

Protective Role of *Alternanthera Sessilis* (Linn.) Silver Nanoparticles and Its Ethanolic Extract against Rotenone Induced Parkinsonism

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Abstract

Biosynthesis of nanoparticles incorporating the herbal extracts can potentially eliminate the toxicity problem. Green synthesis of silver nanoparticles was done by using the ethanolic extract of *Alternanthera sessilis* (Linn.) and Silver nitrate. The anti-parkinsonism activity of ethanolic extract of *Alternanthera sessilis* (Linn.) (EEAS) and its silver nanoparticles (ASAgNPs) was evaluated using oxidative stress model of Parkinsonism using Rotenone. Parkinsonism was induced by administration of Rotenone (12 mg/kg). The effect of EEAS (200 mg/kg) and ASAgNPs (20mg/kg) were assessed by antioxidant assays such as Glutathione and Lipid peroxidation. The behavioural parameters such as rearing, self-grooming, ambulation activity and muscle rigidity were analysed using open field apparatus and bar test. EEAS and ASAgNPs caused significant neuroprotection as evidenced by decrease in catalepsy and muscle rigidity along with a significant increase in locomotion. The reduction in the lipid peroxidation and an increase in the GSH, indicate the reduction in oxidative stress in the brain of animals.

Keywords: Anti Parkinsonism, *Alternanthera sessilis* (Linn.), silver nanoparticles,

I. Introduction

Neurodegenerative disorders are characterized by a slow and progressive degeneration of neurons in specific locations of the central nervous system. The changes in neuronal activity in motor systems will induce motor symptoms such as those seen in Parkinson's disease (PD), Huntington disease (HD) etc.¹ Parkinson's disease (PD) is a progressive, disabling neurodegenerative disorder of unknown cause, characterized by bradykinesia, resting tremor, muscle rigidity, and postural instability²⁻³. It is the second most common neurodegenerative disorder, after Alzheimer's disease⁴. It is due to damage or loss of dopaminergic neurons in this brain region results in the depletion of dopamine from terminals in the striatum. Due to the numerous protective barriers surrounding the CNS, there is an urgent need for effective treatment for patients living with PD.

The current study envisages in evaluating the anti Parkinsonian effect of ethanolic extract of *Alternanthera sessilis* (Linn.) (EEAS) and its silver nanoparticles (ASAgNPs) using oxidative stress model of Parkinsonism using Rotenone. *Alternanthera sessilis* (Linn.) is a commonly known as sessile joy weed, found in humid and warm regions of the world, used to relieve headache and dizziness⁵. The use of nanoparticles in drug delivery therapy holds much promise in targeting remote tissue. Silver nanoparticles used as alternative strategies for drug delivery to Alzheimer brain are able to cross the Blood brain barrier and penetrate into the cell cytoplasm and induce underlying cellular change⁶. EEAS have shown to exert significant antioxidant activity as in FARP and DPPH radical scavenging assay⁷⁻⁸. Hence in the present study an attempt was made to explore the possible anti Parkinsonism activity of *Alternanthera sessilis* (Linn.), keeping in mind its anti-oxidant potential. 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 death⁹.

II. Materials And Methods

Experimental animal

Healthy male Wistar albino rats (150 - 200 gm) were obtained from the animal house of Department of Pharmaceutical sciences, M.G University, Cheruvandoor, Kottayam. They were housed in well ventilated, large spacious hygienic cages under standard animal husbandry conditions (22-28°C) with relative humidity of 55±5 % and alternate 12 hour light-dark cycle. The animals were fed with standard food and water ad libitum. All animals were acclimatized to the experimental environment prior to study.

Plant

Alternanthera sessilis (Linn.) whole plant were collected from Kanjiramattom village of Ernakulum district, Kerala, India and were authenticated at CMS College, Kottayam, Kerala. A voucher specimen is preserved at the Herbarium with collection number. 782.

Drugs and chemicals:

95 % Ethanol, Silver nitrate (3Mm), Rotenone, Syndopa.

Preparation of *A. sessilis* (Linn.) Silver Nanoparticles

20 ml of the plant extract (2 g) was mixed with 80 ml of 3mM of silver nitrate solution¹⁰. The colour changed from yellow to reddish brown colour indicates the formation of silver nanoparticles. The ASAgNPs thus obtained was purified by repeated centrifugation at 7000 rpm for 10 min. The pellet was collected and dried. The chemical tests were carried out in ASAgNPs for Proteins and Vitamin C.

Characterization of biosynthesized silver nanoparticles of *A. sessilis* (Linn.)

UV spectra analysis: The reduction of pure silver ions was confirmed by measuring the UV spectrum of the reaction mixture against blank. Spectral analysis was done using double beam Shimadzu 1800, spectrophotometer at a resolution of 1 nm from 250 to 450 nm.

SEM analysis: Morphological characterization of the samples was done using FE-SEM (JEOL JSM 3600). A pinch of dried sample was coated on a carbon tape further coated with platinum in an auto fine coater and subjected to analysis.

Particle size measurement: Carried out by means of laser diffractometry, using Nano-ZS, Malveren Instrument. Measurements were taken in the range between 0.1 and 10,000 nm.

Acute oral toxicity study

The oral acute toxicity study was carried out in adult female albino rats by the “fixed dose” method of OECD Guideline No.420.

Rotenone model of Parkinsonism

Male Wistar rats were divided into five groups of six rats each. Group-I: Normal control (every 48h for 11days by s.c). Group-II: Disease control (Rotenone 1.5mg/kg, every 48h for 11 days by s.c). Group-III: Rotenone + Syndopa (10mg/kg) p.o daily for 11 days. Group-IV: Rotenone + EEAS (200mg/kg) p.o daily for 11 days. Group-V: Rotenone + ASAgNPs (20mg/kg) p.o daily for 11 days¹¹. The behavioural tests were conducted before

treatment, then regularly at an interval of 6 days post treatment and final behavioural quantification was done 1 hour of the last dose. On 12th day animals were strangulated to death by cervical dislocation and the brain was dissected out for biochemical estimations.

Catalepsy

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

Open field test

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 min and behavioural aspects like ambulation, rearing frequency and self-grooming were noted.

III. Biochemical Estimations

Lipid peroxidation assay

The tissue homogenate was prepared in 0.1N Tris-HCl buffer. 1ml of the homogenate was combined with 2ml of TCA-TBA-HCl reagent and mixed thoroughly. The solution was heated in a boiling water bath for 15 min. After cooling the flocculent precipitate was removed by centrifugation at 1000xg for 10 min. The absorbance of the sample was read at 535nm against a blank.¹²⁻¹³

Reduced glutathione assay

Tissue was homogenized in 5ml precipitating solution. The tubes were incubated for 5 min at room temperature and then filtered through coarse grade filter paper. To 0.2ml filtrate, 3ml of 0.3M phosphate solution and 1ml of 0.04% DTNB was added. The tubes were capped, mixed by inversion and contents were read at 412nm within 4 min.¹⁴

Statistical analysis

The results were analyzed by using graph pad prism version 6.00; one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test.

IV. Results

Characterization of biosynthesized Silver nanoparticles of *Alternanthera sessilis* (Linn.)

UV-vis spectra analysis

Extracts from whole plants under study (*A. sessilis* Linn) showed rapid conversion of silver nitrate into silver nanoparticles indicated by color changes from pale yellow to red-brown within few min of extract addition in 3mM AgNO₃ solution. A representative scheme of biosynthesis and UV-Vis spectrum is given in Fig 1. Synthesized silver nanoparticles primarily characterized by UV-visible spectroscopy. ASAgNPs give typical spectrum having maximum absorption in range of 420-450 nm. This absorption is unique property of metal nanoparticles called SPR (Surface Plasmon Resonance) arises due to conduction of electrons on surface of AgNPs.¹⁵

Scanning electron microscopy

Characterization of ASAgNPs under the study by Scanning electron microscopy revealed that nanoparticles formed by are spherical in shape. (Fig 2)

Particle Size Analysis

Particle size analysis revealed that nanoparticles formed by *A. sessilis* (Linn.) had an average size of 122-396 nm. A well dispersed ASAgNPs were found with respect to intensity in this range. (Fig 3)

Catalepsy Test

Rotenone induced a significant increase in duration of catalepsy compared to vehicle group. EEAS and ASAgNPs were orally administered to male Wistar rats and duration of catalepsy was measured. The duration of catalepsy was significantly decreased on 12th day in EEAS (P<0.001) treated groups; 6th and 12th day in standard treated group compared to Rotenone induced Parkinsonism group. There observed non-significant decrease in duration of catalepsy on 6th and 12th day in ASAgNPs group (Fig 4)

Open field Test

The open field test was done in order to determine the effect of EEAS and ASAgNPs upon spontaneous locomotor activity. Rotenone group showed significant reduction in locomotor parameters compared to vehicle group. Locomotor parameters were significantly (P<0.001) enhanced on 12th day in EEAS, 6th and 12th day in standard group when compared to Rotenone group. EEAS treated group was seen to produce a significant increase in the ambulation (P<0.01), rearing (P<0.01) and self-grooming activity (P<0.05) compared to Rotenone group. ASAgNPs treated group also showed an improvement in locomotor activity compared to Rotenone group (Fig 5a, 5b, 5c)

Biochemical estimation

Lipid peroxidase assay

The oxidative stress marker studies revealed that administration of Rotenone increased the levels of LPO compared to the Vehicle group. The EEAS (200mg/kg) and syndopa treated groups reduced the lipid peroxidative tissue damage as revealed by the significant (P<0.001) reduction in the cellular malonaldehyde levels. The ASAgNPs showed a moderate effect on LPO. (Fig 6a).

Reduced glutathione assay

Rotenone administration induced a significant (P<0.001) decrease in the tissue GSH content as compared to vehicle group. Syndopa and EEAS showed an increase in GSH level as compared to Rotenone treated group (Fig 6b).

V. Discussion And Conclusion

A. sessilis (Linn.) is a commonly known as sessile joy weed, found in humid and warm regions of the world¹⁶. The use of nanoparticles in drug delivery therapy holds much promise in targeting remote tissue. Silver nanoparticles used as alternative strategies for drug delivery to Alzheimer brain are able to cross the blood brain barrier and penetrate into the cell cytoplasm and induce underlying cellular change. Increased generation of oxidative free radicals (OFR) or impaired antioxidant defence mechanism have been implicated in neurodegenerative diseases¹⁷. The extracts of *A. sessilis* (Linn.) have shown to exert significant antioxidant activity as in ferric reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay¹⁸ and it has shown to improve the levels of superoxide dismutase and catalase in the livers of ovariectomized mice¹⁹. Rotenone administration to rats caused a significant increase in duration of catalepsy,

decrease in locomotor and muscle activity. The duration of catalepsy was significantly decreased following EEAS ($P < 0.001$) treatment compared to Rotenone group. (Fig 4). Locomotor parameters were significantly enhanced on 12th day followed by EEAS treatment and this was evidenced from the results of open field test confirming anti-Parkinson's activity (Fig 5a to 5c). Enhancement in Locomotor parameters and decrease in duration of catalepsy was also seen in ASAgNPs.

Rotenone provides a valuable model for studying mechanism of oxidative stress induced dopaminergic damage²⁰. Oxidative stress generated as a result of mitochondrial dysfunction, particularly mitochondrial complex-1 impairment plays an important role in PD 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 it.²¹ Increased levels of the lipid peroxidation have been found in the substantia nigra of Parkinson disease patients²². Similar results were observed in the brain homogenates of Rotenone-treated animals. EEAS showed significant ($P < 0.001$) decrease in lipid peroxidase as compared to Rotenone group (Fig 6a).

A defect in one or more of the naturally occurring antioxidant defences 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.²³ A reduction in GSH levels may impair H_2O_2 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. EEAS showed a significant ($P < 0.001$) increase in level of GSH as compared to Rotenone group (Fig 6b). Increase in levels of GSH and decrease in levels of lipid peroxidase were also seen in ASAgNPs treated group. Rotenone treated group showed a significant increase in the levels of MDA which is an indication of extent of lipid peroxidation, decrease in the levels of 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. Pre-treatment with standard and EEAS resulted in a decrease in lipid peroxidase level and increase in the levels of 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 antioxidants²⁴. Oxidative processes are an important factor in the pathogenesis of several disorders, and postmortem studies have consistently implicated oxidative damage in Parkinson disease pathogenesis²⁵. The results are consistent with others studies which showed protective activity by substances such as alkaloids, poly phenols and flavonoids, known by their antioxidant power in the same experimental model²⁶⁻²⁷. Rotenone causes motor defects; due to the depletion of dopamine in the striatum therefore use of antioxidants could prove beneficial in countering its neurodegeneration²⁸. The change in dopamine level cause neuronal dysfunction, leads to Parkinsonism symptoms. The restoration of behavioural and locomotor alteration in Parkinsonism rats administered with EEAS indicates its potent antioxidant activity and presence of higher levels of flavonoids and phenolics in plant. Further studies are required for screening and evaluation of the particular phytoconstituents present in plant, which might exhibit a protective effect in this study.

FIGURES

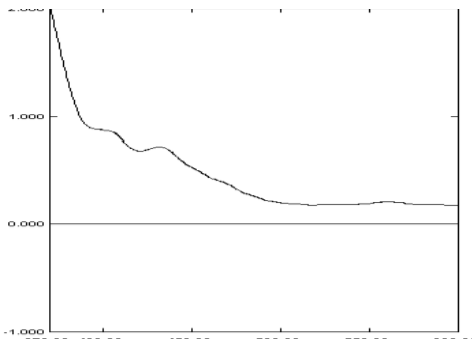


Fig 1: UV absorbance spectra at 444nm.

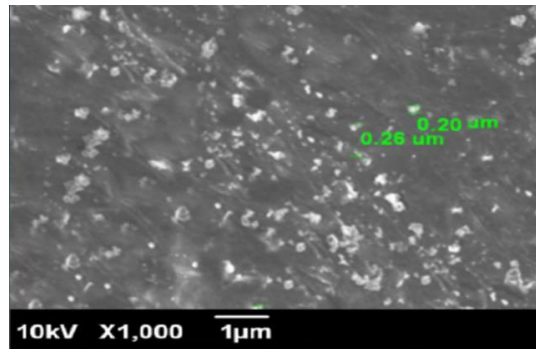


Fig 2: SEM image of ASAgNPs

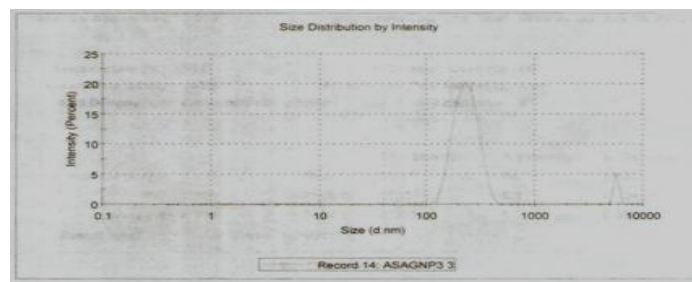


Fig 3: DLS (Size distribution by intensity)

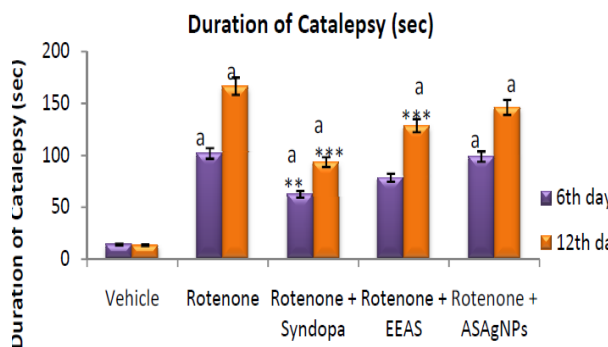


Fig 4. Effect of EEAS and ASAgNPs on Catalepsy test in rotenone model.

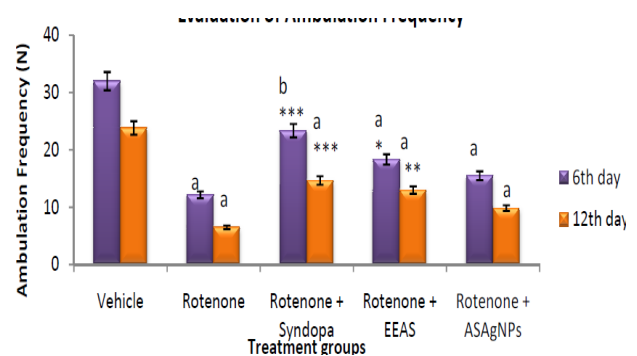


Fig 5a. Effect of EEAS and ASAgNPs in ambulation frequency on Open field test.

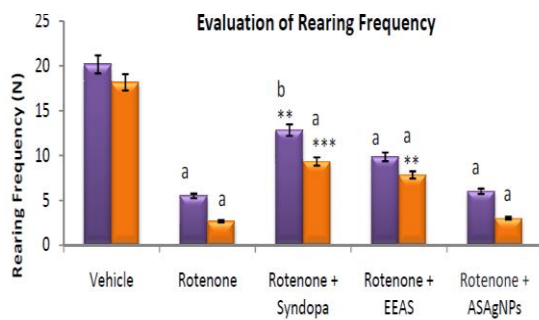


Fig 5b. Effect of EEAS and ASAgNPs in rearing frequency on Open field test.

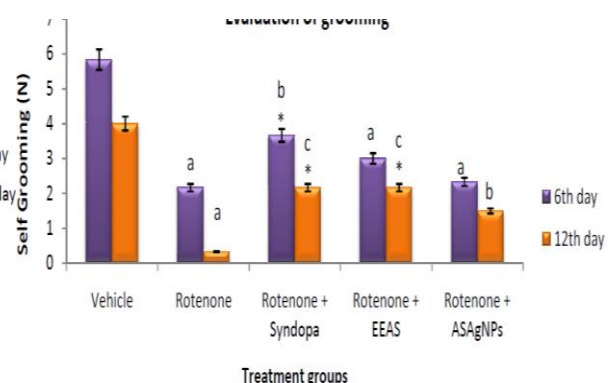


Fig 5c: Effect of EEAS and ASAgNPs in self-grooming on Open field test.

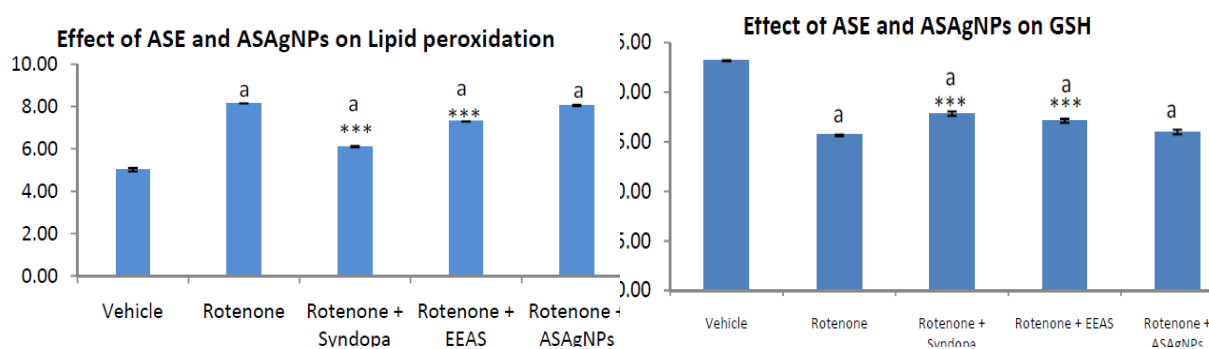


Fig 6a: Effect of EEAS and ASAgNPs on Lipid peroxidation

Fig 6b: Effect of EEAS and ASAgNPs on GSH

Values are expressed as Mean \pm SEM (n=6), analyzed by one-way ANOVA followed by Tukey's multiple comparisons test, * Represents $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ when compared to rotenone group. "a" Represents $P < 0.001$ when compared to vehicle only group.

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