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Mangiferin attenuates MPTP induced dopaminergic neurodegeneration and improves motor impairment, redox balance and Bcl-2/Bax expression in experimental Parkinson's disease mice

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ABSTRACT

Mangiferin, a polyphenol compound of C-glucoside, is well-known for its anti-inflammatory, antioxidant, anticancer, antidiabetic and cognitive enhancement properties. In this study, we investigated the neuroprotective effect of mangiferin against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease (PD), which is most popular and widely used to evaluate therapeutic implications of new protective agents. Male C57BL/6 mice were orally treated with mangiferin (10, 20 and 40 mg/kg body wt.) for 14 days and from 10th day onwards MPTP (30 mg/kg, *i.p.*) was injected for last 5 days. MPTP treatment leads to enhanced oxidative stress, induction of apoptosis (upregulates the expression of Bax, proapoptotic protein and downregulates the expression of anti-apoptotic marker Bcl-2), and loss of dopominergic neurons which results in motor impairments. Results of our study confirmed that mangiferin prevented MPTP-induced behavioral deficits, oxidative stress, apoptosis, dopaminergic neuronal degeneration and dopamine depletion. Taken together, we conclude that mangiferin attenuates the dopaminergic neurodegeneration mainly through its potent antioxidant and antiapoptotic properties.

1. Introduction

PD is the second most common and progressive neurodegenerative disease affecting about 1% of the aged people. The diagnosis is based on the presence of a set of cardinal motor signs that is a consequence of the loss of dopaminergic neurons in substantia nigra (SN) and depletion of dopamine in straitum [1]. Though the cause is not yet completely understood, oxidative stress, mitochondrial dysfunction, inflammation, proteosome dysfunction and apoptosis play a key role in the pathophysiology of this movement disorder [2].

MPTP is a selective neurotoxin of dopaminergic neurons in the SN and has been shown to induce PD symptoms in various experimental animals including monkeys, mice, cats, dogs, rats, goldfish and also humans [3]. Due to its lipophilic nature, it can easily cross the blood–brain barrier and get metabolized to 1-methyl-4-phenylpyridinium (MPP^+) by monoamine oxidase in the glial cells, which is selectively gathered into the mitochondria of dopaminergic neurons by dopamine transporter [4]. It inhibits complex I of the mitochondrial electron transport chain and ultimately leads to the formation of excess reactive oxygen species (ROS) [5]. Downstream effect of ROS results in disturbed mitochondrial membrane permeability, translocation of B-cell lymphoma (Bcl-2) family proteins, increased levels of cytosolic cytochrome C (Cyt-C), activation of caspase-3 and finally lead to apoptotic loss of dopaminergic neurons [6]. There is no proper therapy for PD.

Mangifera indica and its active components have been extensively used in the Indian sub-continent as food additives and in cosmetics and medicines. Mangiferin, a potent glucosyl xanthone compound of *M indica*, has been reported to possess antidiabetic, antioxidant, antiproliferation, immunomodulatory, cardiotonic and diuretics properties [7]. The pharmacology of mangiferin has recently gained great attention owing to its protective function against oxidative injuries as well as its ability to modulate several key inflammatory pathways [8] in various tissues, including the brain. It is able to cross the blood brain barrier and has the real potential to ameliorate the oxidative stress observed in neurodegenerative disorders [9]. It is reported that the administration of

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mangiferin protected N2A cells against 1-methyl-4-phenylpyridine (MPP^+)-induced cytotoxicity by restoring reduced glutathione (GSH) content and down-regulating both superoxide dismutase (SOD) and catalase (CAT) mRNA expression [10]. However its protective effect against MPTP-induced toxicity in mice is not yet investigated.

2. Materials and methods

2.1. Experimental animals

Male C57BL/6 mice (25–30 g) were obtained from Bangalore and maintained in the Central Animal House, RMMC, and Annamalai University. The animals were kept under standard conditions with food and water *ad libitum*. The experimental protocols met with the National Guidelines on the proper care and use of Animals in Laboratory Research (Indian National Science Academy, New Delhi, 2000) and were approved by the Animal Ethics Committee of the Institute (Approval no: 759/2011).

2.2. Chemicals

MPTP, mangiferin, thiobarbituric acid, reduced glutathione and 3,5-dithio-bis-nitrobenzoic acid (DTNB), TH and DAT primary and secondary antibodies were purchased from Sigma Chemical Company, Bangalore, India. All other reagents used were of analytical grade and were procured locally.

2.3. Experimental design

Animals were randomized and divided into six experimental groups ($n = 6$). Group I mice was orally administered with saline (0.5 ml) and were served as control. Group II mice received i.p. injection of MPTP (30 mg/kg body wt.) (Zhao et al., [11]) daily for five consecutive days. Group III mice were injected orally with mangiferin (10 mg/kg body wt.) for 14 days and from the 10th day onwards, MPTP was injected as group II mice. The mice in groups IV and V were injected orally with mangiferin, 20 and 40 mg/kg body wt., respectively, for 14 days. From the tenth day onwards MPTP was injected on the group II mice. Group VI mice were treated orally with mangiferin alone for 14 days, like group III mice. At the end of the experiment, 3 days after the last dose of MPTP (18th day), the behavioral tests described below were performed. Effective dose of mangiferin (40 mg/kg bodyweight) based on the dopamine estimation was used for Phase II (control, MPTP, mangiferin + MPTP and mangiferin).

2.4. Estimation of dopamine and its metabolites

The levels of dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined by the high performance liquid chromatography (HPLC) with an electrochemical detector [12]. Briefly, the straitum (ST) was sonicated in ice-cold. 0.1 M perchloric acid containing 0.01% ethylene diaminetetraacetic acid (EDTA). The supernatant collected after a spin of 10,000 g for 5 min was injected (10 μl) into the HPLC system. Results were expressed in ng/mg weight of brain tissue.

2.5. Behavioral assessment

2.5.1. Open field

A wooden square box (100 × 100 × 40 cm) was used to study the movement and activity of mice in which the floor was covered with a rexin cloth drawn with 25 equal squares. If the animal crosses the central nine squares and sixteen outer squares then it is calculated as central and peripheral movement respectively. Furthermore, rearing (exploratory activity) and grooming (displacement response) were manually scored, while the animal was in the open field for 5 min [13], in normal lighting. The equipment was cleaned with 70% alcohol and water between trials.

2.5.2. Swim test

Swim-test was carried out by placing each animal in water tubs (40 cm length × 25 cm width × 16 cm height) and swim score was measured. Each animal was placed in the water tub to measure the swim score. The depth of water was kept at 12 cm and the temperature was maintained at 27 ± 2 °C. The animals were wiped dry immediately after the experiment using a dry towel and returned to cages kept at 27 ± 2 °C. Swim score scales were: 0, hind part sinks with head floating; 1, occasional swimming using hind limbs while floating on one side; 2, occasional floating/swimming only; 3, continuous swimming [14].

2.5.3. Hang test

Neuromuscular strength was determined by the grid hang test. Mice were lifted by their tail and slowly placed on a horizontal grid (grid 12 cm^2 opening 0.5 cm^2) and supported until they grabbed the grid with both their forelegs and hind paws. The grid was then inverted so that the mice were allowed to hang upside down. The grid was mounted 20 cm above a hard surface, to discourage falling or injury in case of falling. The apparatus was equipped with a 3-inch wooden wall to prevent animals from moving to the upper side of the grid. Animals were allowed to stay on the grid for 30 s and 10 chances were given with intervals of 1 min and the best fall values were recorded. The percentage of success was recorded as maximum time hanging/30 s × 100 [15].

2.5.4. Catalepsy

Briefly, catalepsy was measured as the time the animal maintained an imposed position with both front limbs extended and resting on a 4 cm high wooden bar. The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. If the animal maintained the imposed posture for at least 20 s it was said to be cataleptic and given a point. For every further 20 s that the animal continues to maintain the cataleptic posture an extra point was given. The animal is lifted by its tail and is allowed to place its forepaws on a horizontal wooden bar. The duration taken for the first movement of paws was measured as cataleptic time. The test was conducted accordingly [16] thrice.

2.5.5. Estimation of TBARS

Briefly, the tissue extracts were incubated with 0.2 ml phenyl methosulfate at 37 °C in metabolic water bath shaker. After 1 h of incubation, 0.4 ml of 5% tricarboxylic acid and 0.4 ml of 0.67% thiobarbituric acid were added. The reaction mixture was centrifuged at 4000 rpm for 15 min, and the supernatant was boiled for 10 min. After cooling, the samples were read at 535 nm. The rate of lipid peroxidation was expressed as nmol of thiobarbituric acid reactive substance (TBARS) formed/g tissue [17].

2.5.6. Assay of SOD

SOD activity was assayed using an indirect inhibition assay, in which xanthine and xanthine oxidase serve as a superoxide generator, and nitro blue tetrazolium (NBT) is used as a superoxide indicator. The assay mixture consisted of 960 μl of 50 mM sodium carbonate buffer (pH 10.2) containing 0.1 mM xanthine, 0.025 mM NBT, and 0.1 mM EDTA, 20 μl of xanthine oxidase and 20 μl of the brain supernatant. Changes in absorbance were observed