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An Effective Novel Strategy of Combined Plants Extract for the Treatment of Parkinson's Disease

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ABSTRACT

The aim of present study was an effective novel strategy of combined plants extract for the treatment of Parkinson's disease, using Amla Withania and Tulsi extract in different ratio. The extracts were prepared by using solvents like 60% ethanol for Amla fruit, 50% ethanol for Withania root and pure ethanol for Tulsi aerial part. Haloperidol was used to induce catalepsy and Akinesia. The ability of these combined extracts in inhibition of haloperidol-induced catalepsy and Akinesia in rodent models of Parkinsonian symptoms also Motor co-ordination test was conducted using a rota rod apparatus. It was found that treatment with the extract significantly reduced catalepsy according to dose. In the second experiment haloperidol was used to suppress locomotor activity in test. Treatment with extract significantly increased locomotion reduced by haloperidol according to dose.

Keywords: Amla fruits, Withania roots, Tulsi aerial parts, Extracts, Parkinson's disease.

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INTRODUCTION

Parkinson's disease is a progressive neurodegenerative disorder characterized by massive depletion of dopamine as a result of degeneration of dopaminergic neurons in the substantia nigra. The main symptoms of Parkinson's disease are rigidity, akinesia, bradykinesia, resting tremor and disturbance of posture and gait. Parkinson's disease affects movement, producing motor symptoms Non-motor symptoms, which include autonomic dysfunction, neuropsychiatric problems (mood, cognition, behavior or thought alterations), and sensory and sleep difficulties, are also common.

Tremor is the most apparent and well-known symptom. It is the most common, though around 30% of individuals with Parkinson's disease do not have tremor at disease onset, most develop it as the disease progresses. It is usually a rest tremor, maximal when the limb is at rest and disappearing with voluntary movement and sleep. Bradykinesia (slowness of movement) is another characteristic feature of PD, and is associated with difficulties along the whole course of the movement process, from planning to initiation and finally execution of a movement. Rigidity is stiffness and resistance to limb movement caused by increased muscle tone, an excessive and continuous contraction of muscles. Postural instability is typical in the late stages of the disease, leading to impaired balance and frequent falls, and secondarily to bone fractures. Festination (rapid shuffling steps and a forward-flexed posture when walking) speech and swallowing disturbances including voice disorders, mask-like face expression or small handwriting, although the range of possible motor problems that can appear is large.[1] The pathogenesis of Parkinson's disease remains still unclear. Perhaps, not a single factor but a combination of several factors such as aging, oxidative stress, mitochondrial dysfunction and apoptosis may be responsible for neurodegeneration.[2] Parkinson's disease takes a long course and may last for decades, affecting large populations of the old aged and severely reducing the quality of life. Thus, a long-term therapy is required to repair degenerated neurons and maintain the healthy ones. Therefore, there is a need to develop safe and effective formulation.[3]

Our objectives for the present work are to check whether the combined plants extract would be able to control Parkinson's disease in experimental animals. In this study we explored the potential of extract in haloperidol induced PD in rat model following p.o. administration.

MATERIALS AND METHOD

The crude dried fruits of Amla and dried roots of Withania were procured from jonadumbalaya ayurvedic store, Begumbazar, and fresh aerial parts of the plant Tulsi were collected from Herbal Garden, Sun City, Hyderabad, Telangana. The three plant samples were authenticated by Botanical

survey of India, Deccan regional centre Hyderabad-500048, Telangana, India, with reference number BSI/DRC/2015-16/Tech./734.

Preparation of extracts:

Procured plant materials Amla, Withania and Tulsi were properly washed with distilled water and finally allowed to dry under shade, and then coarsely powdered in a blender. The coarse powder (500 gm each) were subjected to maceration for 72 hours, followed by exhaustive maceration for 48 hours and 12 hours, by using various solvents like 60% ethanol for Amla, 50% ethanol for Withania and pure ethanol for Tulsi. The solvents were decanted and filtered with help of filter paper and recovered by rotary vacuum evaporator. Finally extracts were dried under desiccators.

Animals:

Wistar rats (180-250g) of either sex were used for this study. Animals were obtained from in house facility and housed in the room on an artificial light/dark cycle (12/12 hr, light on from 7 a.m. to 7 p.m.), under standard conditions with free access to food and water. The study was performed in accordance with the guidelines issued by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) an authority regulating animal experiments and was approved by the Institutional Animal Ethics Committee Reg. No. 1864/PO/RE/S/16/CPCSEA formed as per CPCSEA guidelines.

Drugs and dosage:

The test drug, Amla withania and tulsi in the ratio of 125:50:80 were used as a dose of 100,200 and 400mg/kg p.o., Haloperidol 1mg/kg i.p. (Serenace, LPG Life Sciences) solution was diluted in saline. All the solutions were freshly prepared prior to each experiment.

Models:

Haloperidol is widely used to induce Parkinsonism like conditions in the dose 1mg/kg i.p. in rats.[4] The study was performed by using different models as given below-

Wistar rats were randomly divided in 5 sets of experiments having 5 groups each group having 6 animals (N=6).

Group 1: - Normal- Received equivalent volume of saline p.o /kg body wt. as in control except haloperidol and plants extract,

Group 2:-. Control-haloperidol i.p, 1mg/kg body wt.

Group 3:- Treated with combined plants extract 100mg/kg p.o.

Group 4:- Treated with combined plants extract 200mg/kg p.o.

Group 5:- Treated with combined plants extract 400mg/kg p.o.

Rota rod test: [5]

Motor co-ordination test was conducted using a rota rod apparatus (Inco ambala, India) The speed of the rotating rod was set at 20 rpm. Animals were placed on the moving rod prior to the treatment and the rat stayed on the rod without falling for 120 seconds were chosen for the study. The time animals take for falling from the rotating rod was noted before and after the treatment with extract.

Catalepsy:[6]

The bar test was used for measuring catalepsy. In the bar test, animal was placed on the apparatus such that its forepaws rested on the bar and their hind limbs were on the platform. After the animal was positioned properly the experimenter released its hold. Catalepsy was measured by the time the animal maintained its position on the bar. When the animals removed one paw from the bar the stopwatch was stopped and the time noted. Animals were observed for a maximum cutoff time for bar test which was fixed for 5 min. Following assessment the animals were returned to their home cages.

Akinesia: [7]

Akinesia was measured by noting the akinesia (difficulty in initiating movement) in seconds (s) to move all the four limbs and the test was terminated if the akinesia exceeded 120 s. Each animal was initially acclimatized for 5 min on a wooden elevated (100 cm) platform (100×150 cm) used for measuring akinesia in rats. Using a stopwatch, the time taken by the animal to move all the four limbs was recorded.

RESULTS AND DISCUSSION

Table 1: Rota rod test data

S.No.	Group No.	Mean	SD	SEM
1	G1:- Normal saline	120	±2.65	±1.08
2	G2:- Haloperidol (1 mg/kg i.p)	15	±9.95	±4.06
3	G3:- Haloperidol + CPE(100 mg/kg p.o	40	±2.86	±1.16
4	G4:- Haloperidol + CPE(100 mg/kg p.o	55	±10.40	±4.24
5	G5:- Haloperidol + CPE(100 mg/kg p.o	80	±8.27	±3.37

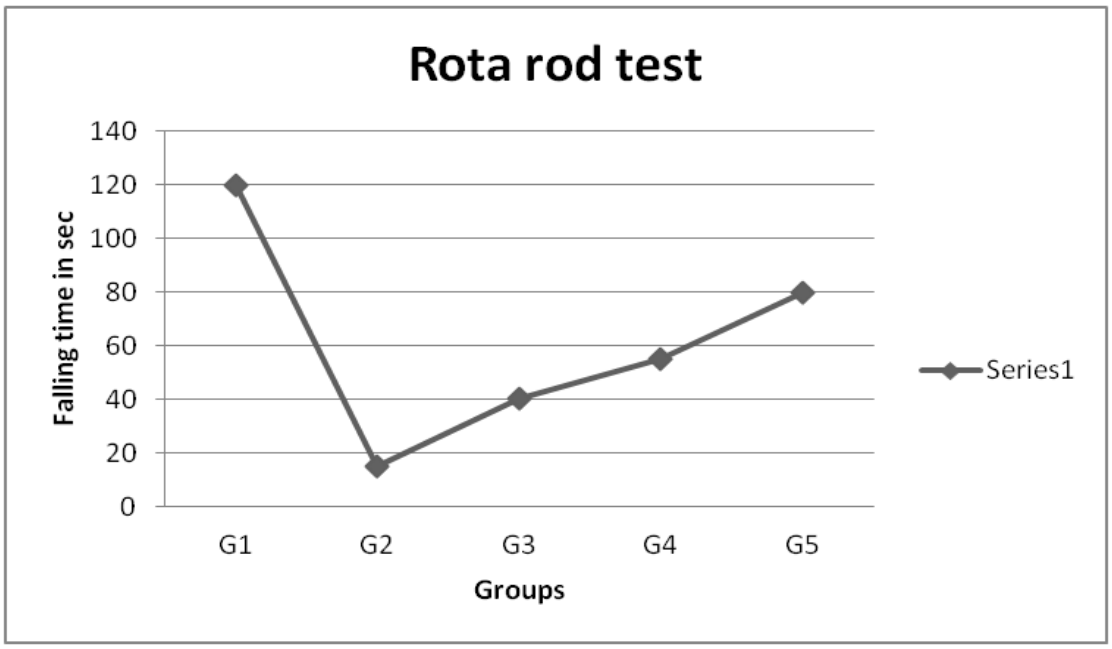

Figure 1: Tests for muscle rigidity using Rota-rod on all rat groups, effect of different concentration of extract on haloperidol induced muscle rigidity in normal rat at the end of 120 min.

Effect of different concentration of extract on haloperidol induced muscle rigidity. Progression of muscle rigidity test performed on 30, 60 and 120 minutes to all groups. Significant at $p < 0.001$ (comparison between saline group G1 and all other rat groups. (comparison between haloperidol-treated groups G 2 and all other rat groups). Values are mean ± S.D (n = 6). G 1 = normal saline. G 2 = haloperidol (1 mg/kg i.p). G 3 = haloperidol + combined plant extracts (100 mg/kg orally). G 4 = haloperidol + combined plant extracts (200 mg/kg orally). G 5 = haloperidol + combined plant extracts (400 mg/kg orally). The combined plant extracts (CPE) contains amla fruit ext, Withania root ext. and tulsi Ariel part ext. in the ratio of 125:50:80.

Table 2: Catalepsy test data

S.No.	Group No.	Mean	SD	SEM
1	G1:- Normal saline	45	±0.51	±0.20
2	G2:- Haloperidol (1 mg/kg i.p)	309	±2.40	±0.97
3	G3:- Haloperidol + CPE(100 mg/kg p.o	205	±7.39	±3.01
4	G4:- Haloperidol + CPE(100 mg/kg p.o	81	±0.51	±0.21
5	G5:- Haloperidol + CPE(100 mg/kg p.o	70	±0.81	±0.33

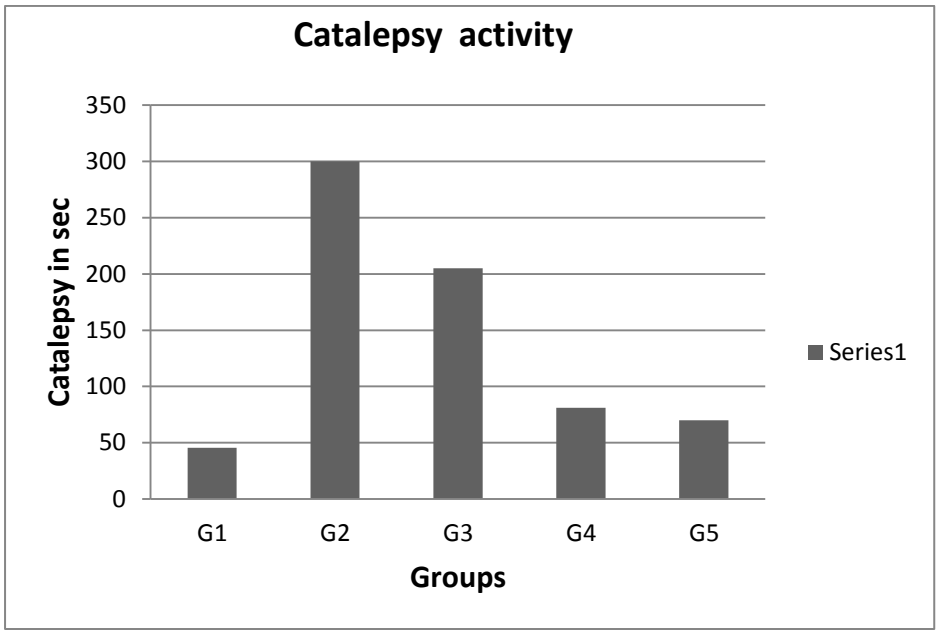

Figure 2: Catalepsy performed on all rat groups, effect of different concentration of extract on haloperidol induced catalepsy in normal rat at the end of 120 min.

Progression of catalepsy test performed on 30, 60 and 120 min to all groups. Significant at $p < 0.001$ (comparison between saline group G1 and all other rat groups., (comparison between haloperidol-treated groups G 2 and all other rat groups). Values are mean ± S.D (n = 6). G 1 = normal saline. G 2 = haloperidol (1 mg/kg i.p). G 3 = haloperidol + combined plant extracts (100 mg/kg orally). G 4 = haloperidol + combined plant extracts (200 mg/kg orally). G 5 = haloperidol + combined plant extracts (400 mg/kg orally). The combined plants extract (CPE) contains amla fruit extract, Withania root ext. and tulsi Ariel part ext. in the ratio of 125:50:80.

Table 3: Akinesia test data

S.No.	Group No.	Mean	SD	SEM
1	G1:- Normal saline	18	±1.04	±0.42
2	G2:- Haloperidol (1 mg/kg i.p)	120	±1.14	±0.46
3	G3:- Haloperidol + CPE(100 mg/kg p.o	100	±4.80	±1.95
4	G4:- Haloperidol + CPE(100 mg/kg p.o	72	±8.01	±3.26
5	G5:- Haloperidol + CPE(100 mg/kg p.o	42	±4	±1.63

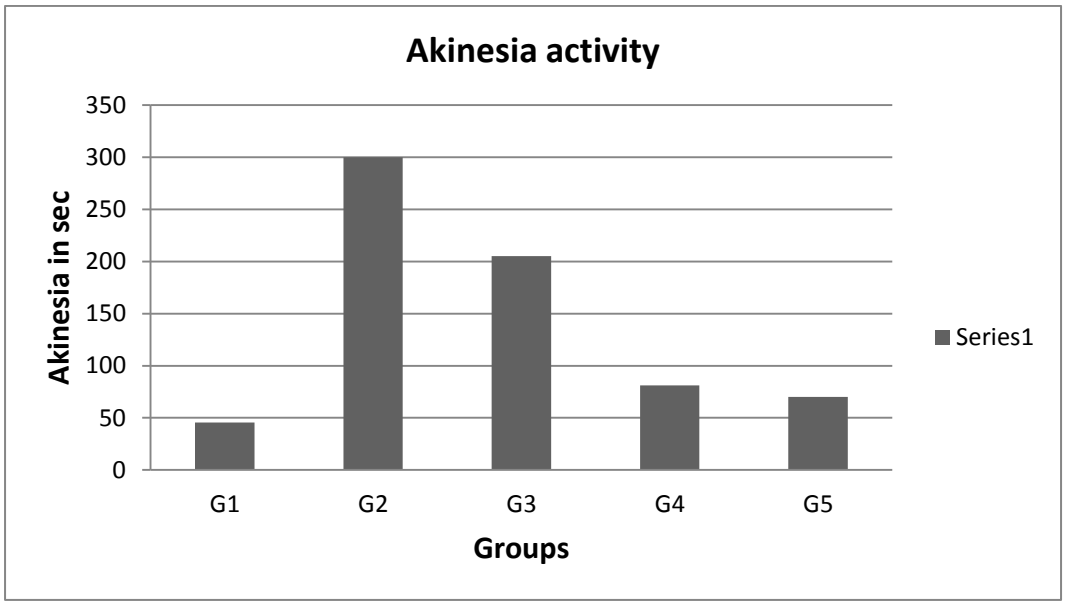

Figure 3: Akinesia performed on all rat groups, effect of different concentration of extract on haloperidol induced Akinesia in normal rat at the end of 120 min.

Progression of Akinesia test performed on 30, 60 and 120 min to all groups. Significant at $p < 0.001$ (comparison between saline group G1 and all other rat groups., (comparison between haloperidol-treated groups G 2 and all other rat groups). Values are mean ± S.D (n = 6). G 1 = normal saline. G 2 = haloperidol (1 mg/kg i.p). G 3 = haloperidol + combined plant extracts (100 mg/kg orally). G 4 = haloperidol + combined plant extracts (200 mg/kg orally). G 5 = haloperidol + combined plant extracts (400 mg/kg orally). The combined plant extracts contains amla fruit ext, Withania root ext. and tulsi Ariel part ext. in the ratio of 125:50:80.

CONCLUSION

The combined plant extract contains the extract of *Emblica officinalis, Withania somnifera* and *Ocimum sanctum*, was found to be suitable for the treatment of Parkinson's disease, so this combination can be used for the preparation of polyherbal formulation.

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