Indian Journal of Natural Products and Resources
Vol. 11(1), March 2020, pp. 9-17

Evaluation of anti-Parkinson's activity of ethanolic extract of *Tridax procumbens* (Asteraceae)

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Received 15 May 2018; Revised 17 February 2020

Parkinson's disease (PD) is the second most common neurodegenerative disease characterized by loss of dopaminergic neurons in the substantia nigra. In the present study was designed to evaluate anti-Parkinson's activity of ethanolic extract of *Tridax procumbens* (EETP) leaves, family Asteraceae. The experimental paradigm included haloperidol-induced catalepsy in rat model and rotenone-induced locomotor impairment in the fruit fly. In the catalepsy model, the rats received treatment of EETP (100 and 200 mg/kg, *p.o.*) followed by haloperidol (1 mg/kg, *i.p.*) for 15 days. The significant (P <0.05) reduction in muscle rigidity, catalepsy at EETP (100 mg/kg) while; improved locomotor activity was found with the EETP (100 and 200 mg/kg, *p.o.*). The catalase and reduced glutathione levels were found to be significantly (P <0.05) increased and decreased lipid peroxidation at EETP (100 and 200 mg/kg). In fruit fly model; rotenone (ROT) 500 µM co-exposed with EETP (0.05% w/v and 0.1% w/v) to flies for 7 days. Treatment with EETP (0.05 and 0.1% w/v) significantly (P <0.05) improved the performances of locomotor activity in flies when compared with ROT treated flies. Thus, the study proved that EETP treatment significantly attenuated motor defects and also protected the brain from oxidative stress.

Keywords: Anti-Parkinson's, Catalepsy, *Drosophila melanogaster*, Haloperidol, Rotenone, *Tridax procumbens*

IPC code; Int. cl. (2015.01)- A61K 36/00, A61K 36/28, A61K 127/00, A61P 25/16

Introduction

Parkinson's disease (PD) is classically defined as a movement disorder, second-most progressive neurodegenerative disease after Alzheimer disease. PD is caused by denervation of dopaminergic neurons in the substantia nigra pars compacta leading to depletion of dopamine at the striatal level. The cumulative effect of increased oxidative DNA damage, lipid peroxidation, mitochondrial dysfunction, excitotoxicity, glutathione depletion, iron deposition and alteration in antioxidant enzymes activities being the main cause of PD[1,2]. The main clinical characteristics of PD include motor symptoms like tremors in the jaw, head, arms or legs; rigidity or stiffness of the limbs and trunk; bradykinesia; abnormalities in postural stability; impaired balance and festinating gait. These symptoms are led by psychological symptoms such as depression and more general non-motor symptoms such as olfactory dysfunction, constipation and sleep disturbances[2].

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Natural products remain an important source of new drugs, new drug leads and new chemical entities for various diseases. Herbal medicines are parts of plants that are used to treat a range of disorders and to enhance wellbeing. One such plant i.e *Mucuna pruriens* (Fabaceae) has proved to be efficacious in Parkinson's diseases management[3]. Herbal medicine has its origins in ancient traditional cultures including those of the Indians, Egyptians, American and Chinese. *Tridax procumbens* is a species of flowering plant in the daisy family (Asteraceae), a common weed and pest plant natively belongs to the tropical region of America[4,5]. It has been introduced to tropical, subtropical, and mild temperate regions all around the world. It is wildly present throughout India growing primarily during rainy season. Also known as coat button or tridax daisy (English), *ghamra* (Hindi), *dagadipala* (Marathi), *jayantiveda* (Sanskrit)[4,5]. It has been found to possess significant medicinal properties, extensively used in Ayurvedic system of medicine for a liver disorder. The plant has various pharmacological activities like anti-hyperglycemic[6], hepatoprotective[7], anti-cancer[8], immunomodulatory[9], hypotensive[10], anti-urolithiatic[11],

anti-bacterial and anti-microbial[12], anti-mycobacterial[13], anti-inflammatory[14], hemostatic activity[5], and anti-trypanosomal[5]. Traditionally in India leaves of *T. procumbens* being used as anti-coagulant[5], hair tonic[15], anti-fungal[13], insect repellent[16], and wound healing[17].

The plants being the natural source have diverse phytoconstituents. The leaves of *Tridax procumbens* have a higher content of lignin viz. galgravin and flavonoids viz. luteolin, kaempferol, ellagic acid, catechin and silymarin. These lignin and flavonoids are reported to have antioxidant, anti-inflammatory and neuroprotective activity[18]. Thus depending on the actives present in *Tridax procumbens* it was selected for evaluation of anti-Parkinson's activity.

Materials and Methods

Drugs and chemicals

Haloperidol (Inj. serenace; RPG Life Sciences), levodopa+carbidopa (Tab. syndopa plus; Sun Pharma.), hydrogen peroxide (Research lab), thiobarbituric acid (Research lab), trichloroacetic acid (Research lab), 5,5-dithiobis-(2nitrobenzoic acid) (HiMedia), ethanol (S.D Fine Chem.), rotenone (Sigma Aldrich).

Collection of plant material

The powder of *Tridax procumbens* leaves was obtained from Ambaji Ayurved Bhavan, Mulund; Mumbai. The powdered drug was identified and authenticated (BSI/WRC/100-1/Tech/2017/H1-34) by Dr Priyanka Ingle, Botanical Survey of India, Pune.

Preparation of plant extract

The powder was packed in a soxhlet extractor and extracted with 95% ethanol at 50 °C. Extraction was carried out for 72 hours. After completion of the extraction process, the filtrate was concentrated to a dry mass by using rotavac evaporator. The extract was stored in a desiccator at room temperature. The percentage yield obtained of EETP was 2.6% w/w.

Phytochemical screening

The EETP (500 mg) was dissolved in 100 mL of its mother solvent ethanol and used for phytochemical screening. Different chemical tests were carried out to detect the presence of sterol, flavonoids, alkaloids, glycosides, tannins and saponins. The test included were salkowski reaction, liebermann's reaction, dragendroff's reaction, mayer's test, wagner's test, hager's test, keller-kiliani test, shinoda test, lead acetate test etc[19].

Animals

Sprague Dawley healthy male rats (180-200 g) were obtained from the Glenmark Research Center, Mahape, Navi Mumbai. The rats were housed in the animal house of Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai at 23±2 °C and 50-65% humidity under a 12:12 hour light and dark cycle. The animals were fed with standard rat pellets and water *ad libitum* throughout the study. The study was approved by the institutional animal ethics committee (Protocol no. BVCP/IAEC/06/2017), and all the animal experiments were carried out according to CPCSEA guidelines.

Drosophila melanogaster wild type, Canton special strain was obtained from the Indian Institute of Science and Education Research, Pune, India. The flies were grown in 2.5 cm × 14 cm vials containing 5 mL of a standard medium (8.3%, w/v maize flour; 5%, w/v glucose; 2.5%, w/v sucrose; 1.5%, w/v agar; 2%, w/v yeast powder; 0.04%, v/w propionic acid; 0.6%, v/w orthophosphoric acid; 0.7%, v/w methyl hydroxybenzoate) at constant temperature and humidity (23±1°C; 60% relative humidity, respectively) under 12 h dark/light cycle. All experiments were performed with the same strain. Studies using flies are exempted from ethical issues.

Experimental design

Haloperidol induced catalepsy in rat

The rats were acclimatized for 7 days before the study. 40 rats were divided into 5 groups with 8 rats per group. Group I as vehicle control [0.5% carboxy methyl cellulose (CMC)], group II as disease control, group III (EETP 100 mg/kg of body weight) and group IV (EETP 200 mg/kg of body weight) as treatment groups and Group V as a standard group (levodopa 100 mg/kg +carbidopa 25 mg/kg of body weight). The treatment was given by peroral route followed by haloperidol challenge (1 mg/kg; *i.p.* route) to all groups except group I daily for 15 days.

After induction of catalepsy with haloperidol, evaluation for behavioural parameters was done on day 7 and 13 for rotarod test; on day 8 and 14 for bar test; and on day 9 and 15 locomotor activity test. On day 15th after behavioural parameters assessment, the animals were sacrificed by exposing them to CO_2 overdose in the euthanasia chamber. The brain was excised out immediately and rinsed with ice-cold isotonic saline. The 10% w/v brain tissue homogenate was centrifuged at 10000 rpm for 15 minutes in 0.1 M phosphate buffer. The supernatant was used for

biochemical estimation of lipid peroxidation, catalase and reduced glutathione[20].

Estimation of the behavioural parameter by rotarod activity

The rotarod test is widely used in rodents to assess their "minimal neurological deficit" which hampers the muscle rigidity and affects motor function and coordination. Each rat was given a prior training before taking actual activity. Animals were placed on the rotating rod with a diameter of 7 cm (speed 12 rpm). Each rat was subjected for rotarod activity at an interval of 30 minutes for 3 hours following cut-off time of 180 seconds was maintained throughout the experiment. The average results were recorded as the fall of time[21].

Estimation of the behavioural parameter by bar test

Catalepsy is characterised as a reduced ability to initiate movement and a failure to correct abnormal posture, was assessed using the bar test. To measure catalepsy, both the front paws of rats were placed on 9 cm horizontal bar in half rearing position which is parallel to the base. Rats were observed to record the time of removal of one paw from the bar. The maximum cut off time for observation was fixed at 180 sec[22].

Estimation of the behavioural parameter by locomotor activity

The locomotor activity was monitored by using photoactometer (activity cage) for measurement of akinesia or hypokinesia. Before subjecting the animals to a cognitive task, they were individually placed in the activity meter and the total activity count was registered for 5 min. The locomotor activity was expressed in terms of total photobeam counts per 5 minutes per animal[23].

Estimation of biochemical parameters: Lipid peroxidation

The quantitative measurement of malondialdehyde (MDA) was used as an indirect measure of lipid peroxidation and was determined by reaction with thiobarbituric acid (TBA). Exactly 1 mL of aliquots of supernatant was placed in test tubes and added 3 mL of TBA reagent: TBA 0.38% (w/w), 0.25M hydrochloric acid (HCl), and trichloroacetic acid (TCA 15%). The solution was shaken and placed for 15 min, followed by cooling on an ice bath. After cooling, the solution was centrifuged to 3500 g for 10 minutes. The upper layer was collected after the formation of pink colour and absorbance taken at 532 nm on spectrophotometer[24]. Results were expressed as nanomoles per mg of protein.

Estimation of biochemical parameters: Catalase (CAT level)

The CAT activity was measured according to the method of Aebi[25]. The assay mixture consists of 0.05 mL of supernatant (10% w/v) of brain tissue homogenate and 1.95 mL of 50 mM phosphate buffer (pH 7.0) in 3 mL cuvette. After adding 1 mL of 30 mM hydrogen peroxide (H_2O_2), changes in absorbance were followed for 30 seconds at 240 nm at 15 seconds intervals. The millimolar extinction coefficient of H_2O_2 (0.071 mmol/cm) was used to calculate and the activity was expressed as micromoles of H_2O_2 oxidized per minute per milligram protein.

Estimation of biochemical parameters: Reduced glutathione (GSH Level)

For the estimation of GSH, the 1 mL of tissue homogenate was precipitated with 1 mL of 10% TCA. To an aliquot of the supernatant, 4 mL of phosphate solution and 0.5 mL of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) reagent were added and absorbance was taken at 412 nm. The values were expressed as nanoMoles of reduced glutathione per mg of protein[26].

Rotenone induced locomotor deficits in *Drosophila melanogaster*

ROT treated flies were exposed to low and high doses of EETP mixed in the culture medium. EETP was added in the medium at final concentrations of 5 mg/mL and 10 mg/mL to give (0.05% w/v and 0.1% w/v). Vials of PD flies without test compound were used as control. Adult flies of 7–8 days old were divided into four groups: group I as Control group, group II as the disease control group (ROT 500 μM), group III as EETP (0.05% w/v) plus ROT, group IV as EETP (0.1% w/v) plus ROT. The total food medium contained a volume of 1% of dimethyl sulfoxide (DMSO), ROT or ROT plus EETP. The flies were exposed to treatments for 7 days before being used for the assay.

Measurement of locomotor deficits

Negative geotaxis assay

The motor function was assessed using a negative geotaxis assay. During their light cycle, 15 flies were transferred into a graduated flat bottom glass vial (length 12 cm; diameter 2 cm) and allowed to habituate for at least 5 minutes. The vial was gently tapped at the bottom and observed for 60 seconds for the climbing activity. Locomotor behaviour was expressed as per cent flies escaping beyond a minimum distance of 10 cm in 60 seconds[27].

Statistical analysis

All the data were expressed as mean±standard deviation (SD). Statistical significance was tested using one way ANOVA followed by Tukey's multiple comparisons test using Prism graph pad version 7.0 (Graph pad software, Inc., CA, USA). *P <0.05 was considered statistically significant.

Results

Phytochemical screening

The preliminary phytochemical analysis of the EETP showed the presence of sterols, alkaloids, tannins, flavonoids and saponins.

Estimation of behavioural parameters

Rotarod activity

Animals treated with a low dose of EETP (100 mg/kg) along with haloperidol (1 mg/kg) for 15 days showed a nonsignificant (P <0.05) activity on 7th and 13th day in the latency of fall when compared to the disease control group. Animals treated with a high dose of EETP (200 mg/kg) along with haloperidol (1 mg/kg) for 15 days showed a nonsignificant activity on 7th day (Table 1) while; significant (P <0.05) improvement was seen on 13th day (Table 1) in the latency of fall after 120 minutes of haloperidol challenge when compared to the disease control group. Animals treated with the standard drug (levodopa+carbidopa 100+25 mg/kg) along with haloperidol (1 mg/kg) for 15 days have shown significant (P <0.05) improvement in the latency of fall after 30 min. of haloperidol challenge on both 7th and 13th day (Table 1).

Bar test

Animals treated with a low dose of EETP (100 mg/kg) along with haloperidol (1 mg/kg) for 15 days showed nonsignificant (P <0.05) activity on 8th and 14th day in the reduction of cataleptic time when compared to the disease control group. Animals treated with a high dose of EETP (200 mg/kg) along with haloperidol (1 mg/kg) for 15 days showed a significant (P <0.05) improvement in reduction of cataleptic time on 8th and 14th day after 90 minutes (Table 2) of haloperidol challenge when compared to the disease control group. Animals treated with the standard drug (levodopa+carbidopa 100+25 mg/kg) along with haloperidol (1 mg/kg) for 15 days have shown significant (P <0.05) improvement in reduction of cataleptic time after 30 minutes of haloperidol challenge on both 8th and 14th day (Table 2).

Locomotor activity

Animals treated with a low dose of EETP (100 mg/kg) along with haloperidol (1 mg/kg) for 15 days showed significant (P <0.05) activity on 9th and 15th day in the locomotor activity after 90 min. of haloperidol challenge when compared to the disease control group. Animals treated with a high dose of EETP (200 mg/kg) along with haloperidol (1 mg/kg) for 15 days showed a significant (P <0.05) locomotor activity on 9th day (Table 3) after 90 minutes of haloperidol challenge, significant (p <0.05)

Table 1 — Effect of EETP on muscle rigidity using the rotarod test

Time (min.)	Vehicle control	Disease control	EETP (100 mg/kg)	EETP (200 mg/kg)	Standard
7thDAY (Activity in seconds)					
30	57.87±10.09	7.50±2.00	9.00±1.60	8.75±1.98	33.37±6.99*
60	64.37±7.80	10.62±1.85	12.50±2.56	14.12±2.80*	38.87±2.60*
90	71.12±12.29	16.25±3.37	13.75±2.71	17.00±2.67	70.12±14.97*
120	62.87±11.00	21.00±3.30	18.75±3.15	20.62±3.29	78.50±10.95*
150	59.75±16.53	23.50±3.78	20.75±4.80	22.12±4.94	88.37±9.77*
180	58.50±12.33	27.25±2.60	23.37±5.26	27.00±4.57	117.62±8.28*
13thDAY (Activity in seconds)					
30	43.63±13.43	8.50±1.41	9.38±2.13	10.13±2.42	46.63±1.85*
60	54.63±13.33	10.25±1.49	11.63±2.13	12.88±2.23	44.25±1.39*
90	50.88±9.31	12.63±1.51	14.13±2.03	14.88±2.30	65.50±1.51*
120	57.00±13.28	15.63±2.13	18.63±2.26	20.63±1.60*	85.75±1.28*
150	56.63±14.56	18.38±2.07	21.38±2.50	24.50±1.51*	104.13±1.46*
180	48.38±13.03	22.38±4.17	27.63±4.03	28.25±4.77*	141.13±2.59*

Values are expressed as mean ± SD; n=8, where n is the number of animals per group. *indicates there is a significant difference with P <0.05 when compared with disease group One-way ANOVA followed by Tukey's multiple comparisons test. Time in minutes indicates recording of activity after haloperidol challenge.

Table 2 — Effect of EETP on cataleptic activity using bar test

Time (min.)	Vehicle control	Disease control	EETP (100 mg/kg)	EETP (200 mg/kg)	Standard
8thDAY (Activity in seconds)					
30	6.25±2.19	180.00±0.00	179.00±2.83	178.75±2.82	168.12±2.53*
60	5.12±1.81	178.87±2.10	178.00±2.83	175.87±4.45	164.12±2.10*
90	4.25±1.49	176.25±3.15	173.12±2.59	170.87±4.16*	155.25±2.23*
120	4.50±1.51	172.87±3.68	167.62±4.90	164.75±4.77*	145.87±2.42*
150	4.12±1.46	167.50±6.02	161.50±5.15	158.00±3.78*	130.87±2.53*
180	4.87±1.55	161.37±6.86	155.75±5.01	152.87±3.09*	116.50±2.67*
14thDAY (Activity in seconds)					
30	5.25±1.75	180.00±0.00	178.75±2.82	179.5±1.41	179.00±2.45*
60	5.00±1.20	178.87±1.46	176.50±2.98	176.12±2.85	175.87±2.17*
90	6.00±2.62	175.75±2.05	173.85±3.14	169.25±2.33*	169.25±2.36*
120	5.62±2.62	173.00±2.45	168.12±6.77	161.87±1.64*	163.12±1.89*
150	6.75±2.71	166.62±6.21	164.25±5.70	158.05±1.64*	156.37±2.72*
180	5.75±1.91	164.37±3.62	157.37±5.42*	152.87±5.41*	148.12±2.36*

Values are expressed as mean ± SD; n=8, where n is the number of animals per group. *indicates there is a significant difference with P <0.05 when compared with disease group by One-way ANOVA followed by Tukey's multiple comparisons test. Time in minutes indicates recording of activity after haloperidol challenge.

Table 3 — Effect of EETP on locomotor activity using actophotometer

Time (min.)	Vehicle control	Disease control	EETP (100 mg/kg)	EETP (200 mg/kg)	Standard
9thDAY (Activity in seconds)					
30	586.00±51.74	39.50±1.60	37.87±2.55	46.75±3.06	62.75±8.81*
60	596.87±28.05	54.25±2.27	58.00±3.58*	57.37±3.72	94.75±3.38*
90	610.37±95.03	63.25±2.47	69.50±2.39*	70.00±2.47*	103.37±5.30*
120	619.87±48.96	69.50±5.29	83.37±4.63*	85.62±2.12*	127.87±6.07*
150	583.25±40.33	80.37±10.20	93.25±8.12*	98.25±3.38*	175.50±16.25*
180	593.62±22.58	88.75±12.74	103.00±8.40*	113.75±1.55*	195.00±2.30*
15thDAY (Activity in seconds)					
30	471.63±42.86	66.88±3.21	66.25±7.61	76.63±5.60	83.13±15.09*
60	434.13±100.81	74.63±7.65	77.25±4.60	87.00±7.30*	100.88±6.24*
90	428.88±3.55	84.50±7.81	97.00±2.07*	111.25±4.83*	116.88±9.96*
120	554.63±67.31	93.63±7.81	112.60±2.07*	119.13±4.83*	127.88±9.96*
150	471.75±3.36	97.00±5.06	122.62±4.99*	136.50±10.79*	140.37±18.54*
180	531.88±11.29	122.38±4.50	139.62±3.24*	153.25±2.14*	188.25±12.45*

Values are expressed as mean ± SD; n=8, where n is the number of animals per group. *indicates there is a significant difference with P <0.05 when compared with disease group by One-way ANOVA followed by Tukey's multiple comparisons test. Time in minutes indicates recording of activity after haloperidol challenge

improvement was seen on 15th day (Table 3) in locomotor activity after 60 minutes of haloperidol challenge when compared to the disease control group. Animals treated with the standard drug (levodopa+carbidopa 100+25 mg/kg) along with haloperidol (1 mg/kg) for 15 days have shown significant (P <0.05) improvement in locomotor activity after 30 min. of haloperidol challenge on both 9th and 15th day (Table 3).

Estimation of biochemical parameters

Administration of haloperidol intraperitoneally resulted in significant changes in biochemical parameters when compared to the vehicle control animals. The administration of haloperidol-induced oxidative stress is indicated by increased MDA level and decreased CAT and GSH levels when compared to vehicle control animals. The treatment with EETP (100 and 200 mg/kg, *p.o.*) showed significant

(P <0.05) decrease in MDA level while; significantly (P <0.05) increased the levels of GSH and CAT in the brain as compared to the disease control group (Table 4).

Rotenone induced locomotor deficits in *Drosophila melanogaster*

Negative geotaxis assay

The results of this study revealed that the response of the PD induced flies was significantly lower than that of control flies. Measurement of the locomotor deficits among flies of ROT treated exhibited severe locomotor impairments as evident by a large number of flies (84.46%) staying at the bottom of the glass vial. The flies co-treated with EETP (0.05 and 0.1% w/v) significantly (P <0.05) improved the locomotor activity when compared with ROT treated flies (Table 5). The number of flies staying at the bottom of the vial was decreased to 51.13% when cotreated with a higher dose of EETP. The flies showed negative geotaxis behaviour treated with EETP dose-dependently; indicating its neuroprotective effect.

Discussion

PD is a chronic neurodegenerative disease caused by depletion of dopamine, due to degeneration of dopaminergic neurons in substantia nigra pars compacta of basal ganglia region of the brain and intraneuronal deposition of Lewy bodies caused by aggregation of α-synuclein proteins[28]. The main clinical characteristics of PD include motor symptoms like tremors in the jaw, head hands, arms, or legs, rigidity or stiffness of the limbs and trunk; bradykinesia, or slowness of movement; and abnormalities in postural stability, or impaired balance and festinating gait[3].

In the present study, we evaluated the neuroprotective effect of ethanolic extract of *Tridax procumbens* on haloperidol-induced catalepsy in rats and ROT induced locomotor deficits in flies of the PD model.

Chronic use of antipsychotics like haloperidol causes extrapyramidal syndrome which affects the motor coordination systems. Haloperidol induced catalepsy is a broadly accepted animal model of PD. Haloperidol is a nonselective D_2 dopamine antagonist which provides a pharmacological model of parkinsonism for experimentation by interfering with the storage of catecholamine's intracellularly, resulting in dopamine reduction in nerve endings[23].

The various phytochemical classes present in EETP have active chemical components which cross the blood-brain barrier to reach the various target sites in the brain. The *Tridax procumbens* is reported to contain many important phytoconstituents having significant pharmacological importance e.g. galgravin, luteolin, kaempferol, ellagic acid, catechin and silymarin[18]. These phytoconstituents are reported to have antioxidant, anti-inflammatory and neuroprotective activity ameliorates to revitalise and strengthen the neurons to perform various cognitive functions. These reported activities of the phytoconstituents suggest its possible role in the treatment of PD.

In the present study, haloperidol (1 mg/kg, *i.p.*) induced significant catalepsy in rats as evidenced by a significant increase in the time spent on the horizontal

Table 4 — Effect of EETP on the levels of lipid peroxidation (MDA), catalase (CAT) and reduced glutathione (GSH) in the brain of haloperidol challenged animals

Groups	MDA (nM/mg of protein)	CAT (μmoles of H_2O_2 used/min/mg protein)	GSH (nM/mg of protein)
Vehicle control	0.57±0.07	3.52±1.43	4.40±0.19
Disease control	1.59±0.11	1.13±0.23	3.45±0.17
EETP 100 mg/kg	1.33±0.03*	2.89±0.27*	4.08±0.27*
EETP 200 mg/kg	1.12±0.02*	3.45±0.27*	4.37±0.18*
Standard	0.88±0.05*	2.75±0.35*	6.24±0.35*

Values are expressed as mean ± SD; n=8, where n is the number of animals per group. *indicates there is a significant difference with P <0.05 when compared with disease group One-way ANOVA followed by Tukey's multiple comparisons test.

Table 5 — Effect of EETP on locomotor activity of *Drosophila melanogaster* as evaluated by negative geotaxis assay

	Vehicle control	Disease control	EETP (0.05% w/v)	EETP (0.1% w/v)
No. of flies escaped beyond 10 cm distance	13.00±1.00 [86.67]	2.34±0.58 [15.54]	5.67±1.53* [37.74]	7.34±0.58* [48.87]

Values are expressed as mean±SD; n=15, where n is the number of flies per group. *indicates there is a significant difference with P <0.05 when compared with disease group One-way ANOVA followed by Tukey's multiple comparisons test. Values in parenthesis are expressed as % protection against rotenone-induced locomotor deficits

bar and caused a significant decrease in locomotor activity and muscle activity as compared to vehicle control animals. Both the doses of EETP were not showing a significant effect on 7 days of treatment; while standard drug showed a significant effect on Day 7. The low and high dose EETP (200 mg/kg) showed a similar protective effect against haloperidol-induced catalepsy exhibited a significant reduction in catalepsy, increase in locomotor activity and muscle rigidity activity after treatment of 15 days. The reduction in catalepsy and improved locomotor activity was comparable to that of protective action exhibited by the standard drug. It can be predicted that the required concentration of EETP to give significant protective effect is achieved as a virtue of cumulative accumulation in the body and hence significant efficacy was found after 15 days of treatment.

Oxidative stress generated as a result of mitochondrial complex-1 dysfunction plays an important role in the pathogenesis of PD. The oxidative stress parameters were measured through the determination of levels of lipid peroxidation, catalase and reduced glutathione in the brain tissue.

Lipid peroxidation, a reactive marker of oxidative stress, was estimated by measuring the levels of MDA. Lipid peroxidation occurs due to attack by reactive free radicals on the double bond of unsaturated fatty acid and arachidonic acid which produces lipid peroxyl radicals and that initiate a chain reaction, which further attacks on other unsaturated fatty acids. As we know, lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes causes impaired membrane function and structural integrity[29], decreased fluidity, and inactivation of some membrane-bound enzymes. In the present investigation, similar results were observed in the brain homogenate of haloperidol treated disease control group. After treatment with EETP (100 and 200 mg/kg) significantly reduction in lipid peroxidation level was found when compared with the disease control group.

Catalase is an antioxidant enzyme which helps in neutralizing the harmful effects of hydrogen peroxide. Catalase converts hydrogen peroxide to form water and nonreactive oxygen species, thus preventing the accumulation of precursor to free radical biosynthesis. Oxidative stress results in a decrease in catalase level. During induction of catalepsy with haloperidol in rats induced oxidative stress, as indicated by a decrease in the catalase levels. After treatment with EETP (100 and 200 mg/kg) significantly increment in catalase level was found when compared with the disease control group.

The depletion of reduced glutathione in the substantia nigra in PD could be the result of neuronal loss due to oxidative stress of free radicals. A positive correlation has been found to exist between the level of neuronal loss and reduction of glutathione[30]. Reduction in the availability of reduced glutathione would impair the ability of neurons to neutralise hydrogen peroxide and increase the risk of free radical formation and lipid peroxidation. A reduction in GSH levels was evident in haloperidol treated disease control animals. After treatment with EETP (100 and 200 mg/kg) significantly increment in GSH level was found when compared with the disease control group.

Thus, the haloperidol-induced disease control group showed a significant increase in the levels of lipid peroxidation and a decrease in the levels of catalase and GSH in the brain as compared to the vehicle-treated control animals. All these indicate an increase in the oxidative stress in the brain of animals treated with haloperidol. Pre-treatment with EETP (100 and 200 mg/kg) resulted in a decrease in lipid peroxidation and increase in the levels of catalase, and GSH, indicating its free radical scavenging effect in the brain of haloperidol treated animals. The activity of EETP at both doses is comparable to the standard drug.

In the Drosophila model, ROT is commonly used for the PD model. It has a high affinity specific inhibitor of mitochondrial (complex I) NADH dehydrogenase. Being highly lipophilic, it crosses the blood-brain barrier rapidly and accumulates in subcellular organelles like mitochondria[31]; where oxidative damage leads to neurotoxicity of dopaminergic neurons and locomotor deficits[32,33]. In ROT (500 μM) induced significant locomotor impairment in flies as indicated by a large number of flies remaining at bottom of vials in the disease control group. The EETP at doses of 0.05 and 0.1% w/v showed a protective effect against ROT induced locomotor impairment exhibited significant improvement in locomotor activity, thus could be proved with possible action on central nervous system indicates that EETP has an ability of free radical scavenging properties helps in neuroprotection of dopaminergic neurons in the brain.

Conclusion

The results of the present study conclusively showed that EETP has free radical scavenging activity and neuroprotective role in experimental models of PD. Hence, the neuromodulatory effect of EETP on behavioural, oxidative stress may be due to its neuroprotective and free radical scavenging properties. Further detailed molecular studies, and also further characterization and isolation of active constituents responsible for the neuroprotective effect should be undertaken.

Acknowledgement

The authors thank the University of Mumbai for funding of this project (No. APD/237/323 of 2018 dated 27th March 2018). The authors also extend thanks to Indian Institute of Science and Education Research, Pune, for providing drosophila culture.

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