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Effect of hydro-alcoholic root extract of *Plumbago zeylanica* l alone and its combination with aqueous leaf extract of *Camellia sinensis* on haloperidol induced parkinsonism in wistar rats

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KEY WORDS

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ABSTRACT

Background: Herbal medicines have been used to treat PD in ancient medical systems in Asian countries such as India, China, Japan and Korea based on their own anecdotal or experience-based theories. Mucuna pruriens commonly known asvelvet beans, or cow itch, are used in case of spasms associated with Parkinsonism. **Purpose:** To investigate the antiparkinsonism activity of hydro alcoholic root extract of *P. zeylanica* L (PZE) alone- and its combination withaqueous extract of *C. sinensis* leaves (AECS) in Haloperidol induced model. **Methods:** Parkinsonism (PD) was induced by intraperitoneal administration of Haloperidol (1 mg/kg). The extracts/drugs being tested were administered orally (p.o) 60 min prior to the administration of the Haloperidol. Catalepsy was measured using the metal bar test. **Results:** Haloperidol induced a time dependent increase in cataleptic score in rats, as compared to vehicle treated groups. All the groups ie L-dopa + carbidopa (syndopa), hydro-alcoholic extract of *P. zeylanica* alone and its combination with *C. sinensis* showed significantly ($P<0.001$) lower scores of catalepsy at all time periods as compared to Haloperidol. Results were analyzed by one way ANOVA followed by Dunnet's multiple comparison tests. **Conclusion:** It is concluded that *P. zeylanica* alone and its combination with *C. sinensis* exert a protective effect against PD, while bi-herbal extracts showed more significant protective effect. Hence it may offer a safer therapeutic approach to the treatment of PD and drug induced dyskinesia.

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Introduction

Parkinson's disease is one of the second most common neurodegenerative disorders.[1] The main features of Parkinson's disease are motor dysfunction including resting tremor, bradykinesia, muscular rigidity and postural reflex impairment. Other manifestations include mood disorders such as anxiety, depression and dysautonomic function such as hypotension, constipation, paresthesias, cramps, olfactory dysfunction and seborrhea dermatitis. As the disease progresses, cognitive ability is found to be decreased. These clusters of all the above mentioned symptoms are collectively described as Parkinsonism. The term is typically used for syndromes of known etiology due to ischemic injuries, exposure to toxins or neuroleptic condition.[2]

Pathologically, the hallmark of PD includes the severe loss (approximately 50–70%) of the dopaminergic neurons in the substantia nigra pars compacta and the presence of proteinacious inclusion called Lewy bodies which is mainly composed of fibrillar α- synuclein and ubiquitinated protein within some remaining nigral neurons. The degeneration of dopaminergic neurons results in threshold reduction of approximately 80% dopamine in the striatum, which leads to the emergence of neuromuscular executive dysfunction, learning problems and mood disorders.[3]

Levodopa has been considered the gold standard drug therapy for Parkinson's disease but it is limited only to relieve symptoms and its long term use may cause serious side effects that include involuntary movements (dyskinesia), the on-off effect may cause Parkinson's related movement problems to appear and disappear suddenly and unpredictably. The side effects of allopathic medicines for PD are highly alarming, hence, the current research is now focusing on herbal and alternative systems of medicine. In addition to several synthetic agents modulating various pathways, certain plant constituents like polyphenols, flavonoids also exhibit some activity against experimentally induced PD.[4] Moreover herbal medicines have been used to treat PD in ancient medical systems in Asian countries such as India, China, Japan and Korea based on their own anecdotal or experience-based theories. *Mucuna pruriens* commonly known asvelvet beans, or cow itch, are used in case of spasms associated with Parkinsonism. Clinical efficacy of seeds of thisplant was confirmed and the efficacy was contributed to its natural L-Dopa content.[11]

The current study envisages evaluation of the anti-parkinsonian effect of hydro alcoholic root extract of *Plumbago zeylanica* alone and its combination with *Camellia sinensis* in a well-established and frequently employed animal models. The studies suggest that free radicals have a key role in neurodegenerative disorders including Parkinsonism.[5,6] This may be due to reduced level of antioxidants such as glutathione that may lead Parkinsonism patient more vulnerable to oxidative stress.[7] *P. zeylanica*[8] and *C. sinensis*[9] have potent antioxidant activity which may reduce the degeneration of neurons associated with PD. These plants contain alkaloids, flavonoid, polyphenoles, catechol, terpenes, which are the most promising candidates for the treatment of PD.[4]

The root of *P. zeylanica* contains several bioactive constituent like L-dopa, plumbagin, droseron, chitranone, triterpinoid, anthraquinone.[10] L-dopa, present in the herbal drugs and reported to provide anti-parkinsonism activity, without producing drug induced dyskinesia, which is the main side effect of synthetic drugs.[11] The dried root extract of *P. zeylanica* may ameliorate Parkinsonism without developing side effects. *P. zeylanica* shown to have a number of actions in the central nervous system including stimulatory[12] and nootropic action.[13] Neuroprotective mechanism ascribed to *P. zeylanica* in these studies may also claim to have beneficial effect in PD.

L-dopa is inactive by itself because it does not cross blood brain barrier, but is the immediate precursor of dopamine. L-dopa is

decarboxylated to dopamine by the enzymes mono amine oxidase and catechol-O-methyl transferase. About 1-2% of orally administered L-dopa crosses the blood brain barrier, is taken up by the surviving dopaminergic neurons, converted to dopamine, which is stored and released. In modern practice L-dopa is administered with peripheral decarboxylase inhibitors which prevent peripheral decarboxylation of L-dopa and increase the availability of dopamine in the brain. This not only reduces the dose but also the peripheral side effects of L-dopa. Vast epidemiology data has shown that green tea consumption reduces the occurrence of neurodegenerative disorders such as Parkinsonism and Alzheimer's disease.[14] In particular, recent literature strengthens the perception that diverse molecular signaling pathways, participating in the neuroprotective activity of the major green tea polyphenol, (-)-epigallocatechin-3 gallate (EGCG), renders this natural compound as potential agent to reduce the risk of various neurodegenerative diseases. Various studies have suggested that EGCG may inhibit COMT-catalyze dmethylation of endogenous and exogenous compounds.[15] We assumed that *C. sinensis* may prevent the metabolism of dopamine present in the *P. zeylanica* root extract by inhibiting the enzyme catechol-o-methyl transferase allowing maximum L-dopa to reach the CNS. COMT inhibition may also preserve dopamine formed in the striatum. The synergistic action of *C. sinensis* extract may reduce the dose and peripheral side effect of L-dopa.

Haloperidol induced muscle rigidity model is the second most important model which is used largely by the researchers. This model being comparatively simpler, is used extensively for examining antiparkinsonism effects.

Methods

Animals

Healthy adult male Wistar albino rats, weighting 150-220 g obtained from the registered animal house of University College of Pharmacy (UCP), Cheruvandoor campus (CVR), Mahathma Gandhi University, Kottayam, Kerala, India and College of Veterinary and Animal Sciences, Mannuthy, Thrissur, India were used for the study. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of UCP, CVR Campus, bearing **IAEC no: [012/MPH/UCP/CVR/13**]. All the animals were housed in polypropylene cages, maintained under standard husbandry conditions (12 h light and dark cycles, room temperature (27 ± 2°c) and 45–55% relative humidity). The rats were provided with standard pellet diet and water *ad libitum* throughout the course of the study.

Drugs

Haloperidol (LPG Life Sciences, Mumbai, India), Syndopa (L-dopa+C-dopa). *P. zeylanica* roots were collected from Madhuvanam botanical garden Calicut, India and *C. sinensis* leaves were collected from Munnar, Idukki, India during the month of March, 2013. The plants were taxonomically identified and authenticated by Dr. Pandurangann, evolutionary science division, Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, Trivandrum, Kerala.

Statistical analysis

The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett' smultiple comparison test. Comparisons were made between haloperidol group and test/standard groups. P-values <0.05 was considered statistically significant. The statistical analysis was done by using Graph pad prism version no: 6.0.

Haloperidol-induced catalepsy

Haloperidol is a first generation anti-psychotic drug and produces typical extra pyramidal side effects widely used as a pharmacological tool to mimic Parkinsonism like conditions at a dose of 1 mg/kg in rats. In rats, Haloperidol produces marked catatonia, muscular rigidity and bradykinesia. The cataleptic behavior was assessed by metal bar test.

Male albino wistar rats of weighing 150–200 g were selected and randomly divided in five groups (n = 6 in each) as mentioned above. The first group received vehicle (distilled water) whiles the second group received Haloperidol (1 mg/kg/i.p). Third group received syndopa (10 mg/kg), fourth group received PZE alone (100 mg/kg) and fifth group received combination of PZE + AECS (100 mg/kg) orally and Haloperidol were given to all groups at a dosage 1 mg/kg after 60 min of the extracts/drug administration. The mean catalepsy score was noted at an interval of 15 min for a period of 4 hr.[16,17]

Behavioral assessment (Metal bar test)

Catalepsy of an individual rat was measured by a scoring method given below.[16]

Step 0-0 Rat moved normally when placed on the table;
Step II-0.5 Rat moved only when touched or pushed;
Step III-0.5 The front paws of the rats were placed alternately on a 3 cm high block. If the rat failed to correct the posture within 15 sec, a score of 0.5 for each paw was added to the score of step 1.
Step IV-1.0 The front paws of the rat were placed alternately on a 9 cm high block. If the rat failed to correct the posture with in 15 sec, a score of 1 for each paw was added to the scores of step I and II. Thus, for an animal, the highest score was 3.5 (cut off score) and that reflects Parkinsonism.

Results

Haloperidol induced a time dependent increase in cataleptic state in rats, as compared to vehicle treated groups. Maximum catalepsy score was noted at a time interval of 120-180 min. All the groups ie standard (L-dopa + carbidopa), hydro-alcoholic extract of *P. zeylanica* and combination of both PZE and AECS showed significant ($P<0.001$) reduction in scores at all time periods. The average scores for the standard and the test drugs were reduced up to half that of the Haloperidol group (Group II). In terms of average scores the combination drug seems to work as good as the Syndopa drug alone.

Discussion

Parkinsonism disease is a neurodegenerative disorder characterized by the selective loss of dopamine neurons of the substantia nigra pars compacta (SNpc). The events which trigger and/or mediate the loss of nigral dopaminergic neurons, however, remain unclear. Current treatment of PD is based on dopamine replacement therapy, but this leads to long term complications, including dyskinesia. Herbal medicines are currently used as a safer alternative to PD. The World Health Organization has also recognized the importance of traditional medicine and has created strategies, guidelines and standards

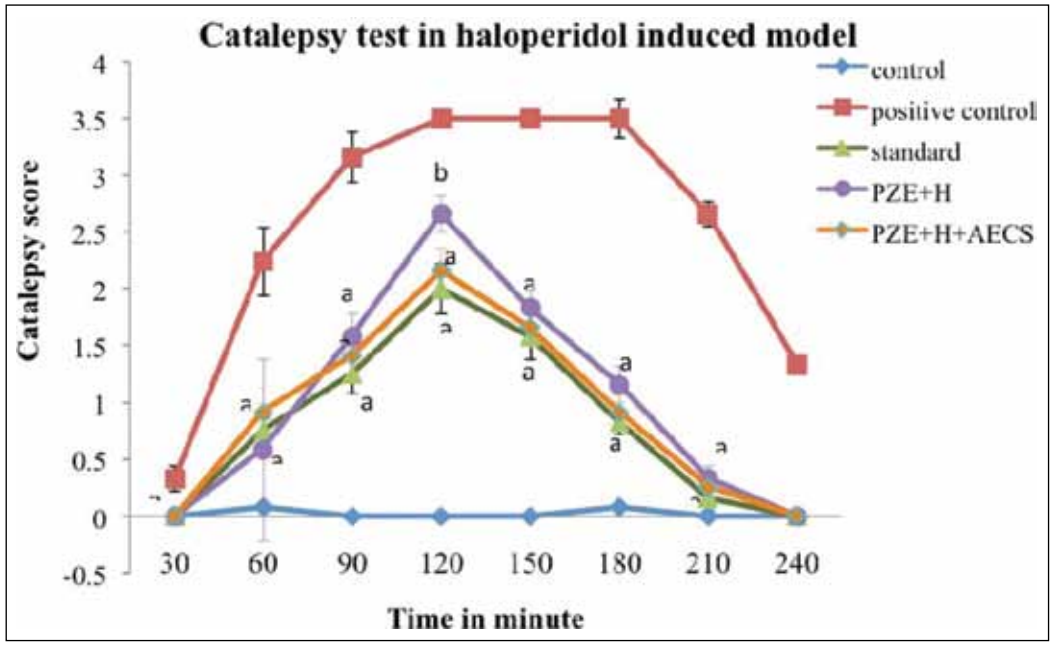

Fig. 1: Effect of *P. zeylanica* and its combination with *C. sinensis* on Haloperidol induced catalepsy test. a-*** and b-**
Values are expressed as Mean ± SEM (n = 6), analyzed by one-way ANOVA followed by Dunnett's post hoc test, *Represents P<0.05, **P<0.01, ***P<0.001 as compared to positive control.

for botanical medicines. *P. zeylanica* (family: Plumbaginaceae) is an annual plant found in India. It has high content of natural dopamine in roots.[10] The plant also contains flavonoids and alkaloid. L-dopa present in the herbal drugs is reported to provide anti-parkinsonism activity without producing drug induced dyskinesia, which is the main side effect of synthetic drugs.[11] The present study, therefore, for the first time, showed that PZE have an anti-parkinsonism activity.

Green tea has been shown to contain polyphenols such as EGCG and EGC, which has shown protective effects in 6-OHDA and MPTP rat model of PD.[15] The mechanisms of neuroprotection described in these studies were antioxident and COMT inhibitory effect of AECS. In the present investigation, *C. sinensis* in combination with PZE, showed neuroprotective and rescue of function effect in Haloperidol induced models PD.

Various animal models (neurotoxin induced, neuroleptic induced and surgery induced) have been employed in both rodents and nonhuman primates that have been used successfully in the evaluation of PD. The most commonly used model of PD is generated through the administration of Haloperidol. 6-OHDA model is well accepted as gold standard model and Haloperidol induced muscle rigidity model is the second most model which is widely used as per literature survey. However 6-OHDA model requires surgical procedure, MPTP is a highly neurotoxic chemical which require stringent safety procedure while Haloperidol model being comparatively simpler cheaper and well accepted lab model for i anti-parkinson study.

Haloperidol is a well- known neuroleptic for the treatment of schizophrenia and other affective disorders, primarily acting as a D_2 receptor antagonist in the mesolimbic mesocortical pathway. Due to its non-selective action, it also produces blockade of post-synaptic D_2 receptors in the nigrostriatal pathway leading to the development of extrapyramidal side effects in humans and catalepsy in animals.[18] Catalepsy has been defined as "an inability to correct an imposed abnormal posture while maintaining the righting reflex". Haloperidol induced catalepsy is also associated with an increase in oxidative stress in the brain.[19] In this study, administration of Haloperidol (1 mg/kg, i.p) induced a time dependent increase in cataleptic state in rats, as compared to vehicle treated groups (Fig. 1).

All the groups ie Syndopa, hydro-alcoholic root extract of *P. zeylanica* and combination of PZE and AECS showed significant (P<0.001) reduction in antagonism at all time periods. This indicates that Syndopa, PZE alone and its combination with AECS show effects within 1 hr itself, which lasts for at least 4 hours. The average scores for the standard and the test drugs were reduced up to half that of the Haloperidol treated group. Decrease in cataleptic score predicts the activity of PZE against Parkinsonism and drug induced dyskinesia (tardive dyskinesia) management therapy. The antiparkinsonism activity might be due to the combined effect of antioxidant properties. Both of them might have restored the alteration in locomotor activity. The combination of PZE and AECS seems to work as good as the Syndopa alone. The effect might be due to the synergistic antioxidant activity and also due the Catechol-O-methyl transferase inhibitory activity of EGCG of *C. sinensis*. This enzyme is responsible for peripheral utilization of L-dopa inhibition of this enzyme increased the dopamine content in the brain by inhibiting peripheral decarboxylation of natural L-dopa present in PZE and also preserved already formed dopamine in SNpc. The antioxidant potential of PZE and AECS may prevent the degeneration of dopaminergic neurons in SNpc.

From the study we concluded that the antiparkinsonism effect was seen in all groups ie Syndopa, PZE and combination of extracts the maximum anti-parkinsonism effect was observed in bi-herbal extracts. Some important classes of phytoconstituents

like natural L-dopa, alkaloids and polyphenols have been reported in this plant which might be responsible for the above behavioral effects. Further studies should be done for screening and evaluation of the particular phyto-constituents present in plants, which might exhibit a protective effect in this study. In addition, further studies are needed to explore other possible mechanisms involved in the neuroprotective effect of *P. zeylanica* alone andits combinationwith *C. sinensis* in other experimental models of PD. Further clinical trials are needed to prove the safety and efficacy of long term administration of extract of *P. zeylanica* alone and its combination with *C. sinensis*.

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