NEUROPHARMACOLOGICAL ACTIVITIES OF METHANOLIC EXTRACT OF LEAVES OF VISCUM CAPITELLATUM SMITH.

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In present study, Viscum capitellatum Sm., hyperparasite was investigated for various neuropharmacological activities such as locomotor activity, skeletal muscle relaxation activity, antianxiety and sleep potentiation using Swiss Albino mice. The effects of methanol extract of V. capitellatum (MEVC) on anxiety, depression, rota rod performance and pentobarbital-induced sleep time were evaluated. The anxiolytic activity of MEVC at lower dose (100 mg/kg) was characterized by increased time spent and number of entries in open arms in the elevated plus maze paradigm as compared to control group. The MEVC (100, 200, and 400 mg/kg, i.p.) showed dose-dependent significant increase in motor coordination in rota rod performance test. The MEVC at the doses 500 mg/kg, i.p. was found to produce a significant prolongation of pentobarbital-induced sleeping time. The phytochemical estimation revealed the presence of significantly high amount of polyphenols. The results of the study showed that the V. capitellatum possesses neuropharmacological activity, confirming the traditional claims.

Key words: Viscum capitellatum, neuropharmacology, Hyperparasitic plants, polyphenols.

INTRODUCTION

Anxiety and depression are the most common psychiatric disorders. There are approximately 50 million people worldwide, 121 million of whom suffer from depression, and approximately 50 million people have epilepsy.¹ In developed countries, herbal medicines are used by 70% to 80% of the population to treat ailments. More than 53% of patients with depression and anxiety utilize complementary and alternative plant-related medicines.² This could be attributable to various factors, including a desire to avoid side effects as well as take advantage of the safety, effectiveness, and greater quality control of today's phytomedicines.³ In the development of pharmaceuticals, medicinal plants have proven to be an important resource. Plants are responsible for approximately 25% of today's prescribed drugs, either directly or indirectly.⁴ A considerable number of herbal medicines are recognized as active in the central nervous system (CNS). As such, some researchers are focusing on medicinal plants to elucidate compounds that are similarly effective but with fewer side effects. It has become an important area of research interest in psychopharmacology during this decade.

There are approximately 100 species of Viscum, which are often native in Africa and Madagascar, with a few in South Asia. The genus Viscum is now classified as a member of family Viscaceae.⁵ & ⁶ Viscum has about 100 species, most of them in Africa and Madagascar and a small number in southern Asia. Viscum capitellatum Smith (Viscaceae) is an herbaceous growing shrub parasitic on Dendrophthoe falcata Linn (Loranthaceae), which is itself parasitic on Mangifera indica.

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Linn (Anacardiaceae). Predominantly, V. capitellatum is distributed throughout the Western Ghats region, India. The only host so far reported is Dendrophthoe falcata Linn. (Loranthaceae). Because of their strange habitat, mistletoes remain the source of their mystical and medicinal use in different cultures. Several other mistletoes have been used and evaluated for its efficacy in CNS diseases in folklore and traditional practice. However, except for the European mistletoe Viscum album, no other mistletoe has been investigated systematically for its CNS related activities. By considering the phylogenetic relationship, we undertook the study to evaluate the neuropharmacological activities of V. capitellatum by using different animal models.

MATERIALS AND METHODS

Collection of plant material
V. capitellatum parasitic plant grown on D. fulcata was collected from the Western Ghats region (Latitude 17° 55' 0N and Longitude 73° 40' 0E and at an altitude of 1352 m). The plant specimen of V. capitellatum (Voucher no. RBJ1311) was authenticated by Dr. C.B. Salunkhe (Department of Botany, Krishna Mahavidyalaya, Rethare, India).

Extraction
The plant material was dried in the dark at room temperature, powered and extracted with methanol (100%) by the cold maceration technique. The liquid extract was dried by removal of the solvent under vacuum. The percent extract was found to be 9.86% w/w. It stored in closed container in desiccator and used in the pharmacological experiments.

Quantitative chemical investigations

Total phenolic content
The total phenolic content (TPC) of methanolic extract of V. capitellatum (MEVC) was determined by the Folin-Ciocalteau phenol reagent colourimetric assay. In brief, 0.1 mL of extract (100 µg/mL) was mixed separately with 0.2 mL of Folin-Ciocalteau reagent (1:5 with distilled water). After this, 2 mL of distilled water and 2 mL of 15% Na₂CO₃ were added to the mixture. After 10 minutes of an incubation period at 50°C in a water bath, absorbance was measured at 660 nm using a UV-visible spectrophotometer (Shimadzu, UV-2450). The total phenolic contents in the extracts were calculated using the gallic acid standard calibration curve. The total phenolic content is expressed as mg/gm of gallic acid equivalents (GAE).

Total flavonoid content
In brief, each 0.5 ml of MEVC (100 µg/mL) were thoroughly mixed with 1.5 mL of ethanol, 0.1 mL of 10% Al (NO₃)₃, 0.1 mL of 0.1M CH₃COONa and 2.8 mL of water. The resultant mixture was incubated for 40 min at room temperature. The absorbance of the reaction mixture was measured at 415 nm using UV-spectrophotometer (Shimadzu, UV-2450). The total flavonoid content (TFC) of the extract was calculated by using the standard quercetin calibration curve. Results were expressed as mg/gm quercetin equivalents (QE).

Total proanthocyanidin content
The total proanthocyanidin content of MEVC was determined using a reported method. In brief, 1ml of extract (100 µg/ml) was mixed with 2 mL of freshly prepared vanillin solution (1% vanillin in 70% H₂SO₄) and maintained for 15 min at 20°C. The absorbance was measured at 500 nm. A calibration curve was drawn using aqueous solution of epicatechin as a reference standard. Total proanthocyanidins were expressed as mg/gm of epicatechin equivalent.

Animals
In both acute toxicity studies and neuropharmacological assays, Swiss albino mice weighing 30-36 gm were used. The animals were housed in community cages and maintained under regular laboratory conditions (23 ± 1°C temperature, 55.5% relative humidity, 12-h light-dark cycle, free access to water and standard rodent chow: Lipton India Ltd). All animals were acclimatized for 3 weeks prior to initiation of the test. The experiments were conducted in a special adjacent noise-free room with controlled illumination.
Acute toxicity study

For the determination of acute toxicity by the oral route, Swiss albino mice were divided into two groups (n=6) and sequentially dosed to determine the oral toxicity of MEVC as per Organization for Economic Co-operation and Development (OECD) guideline 425.14 The test group received MEVC (5-2000 mg/kg body weight) suspended in 0.5% carboxymethyl cellulose (CMC) orally, while the control group received only 0.5% CMC. After the test extracts administration, food or water was withheld for the next 1-2 hrs. Animals were closely observed after dosing of test extracts for the initial 4 hrs after dosing, followed by neurobehavioral, changes for 14 days.

Open Field Test

The open field area was comprised of transparent acrylic walls and a black floor (30 x 30 x 15 cm) divided into nine squares of equal size. One hour before the test, the mice were treated with MEVC (100, 200 and 400 mg/kg, i.p), diazepam (4 mg/kg, i.p., as a positive control) and normal saline (vehicle, i.p., control group). The open field test was used to evaluate the locomotor activity of mice. The observed parameters included the number of squares crossed. The spontaneous motor activity was recorded 30 min after extract/drug administration.15 The results of the treated groups were compared with those of the saline control.

Motor Co-ordination test

The rota rod used in this test was a rubber-coated metallic rod (3 cm in diameter) connected to a motor. The rod was rotated at a constant speed, i.e. 9 rpm, and was placed about 60 cm above the table in order to prevent the mice from jumping off the roller. Mice were exposed to the rota rod as a pretest before the experiment and only those mice included in the study were those that remained on the rod for 5 min at a speed of 9 rpm. All the groups (n=10) were treated with diazepam (4 mg/kg), saline water (10 mL/kg) and MEVC (100,200 and 400 mg/kg, i.p.). Each mouse was exposed for 5 min at 0 and 30 min after the drug administration on the revolving rod and the time spent on the rod was recorded.16

Elevated Plus Maze test

The EPM test is the most frequently employed model for the assessment of the anxiolytic activity of novel substances.17 The maze consisted of a central platform (10 x 10 cm) with two open (12 x 5 cm) and two closed arms (12 x 5 cm) and 12 cm high walls. The maze was elevated 38.5 cm from the room’s floor. The experimental animals were divided into five (n=10). The mice were treated 30 min prior to the test with diazepam (4 mg/kg, i.p., positive control), while normal saline (vehicle, i.p., control group) and MEVC (100,200 and 400 mg/kg, i.p., test group). Each animal was placed at the center of the maze facing one of the open arms. The number of entries and the time spent in the enclosed and open arms were recorded during the 5 min test. After each test, the maze was carefully cleaned with wet tissue paper (10% ethanol solution).

Pentobarbitone induced sleep potentiation

The pentobarbitone-induced sleep potentiation test was performed as per the reported method.18 The mice were divided into five groups (n=10). The MEVC (100, 200 and 400 mg/kg, i.p., test group) and normal saline 1% (vehicle, p.o., control group) were administrated 1 h prior to the test, and diazepam (45 mg/kg, i.p., positive control) was administered 30 min before the test. In this experiment, after the administration of pentobarbitone (30 mg/kg, i.p.,), the mice were placed separately in transparent Plexiglas cages (25 x 15 x 10 cm) to observe the hypnotic effect, which was considered to be the time interval between the disappearance (latency) and reappearace (duration) of the righting reflex.

Statistical analysis

All the data were expressed as mean ± SEM from five animals. The data obtained was analyzed using the one-way ANOVA followed by Dunnett's test for determining the level of significance and p< 0.05 was considered statistically significant.
RESULTS AND DISCUSSION

Quantitative phytochemical analysis

Table 1 displays the total phenolic, flavonoid and proanthocyanidine contents of MEVC. Amongst the all determinations, MEVC found content highest total phenolic content (47.84 ± 4.12 mg/g GAE), followed total flavonoid AEB-ChF (29.25 ± 2.20 mg/g QE) and total proanthocyanidine (15.59 ± 1.81 mg/g EE).

Table 1: Quantitative phytochemical estimations

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>MEVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic content (mg/g of GAE)</td>
<td>47.84 ± 4.12</td>
</tr>
<tr>
<td>Total flavonoid (mg/g of QE)</td>
<td>29.25 ± 2.20</td>
</tr>
<tr>
<td>Total proanthocynidine (mg/g of EE)</td>
<td>15.59 ± 1.81</td>
</tr>
</tbody>
</table>

Values (n=3) are mean ± SD

Open field exploratory test

Mice treated with MEVC (100, 200 and 400 mg/kg), did not showed dose dependent change in the total crossing. 100 mg/kg showed the least changes in the parameters of total crossing compared with the control group (p>0.05). The MEVC at a higher dose has shown significant percent inhibition comparing with the control group (p<0.01). In the case of animals treated with a dose of 400 mg/kg of MEVC exhibited a decrease in the number of total crossings (p<0.01) (Table 2).

Motor Co-ordination test

The data shows that, the animals treated MEVC (100, 200 and 400 mg/kg i.p.) were able to maintain equilibrium on the rotating rod and stayed on longer without falling. The rota rod activity of all groups is shown in table 3. The time spent on the revolving rod was significantly reduced by diazepam, in comparison with the control group.

Table 2: Effect of MEVC on locomotor activity by using Actophotometer.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group &amp; Dose (i.p.)</th>
<th>Crossings</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>‘0’min</td>
<td>‘30’min</td>
</tr>
<tr>
<td>1.</td>
<td>Control (10 ml)</td>
<td>454.3 ± 31.23</td>
<td>349.2 ± 36.35</td>
</tr>
<tr>
<td>2.</td>
<td>Diazepam (4 mg/kg)</td>
<td>361.4 ± 13.81</td>
<td>35.90 ± 7.521</td>
</tr>
<tr>
<td>3.</td>
<td>MEVC (100 mg/kg)</td>
<td>420.2 ± 27.60</td>
<td>257.4 ± 41.60</td>
</tr>
<tr>
<td>4.</td>
<td>MEVC (200 mg/kg)</td>
<td>303.8 ± 38.54**</td>
<td>161.9 ± 13.41**</td>
</tr>
<tr>
<td>5.</td>
<td>MEVC (400 mg/kg)</td>
<td>293.3 ± 35.22**</td>
<td>89.00 ± 13.78**</td>
</tr>
</tbody>
</table>

All results are expressed in terms of Mean ± S.E.M., n=6, Data processed by one-way ANOVA followed by Dunnett’s test, *p<0.05, **p<0.01 compared to vehicle treated group. MEVC- Methanolic extract of V. capitellatum.

Table 3: Effect of methanolic extract of V. capitellatum (VCM) on muscle relaxant activity in rotarod performance.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group &amp; Dose (i.p.)</th>
<th>Fall off time (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before drug</td>
</tr>
<tr>
<td>1.</td>
<td>Control (10 ml)</td>
<td>300</td>
</tr>
<tr>
<td>2.</td>
<td>Diazepam (4 mg/kg)</td>
<td>300</td>
</tr>
<tr>
<td>3.</td>
<td>MEVC (100 mg/kg)</td>
<td>300</td>
</tr>
<tr>
<td>4.</td>
<td>MEVC (200 mg/kg)</td>
<td>300</td>
</tr>
<tr>
<td>5.</td>
<td>MEVC (400 mg/kg)</td>
<td>300</td>
</tr>
</tbody>
</table>

All results are expressed in terms of Mean ± S.E.M., n=6, Data processed by one-way ANOVA followed by Dunnett’s test, *p<0.05, **p<0.01 compared to vehicle treated group. MEVC- Methanolic extract of V. capitellatum.
**Elevated plus maze test**

The anxiolytic diazepam induced a significantly increased number of entries to open arms and the of time spent in the arms in the MEVC, when comparing to control group \( (p < 0.05) \) at lower dose (100 mg/kg, i.p.). Surprisingly the mice treated with MEVC in the dose of 200, 400 mg/kg, showed reduced entries and time in open arm with respect to the control group (Table 4).

**Pentobarbitone induced sleep potentiation**

In the pentobarbitone induced sleep potentiation, the extract at doses, 100, 200 and 400 mg/kg showed a significant reduction in the time of onset of sleep in a dose-dependent manner (Table 4). The effect of the extract (400 mg/kg) on the onset of sleep was comparable to that of the standard. All doses of the extract potentiated the duration of pentobarbitone induced sleeping time in test animals compared to controls (Table 5).

**Table 4: Effect of MEVC on mice in antianxiety activity by using Elevated Plus Maze Apparatus.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Group &amp; Dose (i.p.)</th>
<th>Open arm</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of entries</td>
<td>Avg. time spent (s)</td>
</tr>
<tr>
<td>1.</td>
<td>Control (10 ml)</td>
<td>6.7 ± 1.106</td>
<td>12.0 ± 2.338</td>
</tr>
<tr>
<td>2.</td>
<td>Diazepam (4 mg/kg)</td>
<td>7.7 ± 1.38*</td>
<td>14.8 ± 2.215</td>
</tr>
<tr>
<td>3.</td>
<td>MEVC (100 mg/kg)</td>
<td>7.4 ± 0.65*</td>
<td>10.2 ± 1.482</td>
</tr>
<tr>
<td>4.</td>
<td>MEVC (200 mg/kg)</td>
<td>4.9 ± 0.95*</td>
<td>7.1 ± 1.215</td>
</tr>
<tr>
<td>5.</td>
<td>MEVC (400 mg/kg)</td>
<td>4.1 ± 0.74*</td>
<td>5.4 ± 0.686</td>
</tr>
</tbody>
</table>

All results are expressed in terms of Mean ± S.E.M., n=6, Data processed by one-way ANOVA followed by Dunnett’s test, \( *p < 0.05, **p < 0.01 \) compared to vehicle treated group. MEVC-Methanolic extract of *V. capitellatum*

**Table 5: Effect of MEVC on pentobarbitone induced sleep time potentiation.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Group &amp; Dose (i.p.)</th>
<th>Onset of sleep (min.)</th>
<th>Total sleep time (min.)</th>
<th>% Potentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control (10 ml)</td>
<td>49.30 ± 2.89</td>
<td>81.40 ± 4.08</td>
<td>----</td>
</tr>
<tr>
<td>2.</td>
<td>MEVC (100 mg/kg)</td>
<td>23.90 ± 1.17&quot;</td>
<td>94.40 ± 4.67</td>
<td>51.52</td>
</tr>
<tr>
<td>3.</td>
<td>MEVC (200 mg/kg)</td>
<td>22.10 ± 1.16&quot;</td>
<td>108.9 ± 3.96&quot;</td>
<td>55.17</td>
</tr>
<tr>
<td>4.</td>
<td>MEVC (400 mg/kg)</td>
<td>15.10 ± 0.96&quot;</td>
<td>133.6 ± 2.78&quot;</td>
<td>69.37</td>
</tr>
</tbody>
</table>

All results are expressed in terms of Mean ± S.E.M., n=6, Data processed by one-way ANOVA followed by Dunnett’s test, \( *p < 0.05, **p < 0.01 \) compared to vehicle treated group. MEVC-Methanolic extract of *V. capitellatum*

**Fig. 1:** Histopathological section of test extract mice.

No signs of inflammatory cell infiltration, macro and micro-vesicular steatosis necrosis with loss of radial arrangement of hepatocyte plaques, a pale eosinophilic stain, and pyknotic or absent nuclei
Discussion

Hyperparasitic plants (Mistletoes) are used in both traditional and complementary medicine to treat and control a variety of disorders. Except European mistletoe (Viscum album), no other mistletoe has been investigated systematically for their CNS related activities. No scientific literature is available on CNS related activities for V. capitellatum other species of Viscum. Therefore, we decided to investigate the CNS effects of V. capitellatum using in-vivo models. The present study investigated for the first time the CNS effects of a MEVC in mice. Oxidative stress is an important underlying factor in a number of neurodegenerative and psychiatric disorders. Quantitative estimations revealed that MEVC contains a significant amount of antioxidants like polyphenols like polyphenols, flavonoids and proanthocynidin. The acute oral toxicity of MEVC by cumulative dose study from 5 mg to 2000 mg/kg showed the extract was found safe up to 2000 mg/kg body weight but toxic at 5000 mg/kg. It was validated using computer software—AOT425 StatPgm. Thus, the dose for pharmacological study was selected by the up and down method as 100, 200, and 400 mg/kg bodyweight. Furthermore, liver histopathology of the test group (2000 mg/kg) does not show signs of inflammatory cell infiltration, macro and micro-vesicular steatosis necrosis with loss of radial arrangement of hepatocyte plaques, a pale eosinophilic stain, and pyknotic or absent nuclei and showed normal articulation (Figure 1).

A reduction in locomotor activity is considered an indicator of alertness, and a decrease in it suggests a sedative effect. The MEVC exhibited a decrease in locomotor score and a significant increase in the hypnotic effect. Skeletal muscle coordination and relaxant effect were monitored by the Rota rod apparatus. Diazepam significantly decreased motor activity in the rota rod performance test due to its sedative and strong neuromuscular blocking action. While, MEVC showed dose dependently increased motor coordination.

The EPM is one of the most commonly used tests, and it is very sensitive to the effects of both anxiolytic and anxiogenic drugs that act on the GABAA-benzodiazepine complex. Normal mice would typically choose to spend the majority of their allotted time in the closed arms in EPM. This tendency appears to reflect an aversion to open arms caused by anxieties about open spaces. Anxiolytics are drugs that encourage open arm exploration, such as diazepam, while anxiogenics are the opposite.

In this study, we found that a low dose of MEVC has an anxiolytic-like effect in mice, as it enhanced open arm entries and time spent in the open arms of the EPM as compared to control animals. While, MEVC at higher doses has been found to possess anxiogenic effect. Many neurotransmitters play a role in regulating sleep behavior. Neurons located in the anterior hypothalamus release GABA to inhibit wake-promoting areas in the hypothalamus and brainstem. The barbiturate pentobarbital binds GABA receptors. Benzodiazepines such as diazepam enhance the affinity of GABA for its receptor and thereby increase pentobarbital-induced sleeping time. Studies have revealed the hypnotic effects of a wide variety of herbal medicine components. MEVC demonstrated a dose dependent increase in pentobarbital induced sleep (p< 0.01). It has been reported that flavonoids are able to cross the blood-brain barrier and induce some effects on the central nervous system, including neuroprotective and antioxidant actions. As MEVC contains significant amount of flavonoids, dose dependent sleep potentiation may be exerted on animals.

Quantitative phytochemical estimation revealed the presence of a significant amount of flavonoids. Flavonoids are reported to possess anxiolytic effects and antidepressant effects. Therefore, it is likely that polyphenols present in this extract might be contributing to observed neuropharmacological activity.

Conclusions

V. capitellatum was found to have a substantial influence on locomotor action and sleep potentiation in the current investigation. The extract had minimal effect on spontaneous motor coordination and antianxiety activity, indicating that the extract has a selective effect in treating CNS diseases. Furthermore, it is necessary to isolate, characterise, and screen the active principles responsible for the neuropharmacological activity of V.
capitellatum’s methanolic extract, as well as to determine the specific mechanism by which the plant exerts the above effects.

List Of Abbreviations

ATO- Acute Oral Toxicity; CNS- Central Nervous System; EPM- Elevated Plus maze; GABA - Gamma-Amino Butyric Acid, i.p.-intra-peritoneal MEVC - methanolic extract of Viscum capitellatum; MeOH- Methanol; min-minute.

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الأنشطة الدوائية العصبية للمستخلص الميثانولي لأوراق فيسكم كابيتالم سميث
جاداف فارشا س - جادات فيلاس ب - جادات رامشاندرا ب - سورانان سانجاي ج - كالاشار موكان ج

قسم العقاقير ، كلية الصيدلة ، سوناي ، امدا ناغر ، الهند
قسم العقاقير ، كلية الصيدلة ، مالجاين ، بون ، الهند
معهد باتل للتعليم والبحوث الصيدلانية ، شيربور ، دهول ، الهند

في الدراسة الحالية ، تم فحص فيسكم كابيتالم سم. فرت الطفل لمختلف الأنشطة الدوائية العصبية مثل النشاط الحركي ، نشاط استرخاء العضلات ، مضادات الفلق و القدرة على النوم باستخدام الفنر السويسرية البيضاء. تم تقييم أثار مستخلص الميثانول من فيسكم كابيتالم على الفلق والاكتئاب وأداء قضيب الروتا ووقت النوم الناجم عن البنثوبارتال. تميز نشاط مستخلص الميثانول من فيسكم كابيتالم كمزيل الفلق بجرعة أقل (100 مجم / كجم) زيادة الوقت الذي يقضيه عدد مرات الدخول في نموذج المتاحة المرتفع بالمقارنة بمجموعة المراقبة. أظهر مستخلص الميثانول من فيسكم كابيتالم (2000 و 400 مجم / كجم ، داخل الصفاق) زيادة كبيرة تعتمد على الجرعة في التنسيق الحركي في اختبار أداء قضيب الروتا. مستخلص الميثانول من فيسكم كابيتالم بجرعات 500 مجم / كجم ، داخل الصفاق أدى إلى الإطالة كبيرة في وقت النوم الناجم عن البنثوبارتال. أظهر التقدير الفيتوكيميائي وجود كمية كبيرة من مادة البوليفينول. أظهرت نتائج الدراسة أن فيسكم كابيتالم تمكّن

نشاطًا دوائيًا عصبيًا ، مما يؤكّد الاستخدام الشعبي.