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Modulating effect of *Hypericum perforatum* extract on astrocytes in MPTP induced Parkinson's disease in mice

M. MOHANASUNDARI, M. SABESAN

Department of Zoology, Annamalai University, Annamalainagar 608 002, Tamilnadu (India)

Abstract. – The present study has been evaluated the neuroprotective effect of *Hypericum perforatum* extract on the reaction of astrocytes in mice brain treated with an intraperitoneal injection of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (20 mg/kg with 2 hr intervals). Treatment with Hypericum perforatum extract (HPE) resulted in an inhibition of monoamine oxidase-B (MAO-B) activity and reduced astrocyte activation in striatal area induced by MPTP. The results show that HPE has neuromodulating effect against MPTP induced Parkinson's disease in mice.

Key Words:

Parkinson's disease, Hypericum perforatum extract, MPTP, Monoamine oxidase-B.

Introduction

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin, causes the damage of dopaminergic neurons as seen in Parkinson's disease[1]. MPTP that is accumulated in astrocyte is converted to its toxic metabolite 1-methyl-4-phenylpyridinium ion (MPP^+), by the enzyme monoamine oxidase-B (MAO-B), which is localized predominantly in astrocytes which were also shown to have a high capacity for MPP^+ retention and efflux[2]. Astrocyte, which can be detected by glial fibrillary acidic protein (GFAP) immunoreactivity is critical for maintaining the homeostatic extracellular environment[3]. Astrocytes are strongly activated in response to neuronal damage called *reactive astrocytosis*[3]. The earlier study showed that a novel astrocyte-modulating agent (R)-(-)-2-propyloctanic acid (ONO-2506) has a potent neuroprotective effect in a rat model of cerebral infarction, providing evidence that astrocytes can be the target of neuroprotection[4]. As the synthetic drugs produce long-lasting side effects, there is a need for attention and so the *Hypericum perforatum* (Family: Clusiaceae) therapy has been investigated for the present study. Furthermore, *Hypericum perforatum* extract has been found to be remarkably safe and devoid of serious side effects compared to synthetic medicines[5]. The aim of the present study is to examine the astrocyte modulating effect of *Hypericum perforatum* extract (HPE) on MPTP induced Parkinson's disease (PD) in mice.

Materials and Methods

Male Swiss albino mice (25-30 g) were purchased and housed in the central animal house of the Rajah Muthiah Medical College, Annamalai University. All mice were maintained on a standard 1:1 light/dark cycle and given *ad libitum* access to food and water. The experimental and animal handling procedures were approved by the institutional animal ethics committee (IAEC).

Hypericum perforatum were collected from Nilgris (Western Ghats) in the months of July and August. The plant was botanically authenticated (AC: No. 2456) by the Department of Botany, Annamalai University. The *Hypericum perforatum* plant leaves were dried in the shade, segregated and pulverized by the mechanical grinder and mesh sieve was used for getting fine powder. It was extracted by 100% methanol in Soxhlet apparatus by continuous hot percolation method. After filtration through Whatmann filter paper No. 40, the filtrate was vacuum dried at 40°C. The extracts were stored in screw cap vials at 4°C until further use. The

Corresponding Author: Muthukumar Sabesan, MD; e-mail: sabesan-muthukumar@yahoo.co.in

extractive value of the methanolic extract was 8.13% w/w. The methanolic extract of *Hypericum perforatum* was subjected to preliminary phytochemical screening to find out the presence of active principles. The extracts were suspended in 10% Tween 80[6].

Experimental Design

Mice were randomly divided into four groups (n-6). Group I: treated with 0.5 ml vehicle given orally for a week. Group II: treated with 300 mg/kg HPE given orally for a week. Group III: the four doses of MPTP (20 mg/kg) were administered intraperitoneally with an interval of 2 hrs in the first day of experimental period. Group IV: the HPE (300 mg/kg) was given once in a day for 7 days and the dose on the first day was given 30 min prior to first MPTP injection.

Immunohistochemistry

The mice were anesthetized with pentobarbital (50 mg/kg) i.p. and the brain was perfusion-fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) following a heparinized saline flush. The brain was removed 1 h after perfusion fixation at 4°C and was immersed in the same paraformaldehyde. Paraffin sections, 5 µm in thickness were used for immunohistochemistry. For glial fibrillary acidic protein (GFAP) immunostaining, a polyclonal anti-GFAP antibody (Lab Systems, Helsinkc, Finland) and a Vestastain Elite ABC Kit (Vector Lab, Burlingame, CA, USA) were used. The paraffin sections were washed 3 times for 5 min in 0.01 M phosphate-buffered saline (PBS, pH 7.4) followed by 30 min of pre-incubation with 10% normal horse serum. The brain sections were then incubated with anti-GFAP antibody (1:200) including 0.3% Triton X-100 overnight at 4°C. After 15 min rinse in changes of PBS, the sections were incubated with biotinylated secondary antibody for 1 hr and then with an avidin-biotin peroxidase complex for 30 minutes at room temperature. Immunoreactions are visualized only 0.5% diaminobenzidine 0.2% NiCl and 0.01% hydrogen peroxidase in 0.05% Tris-HCl buffer (pH 7.6). The GFAP positive neurons in striatal region were examined under light microscope[7].

Monoamine Oxidase-B Assay

Monoamine oxidase-B activity was estimated in mitochondrial (P_2) fraction, a 10% (wt/vol) homogenate of striatal tissue from experimental animals was prepared in 0.3 M sucrose made in 10 mM potassium phosphate buffer, pH 7.2. The homogenate was centrifuged at 1,000 g for 10 min. The supernatant was again centrifuged at 17,000 g for 30 min and the pellets were washed twice with equal volumes of the buffer containing 50 mM Tris. Pellets thus obtained were kept at –20°C overnight and resuspended in appropriate volume of 10 mM potassium phosphate buffer, pH 7.2 and sonicated at low energy for 30s. The whole procedure was performed at 4°C. MAO-B was assayed using the fluorimetric procedure[8]. The reaction mixture of 1 ml contained 0.1 ml of enzyme preparation, inhibitors in 0.1 ml and potassium phosphate buffer (pH 7.2). The reaction was started by the addition of 0.3 ml of 3.07 mM kynuramine at 37°C and terminated by the addition of 0.3 ml of cold 0.4 M $HClO_4$. One millimeter of the supernatant was read at 318 nm excitation, 380 nm emission (Farrand, USA; model system 3) in a double volume of 1 M NaOH. The product formed, 4-hydroxyquinoline, was determined from a standard curve prepared from an authentic sample. The enzyme activity was expressed as nanomoles of 4-hydroxyquinoline formed per mg of protein per hour. Protein was assayed by the method of Lowry et al[9].

Statistical Analysis

Results were expressed as mean ± S.E.M. six animals in each group. Student's t test was used for finding significant differences between two means. $p < 0.05$ was considered significant.

Results

Figure 1 illustrates the GFAP immunostained striatal area of control and experimental mice. GFAP positive astrocytes were absent in the striatum of vehicle treated and HPE alone treated mice (Figures 1A and B), whereas, the GFAP positive astrocytes exhibited a ramified form with many fine processes in the striatum which were markedly increased in this area of MPTP treated animals (Figure 1C). In HPE treatment group, the GFAP immunoreactivity is mild in the striatum (Figure 1D).

Figure 2 depicts the results MAO-B activity, the striatal MAO-B activity was significantly decreased by 31% in HPE treated animals as com-

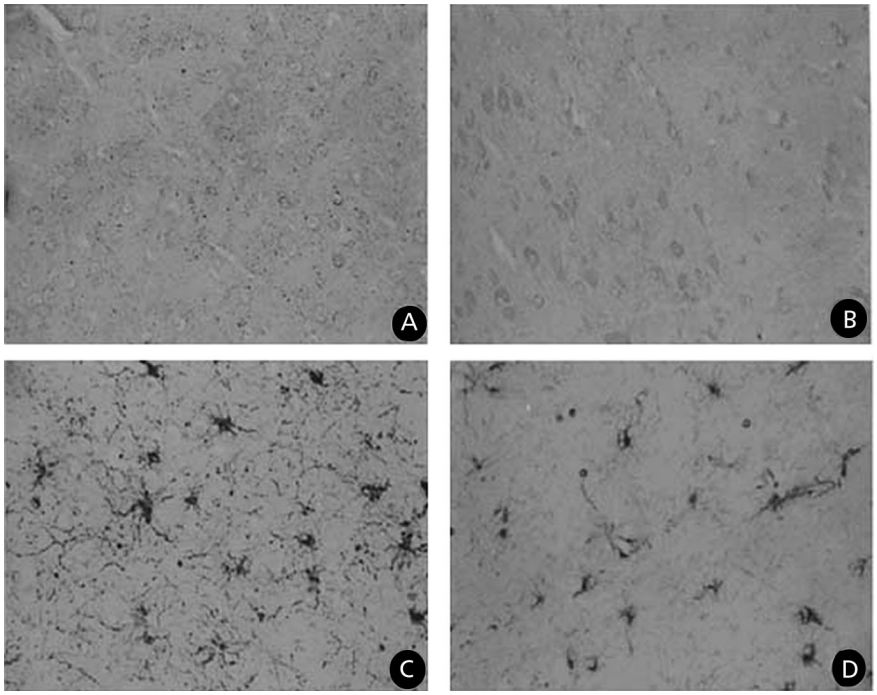

Figure 1. Immuno-histochemical study of GFAP expression in the striatal area of control and experimental animals. ***A,*** Control (20 ×): there is no GFAP positive cells. ***B,*** HPE (20 ×): GFAP positive astrocytes are absent. ***C,*** MPTP (20 ×): Many of the astrocytes and their processes reveal strong positively with GFAP. ***D,*** HPE + MPTP (20 ×): Astrocytes are poorly immunostained for GFAP.

pared to MPTP treated animals. There is no significant difference between control and MPTP treated animals. Control activity of striatal MAO-B was 6.7 ± 0.62 nmol 4-hydroxyquinoline/mg protein/h.

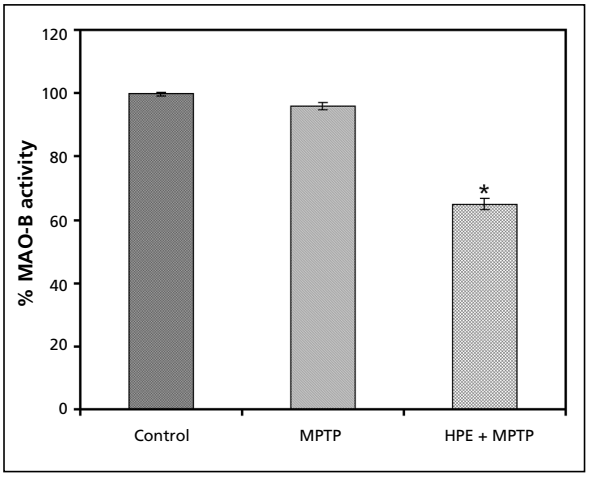

Figure 2. Effect of HPE on striatal MAO-B activity. Mice were treated with MPTP (20 mg/kg × 4 with 2 hr intervals); HPE (300 mg/kg). Results are shown as mean ± S.E.M. *$p < 0.05$ as compared to the MPTP treated animals (n = 6).

Discussion

MPTP is metabolized by monoamine oxidase-B to produce an active neurotoxic metabolite MPP^+.[10] At various stages of MPTP metabolism and biological interaction, generation of Reactive Oxidative Stress (ROS) were observed in the earlier studies[11,12]. Several antioxidants have been shown to protect against MPTP or MPP^+ induced neurotoxicity[10]. In our previous study, *Hypericum perforatum* extract was found to stimulate antioxidant enzymes which scavenged free radicals and also increased the dopamine level in striatal region of MPTP treated mice[13]. The free radical scavenging capacity could be due to quercetin and hyperoside as per the previous report[14]. In the present study, significant inhibition of striatal MAO-B activity was observed in the HPE treated animals. This could be due to flavonoids content of HPE[15]. Another interesting observation is that the astroglial activation (GFAP expression) was mild in HPE treated animals when compared with MPTP treated animals which is due to MAO-B inhibition resulting in blockade of MPP^+ production from MPTP, leading to the inhibition of dopaminergic cell damage and lesser induction of astrogliosis.

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