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Research

Anti-parkinsoniasm Effect Of *Syzygium Cumini Fruit* Attenuates MPTP Induced Parkinsonism In Mice

Pavithra Duraisamy*, Dr. (Prof). V. Suresh, Kannan Raman, Latha Sundarraj, R.Mageswari, D.Padmavathi, I.Sathish

Department of pharmacology, J.K.K.Munirajah Medical Research Foundation, Annai J.K.K. Sampoorani Ammal College Of Pharmacy, Komarapalayam, The Tamil Nadu Dr. M.G.R Medical University, Chennai-600032, India.

*Author for Correspondence: Pavithra Duraisamy
Email:dpavithra191096@gmail.com

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Abstract

The present investigation has been undertaken as study the anti-parkinson activity of ethanolic extract of *syzygium cumini fruit*.The plant *Syzygiumcumini* of family Myrtaceae an ayurvedic herb which is known for its significant medical properties. Experiments were conducted following standard procedures. The ethanolic extract of *Syzygiumcumini* were evaluated for their invivo antioxidant and anti-parkison properties and neurotransmitters level. The antiparkinson activity of EESC was evaluated using MPTP induced parkinson models. Levodopa was used as standard. Extracts treated groups showed higher invivo antioxidant and antiparkinson activities. They also showed higher activity in neurotransmitters level. EESC exhibited better anti-parkinson activity that of standard. The result may be attributed to the chemical constituents such as cyanidindiglycosides present in it which may be due to their individual or cumulative effect that enhanced anti-parkinson activity and provided scientific evidence of the ethnomedicinal futures of *syzygiumcumini fruit*. These findings could justify the inclusion of this plant in the management of parkinson's disease.

Keywords: EESC, anti-parkinson, MPTP, chemical constituents.

INTRODUCTION

Parkinson's disease (PD) was the first neurological disease and characterized by degeneration of neuromelanin-rich dopaminergic neurons in the substantia nigra pars compacta and the frequent deposition of Lewy bodies. Speech impairment has been reported in 60-80% of the PD patients which reaches up to 100% in the later stages. Lazarus et al., subjected PD patients to the Indian Speech and Hearing Association (ISHA) articulation assessment and the Vaghmi software. It was found that 64.7% had slow reading speed, 60.2% hoarseness of voice, 39.8% articulatory defect, and 32.3% jerky speech.

Memory disturbances and dementia are known to occur in later stages of PD. Patients with early PD can have subtle disturbances in neuropsychological testing. To evaluate whether these abnormalities were affected by treatment, PD patients were assessed after 12 weeks of levodopa (LD) therapy.

Several neuropsychiatric manifestations such as depression, anxiety, and sleep disturbances have been described in PD. Depression in PD may be reactive depression as a result of chronic illness as well as a result of neurodegeneration.

Psychosis can also be a part of nonmotor spectrum of PD. It may be disabling to the patients and its presence may also warrant change in treatment strategies. The manifestations include hallucinations and delusions. In a study from India by Amar et al., 40 patients of PD with psychosis were assessed. Pure hallucinations were commonest in these patients (85%) and a combination of delusions and hallucinations was found in 7.5%. Of these visual hallucinations were predominant, amounting to 60%.

Animal models are valuable tools for studying the biology and genetics of human parkinson as well as for preclinical investigation of anti-parkinson therapeutics and parkinson preventive studies. Various animal models have been generated by genetic engineering, graft transplantation, and viral/physical/chemical induction. Studies from animal models of parkinson have been utilized for preclinical investigation of therapeutic efficacy and toxicity of chemicals and biologicals. Tremendous advances have been made in the generation of animal models of parkinson, which have become increasingly sophisticated by application of new technologies and integration of clinical information from patients. The goals are to faithfully recapitulate the human parkinson diseases in the animal models and apply them as preclinical tools, with the hope of successfully translating the basic knowledge into treatment and prevention of parkinson in humans.

The mouse has been the traditional animal model for basic and preclinical studies of parkinson, and other organisms including zebrafish play important and complimentary roles as models of parkinson research. Genetically engineered mouse models of parkinson have been generated by a variety of interventions such as chemical or physical mutagenesis, viral infection, insertion of transgenes, homologous recombination, and the recently developed gene edition. There are numerous publications of research studies regarding generation of animal models of parkinson and their pre-clinical applications. [1]

Neuroprotection suggests preventing or slowing disease progression. Nevertheless, despite advances toward this goal, all current treatments are symptomatic; none halt or retard dopaminergic neuron degeneration. L-dopa treatment produces many distressing side effects, and its possible that metabolism of excess dopamine by the monoamine oxidase enzymes in the brain produces too much H_2O_2. An initial good response to symptomatic pharmacological treatment declines with time, and severe side effects develop and later on surgical interventions are to be used. The progressive neurodegeneration in PD is not arrested by the currently used drug therapies. Hence, recent researches are focusing on finding therapies, preferentially herbal drugs.[2]

Traditional medicine practitioners have described the therapeutic efficacies of many traditional and indigenous plants against diseases. A large number of plants are used by folklore traditions in India for a treatment of parkinsonism. Various research data revealed that plants may work as anti-parkinson by multiple mechanisms. There are several reports stating that the extracts of several plants, used for parkinson therapy. Natural products that are safe, and possess physiological properties are excellent sources of new therapeutics for the treatment of parkinson. Some researchers, therefore, have shifted their focus to the potential anti-parkinson properties of plants. Thereby, the search for new agents is ongoing and natural products become a great target. Various literature and studies show that the active principles triterpenoids, iridoids , and flavonoids are having a crucial role in anti-parkinson treatment. Syzygiumcumini fruit is rich in these types of The purpose of this study is to investigate and evaluate anti-parkinson activity on MPTP induced model by using ethanolic extracts of Syzygiumcumini fruit.

Ramyakubar B et al., (2011) evaluated the neuroprotective effect of various extracts of Prosopischilenis (PC) seeds in MPTP mouse models. PC seed extract was administered at different doses in different groups once a day for seven days and the first dose was given 30 min prior to first MPTP injection (20mg/kg/i.p; 4 injections at 2h intervals).The alcoholic extract at given doses [100,200 and 300mg/kg (p.o)] significantly dose dependently increased the spontaneous motor activity, grip strength and alertness. Alcohol, ethyl acetate and aqueous extract of PC significantly improved the brain dopamine, nor epinephrine, epinephrine and 5-HT at a dose of 200 and 300mg/kg. These results indicate that PC extracts had neuroprotective effect on MPTP induced PD.[3]

PLANT PROFILE[4]

Plant name : Syzygiumcumini fruit
Family : Myrtaceae
Vernacular Names
English: Indian Blackberry
Tamil: Naval
Hindi: Jamun
Telugu: Jambolan
Kannada: Neril

Morphological Characters

As a rapidly growing species, it can reach heights of up to 30 m (100 ft) and can live more than 100 years. Its dense foliage provides shade and is grown just for its ornamental value. At the base of the tree, the bark is rough and dark grey, becoming lighter grey and smoother higher up. The wood is water resistant after being kiln-dried.Because of this, it is used in railway sleepers and to install motors in wells. It is sometimes used to make cheap furniture and village dwellings, though it is relatively hard for carpentry. The leaves which have an aroma similar to turpentine, are pinkish when young, changing to a leathery, glossy dark green with a yellow midrib as they mature. The leaves are used as food for livestock, as they have good nutritional value.

Traditional uses

All parts of the jambolan can be used medicinally and it has a long tradition in alternative medicine. From all over the world, the fruits have been used for a wide variety of ailments, including cough, diabetes, dysentery, inflammation and ringworm. It is also an ancient medicinal plant with an illustrious medical history and has been the subject of classical reviews for over 100 years. It is widely distributed throughout India and ayurvedic medicine (Indian folk medicine) mentions its use for the treatment of diabetes mellitus. Various traditional practitioners in India use the different parts of the plant in the treatment of diabetes, blisters in mouth, cancer, colic, diarrhea, digestive complaints, dysentery, piles, pimples and stomachache. During last four decades, numerous folk medicinal reports on the antidiabetic effects of this plant have been cited in the literature. In Unani medicine various parts of jambolan act as liver tonic, enrich blood, strengthen teeth and gums and form good lotion for removing ringworm infection of the head.[5]

Pharmacological activity

Despite tremendous advancements have been made in the field of diabetic treatments, several earlier investigations have been reported from the different parts of jambolan with antioxidant,anti-inflammatory, neuropsycho-pharmacological, anti-microbial, anti-bacterial, anti-HIV, antileishmanial and antifungal, nitric oxide scavenging, free radical scavenging, anti-diarrheal, antifertility, anorexigenic, gastroprotective and anti-ulcerogenic, behavioural effects and radioprotective activities. Besides the above, the effect of various concentrations of the leaf extracts of the plant on the radiation-induced micronuclei formation was studied by Jagetia and Baliga.[6]

Fig 1: Syzygiumcumini fruit

METHODOLOGY

Collection and authentication of plant

The Szyginiumcumini fruit collected from the surrounding areas of Erode district, Tamilnadu, India during the month of December authenticated certificate from Dr.K.K. Vijayakumar assistant professor in Botany Kandaswami kandar's college vellur, namakkal, Tamil Nadu Soon after collection the fruit were cleaned, driened in shade and crushed to a coarse powder, stored in an air tight plastic container, until further use.

Extraction of Plant Material

The *Syzygiumcumini* fruit was powdered in a conventional mixer and grinder were extracted with ethanol in a Soxhlet apparatus for 48 hours at room temperature. After extraction the extracts were evaporated by using rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4^{o}C for the further analysis.

Table 1: Evaluation Of Anti-Parkinson Study of Ethanolic Extract of *Syzygiumcumini* fruit Selection of animal for MPTP induced Parkinson evaluation

Species	Swiss albino mice
Age	3 month Body weight 25-30 g
No: of animals	30

Male Swiss albino mice 3 month of age, and 25-30 g body weight were offered by annaisampooraniammal College of Pharmacy, Namakkal. All the rats were kept at room temperature and allowed to acclimate in standard conditions less than 12 hr light/ 12 hr dark cycle in the animal house. Animals are fed with commercial pellet diet and water ad libitum freely throughout the study. The experimental procedure was approved by IAEC (Institution of Animal Ethical Committee).

Table 2: Experimental Design For Mptp Induced Parkinson

GROUP (n=6)	TREATMENT
GROUP 1	Control animal receiving vehicle(normal saline) only (p.o)
GROUP 2	Animals receiving disease control(1-methyl -4-phenyl-1,2,3,6-tetrahydropyridine) MPTP of dose 20mg/kg (i.p)
GROUP 3	MPTP(20mg/kg i.p) + levodopa (6mg/kg p.o)
GROUP 4	Animals receiving MPTP and ethanolic extract of Syzygiumcumini 200mg/kg (p.o)

Parkinson[7]

Preparation and induction of MPTP solution

The MPTP was purchased from sigma chemicals, Mumbai, India and was stored according to the manufacturer label (370C) to prevent its decomposition. The MPTP solution was freshly prepared at 25 mg/kg. The MPTP was dissolved in 0.9% sodium chloride solution and injected i.p at the dose of 25 mg/kg body weight, 7 days. MPTP solution is stable only for a period of 24 hours at 40C.

Preparation of Levodopa

6 mg/kg of levodopa was dissolved in distilled water. Levodopa was freshly prepared daily and given via i.p to the standard group.

Preparation of sample

200 mg/kg and 400 mg/kg were dissolved in distilled water and it was prepared freshly and given via oral route to group IV& V respectively for 7 days.

Evaluation Parameters

Motor Co-Ordination Test (Rota Rod Test)

Principle

The rota rod performance test is carried out on a rotating rod that provides forced motor activity in animals. The animals were placed on a rotating rod which is placed horizontally, suspended above a cage floor, which is high enough to induce avoidance of fall. Animals naturally try to stay on the rotating rod avoid falling to the ground. The length of time (duration) the animal stay on the rod without falling, gives a measure of their coordination, balance, physical condition and motor-planning.

Procedure

Motor Co-ordination test was conducted using rota rod apparatus. Animal was placed individually on the rotating rod and trained for 3 min trail at 25 rpm on the day before the first day of testing. A cut off time of 180s was fixed and each animal performed 3 separate trials at 5 min interval. After each trial, 5 min rest period was given to alleviate stress and fatigue. Motor coordination can be tested by comparing the latency to fall on the very first trial between treatment groups. The time taken by animals to fall from the rotating rod was noted.

Open field activity test

Principle

The Open Field task is a simple sensorimotor test used to determine general activity levels, gross locomotor activity, and exploration habits in rodent models of CNS disorders. Assessment takes place in a square, white Plexiglas box.

Procedure

Apparatus

The testing area (40cm x 40cm x 50cm tall) is fabricated from clear Plexiglas on the sides and opaque white Plexiglas on the bottom.

General Procedures

The open-field test is used to provide a qualitative and quantitative measurement of exploratory and locomotor activity in rodents. The apparatus consists of an arena surrounded by high walls, to prevent escape, and the floor of the open field is divided into squares. In the test session, the number of square crossings, rearing, and time spent moving are used to assess the activity of the rodent.

Testing Procedure

1. Clean the testing arena with soap and water. Allow the arena to dry before use.
2. Begin recording. Hold the test card (with the date, test name, and testing conditions) in view. Hold the subject's cage card in view.
3. Place the subject into the center of the arena.
4. After 30 minutes, return the subject to its homecage.

Beam walking test

Principle

Fine motor coordination and balance can be assessed by the beam walking assay. The goal of this test is for the mouse to stay upright and walk across an elevated narrow beam to a safe platform. This test takes place over 3 consecutive days: 2 days of training and 1 day of testing.

Procedure

Apparatus

The testing beam is fabricated using clear Plexiglas. There are four 25-cm long segments in the beam. The first segment is 3.5cm wide. The second is 2.5cm wide. The third is 1.5cm wide. The fourth is 0.5cm wide. The beam's incline is 15°.. A wire mesh (1cm square mesh) is affixed 1cm above the beam. The wire mesh is the width as the Plexiglas beneath it.

General Procedures

All testing occurs during the dark-phase (the active phase) of the light cycle. Testing is conducted under dim white-light illumination (about 150 lux). The subjects are moved from their housing room to the testing room and allowed to acclimate for at least 10min before testing. After testing is completed, the mice are immediately returned to the housing room. The training phase is not recorded. The testing phase is recorded using digital video cameras.

Estimation Of Brain Neurotransmitter[8]

Estimation of dopamine

Reagents

- 0.4 M HCl: 0.34 ml conc. HCl up to 10 mL H2O
- Sodium acetate buffer (pH 6.9): 0.72 mL of 1 M acetic acid (6 µL of glacial acetic acid up to 1000 µL with distilled water) + 6.84 mL of 0.3 M sodium acetate (0.408 g of sodium acetate in 10 mL distilled water) and volume were made up to 25 mL with distilled water. pH was adjusted with sodium hydroxide solution.
- 5 M sodium hydroxide: 5 g of NaOH pellets dissolved in distilled water and volume was made up to 25 mL with distilled water.
- M Iodine solution (in Ethanol): 1 g of potassium iodide + 0.65 g of iodine dissolved in ethanol and volume was made up to 25 mL.
- Sodium thiosulphate solution: 0.625 g Na2SO3 in 2.5 mL H2O + 22.5 mL 5 M NaOH
- 10 M Acetic acid: 14.25 mL of glacial acetic acid dissolved in distilled water and made up to 25 mL.

Procedure

To 1 mL of aqueous phase, 0.25 mL 0.4 M HCl and 0.5 mL of Sodium acetate buffer (pH 6. 9) were added followed by 0.5 mL iodine solution (0.1 M in ethanol) for oxidation. The reaction was stopped after 2 min by the addition of 0.5 mL Na2SO3 solution. 0.5 mL Acetic acid was added after 1.5 min. The solution was then heated to 100°C for 6 min. When the sample reached room temperature, excitation and emission spectra were read from the spectrofluorimeter. The readings were taken at 330-375 nm for dopamine. Blanks for the assay were prepared by adding the reagents of the oxidation step in reversed order (sodium sulphite before iodine). Different concentration of dopamine and nor-adrenaline (1 mg/ml) was used as standard.

Estimation of Serotonin

The serotonin content was estimated by the OPT method Reagents O-phthaldialdehyde (OPT) reagent: (20 mg in 100 ml conc. HCl)

Procedure

To 1.4 mL aqueous extract, 1.75 mL of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at 360-470 nm in the spectrofluorimeter. Concentrated HCI without OPT was taken as blank. Serotonin (1 mg/mL) at different concentration was used as standard.

Estimation of brain GABA content

Preparation of tissue homogenate Animals were sacrificed by decapitation and the whole brain was rapidly removed. 0.5 g tissue was weighed and placed in 5 mL of ice-cold TCA (10% w/v). The tissue was then homogenized and centrifuged at 10,000 rpm for 10 min at 0°C. The supernatant was used for estimation of GABA content.

Reagents

- Carbonate-bicarbonate buffer, 0.5 M (pH 9.95): 1.0501 g sodium bicarbonate and 1.3249 g sodium carbonate dissolved in distilled water and made up to 25 ml. pH adjusted to 9.95 if necessary.
- 0.14 M ninhydrin solution: 499 mg ninhydrin dissolved in 0.5 M carbonatebicarbonate buffer and made up to 20 ml.
- Copper tartarate reagent: 0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid. Procedure: 0.1 mL of tissue homogenate was placed in 0.2 mL of 0.14 M ninhydrin solution in 0.5 M carbonate-bicarbonate buffer (pH 9.95), and kept in a water bath at 60°C for 30 min. It was then cooled and treated with 5 mL of copper tartarate reagent. After 10 min fluorescence at 377/455 nm in a spectofluorimeter was recorded.

In vivo antioxidant activity[9]

Estimation of reduced glutathione (GSH)

Requirements

10%TCA 0.6 mM 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) in 0.2 M sodium phosphate 0.2 M Phosphate buffer, pH 8.0

Principle

DTNB is a disulfide compound, which was reduced by sulphadryl groups present in GSH. This reduction leads to the formation of yellow color, which was measured at 412 nm.

Procedure

To 1 ml of the homogenate, 1 ml of the TCA solution was added and centrifuged. The supernatant was collected and the precipitate formed was removed. To 0.5 ml of supernatant 2 ml of DTNB was added, the volume was made up to 3 ml with phosphate buffer. Then absorbance was read at 412 nm. The amount of glutathione was expressed as µ/mg protein.

Estimation of superoxide dismutase[10]

Principle

The estimation of superoxide dismutase enzyme is carried out by Beauchamp and Fridovich(1971) method. The substrate used for the assay consists of nitro blue tetrazolium chloride (NBT) which reacts with superoxide anions produced upon illumination of riboflavin in the presence of methionine as an electron donor, to produce formazan which is a blue colouredcomplex. The SOD present in the sample will act on the superoxide anions produced by riboflavin and thereby reduce the net superoxide anions in the substrate leading to decreased production of formazan manifested by decreased intensity of the blue colour formed.

The decrease in the formation of formazan is directly proportional to the amount of SOD in the sample. A decline of 50% in the formation of formazan is taken as one unit of SOD.

Procedure

Sample preparation: Prepare 10% tissue homogenate (1g liver in 10mL of 0.4M phosphate buffer, pH 7.0). Centrifuge at 10,000rpm for 15 minutes. The supernatant is used for the assay.

Estimation: For each sample analyzed a corresponding control is maintained. A common standard & blank is kept for each set analysed.

Test (T): 2.5mL Methionine, 0.3 mL Riboflavin, 0.1mL NBT, 0.1 mL liver homogenate.

Control (C): 2.5mL Methionine, 0.3mL Riboflavin, 0.1mL phosphate buffer (pH7. 8), 0.1mL liver homogenate.

Standard (S): 2.5mL Methionine, 0.3 mL Riboflavin, 0.1mL NBT, 0.1 mL phosphate buffer

Blank (B): 2.5mL Methionine, 0.3mL Riboflavin, 0.2mL phosphate buffer (pH 7.8)

The beakers labeled as TEST, STANDARD and CONTROL are subjected to illumination for 10 minutes in an illumination chamber lined with aluminium foil, and fitted with a 15W fluorescent lamp. Following illumination, immediately the optical density of all the reaction mixtures are read at 560nm. Units of enzyme present in the sample are calculated using the formula and expressed as U/mg
protein (Liver homogenate).

Histopathology Techniques

The brain was collected and washed under saline and preserved in 10% buffered formalin. The tissues were trimmed into sections and were subjected to prepare paraffin blocks. 5 microns thickness sections were cut and stained with haematoxylin and eosin (H & E) and observed under the microscope.

Statistical Analysis

The statistical analysis was carried out by using PRISM version 5 software. The data's of allparameters were analysed by means of one way ANOVA followed by Dunnett's test. The results were expressed as mean ±SEM.

RESULTS

Extractive Yield

Percentage Yield of EESC

Coarsely powdered fruit of Szyginiumcumini were extracted with water using Soxhlettechnique and thepercentage yield was found to be 24% w/w.

Preliminary Phytochemical Analysis

Table 3: Phytochemical analysis of EESC

S.No	Phytochemical Constituents	Eesc
1.	Alkaloids	Negative
2.	Flavonoids	Present
3.	Steroids	Present
4.	Triterpenoids	Present
5.	Reducing sugar	Present
6.	Tannins	Present
7.	Glycosides	Present
8.	Protein & amino acid	Present
9.	Saponins	Present
10.	Phenols	Negative

Screening Of Antiparkinsonian Activity Of Eesc
[MPTP Model]

Table 4: Rota Rod apparatus

GROUP	I	II	III	IV	V
Retention time In seconds	**180 ± 5.80**	**8.24± 2.8*** **	**24.83 ± 0.98**$^{###}$	**86.17 ± 0.68** $^{###}$	**134.2 ± 1.77**$^{###}$

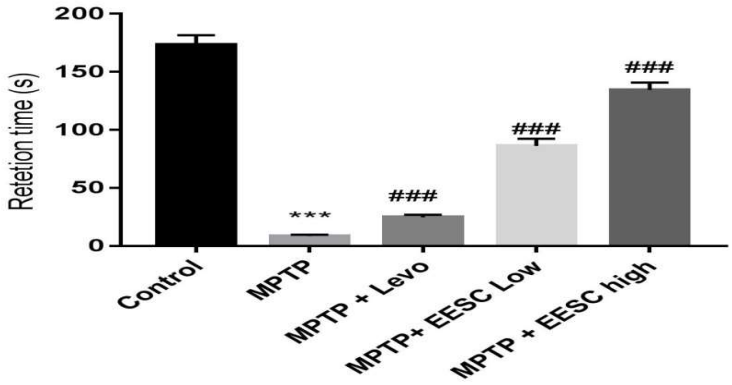


Table 5: Open Field Activity Test

GROUP	I	II	III	IV	V
NO.OF SQUARES CROSSED	**21.33±0.49**	**2.17±0.30*** **	**5.83±0.60** ##	**13.17±0.99** ###	**18.17±1.30** ###
REARING	**17.50±0.42**	**1.17±1.47*** **	**3.83±0.30**$^{###}$	**7.167±0.30** ###	**11.83±0.30** ###
GROOMING	**16.67±0.21**	**2.83±0.40*** **	**4.167±0.40** $^{###}$	**8.50±0.34** ###	**13.33±0.49** ###

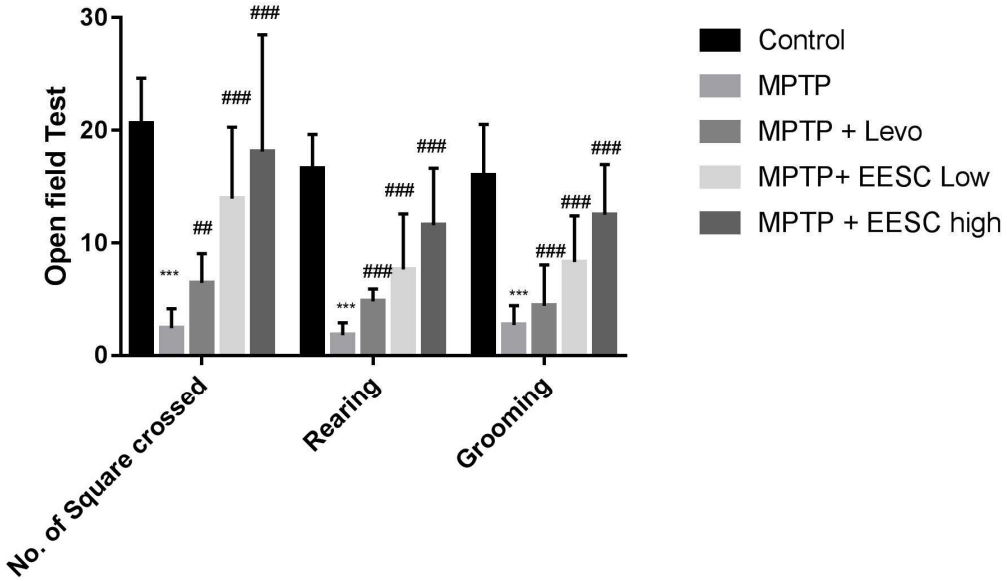
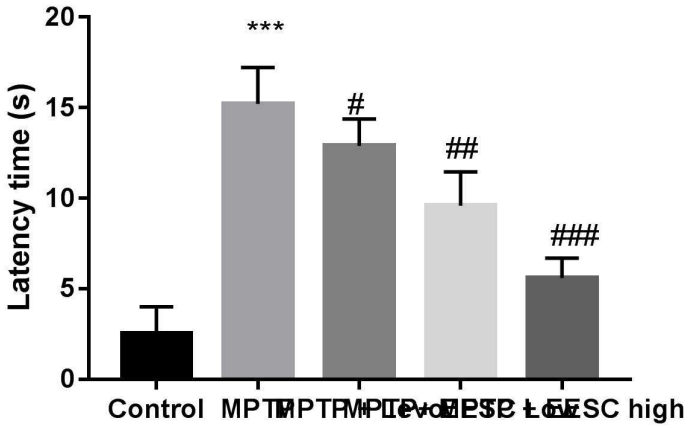


Table 6: Catalepsy Test

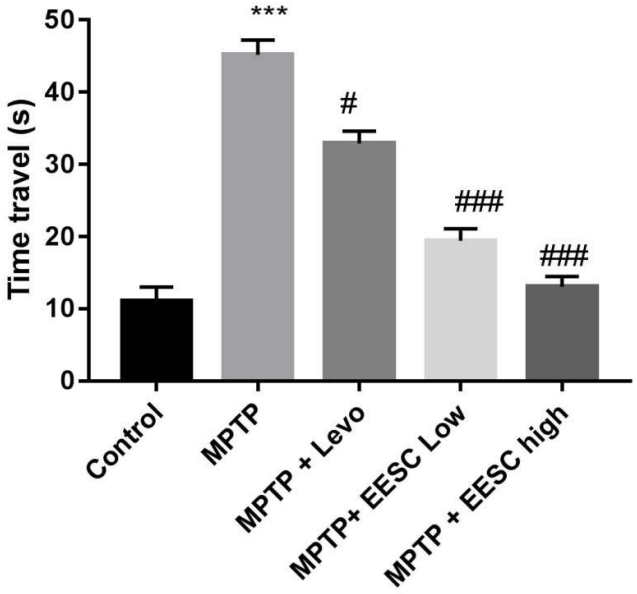
GROUP	I	II	III	IV	V
LATENCY PERIOD(SEC)	2.16±0.16	15.83±0.16***	13.67±0.33 #	9.5±0.21 ##	5.83±0.47 ###



CATALAPSY TEST

Table 7: Beam walking test

GROUP	I	II	III	IV	V
TIME TAKEN TO TRAVEL (SEC)	11.77±0.30	45.33±0.21***	33.50±0.42 #	19.17±0.4014 ###	13.50±0.3416 ###



Estimation of neurotransmitters and metabolic enzyme

Table 8: Estimation of glutamate levels

GROUP	I	II	III	IV	V
µmol/mg of tissue	77.03±0.1500	102.10±0.22**	87.04±0.33 ##	83.53±0.11 ##	74.2±0.15 ##

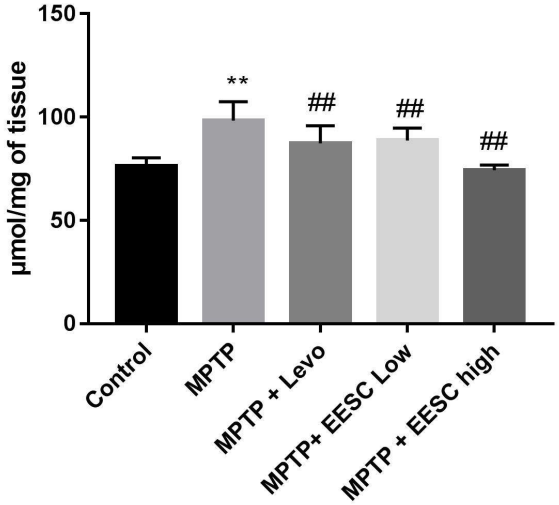


Table 9: Estimation of Dopamine levels

GROUP	I	II	III	IV	V
ng/mg of tissue	1.39±0.10	0.42±1.01 ***	0.54±2.89 #	0.89±1.20 ##	1.21±0.01 ###

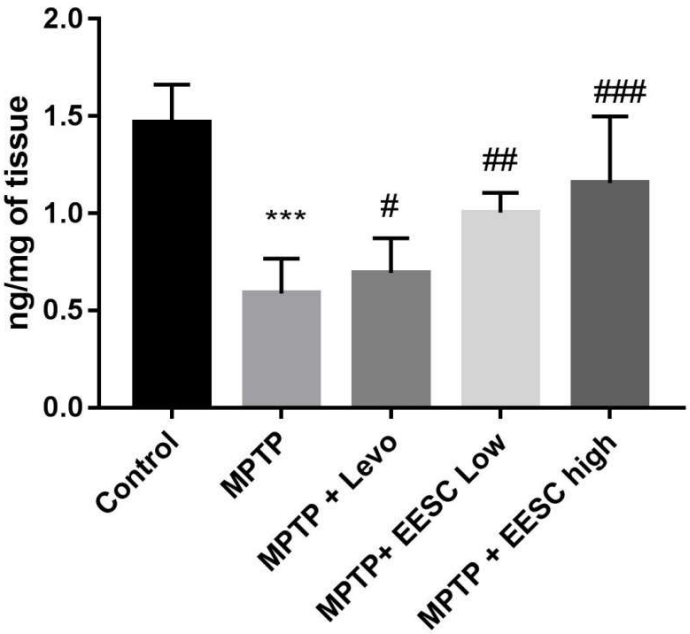


Table 10: Estimation of Serotonin levels

GROUP	I	II	III	IV	V
ng/mg of tissue	0.85±0.06	0.17±1.80 ***	0.58±1.20 ###	0.72±2.45 ###	0.82±2.84 ###

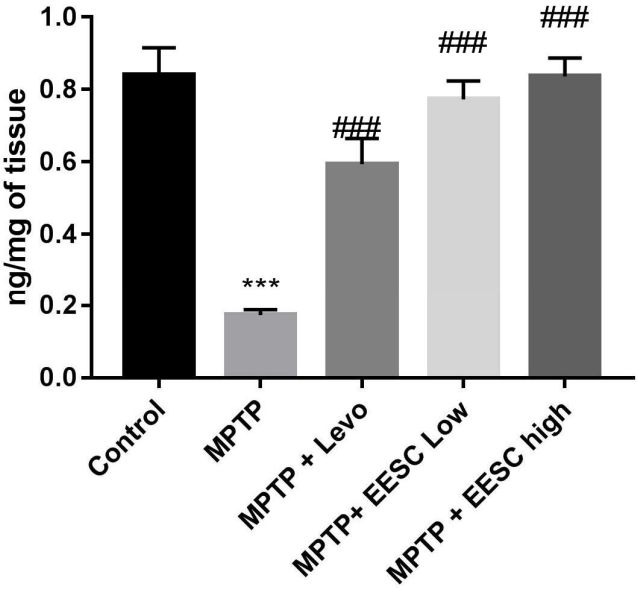
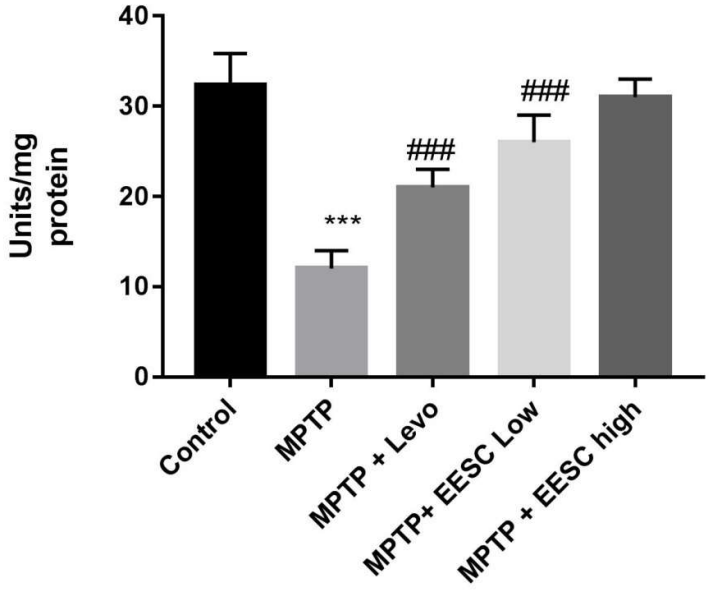


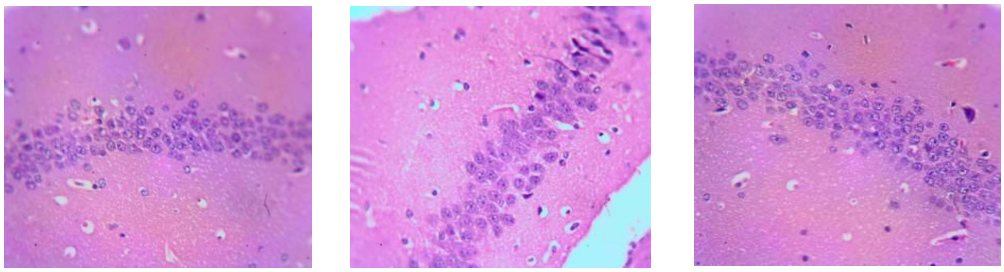
Table 11: Estimation of superoxide dismutase

GROUP	I	II	III	IV	V
Units/mg protein	7.80±0.04	1.72±0.01***	4.64±0.44###	6.45±0.08 ###	7.21±0.12 ###

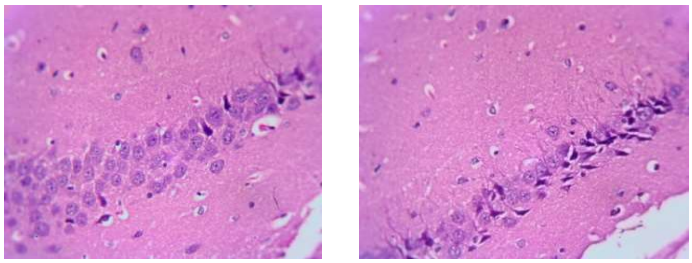
Estimation of glutathione peroxidase



Histopathology of brain



Group –I **Group – II** **Group - III**



Group – IV **Group – V**

Group I- Shows normal neuronal arrangements in brain region.
Group II- Show neuronal cell with spongy arrangement in brain region.
Group III- shows the degeneration of neuronal cells and exhibits the differentiation when compared with normal cells. It also exhibits decrease in number of cell arrangement in striatal region of mice brain when compared to control group.
Group IV- exhibits the regeneration of neuronal cells with the constructive arrangement in brain region.
Group V- shows the increase in neuronal cell arrangement indicating the regeneration and also cell viability in brain region.

DISCUSSION

This study aims to investigate the Neuroprotective effect of Szyginiumcumini on MPTP intoxicated animal models of PD by analyzingbehavior patterns, brain antioxidant, brain neurotransmitters and Histopathological studies. PD is a commonly occurring neurodegenerative disorder that produces muscular rigidity, bradykinesia, tremor in resting limbs and loss of postural balance. The basic neuropathology of PD involves the selective degeneration of dopaminergic cells in specific brain regions like the striatum; when degeneration in these neurons reaches a threshold reduction of 80% dopamine, the motor symptoms of PD emerge. The cerebral cortex, which is abundant with neurons and easily accessible, demonstrates its importance in modelling PD. Szygniumcumini has a long history in herbal medicine in various countries.[11] The fruits of Szyginiumcumini have long been used as a natural medicine in tropics. The fruits of Szyginiumcumini is well known for its different types of pharmacological properties such as Stomach disorder, Urinary infections, fever, Tooth ache, Diuretic, Jaundice, Asthma, Arthritis, Inflammation, Migraine, Dropsy, Leprosy. The major active constituents are the fruits are rich in raffinose, glucose, fructose, citric acid, mallic acid, gallic acid, anthocyanins, delphinidin-3-gentiobioside, malvidin-3-laminaribioside, petunidin-3-gentiobioside, cyanidindiglycoside, petunidin and malvidin. The sourness of fruits may be due to presence of gallic acid. The color of the fruits might be due to the presence of anthocyanins.[12] The fruit contains 83.70–85.80 g moisture, 0.70–0.13 g protein, 0.15–0.30 g fat, 0.30–0.90 g crude fiber, 14.00 g carbohydrate, 0.32–0.40 g ash, 8.30–15.00 mg calcium, 35.00 mg magnesium, 15.00–16.20 mg phosphorus, 1.20–1.62 mg iron, 26.20 mg sodium, 55.00 mg potassium, 0.23 mg copper, 13.00 mg sulfur, 8.00 mg chlorine, 80 I.U. vitamin A, 0.01–0.03 mg thiamine, 0.009–0.01 mg riboflavin, 0.20–0.29 mg niacin, 5.70–18.00 mg ascorbic acid, 7.00 mg choline and 3.00 mcg folic acid per 100 g of edible portion. Since Szyginiumcumini have not been studied for its anti-parkinson activity, the present study was aimed to evaluate the anti-parkinson activity potential of ethanolic extracts of Szyginiumcumini in MPTP inducedswiss albino mice respectively. MPTP was adapted as a PD model in rodents and primates.

These studies showed that 200mg and 400mg of ethanolic extracts of Szyginiumcumini produces better protective effect on MPTP induced PD in Swiss Albino mice than the Standard Anti Parkinson drug Levodopa. Therefore our further studies will be extended to identify which phytoconstituent will be responsible for the better AntiParkinson effect which is comparable to that of standard AntiParkinson Drug Levodopoa.[13]

Effect of Szygniumcumini on MPTP induced PD

Effect on behavioral parameters

Roto rod test

Among MPTP alone treated groups shows significant decrease in retention time as compared to control group. In Levodopa treated group shows significant increase in retention time as compared to MPTP group. EESC 200mg/kg and 400 mg/kg shows significant increase in retention time when compared to MPTP treated as well as levodopa treated group.

Catalepsy bar test

Among MPTP alone treated groups shows significant increase in latency period as compared to control group. In Levodopa treated group shows significant decrease in latency period as compared to MPTP group. EESC 200mg/kg and 400 mg/kg shows significant decrease in latency period when compared to MPTP treated as well as levodopa treated group.

Beam walking test

Among MPTP alone treated groups shows significant increase in time taken to travel as compared to control group. In Levodopa treated group shows significant decrease in time taken to travel as compared to MPTP group. EESC 200mg/kg and 400 mg/kg shows significant decrease in time taken to travel when compared to MPTP treated as well as levodopa treated group.

Open field test

Among MPTP alone treated groups shows significant decrease in no.of square crossed, grooming, and rearing as compared to control group. In Levodopa treated group shows significant increase in no.of square crossed, grooming, and rearing as compared to MPTP group. EESC 200mg/kg and 400 mg/kg shows significant increase in no.of square crossed, grooming, and rearing when compared to MPTP treated as well as levodopa treated group.

Effect on Brain antioxidant levels

The biotransformation of MPTP into MPP+, which is catalyzed by the mitochondrial enzyme monoamine oxidase B, represents the major route for MPTP-mediated neurotoxicity. The conversion of MPTP to MPP+ has been suggested to induce the formation of ROS. This notion is supported by previous studies which showed increased superoxide ($O_2^{\cdot -}$) and hydroxyl radical (·OH) levels during the biotransformation of MPTP. While the damage induced by $O_2^{\cdot -}$ is limited, it can react with nitric oxide (NO) to form peroxynitrite ($ONOO^-$) which readily forms the more reactive ·OH radical. Other studies have shown that MPTP induces toxicity through ATP depletion and mitochondrial dysfunction. Moreover, that ATP depletion plays a major role in MPTP induced neuronal cell death.[14] However, it is likely that MDA can form complexes with other biological components such as protein, lipids, and nucleic acids which can contribute to an underestimation of endogenous lipid peroxidation. On the contrary to our lipid peroxidation data, we also show that MPTP can lead to distinct alterations in endogenous antioxidant defense mechanisms. MPTP treatment has been previously shown to significantly increase Mn-SOD and CuZn-SOD activities in the striatum of C57BL/6 mice, which is suggestive of acute oxidative stress insult. SOD is upregulated in cells when $O_2^{\cdot -}$ is produced in excessive levels. This observation suggests that SOD may play a role in the toxicity observed following acute treatment of MPTP, although ROS formation may not play a major role in MPTP-induced toxicity. CAT is an enzyme that is involved in the detoxification of ROS and the elimination of hydrogen peroxide (H_2O_2) in particular. The increase in both intracellular SOD and CAT activities may therefore represent an adaptive response due to the leakage of free radicals during impaired mitochondrial respiration. Treatment with MPTP also leads to reduced activity of GPx and decreased levels of the essential pyridine nucleotide NAD+, ATP, and GSH in primary human neurons after a 24-hour exposure. The maintenance of GPx activity appears crucial for the maintenance of cell viability during oxidative insult. Moreover, previous studies have shown that MPP+, the metabolite of MPTP induces GSH depletion without increasing the levels of oxidized glutathione disulfide (GSSG)[14]. Reduced GSH levels may occur due to that MPP+ induced decline in intracellular NAD+ and ATP stores which are necessary for GSH anabolism, release, and taken together, our data suggests that MPTP exposure can limit the endogenous antioxidant defense, subsequently increasing the vulnerability of neuronal cells to additional oxidative stress. An imbalance in the function of endogenous antioxidant defense mechanisms can lead to the accumulation of free radicals and ROS and increased susceptibility to oxidative stress, which contributes to the pathogenesis of PD.

Effect Szyginiumcumini on glutathione peroxide level of brain

Among MPTP treated group shows significant decrease in brain GSH level as compared to control group. EESC 200 mg/kg and 400 mg/kg body weight and levodopa pretreated group shows significant increase in brain GSH level when compared to MPTP treated group.

Effect of dopamine assay

The MPTP treated group of animals had decreased dopamine levels when compared to the control group. Treatment and Pretreatment with standard drug, Szyginiumcumini (Group III,IV,V) the dopamine activity showed significance increase in MPTP group. The data showed that the Szyginiumcumini has a very protective role of dopamine in the receptor binding density of mice.

Effect of Serotonin

The MPTP treated group of animals had decreased Serotonin levels when compared to the control group, the serotonin activity was observed in the MPTP group mice when compared to the control group. Treatment and Pretreatment with standard drug, Szyginiumcumini (Group III,IV,V) groups showed significance increase in Serotonin levels.

Effect of glutamate

The MPTP treated group of animals had increased glutamate levels when compared to the control group. The glutamate activity was observed in the MPTP group mice when compared to the control group. Treatment and Pretreatment with standard drug, Szyginiumcumini (Group III, IV, V) groups showed significance decrease in glutamate levels.

CONCLUSION

From the present study, it can be considered that the aqueous extract of Szyginiumcumini exhibited significant anti-parkinsonism activity in MPTP model in mice respectively. The probable mode of action of this plant decreased lipid peroxidation due to the presence of flavonoids, polyphenols and glycosides. All the Parameters of extract treated group animals have shown better results when compared with MPTPinduced group and the standard L-dopa treated group. These findings provide apreliminaryevidence for its potential as anti-parkinsonian medication, including Parkinson's disease prevention and improvement of symptoms.These studies showed that 200mg and 400mg of ethanolic extracts of Szyginiumcumini produces better protective effect on MPTP induced PD in Swiss Albino mice than the Standard Anti Parkinson drug Levodopa. Therefore our further studies will be extended to identify which phytoconstituent will be responsible for the better AntiParkinson effect which is comparable to that of standard AntiParkinson Drug Levodopoa

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