EVALUATION OF SEDATIVE ACTIVITY OF AQUEOUS EXTRACT OF *VIGNA TRILOBATA* (L.) VERDC. LEAVES

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ABSTRACT
The aim of present study is to evaluate the sedative activity of aqueous extract of *Vigna trilobata* (L) verd. Leaves using experimental animal models. In the present study aqueous extract of the *Vigna trilobata* leaves (AEVTL) was used to investigate the sedative activity using Rotarod apparatus and Photoactometer in mice at a dose of 200 and 400 mg/kg of body weight and compared to standard diazepam (5mg/kg, i.p.). The result obtained from this study revealed that AEVTL possessed significant (p<0.05 and p<0.01) sedative activity at a dose of 200 and 400 mg/kg by reducing locomotor activity and fall off time in mice in a dose dependent manner. The results of this study justify the use of the leaves as sedative in traditional medicine. Further studies may be directed at characterizing the bioactive ingredients that are responsible for the observed sedative activity in the plant.

KEY WORDS: *Vigna trilobata*, Sedative, Rotarod apparatus and Photoactometer.

INTRODUCTION
A healthy amount of sleep is paramount to living a healthy life. Good quality sleep is an important part not only for living healthy but also for leading a productive life, physically and mentally. Sleep is absolutely essential for normal, healthy function. Scientists and medical professionals still have much to learn about this complicated physiological phenomenon. According to the National Institute of Neurological Disorders and Stroke, about 40 million people in the United States suffer from chronic long-term sleep disorders each year and an additional 20 million people experience occasional sleep problems.¹

Insomnia is the second most common complaint, after pain, in the primary care. Thirty-five per cent of the general population according to the 1984 report of the National Institutes of Mental Health suffers from insomnia, whereas persistent insomnia affects roughly more than one-third of the population.² Effective treatments such as sedative and hypnotic drug therapy are available but, many patients remain untreated, experience adverse effects of benzodiazepines, barbiturates and other drugs, or do not benefit from full symptom control. There does not seem to be an ideal drug for the treatment of sleep disorder. The search for new drug is still the need of the day. There is high prevalence of usage of alternative traditional system of medicines for the treatment of sleep disorder. Ayurveda offers a unique insight into comprehensive approach to management of sleep disorder. More than 750 medicinal plant species have been used ethno-pharmacologically and traditionally to treat the symptoms of sleep disorder worldwide. The world health organization (WHO) has recognized herbal medicine as an essential building block for primary health care of vast countries like India and China. The extracts of many plants used in traditional medicine contain curative agents that are used in many modern medicines.³

One such plant we have taken as a part of our study is *Vigna trilobata* (L) Verdc. In Traditional Indian medicine i.e. Ayurveda describes *Vigna trilobata* (L) Verdc. having sedative activity of leaves.⁴,⁵ *Vigna trilobata* (L) Verdc. is well known in the traditional medical practice of India. The plant contains friedelin, epifriedelin, stigmasterol and tannins. It also contains methionine, tryptophan, tyrosine, strepogenin, uridine, and diphosphate-galacturonic acid.⁵ Taking into account these findings and in view of alleged sedative activity of the *Vigna trilobata* (L)Verdc., it was decided to evaluate the sedative activity of *Vigna*
trilobata (L.) Verdc. of leaves using experimental animal models.

MATERIALS AND METHODS
Preparation of plant extract
Collection of plant
The plant required for the study *Vigna trilobata* (L.) Verdc. was collected from the botanical garden of college.

The collection of plant was carried out in the month of early January of year 2011. The plant was authenticated by Dr. Minoo H. Parabia, Advisor of Shri R. M. Dhariwal Ayurved College and research Centre, Waghaldhora, Valsad.

Extraction procedure for aqueous extract
Leaves of the plant was collected, cleaned and dried in shade. After 7-days of drying, it was powdered by grinding and sieved with a 40# sieve. The powder was then extracted in a soxhlet apparatus with distilled water for 24 h.

The extract was filtered and concentrated in vacuum under reduced pressure using a rotary flash evaporator. The concentrated mass was used for the study. Yield of extract obtained from leaves was 9.4% w/v.

Animals
Mice (20 to 30g) of either sex were used during whole period of experiments. They were maintained under standard laboratory condition on 12 h day/night cycle and with free access to food and water.

The animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were carried out between 09:00 h to 16:00 h at ambient temperature. The animals were drawn at random for test and control groups. Permission was obtained, prior to the start of experiments, from Institutional Animal Ethics Committee (IAEC) of Rofel, Shri G. M. Bilakhia college of Pharmacy as per CPCSEA guidelines (Reg. no.: 403/01/a/CPCSEA; Rofel/2011/03).

Experimental animal models
Screening of sedative activity using Actophotometer apparatus
Mice weighing 20-25 gm were divided in 4 groups of 6 each. Group 1 received saline (0.25 ml, i.p), Group 2 received standard (Diazepam 5 mg/kg, i.p), Group 3 received test1 (AEVTL 200mg/kg, i.p), Group 4 receives test2 (AEVTL 400mg/kg, i.p).

Each groups of animals were placed in the Actophotometer separately and number of “cut offs” were recorded for 10 minutes duration at the interval of 30 minutes, till the maximum effect of the drug was recorded.6

Screening of sedative activity using Rota rod apparatus
Mice weighing 20-25 gm were selected. They were divided in 4 groups of 6 each. Group 1 receives saline (0.25ml, i.p) Group 2 receives standard (Diazepam 5 mg/kg, i.p), Group 3 receives test1 (AEVTL 200mg/kg, i.p), Group 4 receives test2 (AEVTL 400mg/kg, i.p).

They were placed on rod rotating at 20 rpm after 30 minutes of drug administration. More than one mouse can be placed at a time on the rod which was divided into many sections. “Fall off time” was recorded when the mice fall from the rod for each mouse.6,7

Statistical analysis
All values were expressed as Mean± SEM for the all the models.

Data was analyzed by one way ANOVA followed by Dunnett’s t-test
The result was considered to be statistically significant when p<0.05.

RESULTS
Sedative activity of AEVTL in Actophotometer
The results obtained from this study revealed that aqueous extracts of *Vigna trilobata* (L.) verde. Leaves (AEVTL) significantly decreased the locomotor activity in mice (Table 1). The activity was found to be maximum for aqueous extract at a dose of 400 mg/kg (i.p.); and moderately significant at a dose of 200 mg/kg (i.p) (Figure 1) compared to that of control group. Whereas standard drug diazepam at a dose of 5 mg/kg (i.p.) produced more significantly decreased the locomotor activity in mice compared to that of control group as well as aqueous extract.

Sedative activity of AEVTL using rotarod apparatus
The data obtained from Rota rod test (Table 2) showed that aqueous extracts of *Vigna trilobata* (L.) verde. leaves at doses of 200 and 400 mg/kg (i.p) increased significantly the fall off time and reduced permanence on the bar. The activity was found to be maximum for aqueous extract at a dose of 400 mg/kg; and moderately significant at a dose of 200 mg/kg (Figure 2) compared to that of control group but less than that of standard drug. Whereas standard drug diazepam at a dose of 5mg/kg (i.p.) produced more significantly decreased fall off time due to sedative activity in mice compared to that of control group as well as aqueous extract.

DISCUSSION
This study has established the central nervous system depressant properties of *Vigna trilobata* (L.) Verdc. leaves. Increased locomotor activity is considered as an increase in alertness and decrease in locomotor activity indicated sedative effect. Aqueous extract of *Vigna trilobata* (L.) Verde. Leaves (AEVTL) decreased.
directed at characterizing the bioactive ingredients that sedative in traditional medicine. Further studies may be results of this study may justify the use of the leaves as sedative in traditional medicine. Further studies may be directed at characterizing the bioactive ingredients that are responsible for the observed activities in the plant.

**ACKNOWLEDGEMENT**
I am deeply indebted to my guide Dr. D. D. Santani whose help, suggestion and encouragement helped me in all the time. I am greatly thankful to the Principal of Rofel, Shri G. M. Bilakhia College of Pharmacy, Vapi, Gujarat, for providing necessary facilities. I am also acknowledging to Dr. Minoo H. Parabia, advisor of Shri R. M. Dhariwal Ayurved College and Research Centre, Waghaldhara, Valsad, Gujarat, for the authentication of the plant.

**REFERENCES**
8. Adeyemi OO, Yetmitan OK, Taiwo AE. Neurosedative and muscle relaxant activities of ethyl acetate extract of *Baphia nitida* AFZEL. J Ethnopharmacol 2006; 106: 312-316

**Table 1: Effect of AEVTL on spontaneous motor activity in mice (no. of cut off in actophotometer)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>0.15 ml</td>
<td>324.67±4.64</td>
<td>311.67±2.93</td>
<td>314.67±3.02</td>
<td>308.17±2.64</td>
<td>298.50±4.33</td>
</tr>
<tr>
<td>Standard</td>
<td>5 mg/kg</td>
<td>299.67±5.38</td>
<td>218.17±5.08</td>
<td>192.83±4.62</td>
<td>158.17±4.30</td>
<td>209.67±6.19</td>
</tr>
<tr>
<td>AELTVL (200)</td>
<td>200 mg/kg</td>
<td>311.33±4.26</td>
<td>283.50±4.11*</td>
<td>261.33±3.56</td>
<td>243.17±3.29</td>
<td>275.17±3.27</td>
</tr>
<tr>
<td>AELTVL (400)</td>
<td>400 mg/kg</td>
<td>310.17±4.89</td>
<td>260.83±3.16</td>
<td>229.83±4.12</td>
<td>202.50±4.64</td>
<td>223.67±5.16*</td>
</tr>
</tbody>
</table>

*ns= 6, All values are mean ± SEM; Statistical analysis by one-way ANOVA followed by Dunnett’s t’ test; ns-Non significant *p < 0.05, **p < 0.01, ***p<0.001 compared to control group.
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Table 2: Effect of AEVTL on fall off time in mice (fall of time in rotarod apparatus)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/ kg)</th>
<th>Fall off time (sec) (Mean± SEM)</th>
<th>% decrease in time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Control (saline)</td>
<td>0.15 ml</td>
<td>359.83±3.156</td>
<td>347.67±2.565</td>
</tr>
<tr>
<td>Standard (diazepam)</td>
<td>5 (mg/ kg)</td>
<td>349.67±3.073</td>
<td>135.83±5.659***</td>
</tr>
<tr>
<td>AEVTL (200)</td>
<td>200 (mg/ kg)</td>
<td>352.50±4.759</td>
<td>241.50±3.922**</td>
</tr>
<tr>
<td>AEVTL (400)</td>
<td>300 (mg/ kg)</td>
<td>340.83±3.005</td>
<td>193.50±4.433***</td>
</tr>
</tbody>
</table>

n= 6; Statistical analysis by one-way ANOVA followed by Dunett’s t-test; *p < 0.05, **p < 0.01, ***p < 0.001 compared to control group.

Figure 1: Effect of AEVTL on spontaneous motor activity in mice; n=6±SEM.

Figure 2: Effect of AEVTL on fall of time in mice; n=6±SEM.

Figure 3: Leaves of Vigna trilobata (L.) Verdc.

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