Investigation of antiparkinsonian effect of Aloe vera on haloperidol induced experimental animal model

Harish G Bagewadi1*, Afzal Khan AK2

1Asst. Professor, Department of Pharmacology, MVJ Medical College & Research Hospital, Bangalore- 562114, India
2Professor, Department of Pharmacology, MVJ Medical College & Research Hospital, Bangalore- 562114, India

ARTICLE INFO:
Article history:
Received: 26 February 2015
Received in revised form: 20 March 2015
Accepted: 28 March 2015
Available online: 31 March 2015

Keywords:
A.vera, haloperidol, hanging wire test, tardive dyskinesia test, hole board test.

ABSTRACT
Background: Aloe vera (Family: Liliaceae) has been used for the treatment of diabetes, skin disorders and as an anti-inflammatory agent. There is increased concern about the side effects of conventional medicine in the treatment of Parkinson’s disease (PD). As A. vera has found to have antioxidative property, it may be a safer alternative. Methods: Parkinson’s disease was induced by administering haloperidol (1 mg/kg i.p. daily x 1 week). The mice of either sex were divided into 06 groups (n=12). The 1st day group mice were given distilled water (orally), 2nd group were administered haloperidol (20 mg/kg i.p.). The 3rd, 4th and 5th groups were administered A.vera (100, 200, and 400 mg/kg/day, orally) respectively, along with haloperidol. Group 6- received Levodopa (30mg/kg, i.p.) along with haloperidol. To evaluate anti-Parkinson effect, hanging wire test, tardive dyskinesia test and hole board test were performed on the 1st and 8th day. One way ANOVA was used to detect statistical significance followed by post-hoc Tukey test. Results: A.vera (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 haloperidol group. A.vera (200 and 400 mg/kg, p.o.) was found to significantly increase (p<0.001) the Vacuous Chewing Movements (VCMS) and number of dips and no. of line crossings in hole board test when compared to haloperidol group. Conclusion: The results of the present study conclusively showed that A.vera has beneficial effect in haloperidol induced experimental model of Parkinson’s disease.

Introduction
Plants have been used to treat various diseases and have been an exemplary source of medicine over the years[1]. Aloe vera (Family: Liliaceae), is one such ancient plant whose medicinal properties have been known since centuries [2]. It has demonstrated improved lipid profile status in rats with streptozotocin-induced diabetes [3]. In addition, recent studies reveal the role of A.vera in immunomodulation, inflammatory pain, anti-depressant and memory enhancing properties [4,5].

A recent study has reported that A.vera improves antioxidant activity within the hippocampus and cerebral cortex leading to improvement of the motor and memory behavioral tasks in diabetic mice [6]. Such report suggests that A.vera might have some beneficial effects in the treatment of some central nervous system diseases.

The clinical syndrome of PD results from idiopathic degeneration of the dopaminergic cells in the pars compacta of the substantia nigra [7]. While the cause of the degeneration of the dopaminergic cells in the pars compacta of the substantia nigra is not known, oxidative stress plays an important role [8]. Among different pharmacological treatments, levodopa remains the most efficacious and is still the mainstay of therapy. However, long-term use of levodopa can cause disabling motor complications, particularly dyskinesia’s and motor fluctuations, which limit its usefulness. Because of the concern about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment has been on the rise in the last few decades. Thus, medicines from natural sources having antioxidant and neuroprotective activity may serve as a good alternative 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 [9]. The hang test can evaluate the neuromuscular strength, coordination and is sensitive to a loss of dopamine...
Group II- was treated with haloperidol for 1 week). Previous studies undertaken by us shows that A.vera possess antioxidative properties and showed beneficial effect in rotarod test and catalepsy bar tests which are behavioral models of Parkinson disease [13].The present study was undertaken in order to further strengthen the evidence of protective role of A.vera in haloperidol induced parkinsonism in other animal models like- hanging wire, hole board and tardive dyskinesia test.

Materials and methods

Swiss albino mice of either sex weighing between 25 and 30 g, were used in the study. The animals were housed in polypropylene cages in groups of six to eight mice per cage and kept under controlled environmental condition. Care of animals was according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals. The study was duly approved by the Institutional Animal Ethics Committee, University College of Medical Sciences, Delhi. (Approval No. IAEC/2011/49 dated 10 March 2011).

Plant Material

A.vera extract was obtained from M/s Indo World Trading Corporation, New Delhi (Batch no. IWTC/711/9432). As per the literature provided by the manufacturer, the gel obtained from Aloe vera leaf was mixed with double distilled water in the ratio 1:1, mechanically shaken at room temperature and concentrated in the evaporator, followed by lyophilisation to obtain a brown powder with characteristic odour. The characterization of a sample of the extract by the spectrophotometer (IP66 method) revealed 3.14 % aloin. For the purpose of study, the A.vera powder was dissolved in double distilled water to prepare suspensions of required doses of 100, 200 and 400 mg/kg.

Experimental Design

The animals were divided into 06 groups (n=12).

Group I- was administered distilled water (orally, once per day x1 weeks).

Group II- received haloperidol (1 mg/kg, i.p. daily x 1 week).

Groups III, IV, and V- were treated with A.vera (100, 200, and 400 mg/kg/day, orally), respectively, x 1 week along with haloperidol.

Group VI- received Levodopa (30mg/kg, i.p. once per day x 1 week) along with haloperidol.

The A.vera (100mg/kg, 200mg/kg, 400mg/kg) orally and Levodopa (30mg/kg, i.p.) were given 30 minutes prior to haloperidol administration for 08 days of experimental period. Haloperidol and Levodopa were obtained from Sigma Chemical Co. USA and all the other chemicals used were of analytical grade.

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 (30x 20x 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 *A. vera* on hanging wire test in haloperidol 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. Distilled water (0.5ml/kg, p.o)</td>
<td>30.04±1.23</td>
<td>32.25±1.36</td>
</tr>
<tr>
<td>2. Haloperidol (1 mg/kg, i.p.)</td>
<td>08.90±1.32</td>
<td>06.90±2.14</td>
</tr>
<tr>
<td>3. <em>A. vera</em> (100mg/kg, i.p.) + haloperidol</td>
<td>09.2±1.73</td>
<td>11.8±2.61</td>
</tr>
<tr>
<td>4. <em>A. vera</em> (200mg/kg, i.p.) + haloperidol</td>
<td>13.2±2.03</td>
<td>18.5±3.17</td>
</tr>
<tr>
<td>5. <em>A. vera</em> (400mg/kg, i.p.) + haloperidol</td>
<td>14.7±3.53</td>
<td>22.4±3.29</td>
</tr>
<tr>
<td>6. Levodopa (30mg/kg, i.p.) + haloperidol</td>
<td>24.2±4.75</td>
<td>27.2±3.28</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SD for 12 animals in each group. *p < 0.001 vs. Distilled water- control, *p < 0.001 vs. haloperidol, *p < 0.001 vs. (Levodopa + haloperidol).

It was observed that haloperidol 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 haloperidol treated group. *A. vera* 100 mg/kg, 200 mg/kg and 400 mg/kg pretreated groups did not cause any significant change in hanging time on 1st day. But on 8th day, *A. vera* 200 mg/kg and 400 mg/kg pretreated groups showed significant increase in hanging time (p<0.001) when compared to haloperidol (as shown in table 1). Whereas no significant difference in hanging time was seen when *A. vera* 400 mg/kg treated group compared to levodopa treated group.

Table 2: Effect of *A. vera* on tardive dyskinesia in haloperidol treated mice

<table>
<thead>
<tr>
<th>Groups, (Dose)</th>
<th>VCMs/5 min-1st day</th>
<th>VCMs/5min-8th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Distilled water (0.5ml/kg, p.o)</td>
<td>6.4±1.14</td>
<td>7.8±1.37</td>
</tr>
<tr>
<td>2. Haloperidol (1 mg/kg, i.p.)</td>
<td>48.6±3.66</td>
<td>53.1±3.15</td>
</tr>
<tr>
<td>3. <em>A. vera</em> (100mg/kg,i.p.) + haloperidol</td>
<td>46.7±1.13</td>
<td>38.5±2.86</td>
</tr>
<tr>
<td>4. <em>A. vera</em> (200mg/kg,i.p.) + haloperidol</td>
<td>45.3±3.12</td>
<td>18.3±4.46</td>
</tr>
<tr>
<td>5. <em>A. vera</em> (400mg/kg,i.p.) + haloperidol</td>
<td>43.1±3.53</td>
<td>15.7±2.71</td>
</tr>
<tr>
<td>6. Levodopa (30mg/kg,i.p.) + haloperidol</td>
<td>12.2±4.21</td>
<td>10.2±4.15</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SD for 12 animals in each group. *p < 0.001 vs. Distilled water- control, *p < 0.001 vs. haloperidol, *p < 0.001 vs. (Levodopa + haloperidol).

It was observed that among haloperidol 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 haloperidol treated group. *A. vera* 100 mg/kg, 200 mg/kg and 400 mg/kg pretreated groups did not cause any significant change in (VCMs) on the1st day. But on 8th day, *A. vera* 200 mg/kg and 400 mg/kg pretreated groups showed significant decrease in (VCMs) p<0.001 when compared to haloperidol treated group (as shown in table 2), whereas no significant difference in (VCMs) was seen when *A. vera* 400 mg/kg treated group compared to levodopa treated group.
The results are expressed as mean ± SD for 12 animals in each group. *p < 0.001 vs. Distilled water-control, †p < 0.001 vs. haloperidol, ‡p < 0.001 vs. (Levodopa + haloperidol).

It was observed that haloperidol alone treated group, significantly decreased the no. of head dips and line crossings (p<0.001) on 1st day and 8th day as compared to control group. In Levodopa treated group, significant increase in no. of head dips and line crossings (p<0.001) was seen on both 1st day and 8th day, as compared to haloperidol treated group. A.vera 100mg/kg, 200mg/kg and 400mg/kg pretreated groups did not cause any significant change in no. of head dips and line crossings on 1st day. But on 8th day, A.vera 200 mg/kg and 400mg/kg pretreated groups showed significant increase in no. of head dips and line crossings (p<0.001) when compared to haloperidol (as shown in table 3). Whereas no significant difference in no. of head dips and line crossings was seen when A.vera 400 mg/kg treated group compared to levodopa treated group.

### Discussion

Parkinson’s disease is a chronic neurodegenerative disorder characterized by degeneration of dopamine producing neurons in the substantia nigra, caudate nucleus and putamen leading to resting tremor, bradykinesia, shuffling gait, flexed posture and rigidity.

While the cause of the degeneration is not known, oxidative stress plays avital role. Oxidative stress may arise from the metabolism of dopamine with the production of potentially harmful free radical species [17]. Compared to the rest of the brain, the substantia nigra pars compacta is exposed to a higher rate of reactive oxygen species formation and to higher levels of oxidative stress. This may be related to the energy metabolism of these cells or to their high content of dopamine [18]. Various studies have reported oxidative stress changes in the brain of Parkinson’s disease patients [19].

Haloperidol, a neuroleptic drug, induces tardive dyskinesia’s which is due to a blocking of post synaptic striatal dopamine D2 receptors and many studies have shown reactive oxygen species as a cause of Haloperidol induced toxicity[20,21]. Neuroleptics like haloperidol neurotoxicity has been linked to a blocking of post synaptic striatal dopamine D2 receptors and studies have proposed reactive oxygen species as cause of haloperidol induced toxicity [21]. Drugs which attenuate haloperidol induced motor disorders might reduce the extrapyramidal signs of Parkinson’s disease. In the present study, three behavioral assessment parameters – hanging wire test, tardive dyskinesia, hole board tests were used to assess haloperidol induced Parkinson disease in mice. The mice when pretreated with A.vera (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 [22, 23].

The mice when pretreated with A.vera (200, 400 mg/kg, p.o.) for 8 days, significantly increased the no. of dips and line crossings in hole board test, and 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 [24-28]. A.vera is an important medicinal plant that plays a significant role in protection from oxidative stress. A number of studies have shown that A.vera has significant anti-oxidant properties [29]. It has been hypothesized that antioxidants may be neuroprotective in PD, by preventing neuronal death caused by intracellular free radicals [8].

Inquiries into the role of neuroinflammation in Parkinson’s disease have coincided with increasing interests in determining whether anti-inflammatory medications may be helpful in preventing PD. Experimental evidence and animal models in particular support a preventative role for nonsteroidal anti-inflammatory drugs (NSAIDs) in Parkinson’s disease. For example, studies have demonstrated that anti-inflammatory drugs such as acetylsalicylic acid are protective against haloperidol-induced striatal dopamine depletion in mice [30]. Recently, involvement of inflammatory process has been also reported in the pathogenesis of Parkinson’s disease[18,30]. It is widely accepted that inflammation and oxidative stress are

Table 3: Effect of A.vera on Hole board test in haloperidol treated mice

<table>
<thead>
<tr>
<th>Groups, (Dose)</th>
<th>No. of dips-1st day</th>
<th>No. of dips-8th day</th>
<th>Line Crossings-1st day</th>
<th>Line crossings-8th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Distilled water</td>
<td>40.2 ± 1.6</td>
<td>42.5 ± 2.2</td>
<td>25.4 ± 1.3</td>
<td>27.2 ± 1.9</td>
</tr>
<tr>
<td>2. Haloperidol (1 mg/kg, i.p.)</td>
<td>12.3 ± 1.6*</td>
<td>10.1 ± 2.1†</td>
<td>09.1 ± 1.5†</td>
<td>6.4 ± 1.1†</td>
</tr>
<tr>
<td>3. A.vera (100mg/kg, i.p.) + haloperidol</td>
<td>13.1 ± 1.2†, ‡</td>
<td>18.8 ± 1.4†, ‡</td>
<td>10.2 ± 2.6†, ‡</td>
<td>12.5 ± 1.8†, ‡</td>
</tr>
<tr>
<td>4. A.vera (200mg/kg,i.p.) + haloperidol</td>
<td>15.2 ± 4.8†, †</td>
<td>28.5 ± 3.7†, †‡</td>
<td>13.7 ± 1.9†, †</td>
<td>17.9 ± 3.4†, †‡</td>
</tr>
<tr>
<td>5. A.vera (400mg/kg,i.p.) + haloperidol</td>
<td>16.8 ± 1.5†, †‡</td>
<td>30.1 ± 03.3†, †‡</td>
<td>14.2 ± 2.4†, †</td>
<td>19.6 ± 2.7†</td>
</tr>
<tr>
<td>6. Levodopa (30mg/kg,i.p.) + haloperidol</td>
<td>33.6 ± 2.3*, †</td>
<td>36.8 ± 1.5*, †‡</td>
<td>21.5 ± 2.6*, †</td>
<td>23.1 ± 2.3*, †</td>
</tr>
</tbody>
</table>
interrelated. Oxidative stress can increase inflammatory activity and, conversely, inflammation is known to cause oxidative stress [31].

Several studies have also emphasized the anti-inflammatory properties of Aloe vera in mice and rats. Previous studies show that Aloe vera leaf gel extract was found to have anti-inflammatory property [4, 5, 32]. A vera leaf gel is known to be rich in anthraquinones such as aloe-emodin, aloetic acid, anthranol, aloin A and B. Aloe is known to exert anti-inflammatory activity in the rat colitis, and the present extract of A vera contains relatively high amount 3.14% of aloin. Further studies are needed to prove whether anti-inflammatory and anti-oxidant properties of aloin is responsible for the anti-Parkinson effect or whether the synergy of a number of components viz. barbaloin, glucomannan, acemannan, minerals, flavonoids, tannic acid, etc. is responsible for the observed effects. The behavioral assessment of motor imbalance, tardive dyskinesia and antiparkinsonian activity of A vera has to be further explored in other experimentally induced Parkinsonism models like Reserpine, 6-OHDA (6-Hydroxy dopamine). It can be proposed that apart from the known effects of A vera, it has shown beneficial effects in our present study of experimental models of parkinson disease. Thus further studies are required to clearly establish its role as an anti-parkinson agent.

Conclusion

Parkinson’s disease is a progressive neurodegenerative disease accompanied by preferential loss of dopaminergic neurons of the substantia nigra pars compacta. Haloperidol by blocking D2 receptors is commonly used to create experimental model of Parkinson’s disease. The results of the present study conclusively showed that A vera has beneficial effects in hang wire test, tardive dyskinesia test and hole board test. In this regard, future studies on this topic may provide an elaborate view to use A vera 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


