Evaluation of Antioxidant and Anti-Parkinson Activity of Portulaca oleracea Seed Methanolic Extract

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors DKG and SS helped to collection, authenticate and phytochemical investigation of plant material. Author DSP helped in statistical data analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Evaluation of Antioxidant and Anti-Parkinson activity of Portulaca oleracea seed methanolic extract.

Place: C. U. Shah College of Pharmacy and Research, Wadhwan, Surendranagar, Gujarat, India.

Methodology: Collect plant materials were extracted with methanol. Extract was subjected to qualitative and quantitative investigation and antioxidant properties of extract was determine by Nitric oxide free radical scavenging activity and Reducing power by FeCl₃ method. Anti-Parkinson activity evaluated by two behavioral models namely, haloperidol induced catalepsy, and orofacial dyskinesia both models various behavioral activity/ parameter (catalepsy, vacuous chewing movement and tongue protrusion) were evaluated.

Results: Preliminary qualitative phytochemical screening was to reveal presence of polyphenols, flavanoids, glycoside, alkaloids, carbohydrates and reducing sugar etc. Based preliminary...
Portulaca oleracea (Linn.) family Portulacaceae commonly called as briholloni, ghokli, lona, lonamla, loni, lonika, lunia, Pigweed, little hogweed or pusley [5]. It is an annual succulent in the family Portulacaceae, which can reach 40 cm in height. It is a native of India and the Middle East, but is naturalized elsewhere and in some regions is considered an invasive weed [6]. It has smooth, reddish, mostly prostrate stems and alternate leaves clustered at stem joints and ends. The yellow flowers have five regular parts and are up to 6 mm wide [5]. The flowers first appear in late spring and continue into mid fall. The flowers open singly at the center of the leaf cluster for only a few hours on sunny mornings. Seeds are formed in a tiny pod, which opens when the seeds are ready. Purslane has a taproot with fibrous secondary roots and is able to tolerate poor, compacted soils and drought [7].

Portulaca oleracea contains minerals, proteins, carbohydrates, β-carotene, vitamins and fatty acids [8,9]. Among the bioactive components and biological activities assigned to the Portulaca oleracea, the presence of catecholamine [10] and its antioxidant and anti-inflammatory actions [11,12] deserve to be highlighted. Neuropharmacological actions of the Portulaca oleracea extracts were previously reported in rodent models, effects included the reduction of the locomotor activity, the increase in the onset time of pentyletetrizazole-induced convulsions in mice, the opioid mediated anti-nociceptive and muscle relaxant activities in rats [13]. Biological activities also include anti-inflammatory actions. [14,15].

2. MATERIALS AND METHODS

2.1 Extraction

Air dried & coarsely powdered of Portulaca oleracea seed was carried out for soxhlet extraction using methanol. The extract was concentrated to dryness under reduced pressure and it was preserved in a refrigerator [16,17].

Keywords: Portulaca oleracea seed; alkaloids; antioxidant; Orofacial dyskinesia; vacuous chewing movements and methanolic extract.
2.2 Phytochemical Investigation

2.2.1 Preliminary phytochemical investigation

The methanolic extracts of Portulaca oleracea seed (MLPO) was subjected to various phytochemical tests for identification of secondary metabolites present in them [18].

2.2.2 Determination of total polyphenols

The total polyphenol content (TPC) was determined by spectrophotometry method, using tannic acid as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1. Briefly, 1.0 ml of the diluted sample extract was transferred in duplicate to separate tubes containing 5.0 ml of a 1/10 dilution of Folin-Ciocalteu's reagent in water [5]. Then, 4.0 ml of a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm was measured against water. The concentration of polyphenols in samples was derived from a standard curve of tannic acid ranging from 10 to 50 µg/ml and expressed in terms percentage [19].

2.3 Antioxidant Activity

2.3.1 Reducing power by FeCl₃

Various concentrations of the extracts in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml) and incubated at 50ºC for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power [20].

2.3.2 Nitric oxide free radical scavenging activity

Various concentrations of the extracts in 1.0 ml of methanol were mixed with Sodium nitro prusside (10 mM) in phosphate buffer phosphate buffer and made upto 200 µl with methanol mixture of solution Incubate at room temperature for 150 minutes. After the incubation period 5 ml of Griesss reagent was added then absorbance was taken at 546 nm. A blank was prepared without adding extract. Curcumin at various concentrations was used as standard, % reduction and IC50 were calculated [21].

2.4 Experimental Animals

Adult Swiss albino mice weighing 18-22 gm and wistar rats 200-230 gm of either sex obtain from Zydus Research Centre, Ahmedabad, for experimental purpose were all acclimatized for 7 days under standard husbandry conditions i.e.; room temperature of (25±1)ºC; relative humidity of 45%-55% and a 12:12 h light/ dark cycle.

2.4.1 Acute oral toxicity studies

The acute oral toxicity study of MLPO was carried out in Swiss albino mice (20-25 gm) and wistar rats (200-230 gm), using the Organization for Economic Co-operation and Development (OECD) guidelines (OECD 423). The animals received a single dose of 2000 mg/kg orally by gavages and were observed for toxic symptoms and mortality, continuously for first 4 h after dosing. Finally, the number of survivors was noted after 24 hrs and these animals were then maintained for further 14 days with observations made daily.

2.4.2 Haloperidol induced catalepsy in mice

Albino mice of either sex weighing 20-25 gm were divided into five groups of six animals each (n=6). The animals were allowed to adapt to the box for 2 min. A cataleptic behavior was measured with a “High bar test method”. The standard (L-dopa) drug was administered by intraperitoneal route and test drug was administered by oral route, half an hour prior to the haloperidol administration. Catalepsy score was measured for each hour upto 4 h after Haloperidol administration, by gently placing both the forepaws of the mice over a metal bar (diameter 2-5 mm) suspended 6 cm above the table top. The intensity of catalepsy was assessed by counting time in seconds until the mice brought both forepaws down to the table top, with a maximum cut-off time of 180 seconds [22].

2.4.3 Haloperidol induced orofacial dyskinesia in rat

Dyskinesia is characterized by vacuous chewing & tongue protrusion. Wistar rat of either sex weighing 200-230 gm were divided into five groups of six animals each (n=6). Haloperidol was administered in the rats for a period of 21
days to induce oral dyskinesia. Test agent was administered once daily in the morning for a period of 21 days and behavioral assessments was performed at 7th, 14th, 21st day [22].

2.5 Statistical Analysis

Values were expressed as mean ± SEM from 6 animals. Statistical difference in mean will be analyzed using one way ANOVA followed by Turkey’s multiple comparison tests. P < 0.05 were considered statically significant.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The phytochemical studies of methanolic extract of *Portulaca oleracea* seed (MLPO) revealed presence of carbohydrates, alkaloids, glycosides, saponins, tannins and phenolic compounds proteins & free amino acids, flavanoids, and phenolic compounds.

3.2 Determination of Total Polyphenols

The estimation of polyphenolic content was performed by based on primary phytochemical investigation by Folin- Ciocalteu’s reagent, the extracts exhibit that, the MLPO found to contain 96.38%. Brief results are shown in Table 1.

3.3 Antioxidant Activity

The extracts containing varying quantities of total polyphenols were comparatively studied for their antioxidant potentialities. Two different in vitro methods namely Reducing Power by FeCl₃ and Nitric Oxide Free Radical Scavenging activity were employed, ascorbic acid was used as a standard in Reducing Power by FeCl₃, the MLPO significantly decreased the absorbance and the IC50 value 182.02±9.64 µg/ml was found, to possess more significant antioxidant activity, however the IC50 value is lesser then ascorbic acid.

In Nitric Oxide Free Radical Scavenging activity, Curcumin, was used as a standard, MLPO has offered good free radical scavenging activity by decreasing the absorbance and the IC50 value 667.12±16.02 µg/ml the IC50 value is lesser then Curcumin. IC50 value of different antioxidant activity of *Portulaca oleracea* seed methanolic extract (MLPO) was shown in Table 2.

3.4 Acute Toxicity Studies

The acute oral toxicity (AOT) study of MLPO was observed that it was safe upto 2000 mg/kg body weight and it was not showing any mortality, based on AOT selected dose for Anti-Parkinson activity were 200 mg/kg body weight and 400 mg/kg body weight.

3.5 Haloperidol Induced Catalepsy in Mice

Treatment with L-dopa (10 mg/kg, i.p) showed a significant (P<0.01) reduction in the cataleptic behavior between 60 to 180 min of time interval as compared to the haloperidol treated group. Treatment with *Portulaca oleracea* seed showed a significant (P<0.01) reduction in the duration of cataleptic behavior dose dependently when compared to haloperidol treated group. Brief results are given in Table 3.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Extract</th>
<th>Polyphenols (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Methanolic extract of <em>Portulaca oleracea</em> seed (MLPO)</td>
<td>96.38</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Antioxidant determining method</th>
<th>IC50 value of ascorbic acid (µg/ml) ± S.D</th>
<th>IC50 value of curcumin (µg/ml) ± S.D</th>
<th>IC50 value of extract MLPO (µg/ml) ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Reducing power by FeCl₃</td>
<td>15.78±1.87</td>
<td>---------</td>
<td>182.02±9.64</td>
</tr>
<tr>
<td>02</td>
<td>Nitric oxide free radical scavenging activity</td>
<td>---------</td>
<td>41.37±5.05</td>
<td>667.12±16.02</td>
</tr>
</tbody>
</table>

Value are mean ± S.D.; n=3
Table 3. Effect of *Portulaca oleracea* seed (MLPO) in haloperidol induced catalepsy in mice

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Treatment</th>
<th>00</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>15.64±0.21</td>
<td>15.76±1.34</td>
<td>15.53±0.43</td>
<td>16.36±0.96</td>
<td>13.98±1.18</td>
</tr>
<tr>
<td>2</td>
<td>Haloperidol (1.0 mg/kg i.p.)</td>
<td>16.22±6.50</td>
<td>164.19±4.69&lt;sup&gt;c&lt;/sup&gt;</td>
<td>167.27±5.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>173.62±5.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>143.93±6.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>L-dopa (10 mg/kg, i.p.)</td>
<td>15.17±6.68</td>
<td>57.23±6±2.135&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.30±5.485&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.20±2.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>121.43±3.29&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>MLPO200 (mg/kg, p.o.)</td>
<td>16.48±3.67</td>
<td>118.36±2.25</td>
<td>101.00±3.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>111.24±1.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>139.13±3.18&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>MLPO400 (mg/kg, p.o.)</td>
<td>13.71±2.54</td>
<td>68.26±1.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.39±3.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.73±34.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>124.51±3.51&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The values are expressed as mean ±SEM (n=6); <sup>a</sup>P<0.05, <sup>b</sup>P<0.01 vs Haloperidol group (One way ANOVA followed by Dunnett’s test). <sup>c</sup>P<0.001 vs vehicle group, <sup>ns</sup>-Non significant (Students’t test)

Table 4. Effect of *Portulaca oleracea* seed (MLPO) on haloperidol induced orofacial dyskinesia in wistar rat

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Treatment</th>
<th>Treatment Frequency of movement/15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vacuous chewing movement (VCM)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>0.35±0.29</td>
</tr>
<tr>
<td>2</td>
<td>Haloperidol (1.0 mg/kg i.p.)</td>
<td>2.65±0.32</td>
</tr>
<tr>
<td>3</td>
<td>L-dopa (10 mg/kg, i.p.)</td>
<td>0.96±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>MLPO 200 (mg/kg, p.o.)</td>
<td>1.97±0.62&lt;sup&gt;ms&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>MLPO 400 (mg/kg, p.o.)</td>
<td>172.41±0.35&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The values are expressed as mean±SEM (n=6); <sup>a</sup>P<0.05, <sup>b</sup>P<0.01 vs Haloperidol group (One way ANOVA followed by Dunnett’s test). <sup>ms</sup>P<0.001 vs vehicle group, <sup>ns</sup>-Non significant
3.6 Haloperidol Induced Orofacial Dyskinesia in Rat

L-dopa (10 mg/kg, i.p.) showed a significant (P<0.05) recovery of orofacial dyskinesias as evidenced by decrease in the frequency of vacuous chewing movement and tongue significant (P<0.05) decrease in the frequency of vacuous chewing & tongue protrusion while *Portulaca oleracea* seed (200 mg/kg) was found to be insignificant in this respect. Brief result is shown in Table 4.

Animal models of Parkinson’s disease are widely used to investigate its pathophysiological mechanisms and for exploring treatments. Typically, models of PD are characterized by measures of akinesia, such as in bar test for immobility, such as test for immobility [23,24,5].

Narcoleptics such as haloperidol can produce a sustained but reversible akinesia, due to blockade of dopamine D2 receptors and this neuroleptic-induced Parkinsonism is a major side effect of their use in treatment of schizophrenia. D2 antagonists may act directly to reduce the ability of cortical and basal ganglia motor pathways. Neuroleptics have thus been used as an acute model of Parkinson [25]. The central dopaminergic function and evaluation of dopamine agonistic activity was performed by observing the cataleptic behavior in mice [5]. Haloperidol blocks the dopamine D2 receptors in the brain and precipitates the extra pyramidal side effects that can be measured by “Bar test for catalepsy in mice”.

Oral movement is an important as well as important symptom presented by a series of neuropsychiatric conditions including Parkinson’s disease [26,27]. In addition, a spontaneous aging-induced oral dyskinesias has been extensively described. Thus, oral dyskinesia might be representing the behavioral manifestation, resulting from underlying mechanisms shared by different neuropsychiatric conditions. Tardive Dyskinesia is a motor side effect of long term treatment with typical neuroleptics (such as haloperidol) that involves involuntary movements of the face, mouth and tongue, but other different parts of the body may also be affected [28]. Treatment with L-dopa (10 mg/kg, i.p.) significantly attenuated the dyskinetic behavior in rats. *Portulaca oleracea* seed (MLPO) showed a significant attenuation in the frequency of tongue protrusions & Vacuous chewing movement (VCM) at higher doses tested.

Dopamine metabolites, along with a reduction in dopamine receptor activation. During this process, hydrogen peroxide is thus formed and becomes an important source of oxidative stress in catecholaminergic neuronal systems [29]. Some study states that Haloperidol neurotoxicity to the inhibition of mitochondrial electron transfer with an enhancement of O2 and H2O2 production [30]. Reactive Oxygen Species (ROS) originating from the oxidation of Dopamine (DA) further participates in the pathogenesis of PD. Oxidative stress generated as a result of mitochondrial dysfunction; particularly mitochondrial complex-1 impairment plays an important role in the PD pathogenesis [31]. Probably, there is not a single factor responsible for neurodegeneration; it appears that several factors are acting in concert. Oxidative stress and consequent cell death could occur in the substantia nigra pars compacta (SNpc) under circumstances in which there is (a) an increased dopamine turnover, resulting in excess peroxide formation; (b) a deficiency in glutathione (GSH) content, thereby diminishing the brains capacity to clear H2O2 or (c) an increase in reactive iron species, that can promote hydroxyl radical formation [32].

4. CONCLUSION

The treatment *Portulaca oleracea* seed (MLPO), exhibit significant alterations in Parkinson’s affected rodents with respect to antioxidant concentration hence signifies its antioxidant potential. This antiperoxide action observed in *Portulaca oleracea* seed (MLPO) treated animals might be due to the suppression of the production of reactive oxygen species. The MLPO may be scavenge free radicals, including hydroxyl anions and reduce the level of lipid peroxidation in MLPO animals and Inhibition of oxidative stress may be one of the possible mechanisms for the anti-Parkinson effects of *Portulaca oleracea* seed (MLPO).

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental protocols was approved by Institutional Animal Ethical Committee (IAEC) of C.U. Shah College of Pharmacy and Research, Surendranagar (Gujarat) and were conducted in strict compliance according to ethical principles.
and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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