EFFECT OF HYDRO-ALCOHOLIC ROOT EXTRACT OF PLUMBAGO ZEYLANICA L ALONE AND IT’S COMBINATION WITH AQUEOUS LEAF EXTRACT CAMELIA SINENSIS ON ROTENONE INDUCED PARKINSONISM

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ABSTRACT

The aim of this study was to investigate the anti parkinsonism activity of hydro alcoholic root extract of Plumbago zeylanica L. (PZE) alone and its combination with Aqueous Extract of Camellia sinensis (AECs) leaves in Rotenone induced model. Parkinsonism was induced by administration of Rotenone (12 mg/kg, p.o). The effect of PZE (100 mg/kg) and its synergistic effect with AECs were assessed by the ex vivo antioxidant assays by measuring the various enzymes in the striatum region of brain such as Glutathione, Lipid peroxidation, Catalase and Superoxide dismutase. The various behavioral parameters analyzed were rearing, self-grooming, ambulation activity using open field apparatus and muscle rigidity using bar test. The administration of rotenone produced motor dysfunctions like catalepsy and muscle rigidity along with a reduction in locomotor activity. Oxidative stress in the brain was evident from an increase in the level of TBARS and decrease in the levels of catalase, SOD and GSH. Pretreatment with PZE alone and its combination with AECs resulted in a significant ($p < 0.001$) decrease in catalepsy and muscle rigidity along with a significant ($p < 0.001$) increase in locomotion as compared to the rotenone-treated group. The reduction in the TBARS level and an increase in the GSH, SOD and CAT levels indicate the reduction in oxidative stress in the brain of animals. It was concluded that P. zeylanica alone and its combination with C. sinensis have a protective effect against rotenone induced Parkinson disease, while bi-herbal extracts exhibited more significant activity than single administration. Hence may offer a safer therapeutic approach to the treatment of Parkinson disease.

Keywords: Anti parkinsonism, Plumbago zeylanica L, Camellia sinensis, Rotenone.

INTRODUCTION

Parkinson’s disease is one of the second most common neurodegenerative disorders. The prevalence of Parkinson disease has been estimated in several studies. Over all incidence rates for Parkinson disease in door to door studies ranged from 167 to 5703 / lakh person / year.1 The main features of Parkinson’s disease are motor dysfunction include resting tremor, bradykinesia, muscular rigidity and postural reflex impairment. Other manifestation include mood disorders such as anxiety, depression and dysautonomic function such as hypotension, constipation, paresthesias, cramps, olfactory dysfunction and seborrhea dermatitis.2 Pathologically the hallmark of Parkinson disease are the severe loss (approximately 50-70 %) of the dopaminergic neurons in the substantia nigra pars compacta and the presence of proteinaceous inclusion called Lewy bodies, which is mainly composed of fibrillar α-synuclein and ubiquitinated protein within some remaining nigral neurons.3 The degeneration of dopaminergic neurons results in threshold reduction of approximately 80 % dopamine in the striatum, which lead to the emergence of neuromuscular executive dysfunction, learning problems and mood disorders. The cause of dopaminergic cell death in Parkinson disease remains unknown, but is associated with a number of factors that may cause programmed cell death including calcium influx, reactive oxygen species (ROS) and mitochondrial complex I inhibition.4 One of the models to study Parkinson disease is the administration of the plant-derived pesticide Rotenone, a specific inhibitor of mitochondrial complex I. Several studies suggested that ROS play a crucial role in neurodegenerative diseases. The reduced levels of endogenous antioxidant molecules such as glutathione (GSH), antioxidant enzymes such as superoxide dismutase (SOD), increased metabolism of DA and lipid peroxidation product malondialdehyde (MDA) in the brain could contribute to neuronal death.5 Therapeutic efforts aimed at the removal of ROS or prevention of their formation may be beneficial in Parkinson disease. In this regard, natural products are attractive sources of chemical structures that exhibit potent biological activities with desirable pharmacological profiles. Several reports have suggested that flavonoids and alkaloids could be useful to protect cells from Rotenone toxicity.6 The current study envisages in evaluating the anti parkinsonian effect of hydro alcoholic root extract of Plumbago zeylanica alone and its combination with Camellia sinensis in Rotenone models. The studies suggest that free radicals have a key role in neurodegenerative disorders, including Parkinsonism. Free radicals induce lipid peroxidation that leads to neuronal death. Thiobarbituric acid reactive substance which is a marker of lipid peroxidation is increase in the brain of Parkinsonism patient. This may be due to reduced level of antioxidant such as glutathione that may lead Parkinsonism patient more vulnerable to oxidative stress.8 P. zeylanica and C. sinensis have potent antioxidant activity; this may reduce the degeneration of neurons associated with Parkinson disease. The root of P. zeylanica contains several
bioactive constituent like L-dopa, plumbagin, droseron, chitanone, triterpinoid and anthraquinone. L-dopa present in the herbal drugs reported to provide anti parkinsonism activity without producing drug induced dyskinesia, which is the main side effect of synthetic drugs. So 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 and nootropic action. Neuroprotective mechanism ascribed to *P. zeylanica* in these studies may also claim to have beneficial effect in Parkinson disease. 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 reduce the peripheral side effects of L-dopa. Vast epidemiology data indicated that green tea consumption reduces the occurrence of neurodegenerative disorders such as Parkinsonism and Alzheimer’s disease. In particular, recent literature strengthen the perception that diverse molecular signaling pathways, participating in the neuroprotective activity of the major green tea polyphenol, (-)-Epigallocatechin gallate (EGCG), renders this natural compound as potential agent to reduce the risk of various neurodegenerative diseases. Various studies suggested that EGCG may inhibit COMT-catalyzed methylation of endogenous and exogenous compounds. 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 (COMT) and allows 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. This bi-herbal formulation expected to reduce the Parkinson disease progression without inducing the side effect.

**MATERIALS AND METHODS**

**Animals**
Healthy adult male Wistar albino rats, weighting 150-200 g obtained from the registered animal house of University College of Pharmacy (UCP), Cheruvandoor campus (CVR), Mahathma Gandhi University, Kottayam, 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), 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 ± 20°C) and 45-55 % relative humidity). The rats were provided with standard pellet diet and water *ad libitum* throughout the course of the study.

**Procurement of plants and drugs**
*P. zeylanica* roots were collected from Madhuvanam botanical garden; Calicut, India and *C. sinensis* leaves were collected from Idukki, India during the March, 2013. The plants were taxonomically identified and authenticated by Dr. Pandurangann, Scientist F and Head, Plant Systematics and Evolutionary Science division, Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, Trivandrum, Kerala, India. Herbarium voucher specimen numbers were JNTBGRI/76808/ Apr2014 and JNTBGRI/76809/ APR2014 for *P. zeylanica* and *C. sinensis* respectively. Rotenone (Catalogue no: R8875-1G) was obtained from Sigma Aldrich (St. Louis, U.S.A.) and other chemicals were procured from Otto Kemi (p) Ltd, Mumbai, India and spectrum chemicals.

**Methodology**
Male wistar rats weighing 150-200 g were divided into five groups, each consisting of six rats. Group-I served as control and was administered with the vehicle, olive oil (1 mg/kg, p.o) for 12 days. Group-II was administered orally with rotenone at a dose of 12 mg/kg/p.o (dissolved in olive oil) daily for a period of 12 days. Group III received Syndopa (10 mg/kg, p.o) and rotenone at a dose of 12 mg/kg/p.o for 12 days. Groups IV received PZE at the dose of 100 mg/kg p.o and rotenone (12 mg/kg, p.o) for 12 days. Groups V received combination of PZE + AECS (1:1, p.o.) and rotenone for 12 days. Behavioral assessments were carried out before the start of the treatment, then regularly at an interval of 6 days post treatment and final behavioral quantification was done after 1 h of last dose. Animals were then sacrificed by cervical dislocation and their brains were removed and weighed. A 10 % (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged and the clear supernatant was used for biochemical estimations. The behavioral parameters assessed were duration of catalepsy using bar test and locomotor activity with open field apparatus.

**Catalepsy**
The catalepsy was assessed by placing the animal’s forepaws on a horizontal bar positioned at 9 cm above the bench surface. The duration of catalepsy, which was defined as an immobile posture, keeping both forepaws on the bar, was measured up to a maximum of 180s.

**Spontaneous locomotor behavior and abnormal involuntary movements (open field apparatus)**
Open field apparatus consist of squares (61 × 61) were used for the study. Blue lines were drawn on the floor with a marker. The lines divided floor into sixteen squares. A central square was drawn on the middle of open field. The rats were centrally placed in the open field apparatus and were allowed to walk without restraint inside the area for 5 minutes and following behavioral aspects were noted:
Biochemical estimation

Lipid peroxidation assay (TBARS)
The quantitative measurement of thiobarbituric acid reactive substances (TBARS) is an index of lipid peroxidation in brain. 0.2 ml of supernatant of homogenate was pipette out in a test tube, followed by addition of 0.2 ml of 8.1 % sodium dodecyl sulphate, 1.5 ml of 30 % acetic acid (pH 3.5), 1.5 ml of thiobarbituric acid and the volume was made up to 4 ml with distilled water. The test tubes were incubated for 10 minute in boiling water bath, then cooled and added 1 ml of distilled water followed by addition of 5 ml of α-bufanol pyridine mixture (15:1 v/v). The tubes were centrifuged at 4,000 g for 10 minutes and absorbance of the developed pink color was measured spectrophotometrically at 532 nm. A standard calibration curve was prepared using 1–10 nM 1, 1, 3, 3-tetra methoxy propane. The TBARS value was expressed as nanomoles per mg of protein.

Reduced glutathione assay
In this method 5, 5′- dithiobis-(2-nitrobenzoic acid) (DTNB) was reduced by the –SH groups of GSH to form one mole of 2-nitro-5-mercaptobenzoic acid per mole of –SH. The nitro mercapto benzoic acid anion released has an intense yellow color, which can be used to measure –SH groups at 412 nm. The supernatant of homogenate (1 ml) was mixed with trichloroacetic acid (10 %w/v) in 1:1 ratio. The tubes were centrifuged at 1,000 g for 10 minutes. The supernatant obtained (0.5 ml) was mixed with 2 ml of 0.3 M phosphate buffer. Then 0.25 ml of 0.6mM freshly prepared DTNB was added and absorbance was noted spectrophotometrically at 412 nm. A standard curve was plotted using 10–100 μM of reduced form of glutathione and results were expressed as micromoles of reduced glutathione per mg of protein.

Catalase assay
The supernatant (50 μl) was added to a cuvette that contained 1.95 ml of 50 mM phosphate buffer (pH7.0). 1.0 ml of 30 mM hydrogen peroxide (H₂O₂) was added and changes in absorbance were followed for 30 s at 240 nm at 15-sec intervals. The catalase activity was calculated using the mill molar extinction coefficient of H₂O₂ (0.071 mmol cm⁻¹) and the activity was expressed as micromoles of H₂O₂ oxidized per minute per milligram protein. The formula used was as under:

\[ \text{CAT activity} = \frac{\Delta \text{O.D}}{E \times V \times Wt \times e \times 1} \times \frac{1}{T} \]

Where \( \Delta \text{O.D} \) Change in absorbance/minute; 
\( E = \) Extinction coefficient of Hydrogen peroxide (0.0436 m mol cm⁻¹)

Superoxide dismutase assay
A weighed amount of brain tissue (25 mg) was taken in 5 ml phosphate buffered saline containing 100 μM of NBT at 37°C for 1.5 h. The NBT reduction was stopped by adding 5 ml of 0.5 M HCl. The tissue was minced and homogenized in a mixture of 0.1 M sodium hydroxide and 0.1 % sodium dodecyl sulphate in water containing 40 mg/L diethylene triamine penta acetic acid. The mixture was centrifuged at 20,000 g for 20 minutes and the resultant pellet was suspended in 1.5 ml of pyridine and kept at 80°C for 1.5 h to extract formazan, an adduct formed after reaction of NBT with superoxide anions. The mixture was again centrifuged at 10,000 g for 10 minutes and absorbance of formazan was determined spectrophotometrically at 540 nm. The amount of reduced NBT was calculated using the formula:

\[ \text{Amount of reduced NBT} = \frac{A \times V}{T \times Wt \times e \times 1} \]

where A is absorbance, V is volume of pyridine (1.5 ml L), T is time for which the tissue was incubated with NBT (1.5 h), Wt is blotted weight of tissue (25 mg), e is extinction coefficient (0.72 L/m M per mm), and l is length of light path (1 cm)

Results were expressed as reduced NBT picomole per minute per milligram of wet tissue.

Statistical analysis
The results of studies were expressed as mean ± SEM (standard error of mean). The difference between control and treated means were analyzed using one way analysis of variance (ANOVA). P-values < 0.05 were taken to be statistically significant. Dunnett post hoc test was used for multiple comparisons. The statistical analysis was done by using graph pad prism version 6.0.

RESULTS

Catalepsy test
Hydro-alcoholic extract of P. zeylanica and aqueous extract of C. sinensis were administered orally to male wistar albino rats and duration of catalepsy was measured. The duration of catalepsy was significantly (P < 0.001) decreased on 12th day in Syndopa, PZE (100 mg/kg) and its combination with AECS treated groups as compared to rotenone induced Parkinsonism group. The decrease in duration of catalepsy was also seen on 6th day in PZE and PZE + AECS treated group but it was not statistically significant (Figure 1).

Open field test
The open field test was done in order to determine the effect of administration of P. zeylanica and its combination with C. sinensis upon spontaneous locomotor activity. Locomotor parameters were significantly (p < 0.05) enhanced on 12th day in Syndopa, PZE (100 mg/kg) and its combination with AECS treated groups as compared with rotenone treated group. Bi-herbal extracts was statistically equipotent with that of Syndopa (Figure 2).
Biochemical estimation

Superoxide dismutase assay
The level of SOD in Control group (Group I) animals was found to be 0.62 ± 0.18 units/mg protein. The Syndopa, PZE (100 mg/kg) and its combination with AECS treated groups showed significant (p < 0.001) increase in the level of SOD when compared to the Rotenone treated group. Bi-herbal extracts showed more activity than single administration (Figure 3a).

Lipid peroxidation assay
The TBARS level in control animals was found to be 1.3 ± 0.060 n moles of MDA/mg protein. Administration of Rotenone resulted in a significant increase in TBARS level in brain of animals as compared to control animals. Pretreatment with standard, PZE and AECS + PZE showed a significant (p < 0.001) reduction in the level of TBARS in brain as compared to Rotenone treated animals (Figure 3b).

Reduced glutathione assay
Rotenone administration induced a significant decrease in the tissue GSH content as compared to control group. Syndopa showed an increase in GSH level as compared to Rotenone treated group. Both PZE alone and its combination with AECS caused increase in GSH level. Bi-herbal extracts showed more significant activity than PZE alone (Figure 3c).

Catalase assay
Catalase activity was found to decrease in rotenone treated rats as compared to control groups. Syndopa, PZE alone, PZE + AECS treated groups showed increase in catalase activity, but the results were statistically insignificant (Figure 3d).

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 DA neurons however, remain unclear. Current treatment of Parkinson disease is based on dopamine replacement therapy, but this leads to long term complications, including dyskinesia. Plants possess an important and a safer alternative to the treatment of neurodegenerative disorders including Parkinsonism. The World Health Organization has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. The plant *P. zeylanica* (family: Plumbaginaceae) is an annual plant found in India. It has high content of dopamine in roots. The plant also contains flavonoids and alkaloid. L-dopa present in the herbal drugs reported to provide anti parkinsonism activity without producing drug induced dyskinesia, which is the main side effect of synthetic drugs. The roots of PZE are reported to possess nootropic and CNS stimulant activity. In conclusion, the present study for the first time showed that PZE have an anti parkinsonism activity. Green tea has been shown to contain poly phenols such as EGCG and EGC, which has shown protective effects in 6-OHDA and MPTP rat model of Parkinson disease. The mechanisms of neuroprotection described in these studies were anti oxidant and COMT inhibitory effect of AECS. In the present investigation, *C. sinensis* in combination with PZE showed neuroprotective and neuro rescuing effect in rotenone models of Parkinson disease. The bi herbal extract showed more significant activity than alone optimized its COMT inhibitory activity. The results suggested that the above stated hypothesis regarding the presence of L-dopa in roots of *P. zeylanica* and its synergistic activity with *C. sinensis* due to COMT inhibitory activity was shown to be true. Environmental toxins like rotenone, a specific inhibitor of complex I is employed to increase oxidative stress mediated neuropathology and sporadic Parkinson's disease. It is highly lipophilic and readily gains access to all organs. It causes nigrostriatal degeneration similar to Parkinson disease pathology in a chronic, systemic, in vivo rodent model. Rotenone treatment in rats caused a 45 % loss of tyrosine hydroxylase-positive substantia nigra neurons and a commensurate loss of striatal dopamine. Several genes also have been definitively linked to Parkinson disease. The first to be identified was α-synuclein. In rotenone-treated animals, α-synuclein and poly-ubiquitin positive aggregates were observed in dopamine neurons of the SNpc. Other genes linked to Parkinson disease include parkin, DJ-1, PINK1, and LRRK2. Rotenone thus selectively destroys DA-ergic neurons and produces impaired motor function; the characteristic of Parkinson disease. Rotenone administration to rats caused a significant increase in catalepsy, decrease in locomotor and muscle activity. The current data thus suggested damage to the motor control system (DA-ergic neurons) and development of Parkinson’s disease like behavioral symptoms in rats exposed to rotenone. Two methods are used to assess behavioral alteration in rats. Pretreatment of rats with standard, PZE and its combination with AECS resulted in an insignificant decrease in catalepsy on day 6 and a more pronounced and significant reduction (P < 0.001) in catalepsy on day 12 (Figure 1). Open field test was done to observe changes in exploratory and locomotor activity of rats. The results observed in open field test showed that oral administration of standard, PZE, PZE and its AECS following the administration of rotenone showed significant (P < 0.001) increase in ambulation, rearing, grooming behavior on day 12 in open field paradigm (Figure 2). The behavioral analysis showed that a significant decrease in catalepsy, increase in locomotor activity and increase in muscle activity. This thus proved that the hydroalcoholic extract of *P. zeylanica* possess anti parkinsonism activity and combination of *P. zeylanica* with *C. sinensis* showed more pronounced activity than alone. Rotenone exposure in rodents also provides a valuable model for studying mechanisms of oxidative stress induced dopaminergic damage. Oxidative stress generated as a result of mitochondrial dysfunction, particularly mitochondrial complex-I impairment plays an important role in the Parkinson disease pathogenesis. Rotenone leads to depolymerization of microtubules causing rupture of transport vesicles, which then leads to release of dopamine in or near DA-ergic neurons, oxidation of which further damages DA-ergic neurons.
Figure 1: Effect of *P. zeylanica* and its combination with *C. sinensis* on catalepsy test in rotenone model

*represents $P < 0.05$, **$P < 0.01$, ***$P < 0.001$ as compared to rotenone treated group

Figure 2 (a): Effect of *P. zeylanica* and its combination with *C. sinensis* on the total number of line crossing, in male albino wistar rats in Rotenone induced Parkinsonism model

Figure 2 (b): Effect of *P. zeylanica* and its combination with *C. sinensis* on rearing frequency in male albino wistar rats in Rotenone induced Parkinsonism model

Figure 2 (c): Effect of *P. zeylanica* and its combination with *C. sinensis* on grooming frequency in male albino wistar rats in Rotenone induced Parkinsonism model

Figure 4: Superoxide dismutase assay in Rotenone model

Figure 5: Lipid peroxidation assay in rotene model
Chronic complex I inhibition by rotenone in rats caused oxidative damage to proteins, induced a decrease in GSH level and changed antioxidant enzyme (SOD and catalase) activities, which were blocked by antioxidants, e.g. alpha-tocopherol (vitamin E). Superoxide dismutase enzymes (SOD) act as an antioxidant and protect cellular components from being oxidized by reactive oxygen species (ROS). Superoxide anion is known to denature enzymes, oxidize lipids and fragment DNA. SOD is an enzyme which acts as a catalyst in the process of dismutation of superoxide into oxygen and hydrogen peroxide. It is therefore a critical antioxidant defense which is present in nearly all cells which are exposed to oxygen. It can help neutralize free radicals, and in doing so may limit or stop some of the damage they cause.

Rotenone treated group showed a decrease in the level of SOD in the brain of animals, thus indicative of production of oxidative stress. The oral administration of Syndopa, PZE and bi herbal combination caused a significant increase (P < 0.001) in SOD level while compared to Rotenone group (Figure 3a). The extent of lipid peroxidation was estimated by measuring the levels of thiobarbituric acid, a product of lipid peroxidation. Lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes causes impaired membrane function, impaired structural integrity, decreased fluidity and inactivation of number of membrane bound enzymes. There is substantial evidence of oxidative damage in the brains of Parkinson disease patients. Increased levels of the lipid peroxidation have been found in the substantia nigra of Parkinson disease patients. Similar results were observed in the brain homogenates of rotenone-treated animals. But Syndopa, PZE and bi herbal combination showed significant (P < 0.001) decrease in lipid peroxidation as compared to Rotenone group (Figure 3b). A defect in one or more of the naturally occurring antioxidant defenses particularly GSH is an important factor in etiology of Parkinson disease. A similar defect in GSH has been observed in the nigra of Parkinson disease patients who have been diagnosed to have incidental Lewy bodies which has been established as a preclinical symptom of Parkinson disease. A reduction in GSH levels may impair H2O2 clearance and promote hydroxyl radical formation leading to the generation of pro-oxidant milieu. A reduction in GSH levels was evident in rotenone-treated animals. Syndopa and bi herbal combination showed a significant (P < 0.01) increase in level of GSH as compared to Rotenone group (Figure 3c). Catalase is an enzymatic antioxidant and helps in neutralizing the toxic effects of hydrogen peroxide. Hydrogen peroxide is not reactive enough to cause a chain of lipid peroxidation reactions, but its combination with superoxide radicals produces hydroxyl radical, which is highly reactive and thus initiates lipid oxidation reactions. Catalase converts hydrogen peroxide to water and nonreactive oxygen species, thus preventing generation of hydroxyl radical and protecting the cells from oxidative damage. Oxidative stress results in decrease in catalase level. A significant decrease in the level of catalase was observed in the rotenone treated animals. The level of catalase was found to be increased in Syndopa, PZE and bi-herbal combination treated groups as compared to Rotenone group (Figure 3d). Thus, the rotenone per se group showed a significant increase in the levels of thiobarbituric acid which is an indication of extent of lipid peroxidation, decrease in the levels of CAT, SOD and GSH in the brain as compared to the control animals. All these indicate an increase in the oxidative stress in the brain of animals treated with rotenone. Pretreatment with standard, PZE and bi herbal combination resulted in a decrease in TBARS level and increase in the levels of SOD, catalase and GSH, indicating its antioxidant effect in the brain of rotenone treated animals. A possible underlying mechanism of this protection can be associated with the presence of alkaloids, poly phenols and flavonoids in the extracts, which are an important source of antioxidants.

Oxidative processes are an important factor in the pathogenesis of several disorders, and postmortem studies have consistently implicated oxidative damage in Parkinson disease pathogenesis. Then, compounds with potential antioxidant activity are notable candidates to become new therapeutic agents, since perspectives for
treatment of Parkinson disease in the future could include antioxidant therapies. Therefore, the results are consistent with others studies which showed protective activity by substances such as alkaloids, poly phenoles and flavonoids, known by their antioxidant power in the same experimental model. Finally, the data revealed that *P. zeylanica* and its combination with *C. sinensis* could be a potential therapeutic tool for neurodegenerative diseases. Active components of this extracts have to be determined. The research for substance with neuroprotective activity has increased in recent years. Since, oxidative stress produced in brain due to rotenone toxicity seems to be important in producing motor defects; therefore use of antioxidants could prove beneficial. The present study which thus explored the potential of *P. zeylanica* and its combination with *C. sinensis*, earlier proved to be an antioxidant, showed a promising effect in animals with Parkinsonism disease. Roteneone can cause selective dopaminergic neurodegeneration *in vitro* and *in vivo*. Behavior symptoms on rotenone administration are due to the depletion of dopamine in the striatum, which is caused by the dopaminergic neurodegeneration in substantia nigra. The change in dopamine level cause neuronal dysfunction, leads to Parkinsonism symptoms. The restoration of behavioral and locomotor alteration in Parkinsonism rats administered with hydroalcoholic root extract of *P. zeylanica* indicates its potent antioxidant activity and presence of natural L-dopa in plant roots. As CNS stimulant are useful in treatment of Parkinsonism, findings of present study support the earlier observations of CNS stimulant activity of *P. zeylanica*. Superior effects of bi herbal combination indicate the synergistic antioxidant activity and COMT inhibitory action of aqueous extract of *C. sinensis*. The COMT inhibitory activity prevents the decarboxylation of L-dopa and increased the availability dopamine in the brain and also preserved already formed dopamine in the brain. As previous studies indicate the COMT inhibitory action is due to the presence of green tea poly phenoles especially (-) Epigallocatechin gallate (EGCG) and (-) Epigallocatechin (EGC). We concluded that EGCG, as a potent COMT inhibitor, a mild irreversible inhibitor of dopa decarboxylase, a neuroprotective agent in animal and cell models of Parkinson’s disease, and a possible brain-penetrating chemical, may have beneficial effects in patients with Parkinson’s disease. Regular tea drinking has been reported to be a protective factor against Parkinson’s disease. The study indicated that as expected the combined extracts showed significant and pronounced anti Parkinsonism activity than single administration. This suggested that *C. sinensis* had a COMT inhibitory activity as indicated in earlier studies. From the study we concluded that the anti Parkinsonism effect was seen in all groups i.e., standard, PZE and combination of extracts in all models with the anti Parkinsonism effect being highest in the standard and the bi herbal extracts. Some important classes of phyto-constituents like natural L-dopa, alkaloids and poly phenoles have been reported in this plant which might be responsible for the above behavioral effects. Further studies should be done for the screening and evaluation of the particular phyto-constituents present in plants, which have shown the protective effect in this study. Still further clinical studies are needed to prove the safety and efficacy of long term administration of extract of *P. zeylanica* alone and its combination with *C. sinensis*. In addition, further studies are needed to explore other possible mechanisms involved in the neuroprotective effect of *P. zeylanica* alone and its combination with *C. sinensis* in the experimental models of Parkinson disease.

**REFERENCES**


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