Evaluation of neuropharmacological activity of petroleum ether, methanolic and aqueous extracts of flower heads of Sphaeranthus indicus in mice

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ABSTRACT

The neuropharmacological activity of different doses (100, 200, 400 mg/kg/p.o.) of petroleum ether (SIP), methanolic (SIM) and aqueous extract (SIA) obtained from Sphaeranthus indicus Linn were studied in mice. Sphaeranthus indicus was evaluated for its effect on motor coordination, locomotor activity, cognitive behavior, anxiety, haloperidol induced catalepsy, sodium nitrite induced respiratory arrest, hypoxic stress induced neurotoxicity and convulsions induced by pentylenetetrazol (PTZ) and maximum electroshock (MES). The SIP and SIM 200 and 400 mg/kg showed significant decrease in locomotor activity but no effect on motor-coordination. SIP, SIM and SIA 100 mg/kg showed significant anxiolytic activity. SIM 100, 200 and 400 mg/kg/p.o. showed significant anticonvulsant activity. SIM 400 mg/kg/p.o. found to prolong haloperidol induced catalepsy in mice. SIM 100 and 200 mg/kg/p.o. significantly increases discrimination index in object recognition test. Moreover the SIP and SIM extracts also showed significant analgesic activity in hot plate method. The results suggest that the extract may possess sedative principles with anxiolytic activity, anticonvulsant activity and antistress activity.

INTRODUCTION

Plants have been used by human beings since immemorial times to cure diseases and to promote relief from ailments. There were times when they were the most important sources of medicines for people (Carlini, 2003). On the other hand, such ancient use of plants was a lead for scientists in their search for new substances endowed with therapeutic properties. It is estimated that nearly 25% of the modern drugs directly or indirectly originated from plants (De Smet, 1997). Indian natural products, particularly those from traditional plants which are reported in the classical text like ayurveda and Charak samhita have contributed towards this boom in drug discovery (Bhutani and Gohil, 2010). The majority of herbal remedies indicated for the treatment of psychiatric ailments are crude or semipurified extracts, such as H perforatum, G biloba, P ginseng, Melissa officinalis L., V officinalis, Crataegus oxyacantha L., P incarnata, P methysticum, T Bispinosa, B hispida etc (Carlini, 2003, Ambikar et al., 2010, Ambikar and Mohanta, 2013). The traditional crude form of the remedy has emerged as a standardized herbal extract, its formulations and even composite preparations. Moreover, the particular components responsible for the activity have also been isolated and some of which have been synthesized (Vywahare et al., 2008).

Sphaeranthus indicus (SI) Linn belongs to family Asteraceae. As per Ayurveda, all parts of the plant are medicinally important (Gogate, 2000). In folk medicine, the plant is reportedly used in treating epileptic convulsions, mental illnesses and hemicranias (Kirtikar and Basu, 1987). The alcoholic extract of SI flowers has been reported to possess haemostatic and cathartic action (Srivastava et al., 1971). Moreover alcoholic extract of flowers has been found to have antibacterial activity (Shaikh et al., 1996). Alcoholic as well as aqueous extract of plant were found to be highly available online at http://www.japsonline.com
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effective against *Alternaria solani*, *Fusarium oxysporum* and *Penicillium pinophilum* by preventing their growth to greater extent (Dubey et al., 2000). Methanolic extract and its petroleum ether, chloroform and remaining methanol fraction of flower heads of SI were found to be effective for Immunomodulatory activity (Bafana and Mishra, 2004). The essential oil obtained from the leaves of SI exhibited antifungal activity (Garg and Kasera, 1982). The present study aims at investigating the neuropharmacological activity of flower head extracts of *Sphaerantus indicus* Linn in mice.

**MATERIALS AND METHODS**

**Plant material**

The plant material (flowers of SI) collected from pune region of Maharashtra, India was authenticated by botanical survey of India.

**Preparation of Extract**

Petroleum ether extract (SIP), methanolic extract (SIM) and aqueous extracts (SIA) were prepared by successive extraction method. The flowers of SI were dried in shade and coarsely powdered. The powder was successively extracted with petroleum ether followed by methanol in a Soxhlet apparatus. Powder remaining after methanolic extraction was subjected to aqueous extraction (Tyler et al., 1996).

The aqueous extract was prepared by maceration with distilled water for 24 h. The extracts were concentrated under reduced pressure and were stored at 8–10°C throughout the study. The yield of SIP, SIM and SIA were 2.25% w/w, 3.8% w/w and 4.3% w/w respectively.

**Animals**

Swiss male albino mice (18-22g) were used. These mice were maintained at 25° C ± 2° C and 45-55% relative humidity and under standard environmental conditions (12:12 h L:D cycle). These mice had free access to food and water. They were deprived of food but not water 6 h before the drug administration. Institutional Animal Ethics Committee (IAEC) approved the protocol and entire study has carried out as per standard guideline of IAEC. All experiments were carried out between 12:00-16:00 hours.

**Chemicals and drugs**

Pentylentetrazole and sodium nitrate were purchased from Loba Chemical, Mumbai, India. Piracetam syrup, Diazepam, Pentazocin, Clonazepam and Haloperidol injection were purchased from the local market.

**Acute toxicity test**

Healthy adult male albino mice (18-22g) were subjected to acute toxicity studies as per guidelines (AOT 425) suggested by the organization for economic co-operation and development (OECD). The mice were observed continuously for 2 hours for behavioral and autonomic profiles and for any sign of toxicity or mortality up to a period of seven days.

**Effect on motor coordination**

The motor coordination was assessed using digital rota rod (INCO, Ambala, India). Mice were trained by placing them on a rotating rod (20 rev/ min), twice daily for three consecutive days before the experiment. Thirty min interval was kept between two trails. Only those mice which have demonstrated their ability to remain on the rotating rod for at least 2 min were selected. These selected mice were divided into eleven groups with 6 animals in each group. The mice were then tested for motor coordination to record basal fall of time followed by SIP, SIM and SIA (100, 200, 400 mg/kg/p.o.). One hour following the administration of vehicle or drug, mice were placed again on the rotating rod and the fall off time per 300 sec was recorded. The difference between mean fall of time before and after drug treatment was considered for evaluation. Diazepam (2 mg/ kg/i.p.) was used as a reference standard (Gupta et al., 1999; Jain et al., 2003).

**Locomotor Activity**

The locomotor activity was measured using a digital actophotometer (INCO, Ambala, India). Each mouse was placed individually in the actophotometer for 05 min and basal activity score was obtained. Subsequently animals were divided into eleven groups and treated with SIP, SIM and SIA (100, 200, 400 mg/kg/p.o.). 60 min after dosing; the mice were placed again in the actophotometer for recording the activity score as described earlier. The results were reported as mean change in the locomotor activity. Diazepam (2 mg/ kg/i.p.) was used as reference standard (Turner, 1972; Vyawahare and Ambikar, 2010).

**Object recognition test**

The activity cage (INCO, Ambala, India), illuminated by a 40 W lamp suspended 50 cm above the apparatus was used for study. The object to be discriminated was also made of plywood in different shapes of 10 cm height and colored black. One day before the test, mice were allowed to explore the box without any object for 02 min. On the day of test, in the first trial (T1) conducted 60 min after administration of vehicle (10 ml/kg), SIP, SIM and SIA (100, 200, 400 mg/kg/p.o.) and piracetam (150 mg/kg/p.o.). Two identical objects were presented in opposite corners of the box and the time taken by each mouse to complete 20 s of object exploration was recorded (Exploration was considered as directing the nose at a distance less than 2 cm to the object and/or touching with nose). Second trial (T2) was performed 90 min after first (T1) and a new object replaced one of the objects presented in T1 and mice were left in the box for next 05 min. The time spent for exploring the familiar (F) and the new object (N) was recorded separately and discrimination index (D) was calculated as (N-F)/(N+F). The object was changed randomly and apparatus was cleaned with hydrogen peroxide after each trial to avoid place preference and the influence of olfactory stimuli respectively (Jain et al., 2003).
Anxiolytic activity using elevated plus maze (EPM)

The elevated plus maze apparatus consisted of two open arms (30 x 5 cm) and two closed arms (30 x 5 x 20 cm) emanating from a common central platform (5 x 5 cm). Two pairs of identical arms were opposite to each other. Entire apparatus was elevated to a height of 50 cm above the floor level. Swiss albino male mice (25 ± 2 g) were used. Mice received vehicle (10 ml/kg/p.o.), SIP, SIM and SIA (100, 200, 400 mg/kg/p.o.) and reference standard drug (Diazepam 2 mg/kg/i.p) as per treatment schedule 60 min before start of session.

To start session mouse was placed at the center of maze, its head facing closed arm and allowed to explore maze for 5 min. During this 5 min time spent in open arm, percent entries in open and closed arm and total entries were recorded. An entry was defined as all four paws in the arm. The plus maze was carefully wiped with hydrogen peroxide and dried with sponge after each trial (Lister, 1987).

Analgesic activity

The analgesic effect was studied using digital hot plate (INCO, Ambala, India) method wherein the reaction time (paw licking, jumping or any other sign of discomfort) was recorded at 0, 60, and 120 min after administration of vehicle (10 ml/kg/p.o.), and SIP, SIM and SIA (100, 200, 400 mg/kg/p.o.) Swiss albino male mice (25 ± 2 g) were used. The temperature of the plate was maintained at 55°C ± 01° C. A cut off reaction time of 20 s was chosen in order to avoid injury. Pentazocin (30 mg/kg/i.p) was used as a reference standard (Gupta et al., 1999; Vyawahare and Ambikar, 2010).

Haloperidol induced catalepsy

Mice were divided into eleven groups. The control group received vehicle (10 ml/kg/p.o) whereas the other group received SIP, SIM and SIA (100, 200 and 400 mg/kg/p.o.) 60 min before haloperidol (1 mg/kg/i.p). After the treatment, the forepaws of the mice were placed on rod of 1.0 cm diameter set at 3.0 cm from top. Duration for which the mice retains the forepaws on the elevated rod was noted down at 0, 15, 30, 60, 90 and120 min. The cut off time was 300 sec.

The animals were tested twice at each time interval and only the greater duration of time was recorded. Between measurements, the mice were returned to their home cages (Feere et al., 1990; Khisti et al., 1998).

Pentylenetetrazole (PTZ) induced seizure

Clonic seizures were induced 60 min after respective drug treatment in mice by administering Pentylenetetrazole (80mg/kg/s.c). The latency to the onset of seizures in non-protected mice and lethality during the following 24 h was recorded and compared with those of control mice to assess the anticonvulsant activity of the extract. Clonazepam (0.1mg/kg/i.p.) was used as a reference standard (Bienvenu et al., 2002; Swinyard and Woodhead, 1982).

Maximal electroshock (MES) induced seizures

Tonic clonic convulsions were induced in mice by giving maximal electroshock seizures (MES) (40mA for 0.2sec) using an electoconvulsiometer (INCO, Ambala, India) via crocodile ear clip. Mice were pretreated with either vehicle (10 ml/kg/i.p.), SIP, SIM and SIA (100, 200 and 400 mg/kg/p.o.) or Phenytoin (20 mg/kg/i.p) 60 min before subjecting to electroshock. The number of animals protected from tonic hind limb extension seizure (abolation of tonic hind limb extension within 10 sec after delivery of the electroshock was considered as protected mice) and duration of tonic hind limb extension seizure was determined in each dose group (Swinyard and Woodhead, 1982; Bum et al., 2004).

Hypoxic stress induced neurotoxicity in mice

Mice were subjected to hypoxia by putting them individually in a tightly closed 300 ml glass container which was placed in aquarium of 25°C temperature. The animals had convulsion and died from hypoxia. The latency for death was recorded. Mice received single dose of SIP, SIM and SIA (100, 200 and 400 mg/kg/p.o) before subjecting them for hypoxic stress (Vyawahare and Ambikar, 2010).

Sodium nitrite induced respiratory arrest

Mice were divided into four groups and were treated with vehicle (10 ml/kg/p.o) or SIP, SIM and SIA (100, 200 and 400 mg/kg/p.o). Sixty min later, all mice were subjected to sodium nitrite treatment (250 mg/kg/i.p). The time between injection of sodium nitrite and death was recorded (Vyawahare and Bodhankar, 2007).

STATISTICAL ANALYSIS

The results are expressed as mean ± SEM. Comparison between the groups were made by one way analysis of variance (ANOVA) followed by Dunnett’s test.

RESULTS

Acute oral toxicity test

All extract found to be safe at dose used and all mice were free of any toxicity up to the dose of 2 gm/kg. The behavioral and autonomic profiles were found to be normal.

Effect on motor coordination

All doses of SIP, SIM and SIA (100, 200 and 400 mg/kg/p.o.) were found to be statistically insignificant in reducing the time of fall. The reference standard diazepam (2 mg/kg/i.p.) showed highest reduction in time of fall (P<0.01).

Locomotor Activity

SIP and SIM (400 mg/kg/p.o.) produced significant (P<0.01) reduction in mean change in locomotor activity as compared to vehicle treated mice. Moreover SIP and SIM (100 mg/kg/p.o) also showed significant (P<0.05) reduction in mean
change in locomotor activity. The reference standard Diazepam (2 mg/kg/i.p.) showed significant (P<0.01) action in this regard (Figure 1).

**Object recognition test**

SIM and SIA (400 mg/kg/p.o.) treated mice showed significant (P<0.01) increase in discrimination index when compared against vehicle treated mice. Moreover SIM and SIP (100 mg/kg/p.o.) also showed significant (P<0.05) action in this regard. The reference standard Piracetam (150 mg/kg/p.o.) demonstrated significant (P<0.01) activity (Figure 2).

**Anxiolytic activity using elevated plus maze (EPM)**

SIP (100 mg/kg/p.o.), SIM (100 and 400 mg/kg/p.o.) and SIA (100 mg/kg/p.o.) significantly (P<0.01) increased the % of open arm entries and time spent in open arm, compared with the vehicle treated group. The reference standard Diazepam showed significant (P<0.01) increase in the % of open arm entries and time spent in open arm (Table 1).

**Analgesic activity**

SIP and SIM (400 mg/kg/p.o.) showed significant (P<0.05) increase in reaction time in hot plate analgesic activity. The reference standard Pentazocin (30 mg/kg/i.p.) showed significant (P<0.01) analgesic activity by prolonging the reaction time in hot plate method (Table 2).

**Haloperidol induced catalepsy**

In haloperidol-induced catalepsy, maximum catalepsy was noted at 90 min and 120 min. SIM (400 mg/kg/p.o.) treatment showed marked potentiation of catalepsy from 90 min to 120 min. The SIP and SIA did not show any significant (P<0.01) potentiation at all doses.

**Pentylenetetrazole induced seizure (PTZ)**

SIM 200 and 400 mg/kg/p.o. significantly (P<0.05) delayed onset of convulsion as compare to vehicle treated mice. SIP and SIA were found ineffective in this regard. The reference standard Clonazepam (0.1mg/kg/i.p.) showed significant (P<0.01) anxiolytic activity (Table 3).

**Maximal electroshock induced seizures (MES)**

SIM (200 and 400 mg/kg/p.o.) and SIA (400 mg/kg/p.o.) demonstrated significant (P<0.01 and P<0.05 respectively) anticonvulsant activity by protecting mice from maximal convulsion.
electroshock induced seizures. The reference standard Phenytoin showed significant (P<0.01) anticonvulsant activity (Table 4).

Table 4: Anticonvulsant activity of Sphaeranthus indicus against MES induced convulsion.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animal convulsed/No. of mice used</th>
<th>% mice protected</th>
<th>Duration of tonic hind limb extension (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (10 ml/kg/p.o.)</td>
<td>6/6</td>
<td>0</td>
<td>24.39 ± 1.06</td>
</tr>
<tr>
<td>SIP (100 mg/kg/p.o.)</td>
<td>6/6</td>
<td>0</td>
<td>24.6 ± 0.79</td>
</tr>
<tr>
<td>SIP (200 mg/kg/p.o.)</td>
<td>6/6</td>
<td>0</td>
<td>23.06 ± 1.36</td>
</tr>
<tr>
<td>SIP (400 mg/kg/p.o.)</td>
<td>6/6</td>
<td>0</td>
<td>21.7 ± 2.18</td>
</tr>
<tr>
<td>SIM (100 mg/kg/p.o.)</td>
<td>6/6</td>
<td>0</td>
<td>21.5 ± 0.62</td>
</tr>
<tr>
<td>SIM (200 mg/kg/p.o.)</td>
<td>6/6</td>
<td>0</td>
<td>18.19 ± 0.89***</td>
</tr>
<tr>
<td>SIM (400 mg/kg/p.o.)</td>
<td>6/6</td>
<td>33.33</td>
<td>13.98 ± 0.99**</td>
</tr>
<tr>
<td>SIA (100 mg/kg/p.o.)</td>
<td>6/6</td>
<td>0</td>
<td>20.6 ± 0.82</td>
</tr>
<tr>
<td>SIA (200 mg/kg/p.o.)</td>
<td>6/6</td>
<td>0</td>
<td>21.03 ± 1.69</td>
</tr>
<tr>
<td>SIA (400 mg/kg/p.o.)</td>
<td>6/6</td>
<td>0</td>
<td>19.33 ± 0.57*</td>
</tr>
<tr>
<td>Phenytoin (20 mg/kg/i.p.)</td>
<td>0/6</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. (n = 6). Data was analyzed by one way analysis of variance (ANOVA) followed by Dunnetts test. *P<0.05, **P<0.01.

Hypoxic stress induced neurotoxicity in mice
SIM 100, 200 and 400 mg/kg/p.o. significantly (P<0.05) prolonged the latencies for death following hypoxic stress. The SIP and SIM were found to be insignificant to prolonged latencies for death.

Sodium nitrite induced respiratory arrest
SIM (100, 200 and 400 mg/kg/p.o.) significantly (P<0.01) delayed the onset of respiratory arrest due to sodium nitrite compared to the control mice. The SIP and SIA were found to be insignificant in this regard (Figure 2).

DISCUSSION
The most of drug acting on central nervous system (CNS) may leads to neurotoxicity especially when administered for prolong period. Moreover drugs acting on CNS disorders usually recommended for chronic use (Vyawahare and Ambikar, 2010). In our investigation, the extracts did not produce any significant change in rota-rod exploratory activity. All the mice stayed on the rota-rod for longer than 180 s, suggests absence of impaired motor coordination and neurotoxicity. SIP and SIM demonstrated significant reduction in basal locomotor activity. The extract had no effect on the motor co-ordination, suggesting that the inhibitory effect of the extract might be elicited via central mechanisms, not by peripheral neuromuscular blockade (Perez et al., 1998; Amos et al., 2001). The improvement in discrimination index by the SIM and SIA (200 and 400 mg/kg/p.o.) revealed that SI met major criteria for nootropic activity, improvement of memory in absence of cognitive deficit (Parle et al., 2004). Similar doses delayed the onset of death due to sodium nitrite induced respiratory arrest. Sodium nitrate induced chemical hypoxia and thereby respiratory arrest causes reduction in oxygen carrying capacity and resulted in to death. The drugs improving cholinergic transmission showed delay or abolition in this arrest (Vyawahare and Bodhankar, 2007). The neurological basis of learning and memory established the role of cholinergic system (Vyawahare and Bodhankar, 2007; Parle et al., 2004). The scopolamine induced memory impairment is generally used experiment model of amnesia (Naveen and Kohli, 2003), where facilitation of learning and memory along with increase in central cholinergic transmission has proven the role of cholinergic system in the learning and memory (Khalifa, 2001). The improved discrimination index and delayed respiratory arrest in the present investigation validate its traditional claim of nootropic and indicate...
possible role of central cholinergic transmission. Anxiety is one of the most common mental disorders affecting mankind. Its prevalence is increasing in recent years due to the modern lifestyle (Dhawan et al., 2001). It is well reported that anxiety, an emotional disorder is frequently associated with impairment of learning and memory (Yung, 2004). Mice pretreated with SIP (100 mg/kg/p.o.), SIM (100 and 400 mg/kg/p.o.) and SIA (100 mg/kg/p.o.) showed a significant increase in the time spent in open arm and thereby exhibited anxiolytic action (Pellow et al., 1985). SI pretreatment showed significant anxiolytic action, without any incidence of behavioral toxicity. In fact cognitive abilities were found to be significantly improved. This is most important observation of the present investigation because modern medicine does not have any drug that would be useful in the treatment of anxiety and cognitive deficit simultaneously.

SIP and SIM (400 mg/kg/p.o.) possess significant (P<0.05) analgesic activity in hot plate analgesia. However, the mechanism of this action has not been investigated here. It is not known whether this action is opioid-like in nature or involves acetylcholine or other agents (Vyawahare and Ambikar, 2010). In the present investigation, single dose intraperitoneal administration of pentyleneetetrazole (PTZ 70 mg/kg/s.c.) caused clonic convulsions as well as lethality in mice. Acute pretreatment of mice with SIM 200 and 400 mg/kg/p.o. showed significant (P<0.05) increase in latency to clonic convulsions and decrease in mortality, indicating anticonvulsant activity. The electroshock seizure test is widely applicable, because drugs that are effective against tonic hind limb extension induced by electroshock generally have proven to be effective against partial and tonic clonic seizures in human beings (Vyawahare et al., 2007). SIM (200 and 400 mg/kg/p.o.) and SIA (400 mg/kg/p.o.) demonstrated significant anticonvulsant activity by protecting mice from maximal electroshock induced seizures.

Hypoxia induced stress and thereby neurodegeneration is one of the prime pathological states in clinical practice. In modern life incidence of hypoxic stress are increasing day by day. These factors leave lasting imprints on cognitive behavior via induction of convulsion. In rare case it may result in death too. The major mechanism postulated for the hypoxic stress is increased levels of serotonin level (Thorat and Kulkarni, 1990). SIM at all doses showed antihypoxic activity against hypoxia-induced lethality in mice. To know the exact mechanism further investigation is required especially for antioxidant activity and effect on ischemic stroke. Antihypoxic effects, as well as other activities of SI such as nootropic activity, antioxidant activity make it a suitable candidate for prevention and/or treatment of stroke (Hosseinzadeh and Sadati, 2003).

CONCLUSION

SIP and SIM demonstrated significant anxiolytic and anticonvulsant activity. SIM and SIA showed improvement in discrimination index. Moreover SIM showed antihypoxic activity and delayed sodium nitrate induced respiratory arrest.

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