ABSTRACT

Argyreia speciosa (AS), a long woody climber is commonly known as Vryddhadaru in Sanskrit. It is distributed throughout India upto altitude of 300 m. It is scientifically documented for its antiinflammatory, antiarthritic, immunomodulatory, antistress and aphrodiasiac activities. The roots and seeds are used as restorative in various nervous system disorders. The roots are also reported to possess nootropic, antimicrobial, diuretic properties and used in chronic peptic ulcer, gonorrhoea and rheumatism. In the present study the central nervous system (CNS) activity of the hydroalcoholic extract of Argyreia speciosa roots at different doses (100, 200, 400 mg/kg) was studied using various standard paradigms. The results revealed that the Argyreia speciosa extract significantly increased discrimination index in object recognition test, reduced 5HTP induced head twitches, potentiated haloperidol induced catalepsy, delayed the onset of pentylenetetrazole induced convulsions and antagonised amphetamine induced motor hyperactivity.

Keywords: Argyreia speciosa; anxiety; cognition; convulsions; locomotor activity.

INTRODUCTION

Argyreia speciosa an elephant creeper, a long woody climber found throughout India is commonly used in local folk medical practices in India. It is traditionally used in gonorrhoea, strangury and chronic peptic ulcers. The leaves are used externally in skin diseases such as ringworm, eczema, itch and internally to cure boils, swellings etc. The leaves are also employed as rubefacient and local stimulant. The seeds are reported to be potent antihypertensive and spasmolytic. The roots are indegenously prescribed as an aphrodisiac, diuretic and antirheumatic. The roots are also claimed to be a tonic in disorders of central nervous system. Phytochemically the whole plant contains alkaloids, flavone glycosides, flavonoids steroids, triterpenoids and lipids. Various alkaloids such from the plant namely friedelin, chanclavine I, II, ergine, agroclavine, ergonovine, ergometrinine, ergometrine, isooergine lysergol, isolysergol, penniclavine, chanclavine, molliclavine, steoclavine have been isolated from the plant. It also contains flavone glycosides and flavonoids such as quercetin and kaempferol, along with some steroids, triterpenoids, lipids and other phytochemicals such as scopoletin, hexadecanly p-hydroxyxycinnamate. Despite the extensive phytochemical work on Argyreia speciosa, there is paucity of reports on the pharmacological properties, especially CNS effects of the plant.

The alcoholic and aqueous leaf extracts of Argyreia speciosa are documented for their dose dependant pregnancy interruption action in rats at early stages of pregnancy. Another study has revealed the dose dependent potentiation of delayed type of hypersensitivity reaction by the plant, mediated by enhanced production of circulating antibody titre. It has also been shown that the ethnicolic extract of its roots produced anti-inflammatory and antiarthritic effect in carrageenan and freund's complete adjuvant model respectively. Leaves have been documented for its antidiabetic potential. Vyawahare NS et al have documented the nootropic effect of Argyreia speciosa, however the possible mechanism of its action has not been yet revealed. It is well established that the nootropic activity is associated with modulation of various neurotransmitters that can be guaged by either studying the effect on generalised neuropharmacological behavior or by direct estimation of neurotransmitters. Looking at the complexity of CNS and its transmission it is usually recommended to study neuropharmacological evaluation followed by direct neurochemical evaluation.

In light of the fact that Argyreia speciosa has traditionally been used in cases of nervous disorders, the present study titled “Central nervous system activity of Argyreia speciosa” was undertaken.

MATERIALS AND METHODS

Plant material

The hydroalcoholic extract of roots of AS prepared by the following procedure was received as gift sample (ARG-4019) from Green Chem, Bangalore, India.
Preparation of extract
Roots were extracted with 50% aqueous alcohol and concentrated. The concentrated mass was washed with petroleum ether several times to remove the resinous matter. Then the mass was diluted with 25% aqueous alcohol, filtered and concentrated, dried to get the powdered form of the extract.

Chemicals and drugs
Pentylentetrazole, haloperidol, amphetamine, 5HTP, (Rajesh chemicals, Mumbai) and sodium nitrate, (Loba chemicals, Mumbai) were purchased from respective vendors. Diazepam (Calmpose) and Pentazocin injection (Fortwin), Piracetam suspension (Nootropil), Clonazepam (Clonotril) and Phenytoin (Eptoin) tablets were purchased from the local market.

Preparation of drug solution
Accurately weighed quantity of powdered extract was dissolved in the distilled water to prepare the appropriate stock solution of the drug i.e. 10 mg/ml, 20 mg/ml and 40 mg/ml respectively. The doses were administered orally by selecting the appropriate concentration of the stock solution. Clonazepam and Phenytoin were suspended in 1% w/v acacia.

Animals
Swiss male albino mice (18-22g) were used. They were maintained at 25 ± 2°C and relative humidity of 45 to 55% and under standard environmental conditions (12 hrs light 12 hrs dark cycle). The animals had free access to food (Chakan Oil Mills, Pune, India) and water ad libitum. Diazepam (Calmpose) and Pentazocin injection (Fortwin) approved the protocol. All experiments were carried out between 12:00-16:00 hrs.

Acute toxicity test
Healthy adult male albino mice (18-22 g) were subjected to acute toxicity studies as per guidelines (AOT 425) suggested by the organization for economic co-operation and development (OECD-2001). The mice were observed continuously for 2 hrs for behavioral and autonomic profiles and for any sign of toxicity or mortality up to a period of seven days12.

EXPERIMENTAL DESIGN
Behavioral effects
Behavioral changes of AS (100, 200 and 400 mg/kg) were assessed by visual observation 60 min after administration of vehicle or AS for next 2 hrs. The observation parameters consisted of body position, alertness, reactivity to touch stimuli, righting reflex and lacrimation13.

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. 30 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 five groups with 6 animals in each group. The mice were then tested for motor coordination to record basal fall off time followed by respective drug treatment. 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 off time before and after drug treatment was considered for evaluation. Diazepam (2 mg/kg, i.p.) was used as a reference standard14.15.

Locomotor activity
The locomotor activity (horizontal activity) was measured using a digital actophotometer (Space-lab, India). Each mouse was placed individually in the actophotometer for 5 min and basal activity score was obtained. Subsequently animals were divided into five groups and treated with test drugs. 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) preparation was used as a reference standard16.

Analgesic activity
The analgesic effect was studied using digital hot plate (Columbus - USA) 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) or AS extract (100, 200 and 400 mg/kg). The temperature of the plate was maintained at 55°C ± 01°C. A cut off reaction time of 30 sec was chosen in order to avoid injury. Pentazocin (30 mg/kg, s.c.) was used as a reference standard17.

Elevated plus maze (EPM)
An Elevated plus maze (V. J. Instruments, India) consisting of two open arms (35×6 cm) and two enclosed arms (35 × 6 × 15 cm) was used. The maze was elevated to the height of 40 cm. Mice were placed individually in the center of the EPM facing an enclosed arm. The time spent by the mouse during the next 05 min on the open and enclosed arm was recorded. The animals received vehicle (10 ml/kg) or AS (100, 200 and 400 mg/kg) 60 min before and diazepam (1 mg/kg, i.p.) 30 min before their placement on the maze. Increased exploratory activity in the open arm was taken as an indication of anxiolytic activity18,19.

Object recognition test
The apparatus consisted of white colored plywood (70×60×30 cm) with a grid floor. It was illuminated by a 40 W lamp suspended 50 cm above the apparatus. The object to be discriminated was also made of...
noted down at 0, 15, 30, 60, 90 and 120 min. the cut off mice retains the forepaws on the elevated rod was diameter set at 2.5 cm from top. Duration for which the forepaws of the mice were placed on rod of 0.9 cm before haloperidol (1 mg/kg, i.p). After the treatment, group received AS (100, 200 and 400 mg/kg) 60 min whereas the other group received vehicle (10 ml/kg, p.o.) whereas the other Mice were divided into four groups. The control group Haloperidol induced catalepsy after the onset of head twitches23-25. therefrom, 5-HTP (50 mg/kg, i.p.) was injected in the order to prevent the rapid degradation of 5-HTP . Thirty minutes later, vehicle or AS was administered. 60 min thereafter, the locomotor activity was measured for 5 min duration using digital actophotometer29.

Clonidine induced hypothermia Mice were taken in groups of six each and rectal temperature was recorded using digital telethermometer (Dolphin, India) every 60 min after clonidine (0.1 mg/kg, i.p) till 180 min. vehicle (10 ml/ kg) or AS (100, 200 and 400 mg/kg) were administered 60 min before clonidine30.

Pentylenetetrazole induced seizure (PTZ) Clonic seizures were induced 60 min after respective drug treatment in mice by subcutaneous injection of 80mg/kg pentylenetetrazole. 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 standard31-33.

Maximal electroshock induced seizures (MES) Tonic clonic convulsions were induced 60 min after the respective drug treatment by giving maximal electroshock seizures (MES) (40mA for 0.2sec) using an electroconvulsimeter (INCO, Ambala, India) via crocodile ear clip 60 min after administration of either vehicle(10 ml/kg) , AS (100, 200 and 400 mg/kg) or Phenytoin (20 mg/kg, i.p). The number of animals protected from tonic hind limb extension seizure (abolition 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 group31,34.

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 mice were free of any toxicity as per acceptable range given by the OECD guidelines up to the dose of
2000 mg/kg. From this data and pilot study reports; three different doses 100, 200, 400 mg/kg were selected for further study.

**Behavioral assessment**
The mice were observed for a period of 2 hr; 60 min after oral administration of vehicle or AS (100, 200, 400 mg/kg). The observations are summarised in Table 1.

**Table 1. Behavior assessment of AS extract in mice**

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Vehicle (100 mg/kg)</th>
<th>AS (100 mg/kg)</th>
<th>AS (200 mg/kg)</th>
<th>AS (400 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Hindpaw</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Reactivity to touch</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Righting reflex</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Locomotion</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

- : Normal, : Increased, : Decreased.

**Effect on motor coordination**
All the doses of AS (100, 200, 400 mg/kg) were found to be statistically insignificant towards the mean fall off time while diazepam (2 mg/kg, i.p) showed a significant reduction (P<0.01) in fall off time recorded as mean change in fall off time (Fig 1).

![Fig. 1. Effect of AS extract and diazepam on motor coordination in mice. n=6, Data analysed by ANOVA followed by Dunnetts test. **P<0.01.](image1)

**Effect on Locomotor activity**
None of the doses of AS (100, 200, 400 mg/kg) showed any significant alteration in locomotion. Diazepam 2 mg/kg significantly (P<0.01) reduced the locomotor activity (Fig 2).

![Fig. 2. Effect of AS extract and diazepam on motor performance in mice. n=6, Data analysed by ANOVA followed by Dunnetts test. **P<0.01](image2)

**Elevated plus maze**
The time spent in open and enclosed arm by vehicle treated control mice were 67.36 ± 4.01 sec and 219.56 ± 8.11 sec respectively. Diazepam (1mg/kg, i.p) significantly increased (P<0.01) time spent in open arm and thereby showed anxiolytic action while AS treatment did not show any significant effect on the time spent in open or enclosed arm by mice when placed on EPM. (Table 2).

**Table 2: Effect of AS extract and diazepam on anxiety induced using elevated plus maze apparatus.**

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Time spent in second (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (100 mg/kg)</td>
<td>67.36 ± 4.01</td>
</tr>
<tr>
<td>AS (100 mg/kg)</td>
<td>73.24 ± 4.53</td>
</tr>
<tr>
<td>AS (200 mg/kg)</td>
<td>70.92 ± 5.76</td>
</tr>
<tr>
<td>AS (400 mg/kg)</td>
<td>75.35 ± 4.66</td>
</tr>
<tr>
<td>Diazepam (1 mg/kg)</td>
<td>106.11 ± 4.02**</td>
</tr>
</tbody>
</table>

n = 6 Data analyzed by one-way ANOVA followed by Dunnett’s test. **P<0.01.

**Object recognition test**
In this test, AS (200 and 400mg/kg) and piracetam (150 mg/kg) treated mice required significantly less time against control mice to explore the familiar objects as compared with the new object and thus significantly improved the discrimination index. The AS 400 mg/kg was found to be more significant (P<0.01) than AS 200 mg/kg (P<0.05) (Fig 3).

![Fig. 3. Effect of AS extract and piracetam on discrimination index in object recognition test in mice. n=6, Data analysed by one way ANOVA followed by Dunnett’s test. **P<0.05, ***P<0.01](image3)

**Double unit mirrored chamber**
Pre treatment with different doses of AS did not affect latency to first entry or time spent in mirror chamber when compared to vehicle treated mice. Diazepam (1 mg/kg, i.p), a reference standard significantly increased the time spent (P<0.01) and
latency to the first entry (P<0.05) in mirror chamber and thereby showed significant anxiolytic effect (Data not shown).

**5HTP-induced head twitches in mice**

5HTP induced 15.40±1.43 head twitches in 10 minutes in vehicle treated control mice. Pretreatment with AS (100, 200 400 mg/kg) significantly reduced the number of head twitches when compared to the control values. AS 100 mg/kg was found to be less effective (P<0.05) than AS 200 and 400 mg/kg (P<0.01) (Fig 4).

**Haloperidol induced catalepsy**

In haloperidol-induced catalepsy, maximum catalepsy was noted at 120 min. AS 200 and 400 mg/kg showed a significant (P<0.01) increase in the duration of catalepsy from 90 min to 120 min while AS 100 mg/kg reported a significant increase in duration of catalepsy (P<0.05) at 120 minute only (Table 3).

**Table 3:** Effect of AS on duration of haloperidol induced catalepsy in mice.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Vehicle</th>
<th>AS 100 mg/kg</th>
<th>AS 200 mg/kg</th>
<th>AS 400 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15 ± 1.24</td>
<td>20 ± 1.45</td>
<td>25 ± 1.67</td>
<td>28 ± 1.89</td>
</tr>
<tr>
<td>90</td>
<td>15 ± 1.24</td>
<td>20 ± 1.45</td>
<td>25 ± 1.67</td>
<td>28 ± 1.89</td>
</tr>
<tr>
<td>180</td>
<td>15 ± 1.24</td>
<td>20 ± 1.45</td>
<td>25 ± 1.67</td>
<td>28 ± 1.89</td>
</tr>
</tbody>
</table>

**Sodium nitrite induced respiratory arrest**

None of the doses of AS (100, 200, 400 mg/kg) significantly altered the onset of respiratory arrest (Fig 5).

**Amphetamine antagonism**

AS 100, 200 and 400 mg/kg showed significant reduction in amphetamine induced increased locomotor activity score. The dose of 100 mg/kg was found to be less effective (P<0.05) than the subsequent two doses (200 and 400 mg/kg) of AS (P<0.01) (Fig 6).

**Clonidine induced hypothermia**

Clonidine induced hypothermia was not affected by the pre treatment of AS. However, a non significant potentiation of hypothermic effect was seen (Data not shown).

**Pentylenetetrazole induced seizure (PTZ)**

In vehicle treated control mice, convulsions were produced after 191.50 ± 6.22 sec. The pretreatment with AS (200 and 400 mg/kg) delayed this onset up to 219.50 ± 6.74 sec and 218.33 ± 4.61 sec respectively, thus showed significant delay (P<0.05) in the onset of the first clonus. Also, AS 400 mg/kg treatment showed survival of one mouse while standard clonazepam showed 100% protection (Table 4).

**Table 4:** Effect of AS extract and clonazepam on Pentylenetetrazole induced convulsions in mice.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Onset of first clonus (sec)</th>
<th>No. mice</th>
<th>Percent survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>191.50 ± 6.22</td>
<td>6/6</td>
<td>0 %</td>
</tr>
<tr>
<td>AS 100</td>
<td>219.50 ± 6.74</td>
<td>6/6</td>
<td>100 %</td>
</tr>
<tr>
<td>AS 200</td>
<td>218.33 ± 4.61</td>
<td>6/6</td>
<td>100 %</td>
</tr>
<tr>
<td>AS 400</td>
<td>215.20 ± 4.81</td>
<td>1/6</td>
<td>16.67 %</td>
</tr>
</tbody>
</table>

**Maximal electroshock induced seizures (MES)**

All the doses of AS failed to prevent tonic hind limb extension (THLE), however a non significant reduction in duration of THLE was observed. Phenytoin showed 100% protection in this regard (Table 5).
Table 5: Effect of AS extract and phenytoin on mes induced convulsions in mice.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Duration of hind limb extension (second)</th>
<th>Mice convulsions/min used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>23.57 ± 1.85</td>
<td>9/6</td>
</tr>
<tr>
<td>AS (100)</td>
<td>20.33 ± 1.43</td>
<td>6/5</td>
</tr>
<tr>
<td>AS (200)</td>
<td>15.20 ± 1.00</td>
<td>6/5</td>
</tr>
<tr>
<td>Phenytoin (5)</td>
<td>15.50 ± 1.03</td>
<td>6/5</td>
</tr>
</tbody>
</table>

n=6 Data was analyzed by one-way ANOVA followed by Dunnett’s.

DISCUSSION

The present study investigated the putative central effect of the hydroalcoholic extract of roots of Argyreia speciosa, a plant used in Indian traditional system of medicine. The study provided data on the effects of AS on the central nervous system that is useful to explore possible mechanism of its nootropic action.

The object recognition test is widely accepted model to assess facilitation of learning and memory process in absence of cognitive deficit. The single dose oral administration of AS showed significant improvement in discrimination index and thereby supported the traditional claim. The results also suggest possible use of extract in slow learners wherein cognitive deficit is not reported.

The rota rod and actophotometer were used to assess the motor co-ordination and locomotor activity respectively. Performance observed in mice treated with AS was similar in relation to the control group suggesting absence of impaired motor co-ordination and locomotion due to the AS treatment. Absence of motor activity was also observed in elevated plus maze and double unit mirror chamber, where AS treated mice performed similarly to the control mice. In this way, we can accept absence of deleterious effect of AS on motor activity and co-ordination. Thus AS meet major criterion of the drug that acts on CNS. The absence of anxiolytic activity screened using elevated plus maze and double unit mirrored chamber can be helpful to boost nootropic activity because many anxiolytic agents used in current pharmacotherapy have shown adverse effects on learning and memory processes suggesting inverse relationship between anxiety and cognition. The 5HTP induced head twitches are due to increase in serotonin levels in CNS. The AS treatment significantly reduced head twitches and thereby suggested reduction in central serotonergic transmission. The increase in serotonin level has been reported to interfere with learning acquisition and memory consolidation. The above mentioned results of head twitches point out the possible role of reduced serotonergic transmission in the nootropic action of AS and thereby support the previous findings.

Noradrenergic transmission has been reported to play diverse role in the cognition. The augmentation as well as retardation of its transmission has been linked with the process of cognition. AS extract demonstrated significant antagonism of amphetamine induced hyperactivity which may be due to either its antidopaminergic or antinoradrenergic action. Contrary, AS treatment significantly facilitated haloperidol induced catalepsy that appears to be due to blockade of dopamine transmission suggesting antidopaminergic activity by the extract. Moreover, clonidine induced hypothermia was not significantly antagonised by the extract which further postulated the inhibition of noradrenergic transmission by the extract. Sodium nitrite induced respiratory arrest is widely accepted indirect method to check involvement of cholinergic transmission. Although cholinergic transmission play vital role in cognition but earlier studies have also documented nootropic activity without any modulation in the cholinergic neurotransmission. The anticonvulsant activity evaluated against PTZ and MES induced convulsions using widely accepted methods with high predictive value for detection of clinically effective drugs revealed effectiveness of the extract towards PTZ model only. PTZ test identifies drugs with efficacy against non convulsive absence or myoclonic seizures due to increase of the seizure threshold while the MES test identifies agent active against generalised tonic-clonic seizures due to blocking of seizure spread. The AS was able to modify the progress of convulsive episodes induced by PTZ and thereby suggest possible use of the extract in the treatment of absence seizures. In addition, it can be ideal option to control the cognitive deficit associated with convulsion and post convulsive cognitive deficit.

CONCLUSION

In conclusion, the study provides evidence that the hydroalcoholic extract of the roots of AS possess significant nootropic activity which is not accompanied by motor incoordination. The study further revealed that inhibition of dopaminergic, serotonergic as well as noradrenergic transmission could be the possible mechanism of action. However, further studies involving direct neurochemical estimations are necessary to confirm and extend these results.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Rajendran, Green Chem. Bangalore, India, for providing gift sample of hydroalcoholic extract of Argyreia speciosa. Principal Dr. D. M. Sakarkar, S.N. Institute of Pharmacy, Pusad and Dr. K. G. Bothara, Principal AISSMS College of Pharmacy, Pune for providing the necessary support.

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