

Neuroprotective action of *Piper longum* against MPTP-induced changes in mouse brain

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ABSTRACT

Background: Parkinson's disease is a neurodegenerative disorder characterized by the progressive and selective loss of dopaminergic neurons. **Purpose:** The study was designed with the aim of investigating the effect of *Piper longum* in experimental model of Parkinson's disease. **Methods:** Swiss albino mice were divided into the following 4 groups: group I received vehicle saline (0.25 ml); group II was treated with 20 mg/kg body weight of MPTP (2 dose with 2 h intervals); group III received MPTP along with 50 mg/kg body weight of *Piper longum* and group IV received 50 mg/kg body weight *Piper longum*. **Results:** MPTP induced mice showed vascular degeneration and cytoplasmic vacuolation in the brain tissue. In group II the specific activities of superoxide dismutase and, catalase were increased with increase in lipid peroxidation along with reduction in the activities of adenosine tri phosphatase. **Conclusion:** The oxidative stress and related biochemical alteration by MPTP were altered in *Piper longum* treatment. However, further studies are needed to assess the precise mechanism to support the clinical use of the plant as a source of antiparkinsonian drug.

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Introduction

Parkinson's disease is one of the most common neurodegenerative disorders, characterized by a relatively selective degeneration of dopaminergic neurons in the substantia nigra pars compacta.¹⁻³ However, pathological cell death within this nucleus is not uniform. The symptoms include resting tremor, bradykinesia, postural instability, and rigidity.⁴⁻⁶ 1, methyl, 4 phenyl 1,2,3,6 tetra hydro pyridine (MPTP) is a potent neurotoxin that induces Parkinson's disease in various experimental animals including monkeys, mice, cats, dogs, rats and goldfish.⁷ MPTP is a highly lipophilic molecule after systemic administration it crosses the blood-brain-barrier immediately. Within the brain, MPTP is rapidly converted to the hydrophilic metabolite 1-methyl-4-phenylpyridinium ion (MPP⁺)⁸ and is responsible for the dopaminergic neuron loss. MPTP was used in the current study to mimic PD in mice which was then subjected to neuroprotective treatment. *Piper longum* L. (PI Piperaceae) is also called as long pepper. It is a slender aromatic climber, is a native of the Indo-Malayan region and grows wild in the tropical rain forests of India.⁹ The extract of the crude drug "Piperis Longi Fructus," the fruits of *Piper longum* (PI), is frequently used in folk medicine to treat various disorders.¹⁰⁻¹³ Piperine was the first amide isolated from Piper species and was reported to display central nervous system depression, antipyretic, and anti-inflammatory activity. In the present study the neuroprotective action of PI was investigated on MPTP mouse model.

Methods

Healthy male Swiss albino mice (8-14 weeks old) 25-30g of weight were used throughout the study. Animals were purchased from Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Madhavaram, Chennai, India and maintained under constant temperature with free access to food (pellet diet Gold Mohor rat feed, Hindustan Lever Ltd., Bangalore, India) and water. This study was conducted

according to the Animal Ethics Committee Guidelines of our Institution.

Chemicals

MPTP-HCl was purchased from Sigma-Aldrich, USA. Plant drug used for the study, *Piper longum* (PI) was obtained from local authorized dealers. All the chemicals used for the study were of analytical grade.

Experimental protocol

The experimental mice were divided into 4 groups with 6 animals in each group; group I received 0.9% NaCl (control); group II received MPTP-HCl at the dosage of 20 mg/kg of two doses at 2 h interval and served as PD model; group III received MPTP along with 50 mg/kg body weight of *Piper longum* (treatment group); group IV received 50 mg/kg body weight of *Piper longum* (drug control group). Animals were sacrificed on the 7th day of experimental protocol.

Tissue Collection

At the end of the experimental period, the rats were sacrificed by cervical decapitation. The brain tissue was excised immediately, rinsed in ice-cold saline, dried, weighed and homogenized in 0.1 M Tris-HCl buffer of pH 7.4 using a Teflon homogenizer. The tissue homogenate was then centrifuged in a refrigerated centrifuge at 1500 × g to remove the debris and the supernatant was analysed for various biochemical parameters. The tissue homogenate was stored at -20°C until further use.

Histopathological analysis

Mid portion of brain specimens obtained from all groups of animals were fixed in 10% formalin. The tissue sections were embedded in paraffin wax and sectioned at 5-6 μm thickness and sections were stained with haematoxylin-eosin for photo microscopic observations of the brain histopathological architecture.

Biochemical analysis

The protein content was determined by the method of Lowry *et al.*¹⁴ Superoxide dismutase was assayed following the method of Misra and Fridovich.¹⁵ Catalase was estimated as per the method of Takahara *et al.*¹⁶ The level of lipid peroxides were estimated in mid brain by the method of Ohkawa *et al.*¹⁷ Reduced glutathione was studied by the method of Staal *et al.*¹⁸ Total ATPase activity was determined spectrometrically quantifying inorganic phosphorus produced by ATP hydrolysis. Inorganic phosphorus (Pi) was determined by a colorimetric assay of Taussky and Shorr;¹⁹ the activity of Mg²⁺ ATPase was determined by the method of White and Ralston.²⁰ Ca²⁺ ATPase was determined by the method of Hejerten and Pan.²¹

Statistics

All the grouped data were evaluated with SPSS/10 software. Hypothesis testing methods included one way analysis of variance followed by Least Significant Difference test. P values of less than 0.05 were considered to indicate statistical significance. All these results were expressed as mean \pm SE for six animals in each group.

Results

Fig. 1 illustrates the histopathological changes in control and experimental mice. Degenerative changes could be seen in MPTP mice, which included cellular inflammation, vascular degeneration and cytoplasmic vacuolation which can be altered on PI treatment.

Increased reactive oxygen species, formation and alteration of endogenous antioxidant enzyme activities were reported in the

MPTP induced neurodegeneration and *Piper longum*

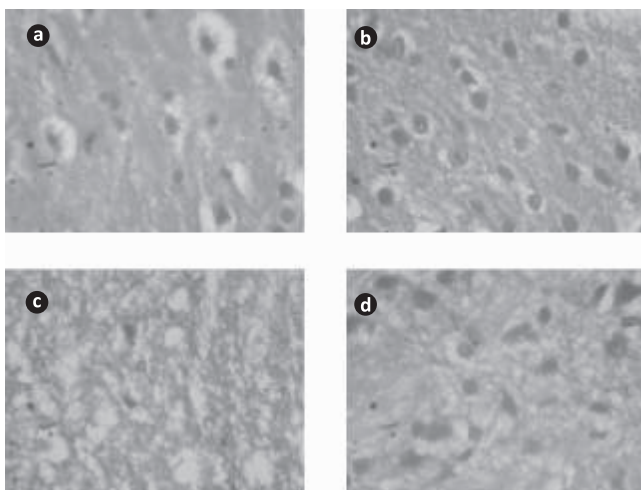


Fig.1: Histopathological studies of mid brain viewed under light microscope in control and experimental animals. Hematoxylin/Eosin staining of paraffin sections (100X H & E). a: Section of brain from mice treated with 0.95% NaCl for 7 days showing normal architecture. b: Section of brain from mice treated with 50mg kg/body weight of PI for 7days showing normal architecture. c: Section of brain from mice treated with MPTP for 7 days showing pathological changes like cell swelling, vascular degeneration and cytoplasmic vacuolation. d: Section of brain from mice treated with MPTP for 7 days followed by PI showing marked reduction of degeneration and vacuolation.

brains of MPTP-treated mice.²² Like these reports, the present study also showed a similar change of activities of antioxidant enzymes in the brain of mice treated with MPTP along with elevated LPO.

Table I shows antioxidant status; the activities of SOD, CAT and LPO in the midbrain were significantly elevated ($p < 0.05$) and decreased GSH ($p < 0.05$) in MPTP-treated animals (group II) relative to the controls. PI administration of MPTP-treated mice (group III) tends to bring the SOD and CAT close to normal values. No significant changes ($p < 0.05$) were found in mice treated with PI alone (group IV).

Table 1: Oxidative stress levels in the brain of control and experimental mouse.

Groups	SOD Units/min/mg protein	Catalase μ Moles of H ₂ O ₂ consumed/ min/mg protein	LPO nmol of MDA released/ mg protein	GSH g/mg protein
Group I	1.55 \pm 0.31	0.98 \pm 0.10	23.31 \pm 0.54	2.54 \pm 0.27
Group II	3.41 \pm 0.45 ^a	2.70 \pm 0.35 ^a	64.96 \pm 0.81 ^a	1.28 \pm 0.18 ^a
Group III	1.57 \pm 0.18 ^b	1.05 \pm 0.34 ^b	24.04 \pm 0.75 ^b	2.49 \pm 0.36 ^b
Group IV	1.45 \pm 0.26 ^{ns}	1.20 \pm 0.32 ^{ns}	27.07 \pm 0.55 ^{ns}	3.41 \pm 0.57 ^{ns}

Statistical significance was represented as $p < 0.05$. ^a Group I were compared with Group I, & ^b Group II were compared with Group III, ns-non significant. [Values are expressed as the mean \pm SE]. Swiss albino male mice were treated with saline (0.95%) served as control. MPTP (20 mg/kg body weight 2 dose for 2 hr intervals) and *Piper longum* 50 mg/kg body weight. 6 animals treated in each group.

Table 2 shows activities of ATPases of brain tissue from experimental animals. The levels of these enzymes were reduced ($p < 0.05$) in MPTP mice group when compared with control group. MPTP induced elevation of SOD, CAT were reduced almost to that of normal mice, while drug control group also showed similar values as that of control.

Table 2: Changes in the level of ATPase in the brain of control and experimental mouse.

Groups	Mg ⁺⁺ ATPase	Ca ⁺⁺ ATPase	Total ATPase
Group I	4.86 \pm 1.72	2.26 \pm 1.35	6.41 \pm 1.67
Group II	1.63 \pm 1.13 ^a	1.72 \pm 1.36 ^a	3.25 \pm 2.26 ^a
Group III	4.67 \pm 1.94 ^b	2.42 \pm 1.76 ^b	5.68 \pm 2.29 ^b
Group IV	4.90 \pm 2.14 ^{ns}	2.50 \pm 1.66 ^{ns}	6.23 \pm 2.03 ^{ns}

Statistical significance was represented as $p < 0.05$. Group I were compared with Group II & Group II were compared with Group III, ns-non significant. [Values are expressed as the mean \pm SD].

Note : Swiss albino male mice treated with saline (0.95%) served as control. MPTP (20 mg/kg body weight 2 dose at 2 hr intervals) and *Piper longum* 50 mg/kg body weight. 6 animals treated in each group.

Discussion

Oxidative stress changes in the brain of Parkinson's disease patients^{23, 24} and MPTP-mouse model and other animals are the importance of oxidative damage and the involvement of free radicals in the pathogenesis of this movement disorder.²⁵⁻²⁷

Inhibition of mitochondrial respiration *in vivo* and the generation of $\cdot\text{OH}$ due to MPTP metabolites in rodents have been showed in the literature.^{28,29}

There have been several reports during the past decade describing increased lipid peroxidation,³⁰ a decrease in GSH content³¹, and changes in antioxidant enzyme activity^{32,33} in the brains of both parkinsonian patients and MPTP monkey models³⁴⁻³⁶ emphasising the importance of oxidative damage and the involvement of free radicals in the pathogenesis of this movement disorder.

The present study also supports these observations and provide a strong evidence for the involvement of $\cdot\text{OH}$ in the neurodegeneration caused by MPTP. A significant increase in the level of LPO was observed in the MPTP treated groups in mid brain when compared to control group. Lipid peroxidation induced by free radicals is believed to be one of the major causes of cell membrane damage leading to lysis of cell.³⁷

SOD protects tissues by catalyzing the removal of superoxide radical that damage the membrane and its function. Catalase is responsible for the detoxification of significant amount of H_2O_2 .³⁸ MPTP mice showed increased activities of SOD, CAT in group II due to from the generation of $\cdot\text{OH}$ to eliminate H_2O_2 formed in the brain³⁹ in addition to the depletion of GSH.

The major observation of this study is the recovery of free radical induced damage in mid brain cells by PI. A significantly decreased activity of ATPase seen in MPTP mice caused due to the inhibition of complex I in the mitochondrial electron transport chain (induced by 1-methyl-4-phenylpyridinium (MPP⁺), results in impaired ATP production, loss of mitochondrial membrane potential, and formation of reactive oxygen species.⁴⁰ The repeated administration of PI was found to reverse the influence of MPTP on SOD, CAT, GSH, LPO and ATPase activities.

The treatment of PD represents a significant challenge. In the later stages of PD, there is an urgent need for novel anti-PD treatment, without inducing severe drug-related involuntary movements. Finally, the challenge of developing an effective neuroprotective therapy for PD remains an exciting, if not elusive goal.

Thus, the treatment with PI holds promise to rectify the deleterious changes induced by MPTP. A detailed investigation about the exact role of PI against neurodegeneration needs further investigation.

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