ANTI-PARKINSON ACTIVITY OF AQUEOUS EXTRACT OF LEAVES OF *MURRAYA KOENIGII* AGAINST PARAQUAT-INDUCED PARKINSONISM IN WISTAR RATS

MAHESWARI REDDY B[1], DHANAPAL CK[2]*, LAKSHMI BVS[1]

[1]Department of Pharmacology, Malla Reddy College of Pharmacy, Medchal, Telangana, India. [2]Department of Pharmacy, University Institute of Pharmaceutical Technology, Faculty of Engineering and Technology, Annamalai University, Chidambaram, Tamil Nadu, India. Email: ckdpal@gmail.com

ABSTRACT

Objective: The current study evaluates anti-Parkinson's activity of aqueous extracts of leaves of *Murraya koenigii* (MK) (AEMK) against paraquat (PQ)-induced Parkinsonism in rats.

Methods: In this study, effects of MK (100, 200, and 400 mg/kg, p.o.) were studied using *in vivo* behavioral parameters such as catalepsy, muscle rigidity, and locomotor activity and its effects on neurochemical parameters malondialdehyde, catalase (CAT), glutathione (GSH) reductase, GSH peroxidase, and GSH in rats.

Results: Parkinson's disease was induced by administering PQ 10 mg/kg b.w/i.p once in a week for 4 weeks. The increased cataleptic scores were significantly ($p<0.001$) found to be reduced, with the AEMK in a dose-dependent manner. Chronic administration of PQ significantly induced motor dysfunction (muscle rigidity and hypolocomotion), showed a significant increase in lipid peroxidation level, and depleted the levels of GSH, CAT, and reduced GSH. Daily administration of AEMK significantly improved motor performance and also significantly attenuated oxidative damage.

Conclusion: The study proved that MK treatment significantly attenuated motor defects and also protected the brain from oxidative stress.

Keywords: Parkinson's disease, Paraquat, Antioxidant parameters, Behavioral parameters, *Murraya koenigii*.

INTRODUCTION

Neurodegenerative disorder (ND) results in a loss or death of neurons [1]. It occurs due to oxidative stress, neurotoxins, serious trauma, and few long-term treatments with medications [2]. Parkinson's disease (PD) is a chronic progressive NO [3] of the central nervous system. *Murraya koenigii* (MK) is used as a stimulant, stomachic, febrifuge, analgesic and for the treatment of diarrhea, dysentery, insect bites, anti-tumor, anti-microbial, anti-inflammatory [4], antibacterial, and antifungal activity [5]. MK is used in the treatment of neurodegenrative disorders like PD. Hence, MK is evaluated for its anti-Parkinson's effect using neurotoxin-induced Parkinson's model in rats. The novelty of this work was to analyze the aqueous extract of MK (AEMK) against PQ-induced Parkinsonism in rats to avoid shortcomings of conventional dosage forms.

METHODS

Materials
Paraquat (PQ) was obtained from Sigma Aldrich (St. Louis, Mo, USA). All other solvents were in analytical grade.

Collection and authentication
The fresh leaves of MK were obtained from the outskirts of Maisammaguda situated in the state of Telangana (India), identified, and authenticated by Dr. H. Ramakrishna, H.O.D, Department of Botany, Osmania University, Telangana, India. Aqueous extract of leaves of MK was prepared by maceration method.

Animal study protocol
Thirty-six male and female Wistar albino rats weighing 150–250 g and of age 4–6 weeks old were obtained from Sanzyme Ltd, Gaghan Pahad, Hyderabad. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 24±1°C and 65±10% humidity). Pelletized feed and water were given. All the pharmacological experiment protocols were approved by the Institutional Animal Ethics Committee with Reg. no 1217/PO/Re/S/08/CPCSEA.

Experimental design
Animals were divided into six groups of six animals each. Group 1 includes the control group received distilled water, in Group 2, PQ-treated group-injected with PQ at a dose of 10 mg/kg b.w/i.p once a week for 4 weeks, in Group 3, Madopar-treated group-injected with PQ 10 mg/kg b.w/i.p+Madopar10 mg/kg b.w/p.o., in Group4, AEMK+PQ-treated group-injected with aqueous extract of leaves of MK 100 mg/kg b.w/p.o and PQ 10 mg/kg b.w/i.p., in Group 5, AEMK+PQ-treated group-injected with aqueous extract of leaves of MK 200 mg/kg b.w/p.o and PQ 10mg/kg b.w/i.p., and in Group 6, AEMK PQ-treated group-injected with aqueous extract of leaves of MK 400 mg/kg b.w/p.o and PQ 10 mg/kg b.w/i.p. All above treatments were carried out for 4 weeks to check disease development and the effect on its treatment. At the end of the experiment, behavioral studies were observed to understand motor skill abnormalities.

Neurobehavioral parameters
PQ-induced PD

Akinesia was determined by holding the tail of the animal and putting the front paws on the platform and let the animal walk while holding.

Catalepsy

The animals were placed with their forepaws on a wooden box [6] on height 9 cm and the time spent without deliberate move to step down was determined.

Muscle rigidity

Muscle rigidity was evaluated in an animal model by rotarod [7,8]. Five trials were taken before the main reading to all the groups by adjusting the rate of rotation at 30 rpm.

On the 28th day, immediately after behavioral assessments, the animals were sacrificed by CO_2 inhalation through the euthanasia chamber. The brain was removed, then rinsed with suitable buffer and supernatant collected with suitable dilution for biochemical estimation.

Biochemical Estimation [9,10]

Ellman method is used to estimate glutathione (GSH) level, enzymatic antioxidant catalase (CAT) activity was done according to Aebi *et al.*, method. GSH peroxidase (GPx) was taken of the previous report with some modifications. Malondialdehyde (MDA) level was analyzed by estimation of the produced thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa *et al.*, GSH reductase (GR) activity was measured by the following previous reports.

Histopathological studies

The brains from control and experimental groups were fixed with 10 % formalin and embedded in paraffin wax and cut into a longitudinal section of 5 μm thickness. The sections were stained with hematoxylin and eosin dye for histopathological observation.

Statistical analysis [11-13]

One-way analysis of variance (ANOVA) is followed by Dunnett's test using the graph pad statistical software for comparison between different experimental groups for statistical analysis. $p < 0.001$ is taken statistically significant. Data were analyzed using one-way analysis of variance (ANOVA)* ($p < 0.05$), # ($p = 0.0001$), and ^ ($p = 0.001$) in comparison of Group II versus Group I, **($p < 0.001$), ## ($p < 0.001$), and ^^($p < 0.001$) in comparison of Group – IV-VI versus Group – II; ***($p < 0.0001$) ###($p < 0.0001$) ^^^($p < 0.0001$) in comparison of Group III versus Group II.

RESULTS AND DISCUSSION

Table 1 suggests that the body weight and brain weight of rats treated with PQ were significantly decreased when compared with normal control group animals. A dose-dependent protection of the activities was observed after treatment with aqueous extract of leaves of MK before PQ intoxication.

Treatment with PQ led to a significant ($p < 0.001$) decrease in time of fall in rotarod test and significantly ($p < 0.001$) increased catalepsy score in catatonia test (Table 2).

The results indicate that animal treated with AEMK at doses 100, 200, and 400 mg/kg b.w ($p < 0.001$) significantly reduced the degree of catalepsy in a dose-dependent manner when compared with PQ-induced group (Table 3).

The aqueous extract of leaves of MK-treated animals showed better performance in the form of significant latency periods when compared to PQ-treated animals (Table 4).

The number of rears was significantly decreased in PQ-treated animals, while there was an increased number of rears in aqueous extract of leaves of MK-treated animals (Table 5).

Treatment with aqueous extract of leaves of MK before PQ intoxication, there was dose-dependent and significant ($p < 0.0001$) reduction in MDA level when compared with PQ-treated animals. In the toxin group, reduced GSH, GR, GP_x, and CAT content was found to be significantly lowered. In AEMK leaves pre-administration groups, there was dose-dependent and significant ($p < 0.001$) normalization of the contents in brain tissues and recovered their activities near to normal values when compared with PQ-treated animals (Table 6).

PQ inoculation in rats induced oxidative stress, as indicated by a decrease in the CAT level, GSH, GR, GP_x, and increase in the levels of

Table 2: Effect of AEMK on number of falls using rotarod apparatus in PQ-induced Parkinsonism in rats

Group	Number of falls in 5 min.±SEM
I	1.50±0.29
II	15.25±0.85*
III	3.50±0.48***
IV	7.25±0.41**
V	5.50±0.29**
VI	4.50±0.29**

All the values are expressed as Mean±SEM, n=6, AEMK: Aqueous extract of *Murraya koenigii,* PQ: Paraquat

Table 3: Effect of AEMK on catalepsy in PQ-induced Parkinsonism in rats

Group	Time (s)±SEM
I	16.75±1.38
II	86.50±2.72#
III	36.25±3.86***
IV	55.25±1.11*
V	45.25±1.49*
VI	32.00±1.08**

All the values are expressed as Mean±SEM, n=6. AEMK: Aqueous extract of *Murraya koenigii,* PQ: Paraquat

Table 4: Effect of AEMK on Akinesia time in PQ-induced neurotoxicity in rats

Groups	Time (s)±SEM
I	33.25±1.38
II	142.25±4.09#
III	48.75±1.65***
IV	96.75±2.72*
V	60.25±2.95**
VI	52.50±1.85**

N.B. All the values are expressed as Mean±SEM, n=6. AEMK: Aqueous extract of *Murraya koenigii,* PQ: Paraquat

Table 5: Effect of AEMK on rearing behavior in PQ-induced Parkinsonism in rats

Groups	Number of rears in 5 min.±SEM
I	20.00±0.91
II	3.00±0.41#
III	16.25±0.48***
IV	10.75±0.48*
V	13.25±0.63**
VI	15.75±0.63**

N.B. All the values are expressed as Mean±SEM, n=6. AEMK: Aqueous extract of *Murraya koenigii,* PQ: Paraquat

Table 1: Effect of AEMK leaves on body weight and organ weight in PQ-induced Parkinsonism in rats

Groups	Original body weight (g)±SEM	Final body weight (g)±SEM	Brain weight (g)±SEM
I	182±1.78	180.25±1.11	1.997±0.056
II	180.25±2.06*	162±2.20#	1.53±0.04^
III	181.5±0.65***	179.5±10.4###	1.88±0.016^^^
IV	185.25±1.49**	168.75±3.15##	1.65±0.0499^^
V	180.25±2.56**	172±1.76##	1.71±0.191^^
VI	186.21±2.12**	175.75±7.70##	1.727±0.029^^

N.B. Values are expressed as mean±SEM, n=6. AEMK: Aqueous extract of *Murraya koenigii,* PQ: Paraquat

Table 6: Effects of AEMK PQ-induced PD in MDA, GSH, CAT, GR, and GPx Level

Groups	TBARS (nM/mg tissue)±SEM	GSH (µgm/ml)±SEM	Catalase (K/min)± SEM	GR (u/ml)±SEM	GPx (nM/g tissue)±SEM
I	202.312±1.82	27.4±1.875	20.2±1.11	20.35±1.005	38.30±0.756
II	558.99±7.56*	14.79±0.865^	11.34±0.76#	10.26±0.59*	20.89±0.623*
III	221.79±6.08***	28.64±0.843^^^	13.47±1.547###	17.42±0.95***	27.21±0.506***
IV	291.34±3.67**	36.23±1.33^	19.76±0.96#	24.69±0.884**	35.62±0.599**
V	262.17±3.56**	32.57±1.001^	16.85±0.884##	21.01±0.47**	33.79±0.63**
VI	222.11±7.31**	29.81±0.322^	15.37±0.46##	19.83±1.16**	29.34±1.21**

N.B. Values are expressed as mean±SEM, n=6. AEMK: Aqueous extract of *Murraya koenigii,* PQ: Paraquat, GSH: Glutathione, PD: Parkinson's disease
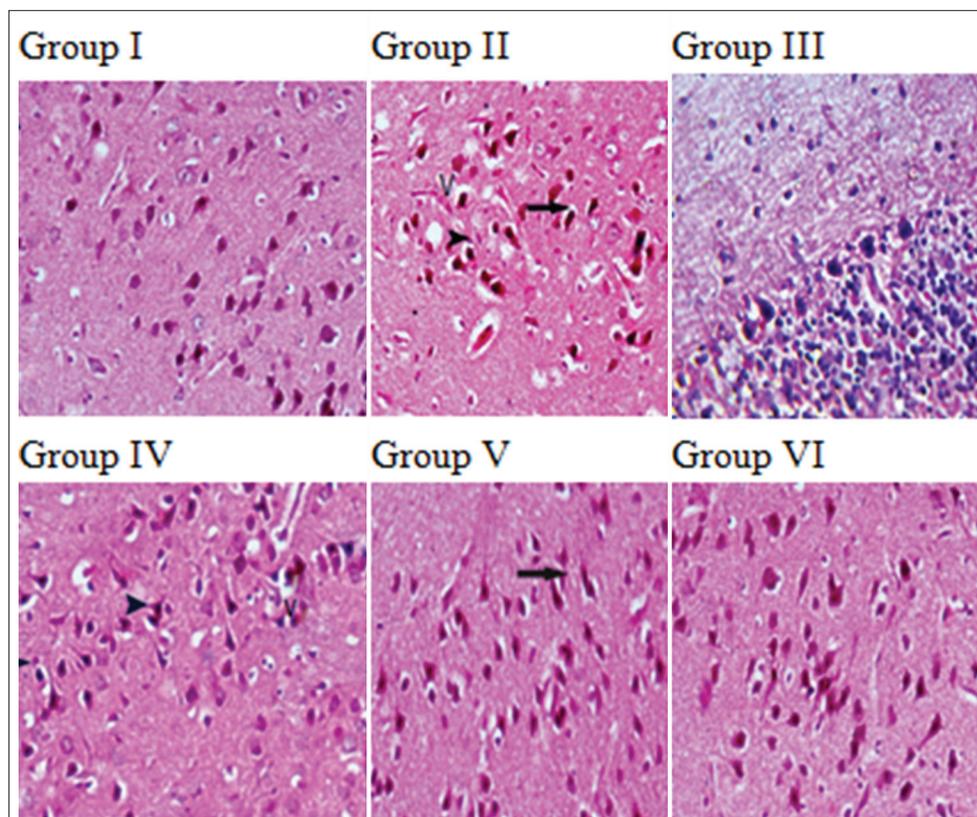

Fig. 1: Effect of *Murraya koenigii* leaves on histopatholological structure of brain

TBARS. Pre-treatment with different doses of AEMK (100 mg/kg, 200 mg/kg, and 400 mg/kg) resulted in a decrease in TBARS level and an increase in the levels of GR, GPx, CAT, and GSH, indicating its antioxidant effect in the brain of PQ-treated animals.

Histopathological findings showed that AEMK-treated animals had decreased infiltration of neutrophils, reduced intracellular space, increased density of cells, and regained normal architecture and moderate necrosis in the striatum region of the brain [Fig. 1].

Group – I: Showed normal neurons with normal nuclei. Group-II: Showed neurodegeneration with distorted, abnormal darkly stained neurons, and reduced number of neurons. Group – III: Showed nearly normal morphological appearance with little neuronal degeneration. Group – IV: Showed inflammatory cell infiltration in the substantia nigral neuron. Group – V: Showed mild neuronal degeneration and disorganization as well as reduced number of apoptotic cells. Group-VI: Showed mild neuronal cell degeneration with less apoptotic cells.

CONCLUSION

It was observed that aqueous extract of MK showed to be an antioxidant and also showed a promising effect in animals with PD. The molecular studies with this plant in anti-Parkinson's pharmacology and toxicology and also characterization of active constituents responsible for the neuroprotective effect.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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AUTHORS' CONTRIBUTIONS

All the authors are equally contributed to the research work.

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