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Original Research Article (Experimental)

Neuroprotective potential of *Myrica esulenta* in Haloperidol induced Parkinson's disease

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ABSTRACT

Background: *Myrica esculenta* is a notable therapeutic plant widely utilized in Indian system of medicine. Ayurvedic literature reported fruit and bark of this plant is used in *gulma, jvara, arsa, grahani, pandu roga, hrillasa, mukha roga, kasa, svasa, agnimandhya, aruchi, meha*, and *kantharoga*.
Objective: The present study aimed to investigate the neuroprotective potential of "Himalayan Bayberry" (*Myrica esculenta* Buch.-Ham. ex D. Don) leaves methanol extract in Parkinson's disease induced by haloperidol.
Materials and methods: The present investigation was completed in wistar rats, in which Parkinson's disease (PD) was induced with haloperidol 1 mg/kg, intraperitoneally. The rats were randomly divided into six gatherings and the test animals received the methanolic extract of *M. esculenta* (MEME) at a dose of 50, 100 and 200 mg/kg, orally for one week. Various behavioural, biochemical and histopathological parameters were estimated in haloperidol exposed rats.
Results: MEME demonstrated significant and dose-dependent increment in behavioural activity and improved muscle coordination. The significant diminution in malonaldehyde level while improved the level of antioxidant enzymes like catalase, superoxide dismutase and reduced glutathione in extract treated group were observed as compared to the control group. Histopathological changes revealed MEME significantly reduced haloperidol-induced damage in the *substantia nigra* and there was very little neuronal atrophy.
Conclusion: The outcomes showed the defensive role of *M. esculenta* against PD. The mechanism of protection may be due to an escalation of cellular antioxidants.

1. Introduction

Parkinson's Disease (PD), is an age-dependent, incessant, dynamic neurodegenerative illness a synucleinopathy - next to Alzheimer's disease [1,2]. The pathological indication of PD is a constant and dynamic cellular misfortune within the substantia nigra that primarily affects the ventral segment of pars compacta and a decrease in dopamine levels in the striatum (caudate and putamen) of the basal ganglia [3,4]. Accordingly, the capacity of dopaminergic neurons is low, while the capacity of cholinergic neurons turns out to be generally predominant, which creates the advancement of movement disorders [5–7]. Clinically, PD is

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described *via* cardinal motor symptoms, such as, bradykinesia, resting tremors, unbending nature, and postural instability [8], notwithstanding non-motor side effects that incorporate neuropsychiatric indications, rest issue, dysautonomia, gastrointestinal side effects, and tactile protestations [9].

Global epidemiological study data described that in 2016, 6.1 million (95% uncertainty range [IU] 5.0-7.3) had Parkinson's disease globally, compared to 2.5 million (2.0-3.0) in 1990 [10]. Europe and North America than in West Africa and Asia. Its prevalence also varies within countries [11]. The prevalent incidence of PD for India is the lowest in the world (70 per 100,000 normal populations). However, the Parsi community of Mumbai (a district in India) represents the highest incidence of PD in the world (328 per 100,000 inhabitants) [11].

Drug-induced parkinsonism (DIP) is the second-most-fundamental etiology of parkinsonism in the elderly after PD [12]. Neuroleptic drug such as haloperidol (HP) is one of the main reasons for drug-induced Parkinson's worldwide. Haloperidol (HP) is a first-generation antipsychotic commonly used in the treatment of schizophrenia [13]. The use of haloperidol is limited by the tendency of the drug to show a series of extrapyramidal symptoms such as parkinsonism and tardive dyskinesia. The exact pathophysiology of haloperidol-induced extrapyramidal symptoms has not yet been clarified [14,15]. Oxidative stress caused due to increased production of reactive oxygen species (ROS) and a decrease in antioxidant defense mechanisms is proposed as a pathogenetic mechanism. Treatment with HP causes blockage of the dopamine receptor which increases dopamine turnover rate. This can lead to the generation of ROS as by-products of their metabolism [15–17]. In addition to the production of free radicals, the administration of HP is also associated with a significant decrease in antioxidant glutathione levels [18].

For decades, levodopa, combined with a peripheral decarboxylase inhibitor, has been viewed as the gold standard for the management of PD [19]. Levodopa and other drugs including carbidopa, orphenadrine, benztropine, and selegiline act as dopamine precursors and reversing the PD symptoms [20–22]. Long-term therapy of these drugs frequently leads to disabling side effects and common reactions such as nausea, vomiting [23,24], respiratory disturbances [25], hallucinations [26], mania, dyskinesia, convulsions, anxiety, and many more [27,28]. The existing pharmacological agents used in PD are with several side effects, and cannot diminish the degenerative process of dopaminergic neurons [27]. Thus, the demand for natural products having antiparkinson activity has been increased in recent years owing to their lower side effects and lower cost.

Myrica esculenta Buch.-Ham. ex D. Don. Commonly known as 'Himalayan Bayberry', 'Hairy Bayberry', 'Kaiphal', 'Katphala' is a significant medicinal plant native to India and widely used in Ayurveda [28–30]. Ayurvedic literature reported fruit and bark of this plant is used in *gulma, jvara, arsa, grahani, pandu roga, hrillasa, mukha roga, kasa, svasa, agnimandhya, aruchi, meha*, and *kantharoga* [31]. Traditionally, different parts of this plant are utilized in the treatment of jaundice [32], inflammation of vocal cord, fever [33], toothache [34,35], headache [36]. sprain [38], paralysis [39], dysentery [40], mental illness [41], skin disorders [42], cholera [43,44], cardiac debility [44], ulcer [44,45], and body ache [45].

Our previous study reported that qualitative phytochemical screening of *M. esculenta leaves* showed the presence of alkaloids, sugars, phenolic compounds, flavonoids, glycosides, and tannins [29]. Its antioxidant, antimicrobial, anti-inflammatory, antiallergic, antidiabetic, antiasthmatic, antifungal, anthelmintic, and nitrate reductase activity modulatory action have been reported [28,30,46]. Nevertheless, there is no work has been done on the neuroprotective activity. Therefore, the purpose of the present investigation was to explore the neuroprotective potential of *M. esculenta leaves* in Haloperidol induced PD model rats.

2. Materials and methods

2.1. Drugs and chemicals

Carboxymethyl cellulose (CMC), trichloroacetic acid (TCA), thiobarbituric acid (TBA), hydrogen peroxide was obtained from SD fine chemicals Ltd. Mumbai. Haloperidol and glutathione were purchased from Sigma Aldrich, Bangalore. Potassium dihydrogen phosphate, sodium dihydrogen phosphate, tris buffer, and all other reagents used were of analytical grade.

2.2. Plant materials

M. esculenta leaves were collected from outskirt area of Chail Chowk, Mandi, Himachal Pradesh. The plant was authenticated by the Department of Botany of the Abhilashi Institution Group a voucher specimen (AGI/2016/1220) is maintained in the institute.

2.3. Preparations of MEME

Firstly, the plant leaves were washed with water to remove dirt and other foreign matters were separated and shade dried. Dried leaves were then milled to a coarse powder and then passed over sieve No. 14. The obtained dried powdered leaves of *M. esculenta* (50 g) were placed in the tube of Soxhlet apparatus in the form of a thimble and extracted with methanol (500 mL) at 60–65 °C for 3–4 h. The obtained extract was filtered while hot and dried by evaporation using a rotary vacuum evaporator and the final dried extract sample was kept at -18 °C for further study. The residue obtained from methanolic extract was dissolved in the same solvent for further analysis.

2.4. Acute toxicity study

The acute oral toxicity was studied in Wistar albino rat as per OECD guideline 423. The extract was administrated orally in an increasing dose of up to 2000 mg/kg. Vehicle (0.5% w/v) was administered to the control group. The general behaviour of the rat was continuously monitored for 1 h after dosing, periodically during the first 24 h with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. Changes in the normal activity of rat and their body weights, food, and water intake were monitored and the time at which signs of toxicity or death appeared recorded. The acute toxicity studies showed that there were no toxic signs up to the dose level of 300 mg/kg but at dose level, 2000 mg/kg animals showed signs of toxicity.

2.5. Experimental animals

Wistar rats of both sexes, weighing between 230 and 250 g and 2–3 months age, were housed in colonial cages and kept in standard laboratory environmental conditions; temperature 25 ± 2 °C, 12 h of light: 12 h of dark cycle and 50 ± 5% of relative humidity with free access to food and water ad libitum. The animals were adapted to the laboratory conditions before testing. Each group consists of six (n = 6) animals. Each of the tests was performed in

the light time period (08: 00–16: 00 h). The investigations were conducted as per the standards provided by the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) of the Pinnacle Biomedical Research Institute (PBRI), Bhopal, under Reg. No. 1824/PO/ERe/S/15/CPCSEA.

2.6. Experimental design

The animals were divided into six groups of 6 rats each and treated as follows (a) Group 1: Received 0.5% carboxy methylcellulose (orally, once/day for one week) (b) Group 2: Received Haloperidol (1 mg/kg, i.p. daily for one week) (c) Group 3: Received MEME 50 mg/kg and Haloperidol (1 mg/kg i.p.) for one week (d) Group 4: Received MEME 100 mg/kg and Haloperidol (1 mg/kg i.p.) for one week (e) Group 5: Received MEME 200 mg/kg and Haloperidol (1 mg/kg i.p.) for one week and (f) Group 6: Received levodopa (30 mg/kg, i.p, once per day for one week) along with haloperidol (1 mg/kg i.p.).

Test drug MEME (50 mg/kg, 100 mg/kg, 200 mg/kg) orally and standard drug levodopa (30 mg/kg, i.p.) were administered 30 min preceding infusion of haloperidol for one week [47].

2.7. Neurobehavioral studies

2.7.1. Catalepsy test

Haloperidol-induced catalepsy was induced and assessed at 30 min intervals until 180 min on a standard bar test. To test of catalepsy, animals were positioned so that their hindquarters were on the bench, and their forelimbs rested on a 1 cm diameter horizontal bar, 6–9 cm above the bench. The length of time that animals maintained this position was recorded by stopwatch (mean of three consecutive trials; interval: 1 min). Animals would determine judge to be cataleptic if they maintained this position for 30 s or more [48].

2.7.2. Hang test

This task has been used as a measure of muscle strength and motor neuron integrity. The rats used the front limbs to suspend their body weight on a wire stretched between two 30 cm poles and hang 70 cm above a foam cushion [37]. The time (in seconds) before the rat fell was recorded. A zero score was awarded if the rat fell immediately, and the 60s were the waiting period. Three trials were performed for each rat [49].

2.7.3. Tardive dyskinesia test

Tardive dyskinesia is known as vacuous chewing movements (VCM) in rodents. On the day of the test, the rats were placed individually in a small plexiglass cage (30 × 20 × 30 cm) for the evaluation of oral dyskinesia. The animals were allowed 10 min to get used to the observation cage before the behavioural assessments. In this study, chewing movements under vacuum are called single-mouth openings in the vertical plane not directed towards the physical material. If protruding tongue movements and vacuous chewing occurred during a grooming period, these were not considered. The mirrors were positioned under the floor and behind the rear wall of the cage to allow observation of oral dyskinesia when the animal was away from the observer. The behavioural parameters of oral dyskinesia were measured continuously over a 5 minutes [50].

2.7.4. Hole board test

Head dipping is an exploratory behavior of animals in the hole board test which is considered an indicator of anxiety. The rats were placed in a black perspex box (50 × 50 cm, 30 cm high walls) with 16 equidistant holes (2.5 cm in diameter, 10 cm apart) on the floor and the box was raised to a height of 25 cm the earth. An animal was placed in the center of the hole-board table and allowed to freely explore the apparatus for 5 min. The total number of crossed lines and the number of head dipping has been recorded. The head dip was scored if both eyes disappeared into the hole [51].

2.8. Biochemical estimation

Oxidative parameters in brain tissue homogenate for the evaluation of malondialdehyde (MDA) [52] and reduced glutathione (GSH) [53] level, superoxide dismutase (SOD) [54], and catalase (CAT) [54] enzyme activities were calculated as per the reported protocol.

2.9. Histopathological studies

The brain from control and trial groups were fixed in formalin 10%, embedded in paraffin wax, and cut into thin longitudinal sections of 5 μm thickness. The sections were stained with hematoxylin and eosin dyes before histopathological examination [55].

2.10. Statistical analysis

All values have been reported as mean ± SEM (standard error of the mean). Statistical assessment of the data was performed by one-way ANOVA (between control and pharmacological treatments) followed by the Dun Dunnett test for multiple comparisons and two-way ANOVA followed by the Bonferroni multiple comparison tests, using the Graph-Pad Prism 7.0 version. The statistical significance has been established accordingly.

3. Results

3.1. Neurobehavioral studies

3.1.1. Catalepsy test

Haloperidol (1 mg/kg) resulted in a significant increase in the catalepsy, as it appeared following a dynamic increase in latency to venture down the bar after some time compared to controls (p, 0.001) (Fig. 1). A significant decrease ($P < 0.001$) in the catalytic score was observed during the observation period, compared to haloperidol treated with the standard drug (levodopa) 10 mg/kg and the drug test MEME at the doses tested (100 and 200 mg/kg).

3.1.2. Hang test

Haloperidol alone treated gathering, altogether diminished the hanging time ($p < 0.001$) compared to the vehicle control group (Fig. 2). Levodopa 10 mg/kg and the test drug MEME at all dosages tried (50, 100 and 200 mg/kg), a significant increase in drop time was observed ($p < 0.001$) compared to the haloperidol group.

3.1.3. Tardive dyskinesia test

It was observed that the haloperidol treated group significantly expanded ($p < 0.001$) in burst and chewing movement was seen when contrasted with the vehicle control group (Fig. 3). In the

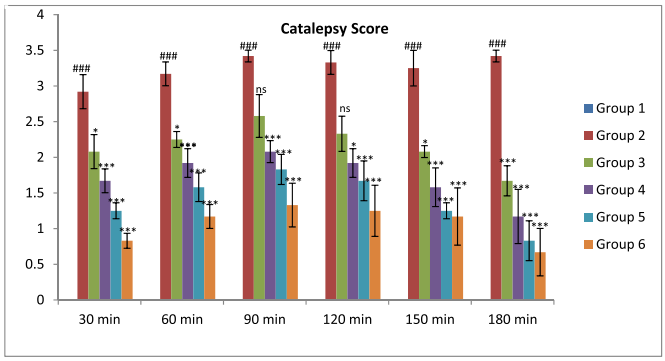

Fig. 1. Catalepsy test. Values are mean ± SEM; n = 6 in each group. ###P < 0.001 when compared with vehicle control group; nsNonsignificant; *P < 0.05; ***P < 0.001 when compared with haloperidol control; One-way ANOVA followed by Bonferroni multiple comparisons test.

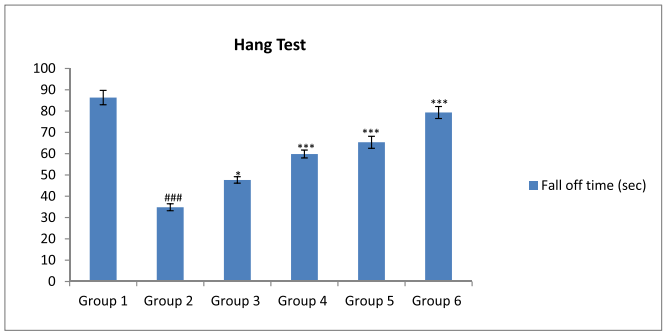

Fig. 2. Hang test. Values are mean ± SEM; n = 6 in each group. ###P < 0.001 when compared with vehicle control group; nsNonsignificant; *P < 0.05; ***P < 0.001 when compared with haloperidol control; One-way ANOVA followed by Bonferroni multiple comparisons test.

levodopa treated group and the test drug MEME at doses (100 and 200 mg/kg) significantly diminish ($p < 0.001$) in burst and chewing movement was observed compared to the haloperidol treated group.

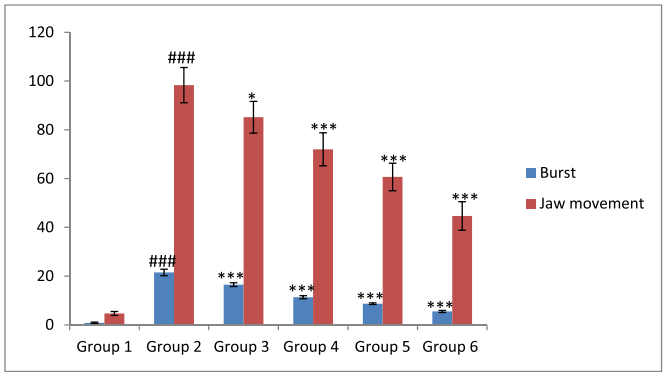

Fig. 3. Tardive dyskinesia test. Values are mean ± SEM; n = 6 in each group. ###P < 0.001 when compared with vehicle control group; nsNonsignificant; *P < 0.05; ***P < 0.001 when compared with haloperidol control; One-way ANOVA followed by Bonferroni multiple comparisons test.

3.2. Biochemical estimation

Administration of haloperidol resulted in significant changes in biochemical parameters when contrasted with vehicle control animals. The inoculation of haloperidol-induced oxidative stress in the brain, as indicated by decreased MDA content and increased CAT and SOD antioxidant enzyme activities as well as GSH levels compared to vehicle control animals. The treatment with MEME showed a significant ($p < 0.001$) increase in MDA (50,100 and 200 mg/kg) compared to haloperidol treated rats. Similarly, daily administration of MEME attenuated the increase in SOD (50,100 and 200 mg/kg), CAT enzyme activities (100 and 200 mg/kg) and GSH level compared to haloperidol treated group [Table 1].

3.3. Histopathological studies

The histopathological study confirmed the neuroprotective activity of MEME as a significant recovery of neuronal damages and decreased necrosis was clearly evidenced [Fig. 4].

4. Discussion

The PD is normally analyzed as a neurodegenerative disorder, represented by the degeneration of neurons that discharge dopamine in the substantia nigra, which causes tremor, bradykinesia, change of pace, flexed position and firm nature. While the aspect of controlling the dopaminergic neuronal passage in PD has not been resolved, it is widely accepted that oxidative stress is at the root of the particular weakness of these neurons [56,57]. Besides, numerous preclinical and clinical studies have proposed the uncontrolled formation of reactive oxygen species (ROS) as a reason for haloperidol-activated lethality [58]. Likewise, the dopamine catabolism by monoamine oxidase-B can create a large amount of ROS, which can go into cycles of Fenton-type free radical generating reactions with ferric particles present in large quantities in the nigral cells [59].

In the present experimental study, three behavioural evaluation parameters were used: catalepsy score, hang test, tardive dyskinesia test to examine haloperidol-induced PD in rats. The rat when pre-treated with MEME at 50,100 and 200 mg/kg and standard drug levodopa at 10 mg/kg for 7 days, the significant reduction (P < 0.001) in the cataleptic score and tardive dyskinesia were observed throughout the period of observations, compared to haloperidol-treated rats. Neuromuscular strength was increased significantly ($p < 0.001$) by the test drug MEME (100 and 200 mg/kg) and levodopa (10 mg/kg). The overall greatest neuroprotective impact was observed with MEME-treated rats (at 200 mg/kg), resulting in a comparable effect to the levodopa treatment in the control group.

Leaves are used as the starting material in this research because our earlier study reported on LCMS analysis of MEME showed that leaves contain flavonoids, and arylheptanoids. Earlier research also supported that several phenolic compounds, viz. simple phenolics, phenolic acids, anthocyanins, and flavonoids present in plants have witnessed a great interest owing to their rich antioxidant potential, which includes free radicals scavenging, and ameliorating effects against mutagens, carcinomas, and inflammatory pathological processes [60,61]. Earlier studies also suggested that due to its redox properties, phenolics compounds also serve as reducing agents, hydrogen givers, singlet oxygen inhibitors, and effective metal chelators [61].

Table 1
Effect of Myrica esculenta on the level of MDA, SOD, GSH, CAT, and glucose in haloperidol treated rats.

Groups	MDA	SOD	GSH	Catalase
Group 1	7.78 ± 0.324	345.23 ± 5.411	10.32 ± 0.0329	43.45 ± 4.704
Group 2	41.11 ± 0.793$^{###}$	193.73 ± 4.878$^{###}$	0.68 ± 0.0303$^{###}$	13.42 ± 0.409$^{###}$
Group 3	34.06 ± 0.617***	238.13 ± 3.679***	2.60 ± 0.0165***	17.91 ± 0.651^{ns}
Group 4	23.06 ± 0.757***	270.77 ± 3.988***	4.58 ± 0.0193***	24.89 ± 1.332*
Group 5	19.17 ± 0.435***	287.78 ± 2.883***	5.86 ± 0.0572***	28.70 ± 1.118***
Group 6	12.58 ± 0.556***	316.26 ± 4.151***	8.77 ± 0.0354***	34.10 ± 1.889***

Values are mean ± SEM; n = 6 in each group. $^{###}$P < 0.001 when compared with vehicle control group; ^{ns}Nonsignificant; *P < 0.05; ***P < 0.001 when compared with haloperidol control; One-way ANOVA followed by Bonferroni multiple comparisons test.

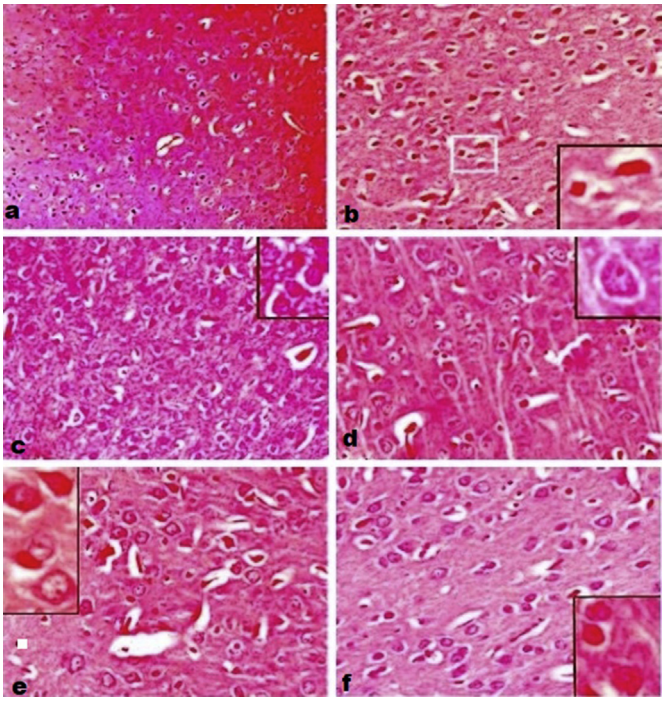

Fig. 4. Effect of MEME on histopathological changes in the brain of normal and Haloperidol treated animals (H&E staining; original magnification, 40×). (a) Normal control showing normal neuronal density and normal brain architecture. (b) Rat treated with Haloperidol showing degeneration of neurons. (c) Rats treated with Haloperidol and MEME (50 mg/kg) showing mild decrease in neurons and cellular hypertrophy. (d) MEME (100 mg/kg) and (e) MEME (200 mg/kg) treated rats showing minimal changes in neuronal cell populations. (f) Rats treated with Haloperidol and Levodopa (30 mg/kg) showing minimal changes in neuronal cell integrity and architecture.

Astudy performed by Chen and his co-workers on neuropeotective potential of *Myrica rubra* leaf extract also supported that main and typical constituent in *Myrica rubra* leaf were flavonoids and cyclic diarylheptanoids [62]. They had both been reported to exhibit neuroprotective activity [63–68].

M. esculenta is a medicinal plant that plays an important role in protecting against oxidative stress. Several tests have shown that *M. esculenta* has important antioxidant properties [17]. It has been hypothesized that antioxidants could be neuroprotective in PD, envisioning neuronal demise caused by intracellular ROS production [54].

In this manner, in addition to neuroprotective exercises, it is believed that antioxidants and might be in charge of antiPD impacts. The above behavioral and biochemical features evidenced that *M. esculenta* has a great potential to improve PD symptoms, at least in part, by restoring the level of dopamine and by the regulation of the antioxidant system. Consequently, *M. esculenta* might be valuable as a neuroprotective preparation in the treatment of PD. The advantageous impacts of *M. esculenta* here observed might be credited to the specific antioxidant substance(s) such as flavonoids, glycosides, saponins, and tannins accumulated in MEME.

5. Conclusion

According to the present results, we can conclude that MEME exerted a significant protective effect against haloperidol-induced PD comparable to the standard drug levodopa. Our study indicates that *Myrica eculenta* could be used as an alternative and/or adjuvant drug to prevent and treat extrapyramidal side effects of antipsychotic agents in clinical practice. Future work needs to be done in the direction to elucidate the molecular mechanism of MEME leaves in neuroprotection. A systemic research is needed to produce a nutraceuticals drug from leaves of *M. esculenta* for neuroprotection.

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None.

Conflict of interest

None

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