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Neuroprotective Evaluation of *Tephrosia purpurea* on Chlorpromazine Induced Parkinson Disease in Animal Model

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ABSTRACT

Aim: The present study was performed to describe the neuroprotective effect of ethanolic extract of *Tephrosia purpurea* (EET) in chlorpromazine (CPZ) induced experimental animal model.

Methods: In this study effects of *Tephrosia purpurea* (200 and 400 mg/kg, i.p.) were studied using various behavior parameters like catalepsy (Bar test), muscle rigidity (Rotarod test), locomotor activity (Open field) and histopathological studies. Chlorpromazine significantly induces motor dysfunctions (catalepsy, muscle rigidity, and hypolocomotion) in experimental animals.

Results: The EET at a dose of 200 and 400 mg/kg, i.p. significantly and dose dependently reduced catalepsy, muscle rigidity and significantly increased locomotor activity in rats. The maximum decline was observed at a dose of 400 mg/kg (i.p.).

Conclusion: Thus the present study showed the neuroprotective effect of EET against CPZ induced Parkinson's disease-like symptoms.

Keywords: Catalepsy, Chlorpromazine, Locomotor activity, *Tephrosia purpurea*.

INTRODUCTION

Parkinson's disease is a chronic neurological disorder. In ventral midbrain, particularly in substantia nigra pathological features show that dopaminergic neurons progressively degenerate, which causes a consequent reduction of dopamine (DA) levels in the striatum. The functions of acetylcholine neurons and dopaminergic neurons in striatum are out of balance, which leads to PD. The patients have some characteristic symptoms, such as tremor, myotonia, and dyskinesia, etc [1]. Pathologically, Parkinson disease may cause depletion of dopamine in brain due to the presence of intracytoplasmic inclusions known as Lewy bodies. It is not clear why Lewy body formation causes neuronal cell death. These pathological changes are also seen in the locus coeruleus and parasympathetic as well as sympathetic postganglionic neurons, pedunculopontine

nucleus, raphe nucleus, and dorsal motor nucleus of the vagal nerve [2].

The Parkinson disease can be treated with various drugs including levodopa, carbidopa, orphenadrine, benztropine, selegiline, pergola, and many more which act by reversing the symptoms of Parkinson's disease but these drugs possess various side effects like nausea and vomiting, respiratory disturbances, hallucinations, orange discoloration of saliva and urine, mania, dyskinesia convulsions and anxiety, arrhythmia, mydriasis, dry mouth, sore throat, and transient dizziness on long term use [3].

Tephrosia purpurea is a polymorphic, much-branched suberect perennial herb, popularly known as "Sarapunkha" in Sanskrit, "Purpule tephrosia" in

English and “Vempali” in telugu. Experimental studies have demonstrated its analgesic [4], antihyperglycemic [5], antiulcer [6] and hepatoprotective effects. To our best knowledge there was no scientific data on neuroprotective effect of *T. Purpurea* in Chlorpromazine (CPZ) induced Parkinsonism. Hence, the present study was designed to evaluate anti-parkinson effect of *T. purpurea* in CPZ induced Parkinson's rats.

MATERIALS AND METHODS

Animals:

Experiments were carried out on Wistar rats (150–200 g) of either sex and were obtained from the Raghavendra enterprises (Bangalore, India). They were housed under standard light/dark cycle and fed with standard pellet diet and water *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Chemicals:

Chlorpromazine, levodopa, and carbidopa were purchased from Sigma Aldrich, Bangalore. All chemicals are of analytical grade and were obtained from SD fine chemicals Ltd Mumbai.

Plant Material:

The roots were collected from Kadapa, Andhra Pradesh. It was identified and authenticated by Dr.K.Madhava Chetty, Assistant Professor, Department of Botany, S.V University, Tirupati. The voucher specimen of the plant was kept in Department of Pharmacology, PRRM College of Pharmacy, Kadapa, Andhra Pradesh. The roots were cleaned, then by cutting them into small pieces and it was dried in shade and then subjected to coarse powdering and passed through a sieve #44 to get uniform powder size. The collected powder was extracted with 95% of alcohol by soxhlet apparatus. After the extraction, solvent was distilled, dried by lyophilization and stored in air tight container under refrigeration.

Methodology

Rats were randomly allocated into five groups each containing 6 animals. Animals of Group I were administered with 1% gum acacia as a vehicle and served as normal group. Group II animals were administered with chlorpromazine 3 mg/kg, i.p. (dissolved with 1% gum acacia in distilled water suspension) on 21st day, and served as the control group. Group III animals received the combination of carbidopa + levodopa (100mg+25mg/kg p.o.) and served as standard group. Group IV and Group V

animals were administered with EET 200 mg/kg i.p. and 400 mg/kg i.p., respectively, for a period of 21 days. Chlorpromazine was given 30 minutes prior to standard and test drug. Behavioral assessments were carried out at the end of experiment. Various parameters like catalepsy, locomotor activity, and muscle activity were measured in all animals [7].

Histopathological studies:

The brains from control and experimental groups were fixed with 10% formalin and embedded in paraffin wax and cut into longitudinal section of 5 μ m thickness. The sections were stained with haematoxylin and eosin dye for histopathological observation.

Statistical Analysis:

Data were analyzed with one-way ANOVAs followed by Tukey's test, with significance set to $P < 0.05$.

RESULTS

Selection of dose:

Tephrosia purpurea at a dose of 200 mg/kg and 400 mg/kg were used for the present study according to the earlier studies of Rambabu *et al.*, reporting anti-inflammatory and analgesic activity of *T. Purpurea* [8].

Effect of EET on Chlorpromazine Induced Catalepsy in Rats

Standard group showed a significant reduction in the cataleptic behavior on 90 min ($p < 0.05$), 120 min, 150 min ($p < 0.01$), 180 min, 210 min and 240 min ($p < 0.001$) as compared to the control group. Treatment with *T.purpurea* showed a significant reduction in the duration of cataleptic behavior dose dependently when compared to CPZ treated group. But interestingly the high dose (400 mg/kg, p.o.) shows good significant decrease in immobility and muscular rigidity at 120 min, 150 min ($p < 0.05$), 180 min ($p < 0.01$), 210 min and 240 min ($p < 0.001$) than low dose (200 mg/kg, p.o.) [180 min ($p < 0.05$), 210 min and 240 min ($p < 0.01$)] (Table 1) (Figure 1).

Effect of EET on Chlorpromazine Induced Muscle Rigidity in Rats

CPZ treated group shows significantly decreased in motor coordination and body balance when compared to normal rats. Pretreatment with EET (200 and 400 mg/kg, p.o.) in CPZ induced rats significantly improved the motor coordination and body balance and showed increase in latency to balance on the beam significantly (Table 2) (Figure 2).

Effect of EET on locomotor activity

In locomotor activity, a significant ($P < 0.001$) decrease in Peripheral movements, Central movements,

Table 1: Effect of EET on catalepsy in rats

Groups	Catalepsy Score (mean $\pm$ SEM) on 21st day								
	0 Min	30 Min	60 Min	90 Min	120 Min	150 Min	180 Min	210 Min	240 Min
I	5.75 $\pm$ 1.79	3.50 $\pm$ 0.64	4.00 $\pm$ 0.91	4.50 $\pm$ 0.64	2.25 $\pm$ 0.85	3.00 $\pm$ 0.40	6.0 $\pm$ 0.91	3.0 $\pm$ 1.08	4.25 $\pm$ 2.39
II	4.25 $\pm$ 0.75	54.00 $\pm$ 5.44 ^{##}	98.00 $\pm$ 5.44 ^{###}	132.5 $\pm$ 5.51 ^{###}	139.3 $\pm$ 1.93 ^{###}	149.0 $\pm$ 6.98 ^{###}	162.8 $\pm$ 6.78 ^{###}	166.0 $\pm$ 7.64 ^{###}	162.3 $\pm$ 6.96 ^{###}
III	4.25 $\pm$ 0.85	43.50 $\pm$ 4.19	82.50 $\pm$ 5.05	112.8 $\pm$ 3.56 [*]	123.3 $\pm$ 3.35 ^{**}	122.3 $\pm$ 3.44 ^{**}	76 $\pm$ 4.93 ^{***}	46.0 $\pm$ 2.19 ^{***}	39.0 $\pm$ 2.27 ^{***}
IV	1.75 $\pm$ 0.47	46.00 $\pm$ 4.50	93.75 $\pm$ 6.75	130.5 $\pm$ 4.66	127.3 $\pm$ 4.51	128.8 $\pm$ 2.78	137.8 $\pm$ 2.81 [*]	111.8 $\pm$ 5.4 ^{**}	123.3 $\pm$ 2.27 ^{**}
V	2.51 $\pm$ 0.50	47.75 $\pm$ 3.14	99.25 $\pm$ 6.48	124.8 $\pm$ 2.17	124.8 $\pm$ 2.68 [*]	124.8 $\pm$ 2.09 [*]	98.75 $\pm$ 4.21 ^{**}	71.50 $\pm$ 2.25 ^{***}	74.50 $\pm$ 3.75 ^{***}

All values were expressed as mean $\pm$ SEM ($n=6$). ^{###} indicates $P<0.001$; ^{##} indicates $P<0.01$ vs. normal

^{***} indicates $P<0.001$; ^{**} indicates $P<0.01$; ^{*} indicates $P<0.05$ vs. control

Table 2: Effect of EET on muscle rigidity in rats

Groups	Fall of time (Sec) (mean $\pm$ S.E.M) on 21st day								
	0 Min	30 Min	60 Min	90 Min	120 Min	150 Min	180 Min	210 Min	240 Min
I	178.3 $\pm$ 1.18	180.0 $\pm$ 0.0	169.0 $\pm$ 5.21	174.8 $\pm$ 5.25	180.0 $\pm$ 0.0	168.3 $\pm$ 3.79	174.0 $\pm$ 3.83	180.0 $\pm$ 0.0	172.3 $\pm$ 7.75
II	180 $\pm$ 0.0	116.5 $\pm$ 2.63 ^{##}	86.50 $\pm$ 2.98 ^{##}	54.75 $\pm$ 2.09 ^{###}	37.75 $\pm$ 3.59 ^{###}	28.5 $\pm$ 4.71 ^{###}	24.75 $\pm$ 1.31 ^{###}	29.25 $\pm$ 1.54 ^{###}	29.5 $\pm$ 2.21 ^{###}
III	174.0 $\pm$ 4.49	138.0 $\pm$ 6.55	89.75 $\pm$ 5.02	77.0 $\pm$ 6.78 [*]	61.50 $\pm$ 5.37 ^{**}	61.5 $\pm$ 4.05 ^{**}	66.25 $\pm$ 2.81 ^{***}	116.8 $\pm$ 3.96 ^{***}	114.0 $\pm$ 4.23 ^{***}
IV	164 $\pm$ 7.67	112.5 $\pm$ 6.99	69.0 $\pm$ 5.87	56.25 $\pm$ 1.93	32.37 $\pm$ 5.26	41.25 $\pm$ 3.25 [*]	37.25 $\pm$ 1.93 [*]	49.25 $\pm$ 1.54 ^{**}	61.5 $\pm$ 3.43 ^{**}
V	180.0 $\pm$ 0.0	122.0 $\pm$ 5.32	77.50 $\pm$ 5.12	68.75 $\pm$ 2.89	56.0 $\pm$ 3.69 [*]	54.75 $\pm$ 2.52 ^{**}	43.0 $\pm$ 2.04 ^{**}	74.5 $\pm$ 4.51 ^{***}	128.5 $\pm$ 2.36 ^{***}

All values were expressed as mean $\pm$ SEM ($n=6$). ^{###} indicates $P<0.001$; ^{##} indicates $P<0.01$ vs. normal

^{***} indicates $P<0.001$; ^{**} indicates $P<0.01$; ^{*} indicates $P<0.05$ vs. control

Figure 1: Effect of EET on catalepsy

Figure 2: Effect of EET on muscle rigidity

Table 3: Effect of EET on Locomotor activity

Groups	On 21st day (mean $\pm$ S.E.M)			
	Peripheral movements (5 min)	Central movements (5min)	Rearings (5 min)	Groomings (5 min)
I	409.8 $\pm$ 18.17	319.8 $\pm$ 9.81	36.75 $\pm$ 2.39	16.50 $\pm$ 0.95
II	196.3 $\pm$ 12.18 ^{###}	153.8 $\pm$ 6.32 ^{###}	13.25 $\pm$ 1.88 ^{###}	4.5 $\pm$ 0.86 ^{###}
III	369.5 $\pm$ 19.53 ^{***}	266.8 $\pm$ 10.98 ^{***}	29.75 $\pm$ 1.75 ^{***}	14.75 $\pm$ 0.47 ^{***}
IV	303.00 $\pm$ 21.84 ^{**}	212 $\pm$ 9.29 ^{**}	23.75 $\pm$ 1.61 ^{**}	10.25 $\pm$ 0.85 ^{**}
V	335.5 $\pm$ 21.03 ^{***}	246 $\pm$ 8.33 ^{***}	27.50 $\pm$ 1.44 ^{***}	13.50 $\pm$ 1.19 ^{***}

All values were expressed as mean $\pm$ SEM (n=6). ^{###} indicates P<0.001; ^{##} indicates P<0.01 vs. normal

^{***} indicates P<0.001; ^{**} indicates P<0.01; ^{*} indicates P<0.05 vs. control

Group I **Group II** **Group III** **Group IV** **Group V****Figure 3: Histopathology of hippocampus region**

C = Congestion
 NC = Neural cells
 DC = Degenerative changes
 RC = Regenerated Cells

Rearings, and Groomings was seen in control group as compared with normal group and EET treatment increased it significantly ($P < 0.001$) (Table 3).

Histopathological Studies

The histopathological study confirmed the neuroprotective activity of EET as a significant recovery of neuronal damage and decreased necrosis was evident (Figure 3).

DISCUSSION

Parkinson is a brain disorder which is clinically characterized by bradykinesia, resting tremor, and postural instability. In Parkinson disease the depletion of dopamine (DA) may occur. Parkinson disease occurs due to the degeneration of dopaminergic neurons in the substantia nigra pars compacta [9]. Various plants have been used for the treatment of neurodegenerative disease like Parkinson disease. Various animal models have been developed for the evaluation of Parkinson's disease and are generated through the administration of toxins. The purpose of the present study was to evaluate the antiparkinson activity of *T.purpurea* in rats treated with chlorpromazine. Chlorpromazine is one of the antipsychotic drugs to treat both acute psychosis and chronic psychosis. It has been associated with side effects such as antidopaminergic extra pyramidal syndromes [10]. Chlorpromazine induced Parkinsonism by interfering with the storage of catecholamines in intracellular granules which may cause monoamine depletion in nerve terminals and in the induction of hypolocomotion and muscular rigidity [11]. There was a significant increase in catalepsy, and a decrease in movements following chlorpromazine administration to rats. The present data suggested that chlorpromazine developed Parkinson's disease-like behavioral symptoms in rats. Treatment with *T.purpurea*, shows dose dependently reduced the catalepsy in chlorpromazine-treated rats. The protective effect of *T.purpurea* at the doses of 200 and 400 mg/kg against chlorpromazine induced catalepsy suggested that this plant has influence on the dopaminergic receptor mediated neurotransmission. The muscular rigidity tested using a rota-rod, is an established method used for the assessment of neurological deficits in rodents [12]. Significantly enhanced muscular coordination was seen in EET treated groups, as compared to control group. Hypolocomotion, a main symptom of PD was studied by open field test by monitoring the locomotor activities of animals. Significant improvement of locomotor activity was observed by increased Peripheral movements, Central movements, Rearings, and Groomings in ethanol extract of *T.purpurea* treated animals. These behavioral parameters reveal an

enhanced motor function, which is usually disturbed in PD.

Histopathological findings showed haloperidol treated group showed the congestion of degenerative changes due to decrease the number of neural cells in SNpc in brain tissue. Pre-treatment with EET (200, 400 mg/kg) rats showed regenerative changes in SNpc in brain. Surprisingly high dose treated rats showed almost normal architecture of SNpc in brain tissue, further substantiates the neuroprotective activity against haloperidol induced Parkinson's disease model.

Conclusion

T.purpurea showed a promising effect in animals with Parkinson's disease. The above findings suggest that *T.purpurea* may offer a safer therapeutic approach to the treatment of Parkinson's disease.

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