Nihal Ahmad2, Nazia Musthaq, Ipshita Chattopadhyaya, Rachna Kumria and Sumeet Gupta. Neuropharmacological profile of extracts of aerial parts of convolvulus pluricaulis choisy in mice model. The open neurology journals. 8, (2014), 11-14

[26] Liu, S., Li, X., Huo, Y., & Lu, F. Protective effect of extract of Acanthopanax senticosus harms on dopaminergic neurons in Parkinson's

disease mice. Phytomedicine, 19(7), (2012). 631-638.

[27] Kin, K., Yasuhara, T., Kameda, M., & Date, I. Animal Models for Parkinson's Disease Research: Trends in the 2000s. International journal of molecular sciences, 20(21), (2019), 5402.

[28] Gaur, V., Bodhankar, S., Mohan, V., & Thakurdesai, P. Neurobehavioral assessment of hydroalcoholic extract of Trigonella foenum-graecumseeds in rodent models of Parkinson's disease. Pharmaceutical Biology, 51(5), (2013). 550-557.

[29] Saeed, A., Shakir, L., Khan, M., Ali, A., Zaidi, A. Haloperidol induced Parkinson's disease mice model and motor-function modulation with Pyridine-3-carboxylic acid. Biomedical research and therapy, 4(05), (2017). 1305.