PRELIMINARY STUDY ON NOOTROPIC EFFECT OF VANDA TESSELATA ROOTS

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ABSTRACT

The research work deals with the screening of ethanolic extract of Vanda tessellata(AERVT) roots using Elevated plus maze (EPM), Scopolamine and Diazepam induced models in mice. Piracetam was used as reference for the study. The Vanda tessellata root is known to contain saponins, glycosides, alkaloids, steroids and tannins in phytochemical screening. Since root of Vanda tessellata is used as folk medicine for diseases of nervous system, we made an attempt to study for nootropic activity. When this extract is subjected for LD₅₀ studies it did not produced any abnormal behaviour or mortality even at the dose level of 2000 mg/Kg body weight in mice. Three different doses 100 mg/kg, 200 mg/kg and 400 mg/kg doses were selected for the present study with respect to maximum dose tested for LD₅₀ study. In this study with the EPM model, Diazepam and Scopolamine induced amnesic models, the transfer latency is significantly reduced with 200 mg/kg and 400 mg/kg doses of AERVT. The result of the study confirmed that the nootropic effect of ethanolic extract was due to several phytoconstituents like saponins, glycosides, alkaloids which were already reported for their nootropic activity.

KEYWORDS: Vanda tessellata Roxb, Alcoholic extract, Nootropic, Piracetam, EPM, Scopolamine, Diazepam.

INTRODUCTION

Alzheimer’s disease is a progressive neurodegenerative age related brain disorder[1] presents with loss of memory, abnormal behaviour, personality changes leading to death. Being a chronic, progressive disabling organic brain disorder, it is manifested by disturbance in the
multiple and higher cortical functions including memory, orientation, comprehension, judgment and learning.\textsuperscript{[2]}

Nootropics are a class of psychotropic agents used as memory cognitive enhancers, that improve mental functions such as cognitive attention, concentration, motivation, intelligence and memory. It is thought that these drugs enhance memory by increasing brain's supply of neurochemicals like neurotransmitters, enzymes and hormones that improve oxygen supply to brain or stimulate nerve growth.\textsuperscript{[3]}

Nootropic agents like piracetam, pramiracetam, aniracetam and choline esterase inhibitors like Donepezilare being used in this condition to improve memory, mood and behavior, but the adverse effects present with these agents have limited their use. Indian system of medicine emphasizes use of herbs for controlling these neurodegenerative disorders. In Yunnanithe roots of \textit{V. tessellate} (Family-Orchidaceae) is used as laxative, tonic to the liver and brain, useful in rheumatism, piles, lumbago, toothache, allied disorders and diseases of the nervous system. Hence the present study was undertaken to investigate the effects of \textit{V. tessellata}, popularly known as rasna on learning and memory in mice.\textsuperscript{[4]}

**MATERIALS AND METHODS**

**Materials**

Root powder of \textit{Vanda tessellata} (Family-Orchidaceae) collected from Hamdard Ayurvedic shop, Raichur, Indiawas extracted by soxhlet apparatus maintained at 60-80°C for 18 h using 95% ethanol.\textsuperscript{[8]} The crude extract was filtered and concentrated by evaporation on a water bath at 50°C under reduced pressure until a thick paste of extract was prepared. AERVT was diluted in distilled water whereas injections of scopolaminehydrobromide (Buscopan, Cadila Healthcare Ltd, Goa, India), diazepam (Calmpose®, Ranbaxy, India) and piracetam (Micro Labs Ltd, Bangalore, India) were dissolved in normal saline. For oral administration and ip injection volume was fixed as 1 ml/100 g of mice.

**Animals**

Albino mice of either sex (18-22 g) procured from animal house of Shri Venkateswara Enterprises, Bangalore were used for experimental purpose. They were acclimatized to laboratory condition.\textsuperscript{[4,5]} for 7 days in polypropylene cage at 12:12 hight-dark schedule at 26 ± 2°C and 45-55% RH and light-dark cycle of 12:12 h. The rats were fed with standarddiet (Amrut Laboratories Pranava Agro Industries Ltd., Sangli, Maharashtra.) and water was
supplied *ad libitum* under strict hygienic conditions. Institutional Animal Ethics Committee approved the experimental protocol and studies were performed as per Committee for the purpose of control and and supervision of experiments on animals (CPCSEA), Department of Animal Welfare, Government of India. Animals were fasted overnight prior to vehicle, standard and extracts administration and during the experiment. All experiments were carried out during day time from 8:00 to 16:00 hrs.

**Acute toxicity studies**

Three hours before the experiment, food was withheld. The acute toxicity studies were performed according to Organization for Economic Co-operation and Development (OECD) guidelines.[6] AERT was administered and mortality of the animals was observed during 48 hrs study period. Based on this short-term toxicity profile of extract, the dose for the animal was determined. From the LD$_{50}$ dose 1/20 (100 mg/Kg), 1/10 (200 mg/Kg) and 1/5(400 mg/Kg) doses were selected and used in the entire study.

**Elevated plus-maze (EPM)**

The elevated plus mazes served as the exteroceptive behavioural model to evaluate learning and memory in mice.[7] The procedure, technique and end point for testing learning and memory was followed as per the parameters described. Transfer latency (TL) was taken as the time taken by the mice to move into one of the covered arm with all its four legs. TL was recorded on the first day. If the animal did not enter into one of the covered arm within 90 sec., it was gently pushed into one of the covered arms and the TL was assigned as 90 sec.

**Scopolamine induced amnesia**

Scopolamine hydrobromide (0.4mg/kg, ip) was administered on 1$^{st}$ day and TL was recorded after 45 minutes of injection and after 24 hr. Extract (100, 200 and 400 mg/kg) and piracetam (200 mg/kg, po) were administered for 7 successive days. On 7$^{th}$ day, scopolamine was given after 45 minutes of administration of the last dose and TL was noted after 45 minutes and after 24 hr. The inflexion ratio (IR) was calculated as per standard procedure.[9]

**Diazepam induced amnesia**

Amnesia was induced by administration of diazepam (5mg/kg, ip) to young mice and TL was recorded after 45 minutes of injection on 1$^{st}$ day and after 24 hr. Extract (100, 200 and 400 mg/kg) and piracetam (200 mg/kg, po) were administered once daily for 7 days. Diazepam
(5mg/kg, ip) was given after 90 minutes of administration of the last dose on 7th day. The TL was noted after 45 minutes and after 24 hr and the (IR) was calculated.

**Statistical analysis**

All results were expressed as mean ± standard error of mean (SEM). Data was analyzed using one-way ANOVA followed by Dunnett’s ‘t’ test. P <0.05 was considered as statistically significant.

**RESULTS**

**Effect on transfer latency (using elevated plus-maze)**

The transfer latency on elevated plus maze was expressed as IR. In normal control IR was recorded as 0.165. Piracetam (200 mg/kg), different dose levels of AERVT (200 and 400 mg/kg) treated groups have shown a significant increase in IR. A similar effect was not observed with low dose (100 mg/kg) of AERVT (Table No.1, Fig No.1).

**Scopolamine-induced amnesia**

In Scopolamine-induced amnesic model in mice normal control inflexion ratio was recorded as 0.398. When compared to normal control group, scopolamine treated group was noted with impairment of memory and has shown a significant decrease in IR noted as 0.256 as reduction in IR indicates amnesic effect. Piracetam and 200 mg/kg and 400 mg/kg doses of AERVT treated groups have shown a significant increase in IR, and reduced the TL and also reversed the scopolamine-induced amnesia. A similar effect was not observed with low dose 100 mg/kg of AERVT treated groups and the results are statistically insignificant. (Table No.2, Fig No.2).

**Diazepam–induced amnesia**

In diazepam–induced amnesic model normal control inflexion ratio was recorded as 0.392. Diazepam has induced amnesia. When compared to normal control group a decrease in IR was observed in this amnesic model as 0.301. Piracetam, AERVT of (200 and 400 mg/kg) treated groups have shown increase in the IR, reduction of TL and reversed the diazepam-induced amnesia. A similar effect was not observed with of 100 mg/kg of AERVT treated group and the group showed statistically insignificant effect. A significant nootropic activity was observed with 200, 400 mg/kg doses of AERVT on EPM model as TL was significantly reduced i.e., reversed the diazepam induced amnesia. (Table No.3, Fig No.3).
Table 1. Effect of AERVT on inflexion ratio in Elevated plus maze in mice (mean±SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose/kg</th>
<th>Inflexion ratio (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control (DW)</td>
<td>10 ml p.o.</td>
<td>0.165±0.059</td>
</tr>
<tr>
<td>II</td>
<td>Piracetam</td>
<td>200 mg p.o.</td>
<td>0.615±0.021**</td>
</tr>
<tr>
<td>III</td>
<td>AERVT</td>
<td>100 mg p.o.</td>
<td>0.103±0.021**ns</td>
</tr>
<tr>
<td>IV</td>
<td>AERVT</td>
<td>200 mg p.o.</td>
<td>0.371±0.022**</td>
</tr>
<tr>
<td>V</td>
<td>AERVT</td>
<td>400 mg p.o.</td>
<td>0.483±0.049**</td>
</tr>
</tbody>
</table>

Table No 2. Effect of AERVT on inflexion ratio in Scopolamine-induced amnesia in mice (mean±SEM).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose/kg</th>
<th>Inflexion ratio (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control (DW)</td>
<td>10 ml p.o.</td>
<td>0.398±0.06</td>
</tr>
<tr>
<td>II</td>
<td>Scopolamine alone</td>
<td>0.4 mg i.p.</td>
<td>0.256±0.045</td>
</tr>
<tr>
<td>III</td>
<td>Piracetam + Scopolamine</td>
<td>200 mg p.o. + 0.4 mg i.p.</td>
<td>0.695±0.033**</td>
</tr>
<tr>
<td>IV</td>
<td>AERVT + Scopolamine</td>
<td>100 mg p.o. + 0.4 mg i.p.</td>
<td>0.376±0.023**ns</td>
</tr>
<tr>
<td>V</td>
<td>AERVT + Scopolamine</td>
<td>200 mg p.o. + 0.4 mg i.p.</td>
<td>0.49±0.039**</td>
</tr>
<tr>
<td>VI</td>
<td>AERVT + Scopolamine</td>
<td>400 mg p.o. + 0.4 mg i.p.</td>
<td>0.59±0.025**</td>
</tr>
</tbody>
</table>

Table No.3. Effect of AERVT on inflexion ratio in Diazepam induced amnesia in mice (mean±SEM).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose/Kg</th>
<th>Inflexion Ratio (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control (DW)</td>
<td>10 ml p.o.</td>
<td>0.392±0.023</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam Alone</td>
<td>5 mg i.p.</td>
<td>0.301±0.037</td>
</tr>
<tr>
<td>III</td>
<td>Piracetam + Diazepam</td>
<td>200 mg p.o. + 5 mg i.p.</td>
<td>0.666±0.017**</td>
</tr>
<tr>
<td>IV</td>
<td>AERVT + Diazepam</td>
<td>100 mg p.o. + 5 mg i.p.</td>
<td>0.37±0.020**ns</td>
</tr>
<tr>
<td>V</td>
<td>AERVT + Diazepam</td>
<td>200 mg p.o. + 5 mg i.p.</td>
<td>0.551±0.030**</td>
</tr>
<tr>
<td>VI</td>
<td>AERVT + Diazepam</td>
<td>400 mg p.o. + 5 mg i.p.</td>
<td>0.650±0.029**</td>
</tr>
</tbody>
</table>

Note: n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant

AERVT-Alcoholic extract of roots of *V.tessellata*(For all above mentioned Tables)

Fig. 1. Effect of AERVT on inflexion ratio in mice (mean ± SEM)
DISCUSSION

In the present study with EPM model and diazepam, scopolamine induced amnesic models, transfer latency is significantly reduced and a shortened TL in the EPM model indicates an improvement in the memory. The observation has been strengthened by the findings of AERVT (200 and 400 mg/Kg) doses as these have shortened the TL in the EPM model indicating improvement in memory, which is in accordance with the hypothesis of Itoh et al., 1998. But a similar effect was not observed with low dose (100 mg/kg) of AERVT. In scopolamine induced amnesia model, an impairment of learning and memory is noted. Pharmacological data clearly indicate that muscarinic and nicotinic acetylcholine receptors have a role in the encoding of new memories. Scopolamine was reflected by prolonged TL.
from the open arm to the closed arm i.e. a decrease in IR was observed with EPM. The AERVT (200 and 400 mg/kg) doses have decreased TL and increased IR thus confirm their nootropic activity. Soreversal effect of amnesia induced by AERVT (200 and 400 mg/kg) indicates that the extract is acting on Ach receptors because it has shown nootropic activity in presence of scopolamine which is a muscarinic receptor antagonist, but a similar effect was not observed with low dose (100 mg/kg) of AERVT. In diazepam induced amnesia model, diazepam, a GABA mimetic agent induces memory impairment and it was reported that a subsequent inhibition of GABA-B receptors has been found to facilitate learning and memory.[9]

Diazepam (5 mg/kg) prolonged TL and decreased IR. The AERVT (200 and 400 mg/kg) have decreased TL and increased IR, thus confirming their nootropic activity. But a similar effect was not observed with low dose (100 mg/kg) of AERVT. The protective effect offered by the extract against diazepam-induced amnesic model may be due to indirect release of Ach in the brain.

Phytoconstituents like triterpenoid saponins from Panax ginseng, Red ginseng, Bacopa monniera and Rubia cardifolia, Vinca minor and Secale cornutum, alkaloids from Panax ginseng, Red ginseng, flavonoids from Albizzia lebback and Pueraria tuberosa and glycosides from Withania somnifera are already reported for their nootropic activity.[10-14] Phytochemical screening of V. tessellata by simple chemical tests showed the presence of presence of alkaloids, steroids, tannins, saponins and glycosides and hence these may be responsible for the observed nootropic activity of AERVT.

CONCLUSION
Preliminary phytochemical investigations on AERVT were noted with alkaloids, steroids, tannins, saponins and glycosides and no mortality or behavioural abnormality recorded in mice even at the highest dose level of 2000 mg/Kg tested for LD₅₀ studies. The nootropic activity of AERVT was evaluated in EPM model, Scopolamine and Diazepam induced amnesic animal models in mice. In this study with all mentioned models with different doses and standards except with low dose of AERVT, transfer latency is significantly reduced.

Phytochemical constituents like saponins, glycosides, alkaloids were already reported for their nootropic activity and these constituents were present in AERVT. Hence these chemical constituents can be accounted for the observed nootropic activity.
REFERENCES


6. OECD 2001-guideline on acute oral toxicity (AOT) environmental health and safety monograph series on testing and adjustment. No.425.


