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EVALUATION OF NEUROPROTECTIVE ACTIVITY OF TERMINALIA PALLIDA FRUIT EXTRACT ON ALBINO RATS

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ABSTRACT:

Neuroprotective activity of ethanolic extract of Terminalia pallida against Haloperidol induced Parkinsonism was evaluated in the present study. Five groups of 6 animals in each namely Test, Control, Normal and Standard groups were used for the study. L-Dopa and carbidopa in combination were used as standard drugs. Haloperidol was used to induce Parkinson's Disease in rats which acts by blocking dopamine D_2 receptors and produces a state of catalepsy in humans or animals and reduces dopaminergic transmission in basal ganglion. Parameters asserted during the study were Catalepsy, Muscle rigidity, Akinesia, Peripheral movements, Central movements, Rearing's, Grooming's, Salivation score, Lacrimation Score, and Tremors.

1. INTRODUCTION:

Though these drugs are effective in the treatment of Parkinson's Disease, they are associated with certain adverse effects like ulcers, respiratory tract depression, constipation, hypertension etc.[1][2][3] Hence neuroprotective activity remains the interest of researchers, in order to develop a potent neuroprotective agent with nill or minimal adverse effects. Haloperidol is a neuroleptic drug which is also used to induce Parkinson's Disease (dose dependent and age dependent). It acts by blocking dopamine D_2 receptors and produces a state of catalepsy in humans or animals and reduces dopaminergic transmission in basal ganglion.[4] *Terminalia pallida* is the member of Combretaceae plant family. It is used for treating kidney diseases, as it is a mild diuretic. Research has proved that it has anti-allergenic, hypertensive treatment and inflammatory treatment properties, but the main use for Terminalia pallida is for kidney stones and nephritis. It is also used to treat gout, Rheumatism and Diabetes. It is effective against fungal and bacterial infections.[5] The current study was

conducted to evaluate the extract of Terminalia pallida for its neuroprotective activity against haloperidol induced Parkinson's disease in albino rats.

2. MATERIALS AND METHODS:

Collection of Plant Material

The fresh fruits of Terminalia pallida were collected from the market which were identified and authenticated by Dr K. Madhavachetty at Sri Venkateshwara University, Tirupathi, AP. The fruits were further dried for the extraction.

Experimental Animals

Albino rats of either sex weighing 150-200g were procured and maintained in polypropylene cages at a controlled room temperature of 24^0c, under 12 h light and 12h dark cycle. After one week of acclimatization, the experiment animals were divided randomly into 5 groups (n=6). The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC). All the experiments were performed in accordance with IAEC guidelines.

Preparation of Extract

The fruits were collected, and shade dried for 3 days. Coarse powdered material weighing about 100 g was subjected to soxhlet extraction using ethanol for about 8-10 hrs at the temperature of 79ºc. The dried extract was weighed and preserved for further investigations (EETP).[6]

Preliminary Phytochemical Studies

The extract was subjected to phytochemical screening for evaluating the presence of important phytochemicals present in it. The standard tests were used for the detection of alkaloids, tannins, phenols, carbohydrates, saponins, terpenoids, steroids, flavonoids, glycosides, amino acids and proteins[7]

Acute Toxicity Study

Acute toxicity study of EETP was carried out in rats according to OECD-423 guidelines. Different doses of EETP were administered up to 4000 mg/kg b.w. (p.o.) and the rats were observed for a period of 72 hr for behavioral changes, toxic symptoms and mortality.[8]

Experimental Design

The animals were divided into 5 groups n=6, and the grouping was done as follows,

Groups 1:Normal (received vehicle -1% CMC, 10 ml/kg body weight, p.o.),

Group 2: Control(received haloperidol 1 mg/kg, p.o.),

Group 3: Standard (received L-Dopa & Carbidopa 100 mg+25 mg/kg p.o.+haloperidol 1 mg/kg, p.o.),

Group 4: Test group-1 (received low dose of EETP (200 mg/kg, p.o. + haloperidol 1 mg/kg, p.o.),

Group 5: Test group-2(received high dose of EETP 400 mg/kg, p.o + haloperidol 1 mg/kg, p.o.).

The doses were given for 21 days as multiple dose studies. Animals are provided with food and water as usual

before experiment. On 21st day haloperidol (1 mg/kg) was injected 60 min after oral administration for all groups and further parameters like Catalepsy, muscle rigidity, akinesia, open field test, salivation, lacrimation, tremors, were evaluated to determine the potential.

Catalepsy: Catalepsy in rats is defined as a failure to correct an externally imposed, unusual posture over a prolonged period.[9]

A. Block method:

Rat was placed on the table and touched gently on the back. If the rats failed to move then a score of 0.5 was assigned. The front paws of the rats were placed alternately on a 3cm high block. If the rat failed to correct the posture within 15 seconds, a score of 0.5 for each paw was added to the score of step 1.

The front paws of the rat placed alternately on a 9cm high block .if the rat failed to correct the posture within 15 sec, a score of 1 for each paw was added to the scores of step I, step II. Thus, for an animal, the highest score was 3.5(cut off score) and that reflects in total Parkinson's disease[10]

B. Metal Bar test:

A cataleptic behavior was measured with a high bar test method. Catalepsy score was measured for 4 hours at 30 min intervals after haloperidol administration by gently placing both the fore paw of the rat over a metal bar. The intensity of catalepsy was assessed by counting the time in seconds until the rat brought both fore pass down to the tabletop, with a maximum cutoff time of 3 minutes. Finally, scores at different time points (0, 30, 60, 90, 120, 150, 180, 210 & 240 minutes after haloperidol injection) were added and expressed as cumulative catalepsy score for comparison purpose[11]

Muscle Rigidity

Motor integrity and coordination were assessed by the time latency from the placement of the animal on the rotating drum until it fell. Time when the animal fell from the rotating rod was recorded, which was taken as motor integrity and coordination.

Akinesia

The impaired ability to initiate movements are measured by akinesia test. Each rat was held by the tail so that it is standing by its forelimbs and moving on its own. The number of steps taken with both forelimbs was recorded for 30 sec[12]

Open Field Test

The open field test provides simultaneous measures of locomotion, exploration and anxiety.

The number of central square entries and the duration of time spent in the central square are measures of exploratory behavior and anxiety. A high frequency/duration of these behaviors indicates high exploratory behavior and low anxiety levels. The behaviors scored (Brown et al, 1999) included:

Peripheral Square Entries**:** Frequency with which the rat crossed one of the grid lines with all four paws into the peripheral Square

Center Square Entries**:** Frequency with which the rat crossed one of the red lines with all four paws into the central square.

Rearing: Frequency with which the rat stood on their hind legs in the maze.

Grooming: Duration of time the animal spent licking or scratching itself while stationary.

Each animal was then given a score for total locomotor activity that was calculated as the sum of line crosses and number of rears.[13],[14]

Salivation

Reduce movements of swallowing musculature which results in less swallowing in PD. This decrease in spontaneous or reflexive swallowing results in an excess of saliva in the mouth. The excess of saliva formation in the mouth can be measured in the form of scores as follows.[15]

Lacrimation:

In PD, Lacrimation increases. Lacrimation is measured on the basis of score.[16]

Tremor:

The scores for all animals in each group at the 3 observation periods are summarized. The numbers in the treated groups are expressed as a percentage of the number of the control group. Tremor is scored after 30 min of administration of haloperidol (1 mg/kg, p.o.)

Antioxidant Test:

Brains were excised and chopped with surgical scalp into fine slices and were chilled in the cold 0.25 M sucrose, quickly blotted with filter paper. The tissue was minced and homogenized in an ice cold 10 mM tris HCl buffer (to pH 7.4) at a concentration of 10% (w/v) with 25 strokes of tight teflon pestle of glass homogenizer at a speed of 2500 rpm. The prolonged homogenization under hypotonic condition was designed to disrupt as far as possible the ventricular structure of cells so as to release soluble protein and leave only membrane and non-vascular matter in a sedimentable form. It was then centrifuged at 5000 rpm at 20^{o} C temperature and clear supernatant was separated and used to estimate reduced glutathione (GSH), catalase (CAT) and lipid peroxidation (LPO).

Histopathological Studies:

For Histopathological studies, substantia nigra tissue obtained from the excised brain was immediately fixed in 10% buffered neutral formalin solution. The fixed tissues were embedded in paraffin and serial sections were cut. Each section was stained with hematoxylin and eosin (H & E stain). The sections were examined under a light microscope and photomicrographs were taken.

2.7 Statistical Analysis

All the data was expressed as Mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the tukey's test using a computer based fitting program (Prism graph pad 5.03). Statistical significance was set accordingly.

3. RESULTS:

Acute Toxicity Study

EETPwas found to be safe as no animal died at the maximum single dose of 4000 mg/kg when administered orally and the animals did not show any gross behavioral changes. Hence $1/10^{th}$ of this dose i.e. 400 mg/kg were used as high dose and 200 mg/kg were used as low dose in the subsequent study respectively.

Anti-Parkinson's Activity

A) On catalepsy

a) Effect of EETP on catalepsy by block method

Rats treated with haloperidol (G-II) showed a significant increase in immobility and muscular rigidity at 60 min, 90 min (p<0.01), 120 min, 150 min, 180 min, 210 min and 240 mins (p<0.001) when compared to the normal group (G-I). The group (G-III) rats treated with standard drug L-Dopa & Carbidopa showed a significant decrease in immobility and muscular rigidity on 60 min (p<0.05), 90 min, 120 min, 150 min (p<0.01), 180 min, 210 min and 240 min (p<0.001) when compared to the control (G-II).The group-IV and group-V were receiving EETP at two different doses showed a significant decrease in immobility and muscular rigidity when compared to control group. But interestingly the group-V shows good significant decrease in immobility and muscular rigidity at 120 min (p<0.05), 150 min, 180 min (p<0.01), 210 min and 240 min (p<0.001) than group-IV [180 min (p<0.05), 210 min and 240 min (p<0.01)]. The results were shown in Table no: 1 and Graph no: 1

b) Effect of EETP on catalepsy by metal bar test

Administration of haloperidol (G-II) induce a significant increase in immobility and muscular rigidity on 60 min (p<0.05), 90 min, 120 min, 150 min, 180 min, 210 min and 240 mins (p<0.001) when compared to the normal group (G-I). On treatment with L-Dopa&carbidopa showed a significant decrease in immobility and muscular rigidity at 90 min (p<0.05), 120 min (p<0.01), 150 min, 180 min, 210 min and 240 min (p<0.001) when compared to the control (G-II).Groups (IV and V) treated with EETP showed a significant decreases in immobility and muscular rigidity compare to control group (G-II). Surprisingly group-V showed more significant effect [120 min (p<0.05), 150 min, 180 min (p<0.01), 210 min and 240 min (p<0.001)] than group-VI [180 min, 210 min (p<0.05), 210 min and 240 min (p<0.01)]. The results were shown in Table no: 2 and Graph no: 2

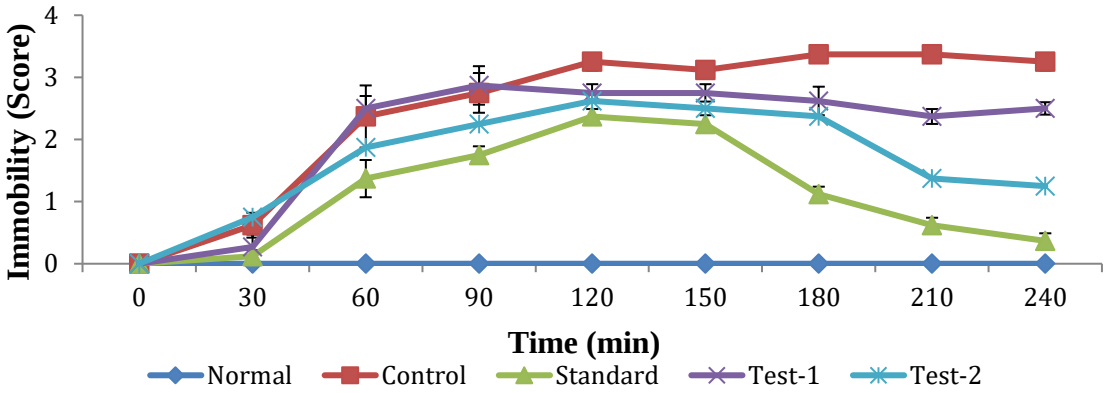
Table no 1: Effect of EETP on catalepsy by block method

Groups	Normal	Control	Standard	Test 1	Test 2
Treatment Immobility (Score) (Mean ± S.E.M) on 21st day	1% CMC (p.o.)	Haloperidol (1 mg/kg, p.o.)	L-Dopa & Carbidopa (100+25 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.)	EETP (200 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.)	EETP (400 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.)
0min	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
30min	0.0 ± 0	0.62 ± 0.2	0.12 ± 0.1	0.27 ±0.2	0.75 ±0.1
60 min	0.0 ± 0	2.37 ± 0.5**	1.37 ±0.3*	2.5 ± 0.2	1.87 ± 0.4
90 min	0.0 ± 0	2.75±0.32**	1.75 ±0.14**	2.87 ±0.31	2.25 ±0.14
120 min	0.0 ± 0	3.25±0.14***	2.37 ±0.12**	2.75±0.14	2.62± 0.12*
150 min	0.0 ± 0	3.12±0.12***	2.25±0.14**	2.75±0.14	2.5 ± 0.2**
180 min	0.0 ± 0	3.37±0.12***	1.12±0.12***	2.62±0.23*	2.37±0.12**
210 min	0.0 ± 0	3.37±0.12***	0.62±0.12***	2.37±0.12**	1.37±0.23***
240 min	0.0 ± 0	3.25±0.14***	0.37±0.12***	2.5±0.1**	1.25±0.14***

n=6 animals in each group; All values were expressed as Mean±S.E.M. *** indicates P<0.001; ** indicates P<0.01 vs. normal

*** indicates P<0.001; ** indicates P<0.01; * indicates P<0.05 vs. control

Graph no: 1 Effect of EETP on catalepsy by block method



Normal = vehicle treated; Control = Haloperidol (1 mg/kg, p.o.); Standard = L-Dopa & Carbidopa (100 + 25 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.); Test-1 = EETP (200 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.); Test-2 = EETP (400 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.).

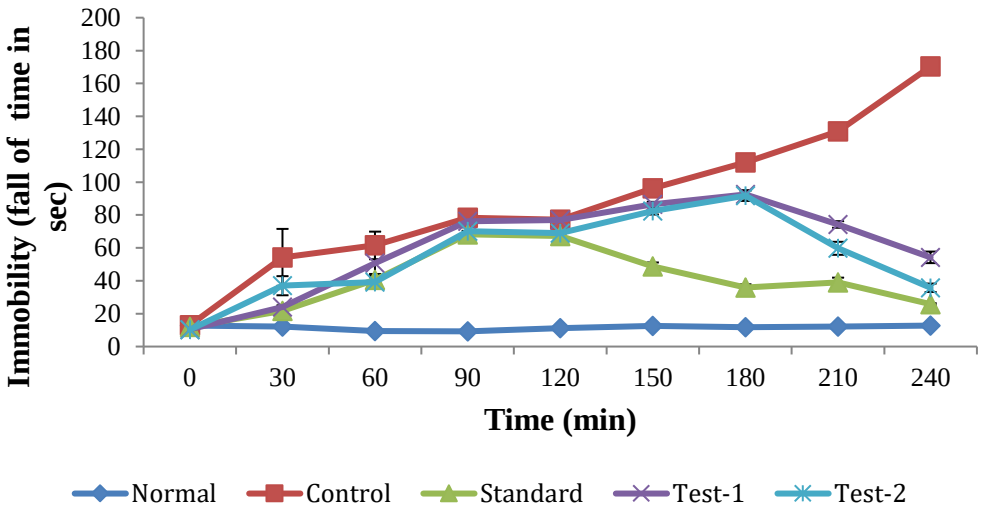
Table no: 2 Effect of EETP on catalepsy by metal bar test

Groups/ Immobility (Fall of time in sec) (Mean ± S.E.M) on 21st day	Normal : 1% CMC (p.o.)	Control : Haloperidol (1 mg/kg, p.o.)	Standard :L-Dopa & Carbidopa (100+25 mg/kg,p.o.)+Haloperidol (1 mg/kg, p.o.)	Test 1 : EETP (200 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.)	Test 2 : EETP (400 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.)
0min	12.75±0.4	13 ±0.4	12 ± 1.2	10.5±1.19	10.25± 1.1
30min	12.25±0.7	54.25±17.3	21.75 ± 1.1	24 ±1.08	37±5.81
60 min	9.5 ± 1.32	61.5±8.4*	40.5±1.6	50.75 ±6.6	39.25 ±3.7
90 min	9.25 ± 1.37	78.25±2.73***	68.25 ± 2.13*	76.25±3.17	70.25 ±2.28
120 min	11.25 ± 0.85	77.25±1.65***	67.25 ±1.65**	77±2.7	69 ± 0.7*
150 min	12.50 ± 0.64	96.25±2.56***	48.75±2.39***	86.50±1.5*	82.50 ±2.21**
180 min	11.75 ± 0.94	112±4.65***	36 ±1.87***	92.5±2.5*	91.75±3.19**

210 min	12.25 ± 1.25	130.8±4.51***	39 ±2.91***	74.25±2**	59.75±3.96***
240 min	12.75± 0.47	170.3±5.1***	25.75±0.62***	54.25±3.51**	35.75±2.56***

EETP: Ethanolic extract of *Terminalia pallida* n=6 animals in each group; values were expressed as Mean±S.E.M. ### indicates P<0.001; ## indicates P<0.01, # indicates P<0.05 vs. normal; *** indicates P<0.001; ** indicates P<0.01; * indicates P<0.05 vs. control

Graph no: 2 Effect of EETP on catalepsy by metal bar test



Normal = vehicle treated; Control = Haloperidol (1 mg/kg, p.o.); Standard = L-Dopa & Carbidopa (100 + 25 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.); Test-1 = EETP (200 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.); Test-2 = EETP (400 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.).

B) Effect of EETP on muscle rigidity

Muscle rigidity was characterized by restriction of the active and passive movements of the joint. Haloperidol (1 mg/kg, p.o.) treated rats (G-II) were showed a significant decrease in active and passive movements at60 min (p<0.01), 90 min, 120 min, 150 min, 180 min, 210 min and 240 mins (p<0.001) when compared to the normal group (G-I). The group (III) rats were treated with standard drug L-Dopa & carbidopa (100mg + 25 mg/kg, p.o.)

showed a significant increase in active and passive movements at 150 min(p<0.05), 180 min (p<0.01), 210 min and 240 min (p<0.001) when compared to control (G-II).

The groups (IV and V) receiving EETP at two different doses (200 mg/kg and 400 mg/kg, p.o) showed a significant increase in active and passive movements when compared to the control group (G-II). But group-V shows more significant effects [150 min (p<0.05), 180 min, 210 min (p<0.01) and 240 min (p<0.001)]than group-VI [180 min, 210 min (p<0.05) and 240 min (p<0.01)]. The results were shown in Table no: 3 and Graph no: 3.

C) Effect of EETP on akinesia

Akinesia was characterized by impaired ability to initiate movements (or) loss of motor functions. The group (G-II) treated with haloperidol (1 mg/kg, p.o.) showed a significant decrease (p<0.001) in the initiated movements when compared to the normal group (G-I) (p<0.001). Treatment with standard drug L-Dopa & Carbidopa (100mg + 25 mg/kg, p.o.) (G-III) showed a significant increase (p<0.001) in the initiated movements when compared to the control (G-II). Treatment with EETP (200 mg/kg and 400 mg/kg) to group IV and V showed a significant and dose-dependent increase in the initiated movements when compared to the control group (G-II) (p<0.01) (p<0.001). The results were shown in Table no: 4 and Graph no: 4

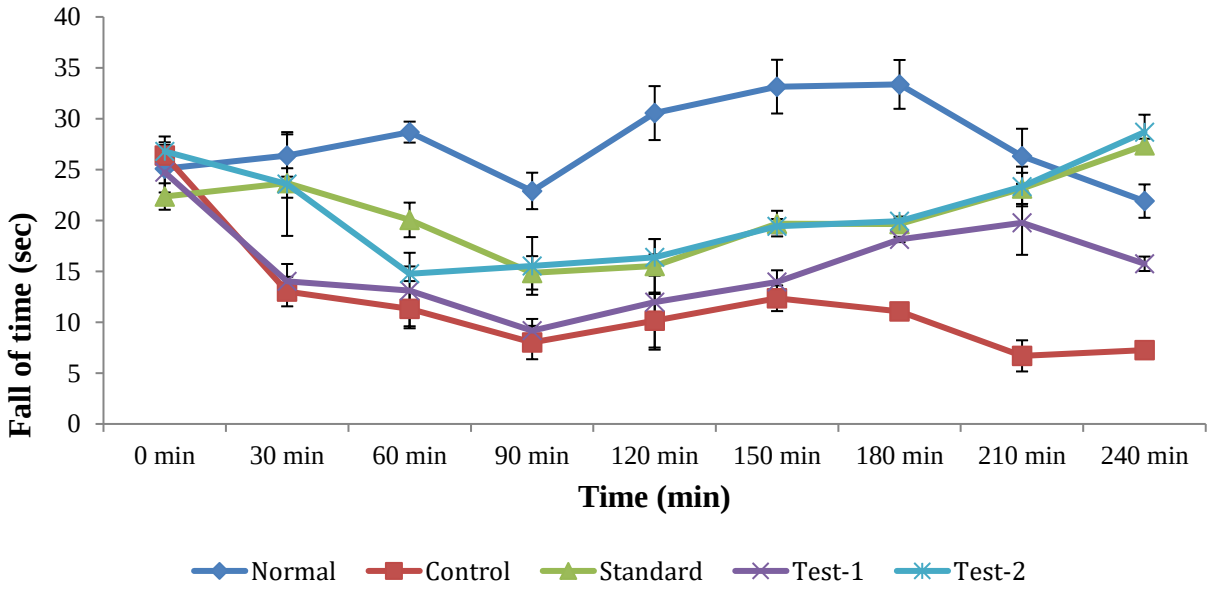
Table no: 3 Effect of EETP on muscle rigidity by using rota rod

Groups/Treatment - Fall of time (Sec) (Mean ± S.E.M) on 21st day	Normal 1% CMC (p.o.)	Control Haloperidol (1 mg/kg, p.o.)	Standard L-Dopa & Carbidopa (100+25 mg/kg, p.o.) +Haloperidol (1 mg/kg,p.o.)	Test 1 EETP (200 mg/kg, p.o.) +Haloperidol (1 mg/kg,p.o.)	Test 2 EETP (400 mg/kg, p.o.) +Haloperidol (1 mg/kg, p.o.)
0min	25.08±2.33	26.38 ± 1.3	22.35±1.79	24.73±2.63	26.78±1.47
30min	26.38±2.08	13.01±1.45	23.68±3.96	14.03±1.69	23.58±5.1
60 min	28.68±1.03	11.3±1.7**	20.05±2.23	13.11±3.71	14.76±0.72
90 min	22.9 ± 1.79	8 ± 1.64***	14.85±0.89	9.16±1.16	15.53±2.84
120 min	30.55 ± 2.65	10.14±2.63***	15.54±2.49	11.98±4.68	16.37 ± 1.8*
150 min	33.15 ± 2.64	12.35±1.26***	19.69±0.92*	13.97±1.13	19.44 ± 0.7*
180 min	33.37 ± 2.4	11.06±0.5***	19.64±2.35**	18.13±0.26*	19.94±0.44**

210 min	26.30 ± 2.71	6.69±1.53***	23.15±1.09***	19.78±3.16**	23.34±1.95***
240 min	21.90± 1.64	7.26±0.64***	27.39±1.12***	15.74±0.71**	28.68±1.71***

n=6 animals in each group; All values were expressed as Mean±S.E.M. ### indicates P<0.001; ## indicates P<0.01, # indicates P<0.05 vs. normal; *** indicates P<0.001; ** indicates P<0.01; * indicates P<0.05 vs. control.

Graph no: 3 Effect of EETP on muscle rigidity by using rota rod



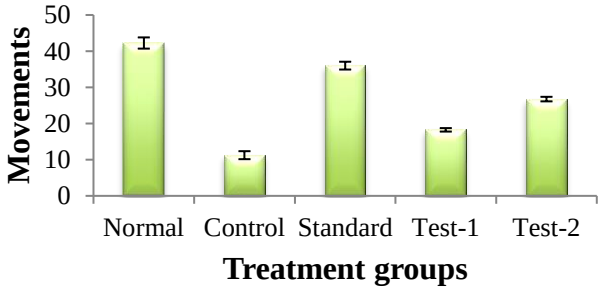
Normal = vehicle treated; Control = Haloperidol (1 mg/kg, p.o.); Standard = L-Dopa & Carbidopa (100 + 25 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.); Test-1 = EETP (200 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.); Test-2 = EETP (400 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.).

Table no: 4 Effect of EETP on Akinesia

Groups	No. of Movements On 21st day
Normal : 1% CMC (p.o.)	42.25 ± 1.54
Control : Haloperidol (1 mg/kg, p.o.)	11.25 ± 1.1$^{###}$
Standard :L-Dopa & Carbidopa (100+25 mg/kg,p.o.)+Haloperidol (1 mg/kg, p.o.)	36 ± 1.08***
Test 1 : EETP (200 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.)	18.25 ± 0.47**
Test 2 : EETP (400 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.)	26.75 ± 0.62***

n=6 animals in each group; All the values indicates P<0.001 vs. normal, *** indicates P<0.001; ** indicates P<0.01 vs. control.

Graph no: 4 Effect of EETP on akinesia



Normal = vehicle treated; Control = Haloperidol (1 mg/kg, p.o.); Standard = L-Dopa & Carbidopa (100 + 25 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.); Test-1 = EETP (200 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.); Test-2 = EETP (400 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.).

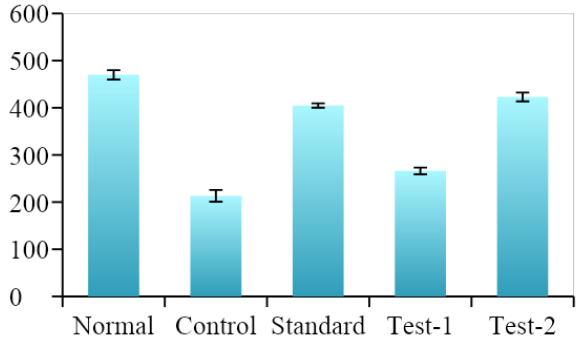
C) Effect of EETP on open field test

Locomotion was measured by an open field test.

a. Peripheral movements

Administration of haloperidol (1 mg/kg, p.o.) induced a significant decrease (p<0.001) in peripheral movements in the control group (G-II) when compared to the normal group (G-I). On treatment with L-Dopa&carbidopa (100mg + 25 mg/kg, p.o.) induced a significant increase in peripheral movements in standard group (G-III) when compared to control group (G-II).Groups (IV and V) treated with EETP induced a significant increase in peripheral movements. Interestingly the group-V showed more significance (p<0.001) than group-IV compared to control (G-II). The results were shown in Table no: 5 and Graph no: 5.

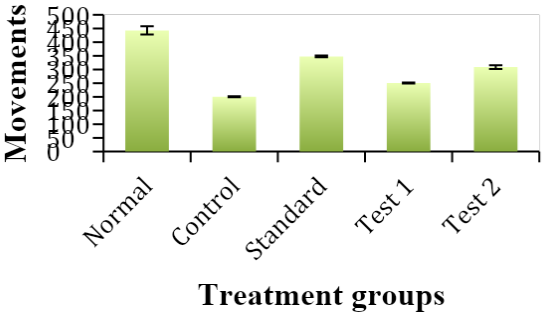
Graph no: 5 Effect of EETP on peripheral movements



b. Central movements

Haloperidol receiving groups (G-II) showed a significant decrease (p<0.001) in central movements when compared to normal groups (G-I). L-Dopa & Carbidopa receiving group (G-III) showed a significant increase (p<0.001) in central movements when compared to control group (G-II).EETP treated groups (IV and V) showed a significant increase in central movements in both doses has shown dose dependent when compared to control group (G-II) (p<0.01), (p<0.001). The results were shown in Table no: 5 and Graph no: 6.

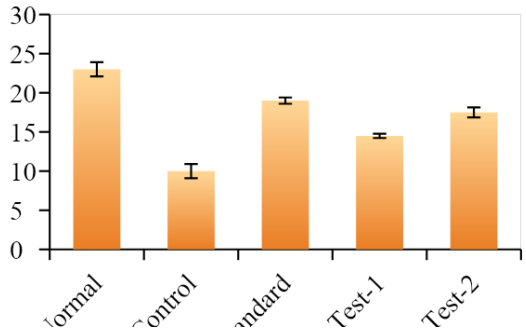
Graph no: 6 Effect of EETP on central movements



c. Rearings

The control group (G -II) receiving haloperidol (1 mg/kg, p.o) showed 10 ± 0.91 decreased Rearings which was more significant ($p<0.001$) compared to normal group (G-I) showed the Rearings as 23 ± 0.91. The standard group (G-III) receiving L-Dopa&carbidopa (100mg + 25 mg/kg, p.o), showed as 19 ± 0.4 a significant ($p<0.001$) increase in the rearings when compared with control group (G- II).The group (G- IV) receiving EETP (200 mg/kg, p.o) showed 14.5 ± 0.28 ($p<0.01$) increased the rearings when compared to control group (G-II), but the group (G-V) receiving EETP (400 mg/kg, p.o), showed 17.5 ± 0.64 increased the rearings which was more significant ($p<0.001$) than group-IV compare to control group. The results were shown in Table no: 5 and Graph no: 7.

Graph no: 7 Effect of EETP on rearings



d. Groomings

A significant decrease in the number of grooming was observed in rats treated with haloperidol (1 mg/kg, p.o) (G-II) ($p<0.001$) when compared to the normal group (G-I). The group-III rats were treated with standard drug L-Dopa & Carbidopa($p<0.001$) showed a significant increase in the number of grooms when compared to the control group (G-II). The groups (IV and V) receiving EETP (200 mg/kg and 400 mg/kg) showed a significant increase ($p<0.01$), ($p<0.001$) in the number of groomings in both doses, which is dose dependent when compared to the control group (G-II). The results were shown in Table no: 5 and Graph no:8

Graph no: 8 Effect of EETP on groomings

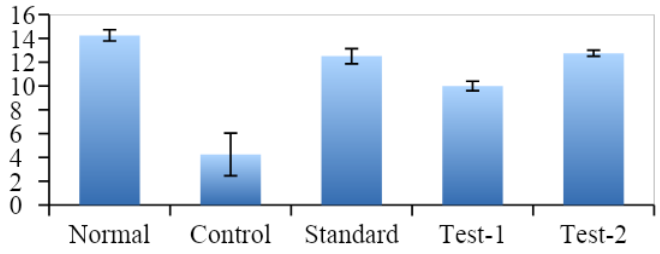


Table no: 5 Effect of EETP on open field test

n=6 animals in each group; All values were expressed as Mean±S.E.M. *** indicates P<0.001 vs. normal; *** indicates P<0.001; ** indicates P<0.01 vs. control

Groups/Treatment - (Mean ± S.E.M) on 21st day	Peripheral movements (PMs/5min)	Central movements (CMs/5 min)	Rearings (5 min)	Groomings (5 min)
Normal: 1% CMC (p.o.)	469.8 ± 9.95	443 ± 14.91	23 ± 0.91	14.25 ± 1.79
Control: Haloperidol (1 mg/kg, p.o.)	213.3 ± 12.34***	200.5 ± 2.21***	10 ± 0.91***	4.25 ± 0.47***
Standard: L-Dopa & Carbidopa (100+25 mg/kg, p.o.) +Haloperidol (1 mg/kg,p.o.)	404.8 ± 4.6***	348 ± 3.3***	19 ± 0.4***	12.5 ± 0.64***
Test 1: EETP (200 mg/kg, p.o.) +Haloperidol (1 mg/kg,p.o.)	266 ± 7.16**	251 ± 1.73**	14.5 ± 0.28**	10 ± 0.40**
Test 2: EETP (400 mg/kg, p.o.) +Haloperidol (1 mg/kg, p.o.)	423 ± 9.29***	309 ± 6.8***	17.5 ± 0.64***	12.75 ± 0.25***

EETP (400 mg/kg, p.o.) + Haloperidol (1 mg/kg, p.o.).

D) Effect of EETP on salivation

Salivation was characterized by dropping of saliva uncontrollably from the mouth. The large volume of saliva was dropped uncontrollably from the mouth in the haloperidol (1 mg/kg, p.o.) treated rats in the control group compared to normal group (G-I). The group-III rats treated with standard drug L-Dopa & carbidopa (100mg + 25 mg/kg, p.o), showed decreasing the drop of saliva from mouth compare to control group.The groups (IV and V) receiving EETP (200 mg/kg and 400 mg/kg, p.o.) showed decreasing the drop of saliva from

mouth compare to control group. Interestingly group-V rats show better capability for decreasing the dropping of saliva from mouth than group-IV compared to control group (G-II) as shown in table no. 6.

E) Effect of EETP on lacrimation

Lacrimation was characterized by excessive secretion of tears. Tears secretion was decreased in Parkinson's disease. The haloperidol (1 mg/kg, p.o.) treated rats showed less amount of tears secretion in eyes when compared to normal group (G-I). The group-III rats treated with standard drug L-Dopa & Carbidopa (100mg + 25 mg/kg, p.o.) showed increasing the tears secretion in eyes compare to control group (G-II).The groups (IV and V) receiving EETP (200 mg/kg and 400 mg/kg, p.o.) showed increasing the secretion of tears in eyes when compared to control group (G-II). Interestingly group-V rats have shown better capability for increasing the secretion of tears than group-IV compared to control group (G-II) as shown in table no.6.

F) Effect of EETP on tremors

Tremor was characterized by involuntary quivering movement of the whole body or any parts of the body. The excessive involuntary movements appeared in control group (G-II) rats treated with haloperidol (1 mg/kg, p.o.) when compared to the normal group (G-I). The group-III rats treated with standard drug L-Dopa & Carbidopa (100mg + 25 mg/kg, p.o.) showed the decreasing of involuntary movements when compared to the control group (G-II).

The groups (IV and V) receiving EETP (200 mg/kg and 400 mg/kg, p.o.) showed the decreasing of involuntary movements at both doses has shown dose dependent when compared to control group (G-II) as shown in table no.6

4. DISCUSSIONS

Neurodegenerative diseases are neurological disorders that are linked to injuries related to the brain. Selective somatic cell loss specially regions of our brain causes differing kinds of neurodegenerative disorders like Alzheimer's disease (AD), Parkinson's malady (PD), stroke, amyotrophic lateral pathology (ALS), and plenty of others.[17]

Haloperidol works by antagonizing dopamine D_2 and, to a limit lesser to D_1 The resultant block of striatal dopamine transmission results in abnormal downstream firing within the basal ganglia circuits that is manifest as symptoms of muscle rigidity and catalepsy within 60 min of haloperidol (0.5– 5 mg/kg-1, i.p.) injection. Although rigidity is a feature of PD, providing this model with some face validity, catalepsy, which is expressed as the inability of an animal to correct itself from an abnormally imposed posture, is not directly associated with PD.[18],[19]

Anti-Parkinson's activity of *Terminalia pallida* was evaluated by measuring following parameters like catalepsy (block method & metal bar test), muscle rigidity, akinesia, locomotion (by open field test) behavioral observations like drooling, lacrimation, tremors. Every result was compared with the standard drugs i.e. L-Dopa & carbidopa (100 + 25 mg/kg, p.o.) to demonstrate anti-Parkinson's activity.EETP at doses of 200 and 400 mg/kg significantly and dose-dependently decreased in muscle rigidity and motor impairment. L- Dopa & carbidopa (100 + 25 mg/kg, p.o) significantly decrease muscle rigidity and motor impairment rats. The catalepsy was measured by block method and metal bar test. In both methods EETP at the dose of 200mg/kg and 400 mg/kg b.w along with haloperidol at a dose 1mg/kg b.w showed a significant and dose dependent

decrease in immobility and muscle impairment when compared to the control group. Amazingly the EETP at 400 mg/kg b.w showed comparable results with standard L-Dopa & carbidopa. Similar results were reported with *Nardostachys jatamansi* reported by Rasheed Ahemad *et al*., 2012. The muscle rigidity was measured by using a rota-rod apparatus. Haloperidol treatment results in muscle rigidity due to depolarizing blockade of neuromuscular transmitters like dopamine. EETP showed significant and dose dependent reduction in fall off time of rats (sec) on revolving rota rod which clearly indicates role of EETP treatment to dopamine enhancement. *Terminalia pallida* at 400 mg/kg b.w showed comparable results with standard L-Dopa & carbidopa.

Akinesia was characterized by impaired ability to initiate movements (or) loss of motor functions.[20] It was measured by standing and movements of forelimbs on their own. The EETP showed significant and dose dependent increase in the movements of forelimbs in 30 sec of time compared to the control group. Thus, impaired ability to initiate movements (or) loss of motor functions may be produced due to depolarizing blockade of neuromuscular transmitters like dopamine.

The locomotion and exploration was measured by open field test. In Parkinson's condition the locomotion movements are decreased because the serotonin levels are decreased in hypothalamus due to decrease of dopamine levels in substantia nigra in the brain. EETP (200 mg/kg & 400 mg/kg) showed significant and dose dependent increase in locomotion as compared to the control group. This may be due to increased levels of serotonin in the hypothalamus in the brain.

The cause of salivation in PD is not fully understood but it is thought to be related to reduced movements of swallowing musculature which results in less swallowing. This decrease in spontaneous or reflexive swallowing results in an excess of saliva in the mouth. A tendency to have lips open and/ or lean forward increases the amount of saliva that leaves the mouth. The EETP (200 mg/kg & 400 mg/kg) showed significant and dose-dependent decrease in salivation by increasing the swallowing movements of musculature as compared to the control group.

In Lacrimation condition the secretion of tears can be reduced in PD. Dry eyes and contrast sensitivity defects seem to be related to the severity of the disease and can be partially corrected by treatment with L-Dopa or the direct dopamine receptor agonist, apomorphine. Interestingly, L-Dopa has recently been reported to improve visual acuity in amblyopic subjects, but the mechanism is not clear. The secretion of tears can be increased significantly and dose dependently by treating with EETP (200 mg/kg & 400 mg/kg) as compared to the control group.

The formation of tremors is more in Parkinson's condition by the degradation of dopamine in basal ganglia or in substantia nigra. Dopamine is the cause for movements. The dopamine turnover causes tremors in hands, limbs and the whole body. The control group rats showed more tremors. The EETP (200 mg/kg & 400 mg/kg) showed significant and dose dependent decrease in the tremors as compared to the control group.

The oxidative stress occurs in PD by the realization of the metabolism of dopamine, by chemical or enzymatic means can generate free radicals and other reactive oxygen species via autoxidation and dopamine oxidation by monoamine oxidase B. The loss of dopaminergic neurons in PD results in enhanced metabolism of dopamine, augmenting the formation of H_2O_2 thus leading to the generation of highly neurotoxic hydroxyl radicals. Low activity of mitochondrial complex- 1 in PD also results in generation of oxygen species.

In nutshell in the present study, following a single injection of haloperidol (1 mg/kg, p.o.) induces catalepsy and rigidity in rats at 22nd day. *T.pallida* of both doses (200 mg/kg, 400 mg/kg) showed remarkable improvement in muscle impairment, loco motor action in various experimental models. This may be due to increasing the dopamine and serotonin levels by *T.Pallida.* At higher doses, the results were comparable with standard treatment. Phytochemical investigations on *Terminalia pallida* have shown the presence of large concentration of flavonoids like quercetin-3 galactoside, quercetin-3-glucoside, quercetin-3- rhamnoside, phenols, antioxidant compounds like vit A, C, E, K, catechin, epicatechin, procyanidin, cyanidin-3- galactoside, coumaric acid, chlorogenic acid, gallic acid, and phloridzin.

5. CONCLUSION

The neuroprotective activity of *Terminalia pallida* could be due to its flavonoid and antioxidant components. These components may exert its anti- Parkinson's by increasing the dopamine and serotonin levels in the brain. The presence of these compounds in ethanolic extract of *Terminalia pallida* may be involved in increasing the dopamine levels in the brain thus it showed anti-Parkinson's properties of this fruit. Taken together, our results strongly support the anti-Parkinson's potential of the *Terminalia pallida* and its use in traditional medicine.

The present study clearly indicates that the fruit of *Terminalia pallida* may be used as an effective anti-Parkinson's agent. Further studies on isolation and structural determination of active principles might be worthy.

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