Elaeocarpus ganitrus: its beneficial antiparkinsonian effect in MPTP induced motor and non-motor impairments in mice

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ABSTRACT

Aim: Elaeocarpus ganitrus (Family: Elaeocarpaceae), has shown beneficial role in the treatment of depression, convulsions and asthma. This study was undertaken to evaluate the antiparkinson effect of E.ganitrus.

Materials and methods: Swiss albino mice of either sex were divided into 06 groups (n = 12). 1st group mice were given 0.5% carboxy methyl cellulose (orally), 2nd group were administered MPTP (2 doses, each dose 20 mg/kg at 2 hr. interval, i.p.). Whereas 3rd, 4th and 5th groups - were administered with E. ganitrus (100, 200, and 400 mg/kg/day, orally), respectively, along with MPTP. Group 6- received Levodopa (30mg/kg, i.p,) along with MPTP. To evaluate anti-Parkinson effect, hanging wire test, tardive dyskinesia test and elevated plus maze test were performed on the 1st day and on 8th day. One way ANOVA followed by post-hoc Tukey test, with p<0.05 was considered statistical significant.

Results: E.ganitrus (200 and 400 mg/kg, p.o.) was found to increase the hanging time significantly (p <0.001) in hanging wire test and significantly decreased (p <0.001) the Vacuous Chewing Movements (VCMs) in tardive dyskinesia test as compared to MPTP group. E.ganitrus (200 and 400 mg/kg, p.o.) was found to significantly increase (p <0.001) the no. of entries and time spent in open arm and significantly decreased the no. of entries and time spent in closed arm (p <0.001) compared to MPTP treated group.

Conclusion: The results of the present study conclusively showed that E.ganitrus has beneficial effect in MPTP induced experimental model of Parkinson’s disease.

Introduction

Plants remain as important source of medicine over the years to treat many diseases [1]. Decoctions prepared from fruits of Elaeocarpus ganitrus (E.ganitrus) have showed better results in the treatment of epilepsy, asthma, liver disorder, dropsy and hypertension [2, 3]. In addition to these benefits, E. ganitrus has found to exhibit many pharmacological activities that include analgesic [4], smooth muscle relaxant effects [5]. Parkinson’s disease is a chronic progressive neurodegenerative disorder resulting from idiopathic degeneration of dopaminergic cells in the substantia nigra pars compacta [6].While the causative factors for the degeneration of dopaminergic cells in the substantia nigra pars compacta is still not well established, oxidative stress might play an important role [7].

Levodopa remains the most efficacious drug in pharmacotherapy of Parkinson’s disease. However, long-term use of levodopa causes disabling motor complications like dyskinesia’s and on-off phenomenon. Because of the concern over the side effects of allopathic medicines, the use of natural products which may serve as better alternative to existing treatment needs to be explored. Thus, strategies employing antioxidant activity with lesser side effects from natural...
sources can be a good approach in improving the treatment of Parkinson’s disease.

Chronic treatment with neuroleptics leads to the development of abnormal oral movements in rodents known as vacuous chewing movements (VCMs). Vacuous chewing movements in rats are widely accepted as an animal model of tardive dyskinesia[8]. The hang test mainly evaluates the neuromuscular strength, coordination and is sensitive to a loss of dopamine[9]. Anxiety symptoms are very common in PD patients and some author’s state that anxiety in PD can be manifested even before the emergence of the first motor symptoms[10,11].

Previous studies undertaken by us shows that E. ganitrus[12] possess antioxidative properties and showed beneficial effect in rotarod test and catalepsy bar tests which are behavioral models of parkinson disease. The present study was undertaken in order to further strengthen the evidence of protective role of E. ganitrus in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) induced Parkinsonism using different behavioral assessment parameters like-hanging wire test, tardive dyskinesia test, elevated plus maze tests. Previous study done by us showed the antiparkinsonian effect of E.ganitrus in haloperidol induced parkinsonian model in these behavioral tests. The different doses of ethanolic extract of E.ganitrus were chosen from other previous studies conducted by Kakalij RM et al [13].

Materials and methods

Swiss albino mice of either sex weighing between 25 and 30 g, were obtained from the central animal house of University College of Medical Sciences and GuruTeg Bahadur Hospital. The mice were housed in cages and kept under controlled environmental condition (temperature 22±2 °C, humidity 50–55 %, natural light/day cycle). Care of animals was given according to the CPCSEA guidelines. Permission was taken from the Institutional Animal Ethics Committee to carry out the study (Approval No. IAEC/2011/49 dated 10 March 2011).

Plant extract and Chemicals: E.ganitrus extract was obtained from M/s Tapovan ayurved sadan, New Delhi. As per the literature provided by the manufacturer, initially the dried fruits of E.ganitrus Roxb were taken and crushed into a fine powder. 200 gms of the drug was soaked in 90% ethanol for 48 hrs. Percolation was carried out by Soxhlet apparatus through suction method. Filtration was performed repeatedly with the help of whatman filter paper No-4 and filtrate was air dried sufficiently. Then the dried extracts were stored at 4°C until its further usage. The yield of the drug was found to be 05.72% (w/w) in terms of dried starting material. To carry out our study, the E.ganitrus powder was dissolved in 0.5% Carboxy methyl cellulose (CMC) to prepare suspensions of different doses of 100, 200 and 400 mg/kg. MPTP and Levodopa was obtained from Sigma Chemical Co. USA.

Experimental Design

Swiss albino mice (6 weeks old) of either sex weighing between 25 and 30 gms, were used for the study. The mice were divided into 06 groups (n =12). Group I- was administered 0.5% carboxy methyl cellulose (orally ×1 week). Group II- received MPTP (2 doses, each dose 20 mg/kg at 2 hr. interval, i.p. daily x 1 week). Groups III, IV, and V- were treated with E.ganitrus (100, 200, and 400 mg/kg/day, orally), respectively for 1 week along with MPTP. Group VI- received Levodopa (30mg/kg, i.p. once per day x 1 week) along with MPTP. The E.ganitrus (100 mg/kg, 200mg/kg, 400 mg/kg, orally) and Levodopa (30 mg/kg, i.p.) were given 30 minutes prior to injections of first dose of MPTP for 7 days of experimental period. The behavioral parameters were assessed on 1st day and on 8th day.

Assessment of behavioral tests

1. Hang test: Neuromuscular strength was determined in the grid hang test. Mice were lifted by their tail and slowly placed on a horizontal grid and supported until they grabbed the grid with both their fore 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 but not leading to injury in case of animal fall. The apparatus was equipped with a 3-inch wall to prevent animals from transversing to the upper side of the grid. Animals were required to stay on the grid for 30 seconds. The animals were tested in the grid hang test for 30 sec and 10 chances were given with 1min interval and maximum hanging time was recorded[14].

2. Tardive dyskinesia test: Tardive Dyskinesia is referred to as Vacuous Chewing Movements (VCMs) in rodents. On the test day mice were placed individually in a small (30× 20× 30 cm) Plexiglas cage for the assessment of oral dyskinesia. Animals were allowed 10 min to get used to the observation cage before behavioral assessments. In the present study vacuous chewing movements are referred to as single mouth openings in the vertical plane not directed toward physical material. If tongue protrusion and vacuous chewing movements occurred during a period of grooming, they were not taken into account. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of oral dyskinesia when the animal was faced away from the observer. The behavioral parameters of oral dyskinesia were measured continuously for a period of 5 min[15].

3. Hole board test: Head dipping is an exploratory behavior of the animals in the Hole Board test which is considered to be an indicator of anxiety. Mice were placed in a black Perspex box (50 x 50 cm, walls 30 cm high) with 16 equally spaced holes (2.5 cm diameter, 10 cm apart from each other) in the floor and the box was raised to a height of 25cms from the ground. An animal was placed in the center of the hole-board and allowed to freely explore the apparatus for 5 min. The total no. of lines crossed and the...
number of head dippings were recorded. A head dip was scored if both eyes disappeared into the hole[16].

Statistical Analysis

Results of the above experiments were expressed as Mean ± SEM, and the difference between means was analyzed by analysis of variance (ANOVA) using graph pad prism followed by post-hoc Tukey test, with P < 0.05 being considered as statistical significant.

Results

Table 1: Effect of E. ganitrus on hanging wire test in MPTP treated mice

<table>
<thead>
<tr>
<th>Groups, (Dose)</th>
<th>Hanging time in (Sec) -1st day</th>
<th>Hanging time in (Sec) -8th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CMC (1ml/kg, p.o)</td>
<td>37.8 ±1.52</td>
<td>39.16 ±1.37</td>
</tr>
<tr>
<td>2. MPTP (20 mg/kg, i.p.)</td>
<td>15.8±1.75</td>
<td>14.7±2.44</td>
</tr>
<tr>
<td>3. E. ganitrus (100mg/kg, i.p.) + MPTP</td>
<td>16.7±1.64</td>
<td>18.5±2.76</td>
</tr>
<tr>
<td>4. E. ganitrus (200mg/kg, i.p.) + MPTP</td>
<td>20.1±3.07</td>
<td>25.8±3.17</td>
</tr>
<tr>
<td>5. E. ganitrus (400mg/kg, i.p.) + MPTP</td>
<td>21.5±3.15</td>
<td>29.2±3.26</td>
</tr>
<tr>
<td>6. Levodopa (30 mg/kg, i.p.) + MPTP</td>
<td>31.5±4.69</td>
<td>34.5±3.74</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SD for 12 animals in each group. *p < 0.001 vs. Carboxy methyl cellulose (CMC) - control, †p < 0.001 vs. MPTP, ‡p < 0.001 vs. (Levodopa + MPTP)

It was observed that MPTP alone treated group, significantly decreased the hanging time (p<0.001) on 1st day and 8th day as compared to control group. In Levodopa treated group, significant increase in hanging time (p<0.001) was seen on both 1st day and 8th day, as compared to MPTP treated group.

Table 2: Effect of E. ganitrus on tardive dyskinesia in MPTP treated mice

<table>
<thead>
<tr>
<th>Groups, (Dose)</th>
<th>VCMs/5 min -1st day</th>
<th>VCMs/5 min -8th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CMC (1ml/kg, p.o)</td>
<td>12.5±1.36</td>
<td>13.3±1.61</td>
</tr>
<tr>
<td>2. MPTP (20 mg/kg, i.p.)</td>
<td>54.3±3.27</td>
<td>59.6±3.75</td>
</tr>
<tr>
<td>3. E. ganitrus (100mg/kg, i.p.) + MPTP</td>
<td>50.1±1.48</td>
<td>44.3±2.32</td>
</tr>
<tr>
<td>4. E. ganitrus (200mg/kg, i.p.) + MPTP</td>
<td>51.3±3.68</td>
<td>24.7±4.36</td>
</tr>
<tr>
<td>5. E. ganitrus (400mg/kg, i.p.) + MPTP</td>
<td>49.5±3.21</td>
<td>21.3±2.31</td>
</tr>
<tr>
<td>6. Levodopa (30mg/kg, i.p.) + MPTP</td>
<td>18.7±4.38</td>
<td>16.8±4.27</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SD for 12 animals in each group. *p < 0.001 vs. Carboxy methyl cellulose (CMC) - control, †p < 0.001 vs. MPTP, ‡p < 0.001 vs. (Levodopa + MPTP)

It was observed that among MPTP alone treated group, significant increase p<0.001 in vacuous chewing movements (VCMs) was seen on 1st day and on 8th day when compared to control group. In Levodopa treated group, significant decrease in (VCMs) p<0.001 was seen on 1st day and 8th day when compared to MPTP treated group. E. ganitrus100mg/kg, 200mg/kg and 400mg/kg pretreated groups did not cause any significant change in hanging time on 1st day. But on 8th day, E. ganitrus 200mg/kg and 400mg/kg pretreated groups showed significant increase in hanging time (p<0.001) when compared to MPTP (as shown in table 1). Whereas no significant difference in hanging time was seen when E. ganitrus400mg/kg treated group compared to levodopa treated group.

Table 3: Effect of E. ganitrus in tail suspension test on 1st day and 8th day

<table>
<thead>
<tr>
<th>Groups, (Dose)</th>
<th>Duration of Immobility (sec) -1st day</th>
<th>Duration of Immobility (sec) -8th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CMC (1ml/kg, p.o)</td>
<td>179.5±2.91</td>
<td>183.5±2.73</td>
</tr>
<tr>
<td>2. MPTP (20 mg/kg, i.p.)</td>
<td>254.7±2.84</td>
<td>262.7±2.52</td>
</tr>
<tr>
<td>3. E. ganitrus (100mg/kg, i.p.) + MPTP</td>
<td>246.3±2.61</td>
<td>241.3±2.19</td>
</tr>
<tr>
<td>4. E. ganitrus (200mg/kg, i.p.) + MPTP</td>
<td>171.2±3.95</td>
<td>97.4±1.37</td>
</tr>
<tr>
<td>5. E. ganitrus (400mg/kg, i.p.) + MPTP</td>
<td>111.6±2.35</td>
<td>88.2±1.54</td>
</tr>
<tr>
<td>6. Levodopa (30mg/kg, i.p.) + MPTP</td>
<td>90.4±1.21</td>
<td>82.3±3.02</td>
</tr>
</tbody>
</table>

(n=12), values expressed as mean±SEM. (p<0.001 vs. normal saline-control), (p<0.01 vs. MPTP), (p<0.001 vs. Levodopa+MPTP)
In group treated with MPTP, animals showed significant increase (p<0.01) in immobility period in tail suspension test, on 1st day and 8th day when compared to control group (as shown in table 3). The group treated with Levodopa as a standard, animals showed significant decrease (p<0.001) in immobility period on 1st day and 8th day as compared to control group (as shown in table 3). When E.ganitrus 200mg/kg and 400mg/kg pretreated groups when compared to control group, animals showed slight decrease in the period of immobility on 1st day which was not statistically significant. Whereas, on 8th day, a significant decrease (p<0.001) in immobility period was noticed (as shown in table 3). But on 8th day there was no statistically significant difference in the immobility period when E.ganitrus 400mg/kg pretreated groups compared to Levodopa treated group (as shown in table 3).

Discussion

Parkinson’s disease is a progressive neurodegenerative disorder, characterized by resting tremor, bradykinesia, flexed posture, shuffling gait and rigidity. The cause for dopaminergic neuronal degeneration is still not well established, but oxidative stress might play significant role [7]. Oxidative stress may result from generation of free radical reactive species during the metabolism of dopamine [17]. The substantia nigra pars compacta is relatively more succumbed to generation of free radical reactive oxygen species amounting to more oxidative stress.

This excess generation of free radical reactive oxygen species might be directly correlated to the high energy metabolism or to more content of dopamine in these cells [18]. Several studies have established the finding that oxidative stress changes are demonstrable in the brain of Parkinson’s disease patients [19]. (MPTP), 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine is a potent neurotoxin used to create an experimental model of Parkinson’s disease in animals. Certain aspects of the Parkinson’s disease such as cataplexy, motor incoordination and bradykinesia can be easily studied in this model. As MPTP is highly lipophilic, makes it enable to cross the blood brain barrier immediately after its systemic absorption. Once MPTP reaches the brain tissue, it is converted to the hydrophilic metabolite 1-methyl-4-phenylpyridinium ion (MPP⁺), the free radical reactive specie in the causation of dopaminergic neuronal loss. It is established that these free radical reactive species play a vital role in the pathogenesis of dopaminergic neuronal loss in Parkinson’s disease [20]. E.ganitrus is an important medicinal plant which has proved its benefits in various neurological diseases caused by oxidative stress. It is convincingly explained that antioxidants might be effective in PD by preventing neuronal death which is caused by intracellular free radicals[7].

The mice when pretreated with E.ganitrus (200, 400 mg/kg, p.o.) for 8 days, significantly increased the hanging time in hanging wire test, decreased the vacuous chewing movements (VCMs) in tardive dyskinesia test and this effect is comparable to that of levodopa group. The above findings of behavioral tests are similar with other previous studies[21,22]. The mice when pretreated with E.ganitrus (200, 400 mg/kg, p.o.) for 8 days, significantly increased the duration of immobility in tail suspension test this effect is comparable to that of levodopa group. The above findings of behavioral tests are similar with other previous studies done on different parkinsonian animal models induced by MPTP and haloperidol [23-25].

The role of neuroinflammation and usage of anti-inflammatory medication in the prevention of Parkinson’s disease still needs to be well established. However, some animal experimental studies have showed preventative role of nonsteroidal anti-inflammatory drugs (NSAID’s) in Parkinson’s disease. Recently, it became evident that inflammatory process contributes significantly to the pathogenesis of Parkinson’s disease [18, 26]. Previous studies have established the finding that anti-inflammatory drugs such as acetysalicylic acid can prevent MPTP induced Parkinson’s disease in mice [27]. It is widely accepted that inflammation and oxidative stress both are interrelated. It is interesting to know that oxidative stress can potentiate inflammatory process and inflammation is found to generate oxidative stress [27]. Anti-inflammatory activity was demonstrated by the several fruit extracts of E.sphaerica like petroleum ether, benzene, chloroform, acetone and ethanol extracts in rats [28]. The key ingredients identified after phytochemical screening of ethanolic extract of fruits were alkaloids, flavonoids, carbohydrates, proteins and tannins[29]. The ethanolic extract of leaves of E.ganitrus isalso known to contain quercetin, gallic and ellagic acids (elaecarpine, isoeelaecarpine)[30]. The major phytoconstituents of E.sphaerica are mainly alkaloids like- elaecarpidine, elaeocarpine[31], and rudrakine[32]. Flavonoids like quercetin[33], phenolics are also important phytoconstituents of E.sphaerica. Increased antioxidants activities and decreased levels of lipid peroxides were seen in the brains of mice pretreated with E.ganitrus convincingly demonstrated that the extract significantly reduces oxidative stress. Our study findings are in accordance with previous studies done by Reddy VVM et al [34] and Bagewadi HG et al [35]. It is also established that Flavonoids content in E.ganitrus leaves has marked antioxidant activity[36].

The anti-Parkinsonian effect of E.ganitrus may be attributed to the presence of alkaloids and flavonoids having marked antioxidant and anti-inflammatory properties. However, further studies should be undertaken to identify and investigate the mechanism of action of active antiparkinsonian compounds in E.ganitrus. The behavioral assessment of motor imbalance, tardive dyskinesia and antiparkinsonian activity of E.ganitrus has to be further explored in other experimentally induced Parkinsonism models like Reserpine, 6-OHDA (6-Hydrox dopamine).
Conclusion

Parkinson’s disease is a progressive neurodegenerative disease accompanied by preferential loss of dopaminergic neurons of the substantia nigra pars compacta. MPTP is a potent neurotoxin, commonly used to create experimental model of Parkinson’s disease. The results of the present study conclusively showed that *E. ganitrus* has beneficial effects in hang wire test, tardive dyskinesia test and elevated plus maze test. In this regard, future studies on this topic may provide an elaborate view to use *E. ganitrus* in clinical medicine for treatment of Parkinson’s disease and its neurological sequel.

Conflict of interest: We declare that we have no conflict of interest.

References

24. Olga Lopatina *et al.* Anxiety- and depression-like behavior in mice lacking the CD157/BST1 gene, a...