EFFECT OF EVOLVULUS ALSINOIDES ROOT EXTRACTS ON ACUTE RESERPINE INDUCED OROFACIAL DYSKINESIA

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ABSTRACT
The roots of Evolvulus alsinoides have been traditionally used in India for various diseases and it acts as a nerve tonic. The methanolic extract of Evolvulus alsinoides roots (MEEA) was evaluated for antidyskinesia activity using acute reserpine treated dyskinesial rats. Effect of extract was evaluated at various concentrations (200,400mg/kg,P.O) for behavioral assessment of acute reserpine treated rats in a plexi-glass cage,transfer latency on elevated plus maze and locomotor activity. The chronic administration of MEEA showed significantly shortened the transfer latency in elevated plus task paradigm and significantly reverses the oral dyskinesia.

Keywords: Dyskinesia, Methanolic extract, Transfer latency, Locomotor activity

INTRODUCTION
From the earliest times herbs have been prized for their pain relieving and healing abilities and today we still rely largely on the curative properties of plants. Over centuries societies around the world developed their own tradition to make sense of medicinal plants and their uses. Brain is a much complex organs of our body and hence no surprise that only a few drugs are approved by regulatory authorities for treating multi-factorial ailments like Alzheimer's disease.

One such plant which claims various medicinal properties is Evolvulus alsinoides one of the popular and important medicinal plant in India. All parts of Evolvulus alsinoides possess valuable medicinal properties. This plant is widely used in ayurveda. Hence in the present study has been taken and evaluated for its efficacy against reserpine induced orofacial dyskinesia.

The plant reported to contain Shankapushpina, Betaine & also contains volatile oils and potassium chloride. It also contains yellow neutral fat, an organic acid & saline substances and extract shows positive result for sterols, alkaloids and shows absence of phenols, tannins, carbohydrates and proteins.

MATERIALS AND METHODS
Preparation of extract
Freshly collected roots of Evolvulus alsinoides were dried in shade and pulverized to get a coarse powder and subjected to hot solvent extraction in a soxhlet apparatus using methanol at a temperature range of 40 - 80°C. The filtrate was evaporated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator. A brownish black waxy residue was obtained. MEEA extracts was tested for presence of phytochemicals.

Experimental animals
Rats were obtained from C.L.Baid Metha College of Pharmacy animal house, Chennai. The animals were acclimatized for 10 days before being used for the experiments. They were housed in a room with controlled temperature (23±20°C) and a 12-hour light/ 12-hour dark cycle.

The animals were fed with standard pellet diet and water ad libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee of institute and conducted according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

Dyskinesia induced by acute reserpine administration in rats
Reserpine (1mg/kg, s.c) was repeatedly administered to rats on alternative days for a period of 5 days (days 1, 3 and 5) to induce oral dyskinesia.

Twenty four rats were divided into four groups of six animals each.
Group 1: The animals received 4%CMC (5ml/kg, p.o.) and served as control.
Group 2: The animals received reserpine (1mg/kg, s.c) injections & treated with MEEA (200mg/kg, p.o.) suspended in 4%CMC.
Group 3: The animals received reserpine (1mg/kg, s.c) injections & treated with MEEA (400mg/kg, p.o.) suspended in 4%CMC.
Group 4: The animals received reserpine (1mg/kg, s.c) injections & treated with MEEA (400mg/kg, p.o.) suspended in 4%CMC.

The above mentioned treatment schedule was followed for the respective group of animals. All animals were subjected to in vivo pharmacological studies during the study period.

Behavioral assessment of acute reserpine treated rats in a plexi-glass cage

The rats were placed individually in a small plexi glass observation chamber for the assessment of oral dyskinesia. The animals were allowed 10 mins to get used to the observation chamber before behavioral assessments. The number of vacuous chewing movements is referred to as single mouth openings in the vertical plane not directed toward physical material. Counting was stopped whenever rat began grooming, and restarted when grooming stopped. Mirrors were placed under the floor and behind the back wall of the chamber to permit the observation of oral dyskinesia when the animal was facing away from the observer. The behavioral parameters of oral dyskinesia were measured continuously for a period of 5 min. In all the experiments the scorer was unaware of the treatment given to the animals.

Transfer latency on elevated plus maze
Cognitive behavior was assessed by the elevated plus maze learning task, it measures the spatial long term memory. Transfer latency, the time in which animal moves from open arm to the enclosed arm was utilized as an index of learning memory process. Elevated plus maze consists of two open arms and two closed arms with an open roof. Animals placed individually at open arms and transfer latency noted.
Table 1: Effect of chronic administration of methanolic extract of Evolvulus alsinoides on acute reserpine induced vacuous chewing movements in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vacuous chewing movements/5mins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd day</td>
</tr>
<tr>
<td>I</td>
<td>8.16±0.962</td>
</tr>
<tr>
<td>II</td>
<td>27.83±2.56***</td>
</tr>
<tr>
<td>III</td>
<td>24.16±4.12 ns</td>
</tr>
<tr>
<td>IV</td>
<td>25.19±1.86 ns</td>
</tr>
</tbody>
</table>

The values are expressed as mean SEM of 6 animals. Comparisons are made between: a-group I with group II, b-group II with group III & IV

Statistical significant test for comparison was done by one way ANOVA followed by Dunnet multiple comparison tests using Graph Pad Prism software.

**p< 0.01, *p<0.05, ns-non significant

Table 2: Effect of chronic administration of methanolic extract of Evolvulus alsinoides on acute Reserpine induced memory dysfunction in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Memory dysfunction in rats in elevated plus maze task paradigm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd day</td>
</tr>
<tr>
<td>I</td>
<td>38.12±2.34</td>
</tr>
<tr>
<td>II</td>
<td>61.23±5.14***</td>
</tr>
<tr>
<td>III</td>
<td>60.17±4.23 ns</td>
</tr>
<tr>
<td>IV</td>
<td>59.67±4.14 ns</td>
</tr>
</tbody>
</table>

The values are expressed as mean SEM of 6 animals. Comparisons are made between: a-group I with group II, b-group II with group III & IV

Statistical significant test for comparison was done by one way ANOVA followed by Dunnet multiple comparison tests using Graph Pad Prism software.

**p< 0.01, *p<0.05, ns-non significant

Locomotor activity
It was monitored using actophotometer. Total activity registered for 10 mins. Activity expressed in terms of total photo beam interruption counts / 10 mins / animal.

RESULTS

Extraction
The yields of the methanol extract of Evolvulus alsinoides roots (MEEA) was 2.85% w/w and stored at 40ºC till further use. Methanolic extract of Evolvulus alsinoides on preliminary phytochemical analysis shown that the presence of alkaloids, sterols, volatile oils which are potent anti-oxidants.

Acute oral toxicity studies revealed the non toxic nature of roots. Methanolic extract of Evolvulus alsinoides there was no lethality observed at a dose of 2000 mg/kg/oral.

Behavioral assessment of acute reserpine treated rats in a plexi-glass cage
Acute reserpine treated animals shown increased frequencies of vacuous chewing movements and tongue protrusions compared with control. Treatment with methanolic extract of Evolvulus alsinoides significantly reversed reserpine induced vacuous chewing movements and tongue protrusion in a dose dependant manner (table1).

Table 3: Effect of chronic administration of methanolic extract of Evolvulus alsinoides on acute Reserpine induced changes of locomotor activity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Locomotor Activity in 10 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd day</td>
</tr>
<tr>
<td>I</td>
<td>37.73±3.07</td>
</tr>
<tr>
<td>II</td>
<td>258.50±3.09a***</td>
</tr>
<tr>
<td>III</td>
<td>286.40±3.16b**</td>
</tr>
<tr>
<td>IV</td>
<td>321.00±2.88b**</td>
</tr>
</tbody>
</table>

The values are expressed as mean SEM of 6 animals. Comparisons are made between: a-group I with group II, b-group II with group III & IV

Statistical significant test for comparison was done by one way ANOVA followed by Dunnet multiple comparison tests using Graph Pad Prism software.

**p< 0.01, *p<0.05 ns-non significant

Transfer latency on elevated plus maze
In present study acute reserpine treatment induced significant memory impairment assessed by recording the latency on the elevated plus maze task paradigm. Chronic administration of MEEA significantly decreased the transfer latency in acute reserpine treated rats (table2).

Locomotor activity
The acute reserpine treatment induced changes in locomotor activity has been reversed by chronic methanolic extract of Evolvulus alsinoides administration in rats⁶ (table3).
EFFECT OF CHRONIC ADMINISTRATION OF MEEA ON ACUTE RESERPINE INDUCED VACUOUS CHEWING MOVEMENTS

Fig. 1: Effect of chronic administration of MEEA on acute reserpine induced vacuous chewing movements

EFFECT OF CHRONIC ADMINISTRATION OF MEEA ON ACUTE RESERPINE INDUCED MEMORY DYSFUNCTION IN RATS

Fig. 2: Effect of chronic administration of MEEA on acute reserpine induced memory dysfunction in rats

EFFECT OF CHRONIC ADMINISTRATION OF MEEA ON ACUTE RESERPINE INDUCED CHANGES OF LOCOMOTOR ACTIVITY

Fig. 3: Effect of chronic administration of MEEA on acute reserpine induced changes of locomotor activity
REFERENCES


