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SMILAX CHINA ETHANOLIC EXTRACT ON ATTENUATED BEHAVIOURAL IMPAIRMENTS, NEUROCHEMICAL DEFICTS AGAINST MPTP INDUCED PARKINSON'S DISEASE IN RATS

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ABSTRACT

The present investigation was aimed to analyse the ethanolic extract of S. china (EESC) on the Behavioural Impairments, neurochemical variables against MPTP induced rat model of Parkinson's disease (PD). Except control group, all animals received 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) daily administered for 7 days through intraperitoneally at doses of (30 mg/ kg of body weight, dissolved in saline, to induce PD. Later the animals, were divided into five groups for each plant extracts as; two test groups, one control group, one negative control and one standard group. The test group received 150 mg/kg of body weight of EESC and 300 mg/kg of body weight of EESC. The total time duration of the study was 14 days for each extract i.e. 28 days to complete the study. Treatment with EESC at doses of 150 and 300 mg/kg of body weight in PD rats improved the behavioral irregularities found in the vehicle and MPTP administered group (P< 0.05). Treatment of EESC at 300 mg/ kg of body weight showed reduced to start movement (akinesia) and muscle rigidity or failure to modify an externally forced position (catalepsy).This was more significant of improvement in locomotor activity on 20th day of treatment with EESC at 300 mg/ kg of body weight when compared between levodopa and carbidopa treated group (P < 0.001) and showed dose dependent efficacy for EESC. On 7th day, EESC at 150 and 300 mg/ kg of body weight administered groups, i.e. groups III and IV, showed significant rise in retention time (P <0.001) when compared with MPTP administered group. The Passive avoidance test was employed to study foot shock stimulus. Repeated treatment with MPTP (group II) has decrease in latency time when compared with control group. Administration of EESC at both doses at 150 and 300 mg/ kg of body weight administered groups, i.e. groups III and IV with MPTP administered rats significantly (P < 0.005) increased latency period when compared with MPTP induced group. Findings of the present study revealed that EESC may help in reducing the risk of developing Parkinson's disease (PD).

KEY WORDS

Parkinson's disease, S. China, akinesia, catalepsy,Passive avoidance test.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease that mainly affects the movement of elderly population. It is characterized by tremor, rigidity, akinesia and postural instability, which is arises largely due to the massive loss of dopaminergic (DA-ergic) neurons projecting from the substantia nigra (SN) to the striatum (ST)[1]. Several neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), rotenone, paraquat, maneb and reserpine are used to induce PD in rodents and showed

physiological symptoms and pathological processes similar to human patients, which further helped to evaluate the possible therapeutic implications [2, 3].

The root of *Smilax china* (Linn) belongs to the Liliaceae family. *Smilax china* (Liliaceae) is a deciduous climber with rounded leaves and red berries. The root tubes of which furnish the drug known as china root. It is found in the south Indian states namely Andra Pradesh, Tamilnadu and Karnataka. It possesses anti-inflammatory, diuretic, anti-diabetic, anti-psoriatic, digestive properties. It is also heptoprotective, nephroprotective and used in cases of infertility. Till now various pharmacological activities had been done on different parts and extracts of the plant *Smilax china* like chronic pelvic inflammatory diseases[4] promoting blood circulation[5] inhibit AA rats's secondary inflammatory swelling, reduce thymus and spleen weights, decrease CD4/CD8, but had little influence on B Cell. It acts regulating cell-mediated immunity, but has little effect on humoral immunity[6] anti microbial[7] chronic pelvic inflammation[8] anti- inflammatory and anti-nociceptive activities[9] anti-inflammatory effects on acute and chronic inflammation[10], inhibitory effects on cyclooxygenase-2 enzyme (COX-2) and production of TNF alpha (tumor necrosis factor alpha) in murine peritoneal macrophages[11] anticancer activity against HeLa cells[12] chronic pelvic anti-inflammatory activity[13], *in vitro* anti-microbial activity[14] anti-hyperuricemic and nephroprotective activity in hyper uricemic animals[15] hepatoprotective activity[16], antidiabetic activity[17], anticonvulsant and neurotoxic effects[18], spermatological activity[19], antioxidant and antimicrobial in food and cosmetic industry[20], anti-HIV-1 activity[21], anti-obesity activity[22], endothelial dysfunction study[23] and anti-metastatic activity[24] .Therefore, the present investigation focused to evaluate the neuroprotective potential of ethanolic extract of root of *Smilax china* in MPTP induced parkinsons diseases in animal models.

MATERIALS AND METHODS

1. Collection and Identification of Plant materials

The roots of *Smilax china* were collected form Marthandam,Kanyakumari District, Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The roots of *Smilax china* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40-mesh sieve.

2. Preparation of Extracts

The above powdered plant materials were consecutively extracted with petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[25] for one day. The marc was dried out and extracted with chloroform and then marc was extracted with ethyl acetate (76-78^{0}C) for one day, then this marc was dried out after that it was extracted with ethanol for one day and then marc was extracted with water. All the three extracts were concentrated by utilizing a rotary evaporator and undergone to freeze drying using a lyophilizer until dry powder was acquired. The ethanolic extract gave more yield and more phytoconstituents were present. So the ethanolic extract of *Smilax china* was selected for the further investigation.

EVALUATION OF NEUROPROTECTIVE ACTIVITY

Experimental

Both the genders of Wister rats with 8 weeks weighed between 150 and 200 g of body weight was used for the present study. Rats were acclimatized for experimental conditions for about two weeks. The rats housed in plastic cages at 25°C with relative humidity of 70% under 12/12 hours day/ night cycle. The experiments were performed according to the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on animals (CPCSEA). The protocol was approved by animal ethics committee [**(Approved number: AU/IAEC/1199/1/18).** Rats were fed with food and water *ad libitum*.

After three days of acclimatization, the rats was randomly divided into the one control group, one negative control group, two test groups and one standard treatment group. Among them, control, negative and standard treatment groups were kept common both the studies. Except control group, all animals received 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) daily administered for 7 days through intraperitoneally at doses of (30 mg/ kg of body weight, dissolved in saline, to induce PD. Later the animals, were divided into five groups for six rats in each group:

Group I: Control group administered with only vehicle, consisted of MPTP dissolved in normal saline solution (30mg/ kg of body weight, i.p).

Group II: Negative control group – administered with MPTP dissolved in normal saline solution (30mg/ kg of body weight, i.p).

Group III: Test Group I – administered with MPTP dissolved in normal saline solution (30mg/ kg of body weight, i.p) + EESC at a dose of 150 mg/ kg of body weight.

Group IV: Test Group II – administered with MPTP dissolved in normal saline solution (30mg/ kg of body weight, i.p) + EESC at a dose of 300 mg/ kg of body weight.

Group V: Standard Treatment group – MPTP dissolved in normal saline solution (30mg/ kg of body weight, i.p) + Levodopa and Carbidopa at a dose of 30 mg/ kg of body weight.

Resting Tremor Scores in MPTP Induced Rats[26]

The resting tremor for Parkinson symptom was assessed in the rats. In a transparent cage rats were kept, which was above from the floor of 0.5 meters and notice for 45 min for the severity of the resting tremor. The resting tremors were scored for every 5 min as follows:

0 = nil observation of resting tremor;

1 = minor resting tremor of postural muscles only;

2 = moderate resting tremor sometimes reaching the head;

3 = obvious resting tremor, but not always involving the head;

4 = continuous resting tremor and no movement of limbs or head; and

5 = continuous resting tremor of the whole body.

Initial Movement Impairment [Akinesia] in MPTP Induced Rats[27]

Akinesia tests shows the straining of rats to initiate the movement in PD induced rats. This test was evaluated by measuring the latency in seconds among the rats to move all four legs and test completed in 180 sec. Prior to the akinesia test individually rats was acclimatized for 10 min in a wooden elevated (100 cm) platform (100 cm × 150 cm). The time taken by the rats to move all the four legs were noted using stopwatch.

Muscular rigidity [Catatonia Test][28]

Muscular rigidity was evaluated by catatonia by providing scores to define activity. Based on the severity of the muscular rigidity were scaled from 0 to 1.

0 = no rigidity;

0.5 = movement caused by pushing the animal in a flat surface and/ or keeping each front limb at 1cm height from the floor;

1 = for the ability to stay in rigidity and keep each forelimb on 1.5 cm height.

Therefore, maximum rigidity score was 3.5. Scoring was noted in at an interval in a period of every 20 seconds.

Locomotor Impairment in MPTP induced Rats [29]

Locomotor behavioural activity model was assessed in among the MPTP induced rats after 5 days from the administration of MPTP and locomotor activity was measured after a study period of 20 days. Cognitive behavioural activity model was studied by using passive avoidance test on 20th day test and 21st day as retention. On 21st day an open field test was assessed. Rats were sacrificed, and brain was isolated by cardiac perfusion in the last day of the study. On 22nd day, brain tissue was rinsed with ice-cold isotonic saline solution and homogenized with 1 mL of 0.1 M phosphate buffer saline (pH 7.4).

Grip strength [Rotarod][30]

Motor and Coordination actions were evaluated by the Rotarod test for a period rats keep their balance on a moving rod. Rats was allowed to modify their posture so as to preserve their balance on a rotating rod at speeds of 5, 10 and 15 rpm 3 times/ day with interval of 30 minutes. The average retention time was calculated.

Movement Impairments [Catalepsy][31]

The Catalepsy means the incapability of an animal to adjust an externally enforced posture. The animal was taken up by lifting its tail and was kept on its forepaws on a horizontal wooden bar. The bar was having a diameter of 1 cm and height of 7.5 cm. The cataleptic time was measured for a duration required to make its 1st movement of any paws. In the present research work maximum declined or inclined latency for a period of minimum 30 seconds and maximum up to 180 seconds were noted as cataleptic time.

Foot Shock Stimulus by Passive Avoidance Test in MPTP Induced Rats[32]

Passive avoidance test was conducted in a step through type device, which was employed to measure the effects of EESC extracts on learning and memory among the MPTP induced rats. The PAT device was divided into two equal chambers with a size 25 cm in length, 15 cm in breath and 15 cm in height. For separating guillotine door was used. Rats were kept in the light chamber at beginning with the door opened. The rats showed probing behavior. Later entered into the dark section. Once rats entered into the dark section the door was closed automatically. Training was provided to the rats

till it enters the dark section within 30 sec (training session). 24 hours after the training session, the rats was kept in the light chamber. When the rats entered the dark section, electric foot shock of about 1 mA was given for 5 seconds via a grid metal floor and door was closed automatically (test session). The rats was repeatedly kept in the dark section. 24 h after the test session and latency time to enter the dark session was noted for 5 minutes (retention session). If the rats did not enter the dark section within the 5 minutes and was allotted a latency value of 5 minutes.

Statistical analysis

Data were presented as mean ± SEM. One-way ANOVA using Tukey's employed for post hoc test for multiple comparisons. The value of $P < 0.05$ was considered statistically significant.

Results and Discussion

Effect of EESC on resting tremor scores in MPTP induced rats

In the present study PD was assessed the signs and parkinsonian severity through resting tremor scores at 1, 7 and 14 days after MPTP induction. The results proved that the MPTP induced group, the resting tremor scores was statistically significant than control group. Both the groups of EESC treated rats revealed dose dependent increase when compared with group II and higher dose i.e. 300 mg/kg of body weight showed maximum effect in reducing the resting tremor impairment at all-time compared with group I ($P < 0.05$). On 14th day of MPTP induced, the EESC at higher dose i.e. 300 mg/kg of body weight showed almost equal efficacy than Levodopa (30 mg/ kg of body weight, i.p.). The data are presented in the Table No.1.

Table:1 Effect of EESC on resting tremor scores in MPTP induced rats

Groups	Scores of Resting Tremor		
	1st Day	7th Day	14th Day
I	0	0	0
II	3.45±0.83^{a,1}	4.12±0.91^{a,1}	4.76±0.96^{a,1}
III	3.93±0.79^{a,1,2}	2.90±0.64^{b,1,2}	1.85±0.61^{c,1,2}
IV	3.96±0.74^{a,1,2}	2.39±0.70^{b,1,2}	1.82±0.69^{c,1,2}
V	2.47±0.59^{c,2}	1.92±0.57^{c,2}	1.38±0.51^{c,2}

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one-way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – group I compared with group II, III, IV & V; 2 – group II compared with group III, IV &V.

In the present research work, it is planned to study in rats' model that MPTP either induce parkinsonism, which was measured behavioral tests for motor function as well as coordination (using rotarod apparatus) and parkinsonism severity (using resting tremor score). Present findings implied that the negative group, i.e. group II showed significant worsening of motor function as well as coordination and severity of parkinsonism when compared with the control group, i.e. group I, which has not administered with MPTP ($P< 0.05$), indicating that the PD model was successful. Treatment with EESC at doses of 150 and 300 mg/kg of body weight in PD rats improved the behavioral irregularities found in the vehicle and MPTP administered group ($P< 0.05$). Especially, EESC at a dose of 300 mg/kg of body weight resulted in the maximum effect of behavioral improvement, which was less statistically significantly ($P < 0.05$) when compared with the standard treatment group i.e. group V.Our study findings are similar to Pennapa Chonpathompikunlert et al., 2018 report.[33]

Effects of EESC on initial movement impairment [Akinesia] in MPTP-induced rats

Impairment in the beginning of movement was calculated by akinesia test. Repeated administration of MPTP triggered reduced ability to begin movement (akinesia) as compared to group I control rats ($P < 0.05$). Initial movement impairment [Akinesia] in MPTP-induced ratswas found to be 50.17±1.05, 8.00±0.26, 15.67±0.88, 33.33±1.02and 41.00±0.82 from group I, II, III, IV and V respectively. Oral administration of EESC both doses statistically significant ($P < 0.01$) reduced MPTP administered akinesia. Both the groups of EESC treated rats revealed dose dependent increase when compared with group I and higher dose i.e. 300 mg/kg of body weight showed maximum effect in reducing the resting tremor impairment at all-time compared with

group I ($P < 0.05$). The data are presented in the Table No.2.

Table:2 Effects of EESC on Akinesia in MPTP-induced rats

Groups	No. of Steps with forelimbs / 3 minutes
I	$50.17\pm1.05^{a,2}$
II	$8.00\pm0.26^{c,1}$
III	$15.67\pm0.88^{b,1,2}$
IV	$33.33\pm1.02^{c,1,2}$
V	$41.00\pm0.82^{c,2}$

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one-way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – group I compared with group II,III,IV &V; 2 – group II compared with group III,IV &V.

In the present study, the administration of EESC at 300 mg/ kg of body weight showed reduced to start movement (akinesia) and muscle rigidity or failure to modify an externally forced position (catalepsy). Akinesia test is used to assess behavior in animal models of PD resembles limb akinesia and posture problems in PD patients[34]. EESC at 300 mg/ kg of body weight abolished particularly dopaminergic neurons and subsequently reduced motor function[35]. In the present research work, reduction of dopaminein brain in MPTP administered rat saffected behavioral defects as noticed in PD patients. Improvement in the levels of striatal dopamine and its regulators including tyrosine hydroxylase by EESC at 300mg/ kg of body weight showed the neuroprotective efficiency of this extract in protecting dopaminergic neurons and reverting to the normal behavior[36].

Effect of EESC on Catatonia Test for Muscle Rigidity in MPTP induced rats

Rats administered with EESC at 150 mg/ kg of body weight that was statistically less significant ($P < 0.01$) showed muscle rigidity when compared with group V, which was administered with levodopa and carbidopa. This was more significant on 20th day of treatment with EESC at 300 mg/ kg of body weight when compared between levodopa and carbidopa treated group ($P < 0.001$) and showed dose dependent efficacy for EESC. When compared with 20th and 25th dayof EESC treatment, which was the first day of administration, significant decrease was observed in muscle rigidity ($P < 0.05$). The data are presented in the Table No.3 and Fig. No.1.

Table:3 Effect of EESC on Catatonia Testin MPTP induced rats

Groups	No. of Steps	
	14th day	20th day
I	$29.67\pm1.67^{a,2}$	$41.09\pm2.56^{a,2}$
II	$7.83\pm0.91^{c,1}$	$10.74\pm1.91^{b,1}$
III	$16.00\pm1.37^{a,1,2}$	$20.92\pm2.98^{b,1,2}$
IV	$23.33\pm1.49^{b,1,2}$	$38.48\pm3.19^{c,1,2}$
V	$26.00\pm1.43^{c,2}$	$39.39\pm3.05^{c,2}$

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one-way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – group I compared with group II, III, IV & V; 2 – group II compared with group III,IV &V.

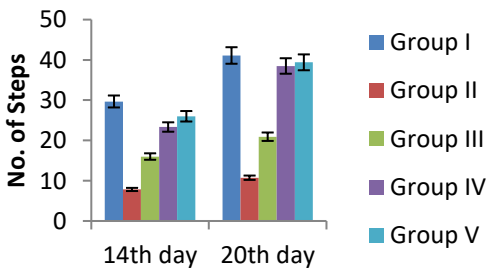

Fig:1 Effect of EESC on Catatonia Testin MPTP induced rats

Catatonia is also called as rigidity or stiffness in the muscle. Catatonia is restriction in the movements of limbs due to high muscle tone and more as well as contraction of muscles. Muscle rigidity will be identical in the PD patients[37]. [Gelb DJ et al., 1999] Cogwheeling is believed to be associated to 2 of the 3 early symptoms of Parkinson's disease, which is combination of tremor and rigidity at higher degree. At the initial stages PD, one can observe asymmetrical rigidity very frequently and affect muscles in neck and shoulder before it affects the face muscles. As the disease progresses, a typical rigidity affects the entire body and decreases the capability to move. In present research work, which EESC at 300 mg/ kg of body weight were showed statistically significant therapeutic effects in catatonic activity when compared with the control group (group I) and standard treatment group (group II). A similar activity was reported by the Sarah Rezaee and Mahsa Hadipour Jahromy, 2018[38].

Effect of EESC on Locomotor Impairment in MPTP induced rats

Rats administered with EESC at 150 mg/ kg of body weight that was statistically less significant ($P < 0.01$) improvement in locomotor activity when compared with group V, which was administered with levodopa and carbidopa. This was more significant of improvement in locomotor activity on 20th day of treatment with EESC at 300 mg/ kg of body weight when compared between levodopa and carbidopa treated group ($P < 0.001$) and showed dose dependent efficacy for EESC. When compared with 5th day and 14th day of treatment with EESC, significant improvement was observed in locomotor activity ($P < 0.05$). The data are presented in the Table No.4.

Table:4 Effect of EESC on Locomotor Impairment in MPTP induced Rats

Groups	No. of Steps	
	5th day	10th day
I	567.33±81.24^{a,2}	582.73±60.37^{a,2}
II	143.17±49.20^{a,1}	166.92±41.89^{b,1}
III	284.33±34.31^{b,1,2}	376.19±56.71^{c,1,2}
IV	418.83±63.46^{b,1,2}	510.22±62.89^{c,1,2}
V	505.17±55.62^{c,2}	561.51±71.29^{c,2}

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one-way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – group I compared with group II,III,IV &V; 2 – group II compared with group III,IV &V.

MPTP can cause damage lipids, proteins and mitochondria lead to change the anti-oxidant enzyme in the brain[39]. Moreover, literature review showed, which is supplementation to antioxidant diminished levels in dopamine and behavioral activity in PD animals[40].

Effects of EESC on Grip strength [Rotarod]in MPTP induced rats

In the present study, it was noticed that MPTP induced, i.e. in group II, significant reduction of retention time ($P < 0.001$) on 0th and 7th day when compared with control group, i.e. group I. In Levodopa and carbidopa administered group shown a significant increase in retention time ($P < 0.005$) was observed on both days i.e. 0th and 7th day when compared with MPTP administered group i.e. group II. On 7th day, EESC at 150 and 300 mg/ kg of body weight administered groups, i.e. groups III and IV, showed significant rise in retention time ($P < 0.001$) when compared with MPTP administered group i.e. group II, while there was less significant ($P < 0.01$) difference in retention time was noticed when compared with levodopa and administered group i.e. group V. The data are presented in the Table No.5 and Fig 2.

Table:5 Effects of EESC on Grip strength [Rotarod] in MPTP induced rats

Groups	No. of Steps	
	0th day	7th day
I	120.67±2.62[a,2]	123.91±3.04[a,2]
II	37.50±1.72[b,1]	42.72±2.77[b,1]
III	45.18±1.40[b,1, 2]	51.38±2.22[b,1,2]
IV	80.66±2.09[b,1, 2]	101.45±3.98[c,1,2]
V	106.34±3.12[c,2]	125.81±3.61[c,2]

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one-way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – group I compared with group II,III,IV &V; 2 – group II compared with group III,IV &V.

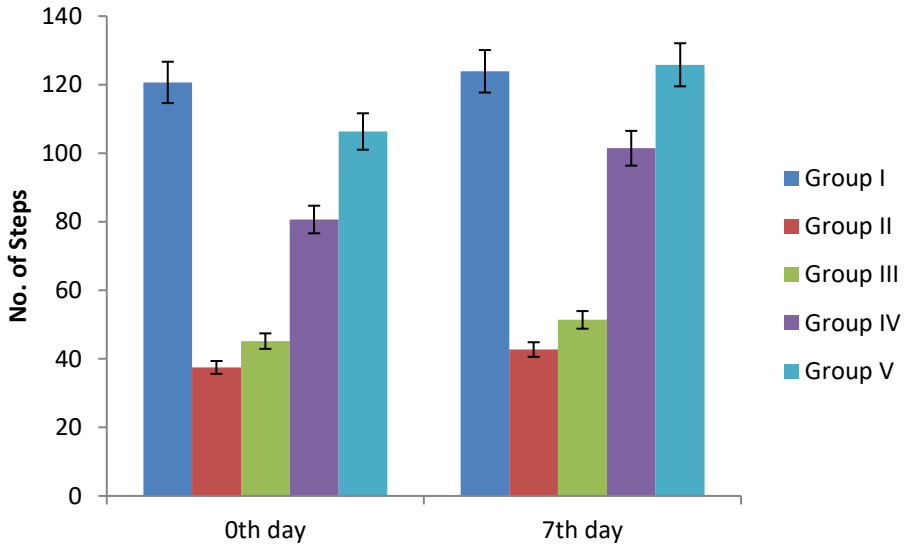

Fig 2: Effects of EESC on Grip strength [Rotarod] in MPTP induced Rats

Effects of EESC on movement impairments [Catalepsy] in MPTP induced rats

Catalepsy test was employed to study the impairment in movement coordination. Repeated treatment with MPTP, i.e. group II, has caused impairment in modification of an externally forced position (catalepsy) when compared with control group i.e. group I. Co-administration of EESC at both doses at 150 and 300 mg/ kg of body weight administered groups, i.e. groups III and IV to MPTP administered rats significantly (P < 0.005) decreased MPTP induced catalepsy when compared with MPTP induced group, i.e. group II.

It was also noticed that among MPTP only administered group, i.e. group II, was significantly increased latency period (P < 0.001) among 0th and 7th day when compared with control group I, i.e. group I. In Levodopa and carbidopa administered group, i.e. group V, significantly reduced in latency period (P < 0.001) on 0th and 7th day was noticed when compared with MPTP alone administered group, i.e. group II. EESC at both doses, i.e. 150 and 300 mg/ kg of body weight treated groups, i.e. groups III and IV, was caused significant (P < 0.01) difference in latency period on 0th day. But 7th day, EESC at both doses, i.e. 150 and 300 mg/ kg of body weight treated groups, i.e. groups III and IV, showed significantly reduced in latency period (P < 0.001) when compared with MPTP administered group, i.e. group II, whereas less significant (P < 0.01) change in latency time was notices when compared with levodopa and carbidopa administered group. The data are presented in the Table No.6 and Fig. No.3.

Table:6 Effects of EESC on Catalepsy in MPTP induced rats

Groups	Days				
	0	1	3	5	7
I	60.73±1.16^{a,2}	61.32±1.30^{a,2}	65.69±1.11^{a,2}	69.15±1.17^{a,2}	70.00±1.16^{a,2}
II	32.29±1.19^{a,1}	39.11±1.26^{a,1}	40.27±1.43^{b,1}	42.24±1.32^{b,1}	45.86±1.57^{c,1}
III	38.54±1.37^{a,1,2}	49.85±1.4826^{a,2,1}	52.56±1.65^{b,1,2}	66.78±1.68^{b,1,2}	78.34±1.39^{c,1,2}
IV	41.66±1.74^{a,1,2}	53.74±1.6126^{a,2,1}	69.04±1.48^{b,1,2}	79.52±1.40^{b,1,2}	86.19±1.81^{c,1,2}
V	38.91±1.88^{c,2}	43.87±1.54^{c,2}	59.18±1.29^{c,2}	65.57±1.46^{c,2}	85.96±1.23^{c,2}

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one-way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – group I compared with group II, III, IV & V; 2 – group II compared with group III, IV & V.

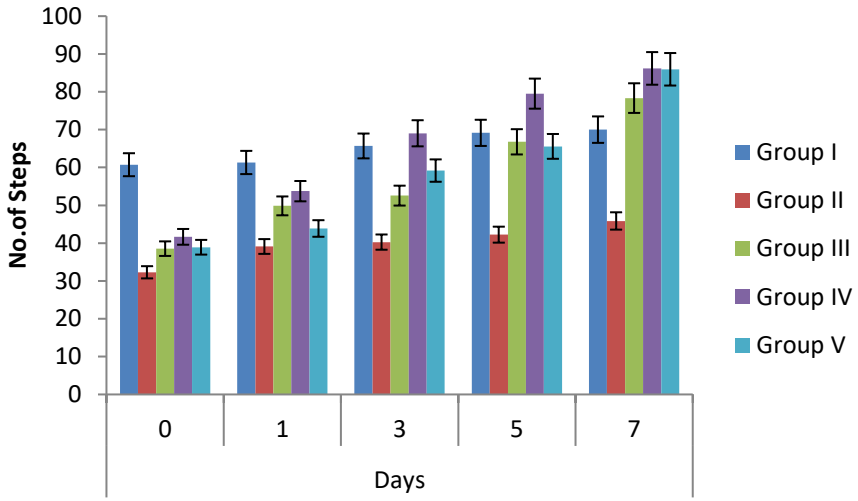

Fig:3 Effects of EESC on Catalepsy in MPTP induced rats

Effect of EESC on Foot Shock Stimulus by Passive Avoidance Test in MPTP induced rats

The Passive avoidance test was employed to study foot shock stimulus. Repeated treatment with MPTP, i.e. group II, has decrease in latency time when compared with control group i.e. group I. Co-administration of EESC at both doses at 150 and 300 mg/ kg of body weight administered groups, i.e. groups III and IV with MPTP administered rats significantly (P < 0.005) increased latency period when compared with MPTP induced group, i.e. group II.

Number of steps was found to be 23.78±1.85, 9.90±1.19, 46.75±1.56, 49.00±1.09 and 62.85±1.47 for groups I, II, III, IV and V respectively on 0th day, while on 2nd day it was noted as 27.09±1.93, 10.31±1.21, 8.75±1.65, 70.49±1.84 and 93.00±2.05 for groups I, II, III, IV and V respectively.

There were significant differences were observed in latency time among the EESC at both doses, i.e. groups III and IV, in the absence the aversive foot-shock stimulus (test trial). The latency period to enter the dark section was significantly (P < 0.001) decreased 1 day after foot shock in MPTP administered group, i.e. group II, when compared with control group, i.e. group I (control rats: 27.09±1.93 minutes; MPTP administered rats: 10.31±1.21 minutes). EESC treated groups at both doses i.e. 150 and 300 mg/ kg of body weight was significantly (P < 0.001) increased latency period (58.75±1.65 minutes and 70.49±1.84 minutes) at 150 and 300 mg/kg of body weight, respectively. The data are presented in the Table No. 7 and Fig. No.:4.

Table No. 7: Effect of EESC on Foot Shock Stimulus in MPTP induced mouse

Groups	Time in Minutes	
	0th day	2nd day
I	23.78±1.85 b,2	27.09±1.93 b,2
II	9.90±1.19 a,1	10.31±1.21 b,1
III	46.75±1.56 b,1,2	58.75±1.65 c,1,2
IV	49.00±1.09 c,1,2	70.49±1.84 c,1,2
V	62.85±1.47 c,2	93.00±2.05 c,2

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one-way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – group I compared with group II, III, IV & V; 2 – group II compared with group III, IV & V.

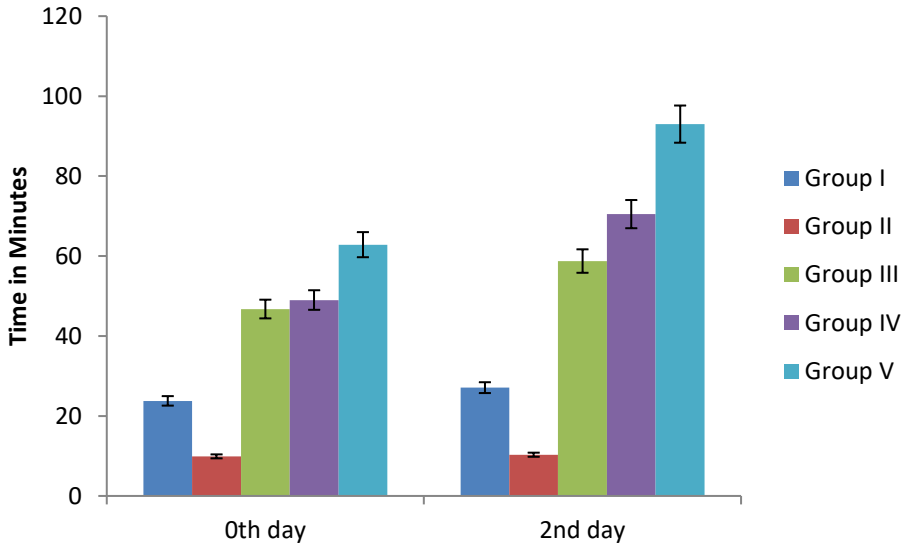

Fig 5.34: Effect of EESC on Foot Shock Stimulus in MPTP induced Rats

In the present research work, an attempt was taken to study the effect of EESC on dopaminergic neuron impairment induced by MPTP. The foot shock stimulus was performed to assess the anti-Parkinson activity of EESC at two doses (150 and 300 mg/ kg of body weight). Stimulus behavior is due to central monoaminergic neurons. D_2 dopamine receptors, which is present in the brain plays a vital role in the modulation of foot shock stimulus behaviour in animal. In foot shock induced stimulus, it is observed that the brain dopamine levels are improved[41]. 0.5 mA current was supplied to the animals. Animals treated with EESC (150 and 300 mg/ kg of body weight, p.o.) highly significant in number of fighting attacks, thus suggesting a probable dopaminergic activity of EESC in foot shock stimulus. Fighting behavior was highly significant in animals treated with EESC when compared with negative control group, group II and less significant with standard treatment group, group V. The above behavioral and biochemical results suggest that EESC have the capability to increase symptoms of Parkinsonism. The anti-parkinsonism effect may be due to reverting back to normalcy in dopamine level and antioxidant property of EESC at high dose (300 mg/ kg of body weight, p.o.). Vandana S. Nade et al., 2014 reported that the present research work similar with this behavioral studies[42].

CONCLUSION

In the present study, EESC (150 mg/ kg and 300 mg/ kg of body weight) administered orally increased learning and memory in rats evaluated by the Grip strength Akinesia, Catatonia Test for Muscle Rigidity, Catalepsy and Foot Shock Stimulus by Passive Avoidance Test. The ethanolic extract of roots of S.china may be help in reducing the risk of developing PD or delaying its onset because of the cumulative antioxidant and mitochondrial protective effects of their Phyto constituents. Hence further research is needed to

confirm the neuroprotective effect of EESC after the onset of disease and also to explore the mitochondrial protective and anti-inflammatory properties of EESC.

REFERENCES

1. Depboylu C, Schafer MK, Arias-Carrion O, Oertel WH, Weihe E,Hoglinger GU. Possible involvement of complement factor C1q in the clearance of extracellular neuromelanin from the substantia nigra in Parkinson disease. J Neuropathol Exp Neurol . 2011; 70:125–132
2. Betarbet R, Sherer TB, MacKenzie G, Garcia-osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci. 2000; 3:1301–1306
3. Cicchetti F, Drouin-Ouellet J, Gross RE. Environmental toxins and Parkinson's disease: what have we learned from pesticide- induced animal models? Trends Pharmacol Sci. 2009; 30:475–483.
4. Cui MF. Observation of curative effect of Jin Gang Ten Syrup on one hundred and twenties patients suffer from pelvic inflammatory. Modern J Integ Tr Chi West Medi 2001; 6:515.
5. Liu Yong Ning, Dong Sheng and Chu Hong. Study on the pharmacological action of *Smilax china* L. on promoting blood circulation. Chi J Hospi Pharm, 2002;9:289-92.
6. Zhong Yao Cai, Chinese Y, Chen D, Deng J and Tian L. Effect of *Smilax china* on adjunctive arthritis rats. Affiliated Xiehe Hospital 2003;26(5):344 – 366.
7. Liu Shi-wang, you bi-gang and Yan-xia 2004. Inhibition Effect of the Ethanol Extract of *Smilax china* L. on Microorganisms. Resource Development and Market 2004;113(1):112 – 115.
8. Huang Si-Hang, Zhao J and Zhang M. Effective Ingredients of *Smilax china* L. for Treating Chronic Pelvic Inflammation. J Mod Food Pharmaceu 2006; 2:321.
9. Xiao SS, Zhong HZ and Xiang LY. Anti-inflammatory and antinociceptive activities of *Smilax china* Linn. J Ethnopharmacol 2006;103(3):327 – 332.
10. Shu XS, Gao ZH and Yang XL. The anti-inflammation effects of *Smilax china* ethyl acetate extract in rats and rats. Zhongguo Zhong Yao Za Zhi 2006;31(3):239 – 243.
11. Shao B, Guo H, Cui Y, Ye M, Han J and Guo D. Steroidal saponins from *Smilax china* and their anti-inflammatory activities. Phytochem 2007;68(5):623-30.
12. Yuan Li, Guo Ping Gan, Hui Zhan Zhang, He Zhen Wu, Chang Long Li, Yong Ping Huang, Yan Wen Liu and Jian Wen Liu. A flavonoid glycoside isolated from *Smilax china* L. rhizome *in vitro* anticancer effects on human cancer cell lines. J Ethnopharmacol 2007; 113: 115 – 124.
13. Chen Dong sheng, Xiao li and Li xiu. The effect of *Smilax china* on chronic pelvic inflammation induced by coliform bacteria. Chi J Hospi Pharm 2007; 2:124 – 126.
14. Shu Xiao S, Jin Hai LV and Tao Jun. Evaluation of the *in vitro* anti-microbial activity of *Smilax china* L. extracts. Indian Journals.com 2010;2(2): 345 – 347.
15. Chen L, Yin H, Lan Z, Ma S, Zhang C, Yang Z, Li P and Lin B. Antihyperuricemic and nephroprotective effects of *Smilax china* L. J Ethnopharmacol 2011;135:399 – 405.
16. Venkidesh R, Subhash C, Mundal, Dilipkumar Pal, Mohana Lakshmi S and Saravanakumar A. Hepatoprotective activity of *smilax chinensis* linn carbon tetrachloride induced hepatotoxicity in rats. Int J Bio Pharmaceu Res 2010;1(2):72 – 75.
17. Rajesh Bhati, Anupama Sing, Vikas Anand Sahara, Veerma Ram and Anil Bhandari. Pharmacognostical standardization, extraction and antidiabetic activity of *Smilax china* L. rhizome. Asian J Tr Medi 2011; 6(5):218 – 223.
18. Vijayalakshmi A, Ravichandiran V, Anbu J, Malarkodi Velraj and Jayakumari S. Anticonvulsant and neurotoxicity profile of rhizome of *Smilax china* linn, in rats. IJP 2011;43(1):27 – 30.
19. Sarawathi CD, Suresh MV, Satyanaryana sreemantula and Venkatakrishna K. effect of *Smilax china* linn on testicular antioxidant activity and spermatological parameters in rats subjected to forced swimming stress. Int Res J Pharm 2012;3(1): 118 – 121.
20. Hye Kyung Seo, Jong Hwa Lee, Hyun Su Kim, Chang Kwon Lee and Seung Cheol Lee. Antioxidant and antimicrobial activities of *Smilax china* L. leaf extracts. Food Sci. Biotechnol 2012;21(6):1723 – 1727.
21. Wei-Xin Wang, Jing-Yi Qian, Xiao-Jing Wang, Ai-Ping Jiang and Ai-Qun Jia. Anti-HIV-1 activity of extracts and phenolics from *Smilax china*. Pak J Pharm Sci 2014;27(1):147 – 151.
22. Park JA, Jin KS, Kwon HJ and Kim BW. The anti-obesity effect of *Smilax china* extract. Ko J Microbio Biotechn 2014; 42(4): 354-360.
23. Victor Ruberio Lincha, Bing-Tian Zhao, Mi-HeeWoo, In-Jun Yang and Heung-Mook Shin. Effects of constituent compounds of *Smilax china* on nicotine induced endothelial dysfunction in human umbilical vein endothelial cells. Biol. Pharm. Bull. 2016;39: 984 – 992.
24. Kyoung Jin Nho, Jin Mi Chun and Ho Kyoung Kim. Anti-metastatic effect of *Smilax china* L. extract on MDA MB 231 cells. Molecular Medicine Reports 2015; 11:499-502.
25. Harborne J.B. Phytochemical methods 11 Edn. In Chapman and Hall. New York, 1999; 4 – 5.
26. Lundblad M, Picconi B, Lindgren H, Cenci MA. A model of L-DOPA-induced dyskinesia in 6-hydroxydopamine lesioned mice: relation to motor and cellular parameters of nigrostriatal function. Neurobiol Dis, 2004; 16: 110 – 123.
27. Fernandez M, Negroa S, Slowing K, Fernandez-Carballido A, Barcia E. An effective novel delivery strategy of

rasagiline for Parkinson's disease. Int J Pharm, 2011; 419: 271 – 280.
28. Sarah Rezaee, Mahsa Hadipour Jahromy. Potential Effects of Pomegranate Juice in Attenuating LID in Mice Model of Parkinson Disease. Pharmacogn J, 2018; 10(4): 700 – 704.
29. Patil SP, Jain PD, Ghumatkar PJ, Tambe R, Sathaye S. Neuroprotective effect of metformin in MPTP induced Parkinson's disease in mice. Neurosci, 2014; 26; 277: 747 – 754.
30. Rozas G, Guerra MJ, Labandeira-Garcia JL. An automated rotarod method for quantitative drug-free evaluation of overall motor deficits in rat models of parkinsonism. Brain Res Brian Res Protoc, 1997; 2: 75 – 84.
31. Tong Q, Wu L, Gao Q, Ou Z, Zhu D, Zhang Y. PPARb/d agonist provides neuroprotection by suppression of IRE1a-caspase-12mediated endoplasmic reticulum stress pathway in the rotenone rat model of Parkinson's disease. Mol Neurobiol, 2016; 53: 3822 – 3831.
32. Shen Z, Wang G, Lin SZ. Two-way shuttle box avoidance conditioning and brain NADH in rats. Physiol Behav,1990; 48: 515 – 517.
33. Pennapa Chonpathompikunlert, Phetcharat Boonruamkaew, Wanida Sukketsiri, Pilaiwanwadee Hutamekalin and Morakot Sroyraya. The antioxidant and neurochemical activity of Apium graveolens L. and its ameliorative effect on MPTP-induced Parkinson-like symptoms in mice. BMC Complementary and Alternative Medicine. (2018) 18:103: 1 – 12.
34. Tseng KY, Kargieman L, Gacio S, Riquelme LA, Murer MG. Consequences of partial and severe dopaminergic lesion on basal ganglia oscillatory activity and akinesia. Eur J Neurosci 2005; 22:2579-86.
35. Samantaray S, Knaryan VH, Guyton MK, Matzelle DD, Ray SK, Banik NL. The Parkinsonian neurotoxin rotenone activates calpain and caspase-3 leading to motor neuron degeneration in spinal cord of Lewis rats. Neuroscience 2007; 146:741-55.
36. Nehru B, Verma R, Khanna P, Sharma SK. Behavioral alterations in rotenone model of Parkinson's disease: Attenuation by co-treatment of centrophenoxine. Brain Res 2008; 1201:122-7.
37. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. Archives of neurology. 1999;56(1):33-9.
38. Sarah Rezaee and Mahsa Hadipour Jahromy. Potential Effects of Pomegranate Juice in Attenuating LID in Mice Model of Parkinson Disease. Pharmacogn J. 2018; 10(4):700-704.
39. Perier C, Vila M. Mitochondrial biology and Parkinson's disease. Cold Spring Harb Perspect Med 2012; 4: 1–19.
40. Khan MM, Hoda MN, Ishrat T, Ahmad A, Khan MB, Khuwaja G, Raza SS, Safhi MM, Islam F. Amelioration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced behavioural dysfunction and oxidative stress by Pycnogenol in mouse model of Parkinson's disease. Behav Pharmacol 2010; 21: 563–571.
41. Yadav AV, Nade VS. Antidopaminergic effect of methanolic extract of Morus alba L. leaves. Indian J Pharmacol. 40(5) (2008) 221-226.
42. Vandana S. Nade, Laxman A. Kawale, Shankar S. Zambare, Pranita P. Dharmadhikari and Priyanka S. Pagare. Ameliorative Effect of Nebivolol in Parkinson's disease. AJPP, 2014; 1(3): 116 – 126.

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