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Anti-Parkinson's and Neuroprotective Activity of *Mentha Aquatica*in 6-Hydroxy Dopamine Lesioned Rat Model

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Abstract

Objective: *Menthaaquatica, plant which is showing monoamine oxidase-B (MAO-B) inhibition and anti-oxidant activity was assessed for anti-Parkinson's activity in 6-hydroxy dopamine (6-OHDA) lesionedwistar rat model.*

Materials and Method: *Menthaaquaticaethanolextract (EMA) was obtained by using alcoholic solvent. Acute oral toxicity of Menthaaquaticaextract was carried out in wistar rats. Rats were treated with 6-hydroxy dopamine (2µg/µl) intra nigrally. After 48 hrs of induction, extract of Menthaaquatica (200 mg/kg/day, p.o.) was given to the treatment groups for 60 days. Levodopa (6mg/kg) was used as the standard drug. The possible pharmacological actions of the plant extract was evaluated by anti-catatonic activity, striatal dopamine (DA) levels, mitochondrial complex-I activity and Superoxide dismutase (SOD) assay.*

Result: *Menthaaquatica ethanol extract significantly ($P<0.001$) improved the muscle coordination, striatal dopamine level, complex-I activity and Superoxide dismutase level compare with 6-OHDA control group.*

Conclusion: *Menthaaquatica ethanol extract produced a significant anti-Parkinson's activity at a dose of 200mg/kg against 6-OHDA induced neurotoxicity.*

Keywords: *Parkinson's disease, 6-OHDA, Menthaaquatica, complex-I*

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder caused by the progressive loss of mesencephalic dopaminergic neurons in the substantia nigra innervating the striatum[1, 2].It was first described by neurologist James Parkinson in 1817 that he called ''Shaking Palsy'', or ''paralysis agitans''. Neurotoxins like 6-OHDA and MPTP used to create PD models which act by inhibiting the electron transport chain (ETC) at complex-I. The effectiveness of levodopa (L-DOPA) in Parkinson's treatment is remaining obscure and most of the PD patients experienced an intolerable adverse effects and the disease condition seemed to be progressed.

Monoamine oxidase (MAO) is a flavin-containing enzyme, is widely distributed in boththe central and peripheral nervous systems and plays a central

role in the control of substrateavailability and activity[3]. MAO catalyzes the oxidation of a variety of amine-containingneurotransmitters to yield the corresponding aldehyde, hydrogen peroxide (H_2O_2), andammonia. MAO exists in two forms, MAO-A and MAO-B, which are distinguished on thebasis of different pharmacological and biochemical characteristics. MAO is a key enzyme incatecholamine metabolism, and increased catecholamine metabolism seen in aging has beenextensively studied. The control on MAO activity may alleviate symptoms and slow theprogression of neurodegenerative disorders. Therefore, inhibition of MAO-Bactivity may improve the quality of life of the elderly and it is used as part of the treatment of Parkinson's patients.

Mentha aquatica[4] is a perennial plant in the genus Mentha and family Lamiaceae. It is also known as Water Mint and distributed through-out Europe except for the extreme north, and also northwest Africa and southwest Asia. The chemical components include (S)-naringenin, oxygenated monoterpenes (+)-pulegone and (+)-menthofuran, viridiflorol. The phytochemical (S)-naringenin showed a potent in vitro inhibitory activity against human brain monoamine oxidase (MAO)-B enzyme. A tea made from the leaves has traditionally been used in the treatment of fevers, headaches, digestive disorders and various minor ailments.

The purpose of the present study to evaluate the possible Anti-Parkinson's activity of *Mentha aquatica*plant which is showing monoamine oxidase-B (MAO-B) inhibition and anti-oxidant activity.

Materials and methods

Chemicals

The chemicals which were used for the present study were procured from Sigma Aldrich USA, Sd-Fine Chemicals Mumbai, Merk chemicals Mumbai.

Plant material

*Menthaaquatica*was collected from local vender from Coimbatore, Tamilnadu, India. The collected plants were authentified by Survey of Medicinal Plants and Collection Unit, Central Council for Research in Homoeopathy, Dept. of AYUSH, The Nilgiris, Tamilnadu.

Plant extract

To prepare the ethanol extract 500 g dried ground plant material was defatted with petroleum ether. After drying, the plant material was extracted with 70% ethanol[4].Percentage yield of the extract was found to be 15.2%w/w.

Animals

Healthy, adult Wistar rats of both sexes (180-220g) were used for the study and maintained under standard laboratory conditions. All the experiments were performed after obtaining prior approval from CPCSEA and IAEC. Approval No.:1143/ac/07/CPCSEA/PCP/IAEC/PhD/132/12.

Acute toxicity

The acute toxicity study of *Mentha aquatica*ethanol extract (EMA) was performed using up and down procedure at a dose level of 2000 mg/kg body weight orally in rats, as per OECD 425 guidelines in two different groups of 3

female each and observed for mortality for 24 h[5].The dose 2000 mg/kg was found to be safe for all animals. From this 1/10th of 200 mg dose was selected for further study.

Induction of Parkinsonism by 6-OHDA

The rats were anesthetized with an intraperitoneal injection of 50 mg/kg of sodium pentobarbital and were fixed in a stereotaxic apparatus (USA)[6, 7]. The needle was inserted into the substantia nigra with the following coordinates: anterior/ posterior: -4.8 mm; medial/lateral: -2.2 mm; ventral/dorsal: -7.2 mm−3.5mm from bregma and injection of 6-OHDA (20 µg of 6-OHDA hydrobromide in 4µl 0.9% saline with 0.02 µg/ml ascorbic acid) was then made over 5 min and the needle was left in place for a further 5 min. Each rat was housed individually following the surgical procedure.

Experimental design

Animals were divided into four groups of both sex and 6 rats in each group. Group I served as sham operated, received normal saline (10 ml/kg,p.o.), Groups II to IV were induced with Parkinsonism as follows: group II served as a 6-OHDA control, received normal saline (10 ml/kg, p.o), Group III served as a L-DOPA control, received L-DOPA (6 mg/kg, p.o.)[8] and Group IV served as EMA treated, received 200 mg/kg, p.o. of EMA respectively. The treatment of animals were started after 48hr of induction with 6-OHDA according to their respective group once a day for 60 days.

Parameters evaluated

The following parameters were evaluated, after the 60th day of treatment.

Catatonia

The major clinical symptom of Parkinson's disease includes difficulty to move and change the posture (akinesia and rigidity) and tremors. Catatonia was assessed using a method reported earlier[9]. Briefly the animals were placed with their forepaws on a wooden box on height 9 cm and the time spent without deliberate move to step down was determined. An average of three trials was taken with each trail commencement up to maximum 30 s.

HPLC measurement of dopamine

The striatal tissues were sonicated in 0.1 M of perchloric acid (about 100 µl/mg tissue). The supernatant fluids were taken for measurements of levels of dopamine by HPLC[10, 11]. Briefly, 20 µl supernatant fluid was isocratically eluted through an 4.6-mm C18 column containing paracetamol (100 mg/ml) as the internal standard with a mobile phase containing 50 mM ammonium phosphate pH 4.6, 25mM hexane sulfonic acid pH 4.04, 5% acetonitrile and detected by a UV detector. The flow rate was 1 ml/min. Concentrations of dopamine was expressed as nenogram per milligram of brain tissue.

Isolation of mitochondrial fractions and Complex I activity assay

Brain tissue was homogenized in a tissue grinder in mitochondrial isolation buffer and suspensions were centrifuged. The supernatant fluids were centrifuged at 13,000 g, 4^{o}C, for 10 min, and the pellets were washed with mitochondrial isolation buffer and centrifuged at 13,000 g, 4^{o}C, for 10 min to obtain the crude mitochondrial fraction. NADH: ubiquinone oxidoreducase (Complex I)

activity was measured in the SN as described earlier[12].

Estimation of Superoxide dismutase (SOD)

Assay mixture contained 0.1ml of supernatant of brain homogenate, 1.2ml of sodium pyrophosphate buffer (pH 8.3; 0.052 M), 0.1ml of phenazinemethosulphate (186 µm), 0.3ml of nitro blue tetrazolium, 300 µM, 0.2ml of NADH (750 µM). Reaction was started by addition of NADH. Reaction mixture was stirred vigorously with 4.0ml of n-butanol. Colour intensity of the chromogen in the butanol was measured spectrophotometrically as described earlier[13].

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test, using statistical package PRISM 5.0 version. The significance of difference between and within various groups was determined. Differences were considered to be significant when $P < 0.01$.

Results

The effect of EMA on catatonia, dopamine estimation using HPLC, Complex I activity and SOD estimation was given in Table 1.

When compared with sham control group the catalepsy score was significantly (P<0.001) increased in the 6-OHDAtreated animals. The EMA and levodopa treated group showed a significant (P<0.001) reduction inthe catalepsy score compared to 6-OHDA control.

When compared with sham control animals, 6-OHDA control showed a significant (P<0.001) reduction in dopamine concentration, but levodopa showed higher degree of dopamine levels. When compare with 6-OHDA control, EMA group significantly (P<0.001) retained the dopamine level.

When compared with sham control group the mitochondrial activity was significantly reduced (P<0.001) for 6-OHDA.While the activity significantly (P<0.001) improved in EMA and levodopa treated animals when compared to 6-OHDA treated groups.

When compared with sham control animals, it showed a significant (P<0.001) reduction for 6-OHDA operated group. But when compared with 6-OHDA operated control, the levodopa and EMA significantly (P<0.001) retained the level of SOD.

Discussion and Conclusion

The efficacy of *Mentha aquatica* in PD has not been well established. In our study, we have demonstrated the anti-Parkinson's and neuroprotective effect of the plant extract.

Catalepsy experiment demonstrated the impairment in the locomotor function and coordinationin Parkinson's rats. Lack of motor coordination andmaintenance of normal limb posture has been reported in PD condition[14]. The treatment withEMA in rats reversed the catalepsy induced by 6-OHDA induction. The pyramidal dopamine facilitatory actions may be the possible action of this test drug.

The EMAretained the equal levels of dopamine as that of levodopa treated groups. This suggests that, apart from dopamine, some other mechanism contribute the anti-Parkinson's activity. So this

issue was addressed by measuring the complex-I activity of mitochondrial. The test drug remarkably improved the complex-I activity, where as levodopa treatment could not. This suggested the protective role of EMA in mitochondrial function of neurons in PD brain.

The turnover of dopamine in nigral cells plays a major role in controlling motor function. In ourstudy, we reported that EMA caused a pronounced increase in dopaminelevels in mid brain regions of 6-OHDA rats and it could a result of protection of dopaminergic neurons by these drugs. The beneficial roles of EMA in retaining dopamine levels demonstrated the protection of nigral neuron by test drug.

Oxidative stress and oxidative damage to critical biomolecule based on the important process mediating cell death in PD[15]. The studies showed the activities of superoxide dismutase was reduced in 6-OHDA group but increased in all other treatment groups and established the neuro protective effect.

In view of the above facts we are concluding that ethanol extract of *Mentha aquatic*plant showed significant anti-Parkinson's activity and we appreciate further detailed molecular studies with this drug in anti-Parkinson's pharmacology and toxicology.

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Table 1. Effect of EMA on catatonia, dopamine estimation using HPLC, Complex-I activity and superoxide dismutase (SOD) levelin rats

Sr.No	Group	Catalepsy score	Dopamine Concentration (ng/mg of protein)	Complex I activity (nmol/min/mg protein)	Superoxide dismutase (U/ml)
1	Sham control	0±0.00	5.654±0.165	64.34 ± 0.321	230.48±4.411
2	6-OHDA control	3.35±0.056***	0.266±0.231***	43.33 ± 1.043***	110.22±0.241***
3	Levodopa	1.463±0.134###	3.412±0.432###	54.0 ± 1.323###	190.41±0.131###
4	EMA	1.01±0.05###	3.357±0.001###	53.44 ± 0.423###	201±0.432###

Values are mean ± SEM; n=6 in each group. ***P<0.001 when compared with Sham control group; ###P<0.001 when compared 6-OHDA control; One-way ANOVA followed by Bonferroni multiple comparisons test.

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