Antiparkinson’s activity of *FRAGARIA ANANASSA LINN* against haloperidol induced Parkinson’s disease model

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**INTRODUCTION**

Parkinson’s disease (PD) is a chronic, progressive, neurodegenerative disease characterized by the classical signs of bradykinesia, rigidity and resting tremor with postural instability developing at a later stage. It involving primarily a degeneration of certain nerve cells in deep parts of the brain called the basal ganglia, and in particular a loss of nerve cells (or) neurons in a part of the brainstem called the substantia nigra (SN). These cells make the neurochemical messenger dopamine, which is partly responsible for starting a circuit of messages that coordinate normal movement [1]. The available treatments are levodopa, carbidopa, apomorphine, Amantadine, orphenadrine, benzhexol, benztropine, selegeline, pergola and many more. This drug effectively reverses the symptoms of Parkinson and improves the level of dopamine. The greatest disadvantage in presently available potent synthetic drugs lies in their adverse effects like constipation, ulcer, respiratory depression and hypertension, toxicity and reappearance of symptoms after discontinuation [2]. Hence search for new neuropharmacological agents that retain therapeutic efficacy and yet devoid of adverse effects are justifiable.

The drug that blocks the action of dopamine may result in Parkinsonism [3]. The neuroleptic drug like haloperidol is one of the major causes for drug induced Parkinson’s worldwide. The incidence of drug induced Parkinson’s progresses with age [4]. It blocks dopamine D2 receptors and produces a state of catalepsy in human or animals by reducing dopaminergic transmission in basal ganglion [5].

*Fragaria Ananassa* is a fibrous high flavanoid, popularly known as “Strawberry” in English,
“Jharaber” in Hindi. Experimental studies have demonstrated its anti-inflammatory and antioxidant properties [6]. To our best knowledge, there were no scientific data on antiparkinson effect of *F Ananassa* in experimental Parkinsonism. Hence, the present study was designed to evaluate the antiparkinson’s effect of *F Ananassa* against haloperidol induced Parkinson’s rats.

**MATERIALS AND METHODS**

**Experimental Animals:**

Experimental animals of either sex weighing 120-180g were obtained from Bharath enterprises, Bangalore. The animals were housed in polypropylene cages at a controlled room temperature of 24°C, under 12h light and 12h dark cycle and given standard laboratory feed and water *ad libitum*. After one week of acclimatization, the experiment animals were divided randomly into 5 groups (n=6). The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee of Santhiram College of pharmacy (1519/PO/a/11/CPCSEA).

**Chemicals:**

Haloperidol, L-Dopa & Carbidopa were purchased from Sigma Aldrich, Bangalore.

**Plant Material:**

The fresh fruits were collected from nandyal, Andra Pradesh. It was identified and authenticated by Botanist. The voucher specimen of the plant was kept in Department of Pharmacology, Santhiram College of pharmacy nandyal, Andra Pradesh. The fruits were cleaned, ground, and filtered through a muslin cloth. The residue was discarded and fresh juice of *F Ananassa* was collected and it was used to assess antiparkinsons activity.

**Experimental design:**

Animals were randomized and divided into five experimental groups (n=6) as follows.

Group 1: Normal was received vehicle (1% CMC, 10ml/kg body weight, p.o.),

Group 2: Control was received haloperidol (1mg/kg, p.o.),

Group 3: Standard was received L-Dopa & carbidopa (100mg+25mg/kg p.o.) and haloperidol (1mg/kg, p.o.),

Group 4: Low dose was received low dose of FFJFA (200mg/kg, p.o.) and haloperidol (1mg/kg, p.o.),

Group 5: High dose was received high dose of FFJFA (400mg/kg, p.o) and haloperidol (1mg/kg, p.o.).

The doses were given for 21 days as multiple dose studies. Animals were provided with food and water as usual before experiment. On 21st day haloperidol (1mg/kg, p.o.) was injected 60 min after oral administration of treatment for all groups except normal group. All the behavioral studies were performed at room temperature in a calm room without any external interference.

**Neurobehavioral Studies:**

**Catalepsy**

Catalepsy in rats is defined as a failure to correct an externally imposed, unusual posture over a prolonged period of time. Catalepsy can be measured by 2 methods

- By Block method
- By Metal bar test

**Measurement of catalepsy by block method**

This scoring method was followed in three steps.

**Step-I**

The rat was taken out of the home cage and placed on a table. If the rat failed to move when touched gently on the back (or) pushed, score of 0.5 was assigned.

**Step-II**

The front paws of the rats were placed alternately on a 3cm high block. If the rat failed to correct the posture within 15 seconds, a score of 0.5 for each paw was added to the score of step I.

**Step III**

The front paws of the rat placed alternately on a 9cm high block. If the rat failed to correct the posture with in 15sec, a score of 1 for each paw was added to the scores of step I, step II. Thus, for an animal, the highest score was 3.5(cut off score) and that reflects in total Parkinson’s disease [7].

**Measurement of catalepsy by Metal bar test**

Catalepsy score was measured for 4 hours at 30 min intervals after haloperidol administration by gently placing both the fore paw of the rat over a metal bar (diameter 2-5mm suspended 6cm above the tabletop). The intensity of catalepsy assessed by counting the time in seconds until the rat brought both fore pass down to the tabletop, with a maximum cutoff time of 3 minute. Finally, scores at different time points were added and expressed as cumulative catalepsy score for comparison purpose [7].

**Muscle rigidity**

Motor integrity and coordination were assessed by the time latency from the placement of the animal on the rotating drum until it falls. First, animals were trained by placing them on a rolling bar and had been to walk
Table no: 1 Effect of FFJFA on Catalepsy by Block method

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>Immobility (Score) (Mean ± S.E.M) on 21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 Min</td>
</tr>
<tr>
<td>1</td>
<td>Normal (1% CMC)</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>2</td>
<td>Control (Haloperidol)</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>3</td>
<td>Standard (L-Dopa &amp; Carbidopa + Haloperidol)</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>4</td>
<td>Test-1 (FFJFA (200mg/kg, P.O + Haloperidol)</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>5</td>
<td>Test-2 (FFJFA (400mg/kg, P.O + Haloperidol)</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

All values were expressed as Mean±S.E.M and n=6
# indicates P<0.05, ## indicates P<0.01, ### indicates P<0.001 when compared to normal group;
* indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001 when compared to Haloperidol induced group(One-way ANOVA followed by Tukey’s test)
### Table no: 2 Effect of FFJFA on Catalepsy by Metal bar method

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>Immobility (Fall of time in sec) (Mean ± S.E.M) on 21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 Min</td>
</tr>
<tr>
<td>1</td>
<td>Normal(1% CMC)</td>
<td>5.33±0.88</td>
</tr>
<tr>
<td>2</td>
<td>Control ( Haloperidol)</td>
<td>5.00±0.57</td>
</tr>
<tr>
<td></td>
<td>Standard ( L-Dopa &amp; Carbidopa + Haloperidol)</td>
<td>4.16±0.94</td>
</tr>
<tr>
<td>3</td>
<td>Test-1 ( FFJFA (200mg/kg,P.O + Haloperidol)</td>
<td>5.50±0.95</td>
</tr>
<tr>
<td>4</td>
<td>Test-2 ( FFJFA (400mg/kg,P.O + Haloperidol)</td>
<td>5.50±0.57</td>
</tr>
</tbody>
</table>

All values were expressed as Mean±S.E.M and n=6

# indicates P<0.05, ## indicates P<0.01, ### indicates P<0.001 when compared to normal group;
* indicates P<0.05, ** indicates P<0.01,*** indicates P<0.001 when compared to Haloperidol induced group(One-way ANOVA followed by Tukey’s test)
Table no: 3 Effect of FFJFA on Muscle rigidity by Rota rod method

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>Fall of time (Sec) (Mean ± S.E.M) on 21st day</th>
<th>0 Min</th>
<th>30 Min</th>
<th>60 Min</th>
<th>90 Min</th>
<th>120 Min</th>
<th>150 Min</th>
<th>180 Min</th>
<th>210 Min</th>
<th>240 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal (1% CMC)</td>
<td></td>
<td>176.7±2.47</td>
<td>180.0±0.0</td>
<td>170.3±3.73</td>
<td>173.7±2.51</td>
<td>171.2±2.62</td>
<td>167.7±5.17</td>
<td>162.0±2.95</td>
<td>180±0</td>
<td>174.0±2.09</td>
</tr>
<tr>
<td>2</td>
<td>Control (Haloperidol)</td>
<td></td>
<td>180.0±0.0</td>
<td>48.83±3.32</td>
<td>62.00±6.55</td>
<td>52.67±1.82</td>
<td>44.83±1.07</td>
<td>39.50±1.31</td>
<td>47.50±2.43</td>
<td>39.33±4.52</td>
<td>42±1.63</td>
</tr>
<tr>
<td>3</td>
<td>Standard (L-Dopa &amp; Carbidopa + Haloperidol)</td>
<td></td>
<td>180.0±0.0</td>
<td>51.83±3.67</td>
<td>63.17±2.67</td>
<td>67.67±2.21</td>
<td>55.33±1.14</td>
<td>58.83±2.40</td>
<td>102.3±2.51</td>
<td>105.8±6.63</td>
<td>116.3±3.28</td>
</tr>
<tr>
<td>4</td>
<td>Test-1 (FFJFA (200mg/kg, P.O + Haloperidol)</td>
<td></td>
<td>180.0±0.0</td>
<td>60.33±2.24</td>
<td>56.67±5.14</td>
<td>53.50±4.03</td>
<td>47.00±2.23</td>
<td>55.83±2.78</td>
<td>57.83±2.37</td>
<td>67.50±3.18</td>
<td>59.00±1.06</td>
</tr>
<tr>
<td>5</td>
<td>Test-2 (FFJFA (400mg/kg, P.O+ Haloperidol)</td>
<td></td>
<td>180.0±0.0</td>
<td>62.67±7.97</td>
<td>50.00±3.03</td>
<td>61.00±3.73</td>
<td>52.33±0.88</td>
<td>59.33±3.10</td>
<td>63.17±1.99</td>
<td>97.83±5.63</td>
<td>127±4.36</td>
</tr>
</tbody>
</table>

All values were expressed as Mean±S.E.M and n=6

# indicates P<0.05, ## indicates P<0.01, ### indicates P<0.001 when compared to normal group;
* indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001 when compared to Haloperidol induced group (One-way ANOVA followed by Tukey’s test)
on it. Then, the animals were conducted to rotarod test with an appropriate speed (20-25rpm). Place the animal one by one on the rotating rod and fall off time i.e., when the animal fall from the rotating rod was recorded, which was taken as motor integrity and coordination [8].

Statistical Analysis:
All the data are expressed in mean ± SEM. The significance of difference in means between control and treated animals was determined by One-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test (graph pad prism 5.03). P<0.05 was considered statistically significant.

RESULTS
Pharmacological studies:
Acute toxicity studies:
The FFJFA was found to be safe at the maximum single dose of 4000mg/kg when administered orally the animals did not show any gross behavior changes hence according to OECD guide lines 423 the dose can be reduced up to 1/10 of the LD$_{50}$ that is 400mg/kg were used as high dose and 1/20 of the LD$_{50}$ that is 200mg/kg were used as low dose in the subsequent study respectively.

Anti Parkinson’s activity:
Neurobehavioral Studies
Effect of FFJFA on catalepsy by block method
Treatment with L-dopa&Carbidopa (100mg+ 25 mg/kg, p.o.) showed a significant reduction in the cataleptic behaviour on 90min (P<0.05), 120 min (P<0.01) 150, 180, 210, 240 min (P<0.001) as compared to the haloperidol treated group. The test-I and test-II groups were receiving FFJFA at two different doses (200mg/kg, 400mg/kg) showed a significant decrease in immobility and muscle rigidity when compared to control group. But interestingly the test-II groups showed good significant decrease in immobility and muscle rigidity at 120min (P<0.05), 150,180(P<0.01), 210 and 240(P<0.001) than test-II [180min (P<0.05)210 and 240(P<0.01)] (Table 1).

Effect of FFJFA on catalepsy by metal bar test
The cataleptic scores of the present study were assessed by metal bar test. On treatment with L-dopa and Carbidopa showed a significant decreased in Immobility and muscle rigidity on 90min. (P<0.05), 120min (P<0.01), 150, 180, 210 & 240min (P<0.001) when compared to the control group.

The test-I and test-II groups were receiving FFJFA at two different doses (200mg/kg, 400mg/kg) showed a significant decrease in immobility and muscle rigidity when compared to control group. But interestingly the test-II groups showed good significant decrease in immobility and muscle rigidity at 120min (P<0.05), 150,180(P<0.01), 210 and 240(P<0.001) than test-II [180min (P<0.05)210 and 240(P<0.01)] (Table 2).

Effect of FFJFA on muscle rigidity
Haloperidol administration significantly (P<0.01) decreases in active and passive movements as compared to normal animals. Pre-treatment with FFJFA (both doses i.e. 200 mg/kg and 400 mg/kg, p.o.) significantly (P<0.01) increase in active and passive movements when compared to control group. But group-V shows more significant effect than group-IV (Table 3).

DISCUSSION
Oxidative stress to dopaminergic neurons of SNpc is believed to be one of the leading causes of neurodegeneration in PD. Antioxidants may play an important role in the prevention of PD and combat against oxidative stress induced progressive neurodegeneration by reactive oxygen species. However, medicinal plants like *Gingko biloba* [18], *Stereospermum aveolens* [19] and *Nigella sativa* have shown neuroprotective activity due to their antioxidant property. Extract of *F. Ananassa* has proven free radical scavenging activity [6].

Animal models of Parkinson’s disease are widely used to investigate its pathophysiological mechanisms and for exploring potential treatments. Typically, models of PD are characterized by measures of akinesia, such as in block method and bar test for immobility. Neuroleptics such as haloperidol can produce a sustained but reversible akinesia, due to blockade of dopamine D$_2$ receptors and this neuroleptic-induced Parkinsonism is a major side effect of their use in treatment of schizophrenia. D$_2$ antagonists may act directly to reduce the ability of cortical and basal ganglia motor pathways to generate descending commands. Neuroleptics have thus been used as an acute model of Parkinson’s [19]. The central dopaminergic function and evaluation of dopamine agonistic activity was carried out by observing the cataleptic behavior in rats. Haloperidol blocks the dopamine D$_2$ receptors in the brain and precipitates the extra pyramidal side effects that can be measured by block method and metal bar test in rats. In both methods FFJFA at the dose of 200mg/kg and 400mg/kg showed a significant and dose dependent decrease in immobility and muscle impairment. Incredibly the high dose of *F. Ananassa* was showed comparable results with standard L-Dopa & carbidopa.

The muscular rigidity tested using a rota-rod, is an established method used for the assessment of
Fig no: 1 Effect of FFJFA on catalepsy by Block method

Fig no: 2 Effect of FFJFA on catalepsy by Metal bar method
neurological deficits in rodents [19]. Significantly enhanced muscular coordination was seen in fresh fruit juice of *F. Ananassa* treated groups, as compared to control group.

**CONCLUSION**

In conclusion, the present study suggests a potential role of fresh fruit juice of *F. Ananassa.* Against haloperidol induced Parkinson’s disease model. Further studies are required for understand the basic mechanism and characterization of active constituents responsible for neuroprotective effect.

**Acknowledgement**

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**REFERENCES**
