Some Neuropharmacological Effects of the Methanolic Root Extract of *Cissus Quadrangularis* in Mice

Viswanatha Swamy A.H.M.1* Thippleswamy A.H.M2, Manjula D.V3 and Mahendra Kumar C.B4

1 KLES College of Pharmacy, Hubli, 580 031, India
2 KLES College of Pharmacy, Belgum, 580 010, India
3 Taranatha Government Ayurvedic Medical College of, Bellary, India
4 Division of Pharmacology, Department of Pharmaceutical Sciences, Jadavpur University, Kolkata, 700032, India

ABSTRACT

In this study, some neuropharmacological effects of methanolic root extract of *Cissus quadrangularis* Linn. (*CQ*) belongs to family Vitaceae were studied in mice using various models. The *CQ* root extract significantly inhibited acetic acid-induced writhings and increased tail flick withdrawal response in mice. The effects of *CQ* on CNS were studied by using, spontaneous motor activity, exploratory behaviour, rota-rod performance and potentiation of pentobarbitone sleeping time in mice. Preliminary phytochemical evaluation and acute toxicity values were also carried out and LD$_{50}$ was found to be 1000 mg/kg by i p route. The extract (50,100 and 200 mg/kg i. p.) produced reduction in spontaneous motor activity, exploratory behaviour and motor coordination and prolonged pentobarbitone sleeping time. Preliminary qualitative chemical studies indicated the presence of triterpenoids, flavonols, sapononis, and alkaloids in the extract. The results suggest that the methanolic root extract of *CQ* contains some active principles, which may be sedative in nature. (Afr. J. Biomed. Res. 9: 69 – 75, May 2006)

Keywords: *Cissus quadrangularis*; Sedation; Spontaneous motor activity, Exploratory Behaviour, Motor coordination, Pentobarbitone sleeping time

*Address for Correspondence: *Address for correspondence: Dept. of Pharmacology and Toxicology, KLES College of Pharmacy, Hubli- 580 031, India. e-mail: vmhiremath2004@yahoo.com, Fax: +918362371694
INTRODUCTION

Cissus quadrangularis Linn (CQ) is a medicinal herb reputed to be of beneficial effect in the traditional system of medicine. The CQ is commonly called as Hajoda (Fam. Vitaceae) is one of the most widely used ingredients in alternative medicine (Ayurveda) for the treatment of piles, anorexia, indigestion, chronic ulcers, asthma, otorrhoea, wounds and in augmenting fracture healing process (Agarwal 1997 and Rajpal, 2002). The alcoholic extract of this plant has evaluated by Udupa et al reported to facilitate healing of fractured bones in albino rats (Udupa, 1965) by intramuscular administration. Phytochemical studies reveal the presence of known flavonols such as quercetin and kaempferol along with resveratrol, piceatannol, palildol, ascorbic acid, ketosteroid and carotene (Saburi, 1999 and Sen, 1966). Flavonoids are some of the widest spread phenolic compounds in the plant world and having a wide range of pharmacological effects. Best of our knowledge, studies on the CQ on central nervous system is not reported. The pilot studies indicated that root extracts CQ have role on CNS. On this view/basis, we investigated the activity of the methanolic extract of the CQ on analgesic activity, motor coordination, spontaneous motor activity; pentobarbital induced sleeping time and exploratory behavior in mice.

Preparation of plant extract

The root of the herb cissus quadrangularis was collected in the month of September and authentication of the plant was done by Dr. Ganesh. R. Hegde, Karnataka University, Dharwad, India. Root extracts were prepared by using methanol in a soxhlet apparatus according to the previously published method. The resultant extract was stored in a desiccator prior to use and it gave a mean yield of 0.76% w/w.

Phytochemical screening

The Preliminary phytochemical studies showes the presence of triterpenoides, flavonols, sapononis, and alkaloids in the extract and were tested using standard procedures.

Animals

The pharmacological experiments were conducted using Swiss Female albino mice weighing 20-25g. Animals were maintained under standard nutritional and environmental conditions of 50 ± 10 % RH and 12 h light and 12 h dark cycle throughout the experiment. The animals were used after an acclimatization period of at least 5 days to the laboratory environment and provided with standard food pellets and water ad libitum. The animals were deprived of food 24h before experimentation. The animal ethical committee clearance was obtained from the institution for the present study.

Acute toxicity test

Mice were divided into groups of ten each and CQ was injected i.p. in doses from 50 to 2000 mg/kg. Death within 24 h was recorded. The LD50 was estimated from the graph of percent mortality against log-dose of the extracts using the Miller and Tainter (1944) method.

Analgesic activity

Analgesic activity was measured against acetic acid induced writhing and tail flick painful stimuli method. The mice were divided into five groups of six each. Group first received normal saline (0.1 ml/10 g i. p.). Group second, third, and fourth received extract of CQ at doses of 50, 100 and 200 mg/kg, by i. p respectively. Group five received aspirin (200 mg/kg p o.). Treatment was given 30 minutes before to writhing were produced by injecting 1ml/100gm of 1% solution of acetic acid i. p to all groups. The writhing response was observed by the method of Turner (1965). The time of writhing and number of writhing in 15 min were noted. A reduction in the writhing numbers as compared to the group first was considered evidence for analgesia.

\[ \text{% inhibition} = \frac{W_C - W_T}{W_C} \times 100 \]

Where,

WC = Mean number of writhes in control group
WT = Number of writhes in test group

Tail flick test

To evaluate the central analgesic effects of methanolic root extract. Tail flick test was performed by time taken for mouse to withdraw the tail when immersed in water maintained at 55±0.5° C was measured (Turner, 1965). The animals were divided into five groups of six mice each. Group one received normal
saline (0.1 ml/10gm) and groups second, third and fourth received CQ extract 50, 100 and 200 mg/kg i.
p. respectively. Group five treated with pentazocine
(10 mg/kg, i. p.).

**Spontaneous motor activity (SMA)**
Spontaneous motor activity was performed using Actophotometer (Techno LE3806, India). Mice were
grouped of six each and treated with saline or the CQ extract (50,100 and 200 mg/Kg i.p.) or received
diazepam 1mg/kg i.p. Activity was automatically
recorded 30 min after treatment and at every 10 min. The experiments were repeated at an interval of 30
min, for a total of 120 min. Results of the treated
groups were compared with those of control group at
each time interval (Amos et al, 2001). SMA
measurements started 30 min after the administration
of the extract and the results were compared with
those of control.

**Exploratory behavioral pattern**
The study was carried out using wooded board
measuring 40x40cm with 16 evenly spaced holes
(Perez et al., 1998). Mice were grouped (n=6) and
treated with saline or extract (dose 50,100 and 200
mg/kg) or received diazepam 1 mg/kg i.p. Thirty
minutes after treatment the mice were placed singly on
an board with 16 evenly spaced holes and the number
of times the mice dipped their heads into the holes
during 5 min trial was counted. Results were
expressed as means for the various treatment groups
at different time intervals.

**Motor coordination**
Rota-rod (Techno, India) biological research apparatus
was used for the test. The instrument (a horizontal
rotation device) was set at a rate of 16 revolutions per
minute (Fujimori and Perez, 1998). Mice were placed
on the rod and those that were able to remain on the
rod longer than 3 min were selected for the study.
Group 1 was treated with saline, while group 2, 3 and
4 received the extract (50,100 and 200 mg/kg i.p.). The
group 5 received diazepam 1 mg/kg i.p. Mouse unable
to remain on the rod at least for three min was
considered as a positive test and the time of its fall was
recorded.

**Pentobarbital-sleeping time**

---

Albino mice were grouped of six each. They were
treated as follows: Group 1 received normal saline,
groups 2, 3 and 4 received the extract (dose 50,100
and 200 mg/kg). Animals were administered with
sodium pentobarbitone (40 mg/kg i.p.) 30 min later
and index of hypnotic effect recorded. The effects
were recorded as follows: Time elapsed between the
administrations of pentobarbital until loss of righting
reflex was recorded of as the onset of sleep, while the
time from the loss to its recovery was considered as
the duration of sleep (Ming-Chin Lu, 1998).

**RESULTS**

**Acute toxicity and general behavioral studies**
The LD$_{50}$ of the extract by i.p. route in mice was 1000
mg/kg. While conducting the toxicity studies animals
were observed continuously for any general behavioral
changes and significant reduction of spontaneous
 locomotor motility, drowsiness and remarkably quiet
were observed.

**Analgesic activity**
Analgesic activity was investigated by the acetic acid-
induced writhing test and tail flick test in mice. Results
of writhing studies in mice are presented in Fig.1. The
maximum writhes were produced by saline treated
mice. Extract of CQ (50,100, and 200 mg/kg, i.p.)
showed a significant dose-dependent reduction in the
number of writhing with approximately 44%, 61% and
84% of inhibition respectively. The maximum
inhibition was observed at the dose of 200 mg/kg,
which was statistically similar to the standard drug
aspirin (100mg/kg). The difference in tail flick
latency (sec) before and after treatment, in saline
treated group was 3.82±0.70 (Table 1). CQ
pretreatment induced dose dependent related changes
in tail-withdrawal latencies when compared to control
group. The maximum analgesic effect reached at 60
min after administration.

---

**Neuropharmacological effects Cissus Quadrangularis**
Neuropharmacological effects Cissus Quadrangularis

Fig. 1
Effect of Cissus quadrangularis (CQ) extract on glacial acetic acid-induced writhing in mice. Fig. 1a shows the number of writhing while Fig 1b shows the percentage inhibition compared with the control values are mean ± SME; n=6 in each group. **Significantly different at P<0.01.

Spontaneous motor activity
CQ produced significant decrease in the spontaneous motor activity in mice. This effect was dose dependent and the effect was observed within 30 min of drug administration and persisted for 120 min (Table 2).

Exploratory behavior pattern
On head dip test in mice treated with different doses of CQ (50, 100 and 200 mg/kg), there was a significant reduction in head dip responses when compared with control (Table 3).

Motor coordination
Results of motor coordination test are presented in Table 4. It was found that, the CQ exhibited a marked reduction in motor coordination in mice and mice were unable to hold on the rotating rod. This effect was dose-dependent and varied with time in mice.

Pentobarbitone induced sleeping time
Prior administration of CQ significantly potentiated pentobarbitone–induced sleeping time in mice. Various sleep time of mice treated with pentobarbitone with or without extract are shown in (Table 5).

Table 1: Effect of CQ extract on tail flick response in mice after immersion in 55°C water bath

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose mg/Kg</th>
<th>Mean reaction time (sec)</th>
<th>Reaction time in sec.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Control Vehicle</td>
<td>0.2ml</td>
<td>3.82 ± 0.70</td>
<td>3.85±0.68</td>
</tr>
<tr>
<td>Extract</td>
<td>50</td>
<td>3.68 ± 0.27</td>
<td>5.11±0.16*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4.82 ± 0.14</td>
<td>5.25±0.14*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>4.85 ± 0.12</td>
<td>5.60±0.20**</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>10</td>
<td>4.90 ± 0.20</td>
<td>6.21±0.13**</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.0418</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

Values are mean ± SME; n=6 in each group. *percentage inhibition when compared to control. **significantly different at P<0.05. ***significantly different at P<0.01.
### Table 2:
Effects of CQ extract on spontaneous motor activity in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug (mg/kg)</th>
<th>Experimental mean time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>Saline</td>
<td>412.50±8.61</td>
</tr>
<tr>
<td>CQ</td>
<td>50</td>
<td>410.00±8.23</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>428.73±6.03</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>438.35±7.58</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1</td>
<td>418.54±5.24</td>
</tr>
</tbody>
</table>


P >0.0518 <0.0001 <0.0001 <0.0001 <0.0001

Values are mean ± SME; n=6 in each group. * significantly different at P<0.05. ** significantly different at P<0.01.

### Table 3:
Effects of CQ extract root on exploratory behavior (head dip test) in mice

<table>
<thead>
<tr>
<th>Treatment (i.p.)</th>
<th>Drug (mg/kg)</th>
<th>Pre-dose</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline 0.1ml/100g</td>
<td>69.16±2.18</td>
<td>67.00±2.89</td>
<td>66.50±2.94</td>
</tr>
<tr>
<td>CQ</td>
<td>50</td>
<td>64.50±2.26ns</td>
<td>42.06±2.83**</td>
<td>28.16±3.61**</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>72.22±3.68ns</td>
<td>36.52±4.50**</td>
<td>18.33±5.42**</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>66.63±6.32ns</td>
<td>24.32±5.68**</td>
<td>13.30±7.42**</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1</td>
<td>63.24±9.90ns</td>
<td>18.66±4.32**</td>
<td>6.21±0.56**</td>
</tr>
</tbody>
</table>


P 0.803 0.0001 0.0001

Values are mean ± SME; n=6 in each group; * significantly different at P<0.05.; ** significantly different at P<0.01.

### Table 4: Effect of CQ extract on motor coordination in mice

<table>
<thead>
<tr>
<th>Treatment (i.p.)</th>
<th>dose (mg/kg)</th>
<th>Experimental mean time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>Saline</td>
<td>195.83±5.32</td>
</tr>
<tr>
<td>CQ</td>
<td>50</td>
<td>197.83±4.23ns</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>196.00±3.96ns</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>196.92±5.22ns</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1</td>
<td>198.28±4.52ns</td>
</tr>
</tbody>
</table>


P 0.9943 0.0001 0.0001 0.0001 0.0001

Values are mean ± SME; n=6 in each group; * significantly different at P<0.05.; ** significantly different at P<0.01.
Table 5:
Effects of CQ extract on pentobarbitone-induced sleeping time in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug (mg/kg)</th>
<th>Onset of sleep (min)</th>
<th>Duration of sleep (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentobarbitone</td>
<td>40</td>
<td>3.47 ± 0.36</td>
<td>39.12 ± 0.33</td>
</tr>
<tr>
<td>CQ</td>
<td>50</td>
<td>2.50 ± 0.22*</td>
<td>67.66 ± 0.47**</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.16 ± 0.16**</td>
<td>70.66 ± 0.49**</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>2.06 ± 0.12**</td>
<td>87.83 ± 0.55**</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.00 ± 0.25**</td>
<td>93.33 ± 0.66**</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.0009</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Values are mean ± SME; n=6 in each group; *duration of sleeping when compared to control. **significantly different at P<0.05. ***significantly different at P<0.01.

The normal sleeping time was found to be 39 min in mice treated with pentobarbitone alone. Prior administration of CQ significantly potentiated onset of action and duration of action of pentobarbitone – induced sleeping time in mice. The maximum duration of sleeping was observed at a dose of 200 mg/kg of CQ and was approximately 87%.

**DISCUSSIONS**

The present study reports some neuropharmacological activities of methanolic root extract of *Cissus quadrangularis* in mice. Results indicated that the CQ significantly reduced acetic acid induced writhings in mice and increased in tail flick withdrawal response. The dose-dependent inhibition of acetic acid induced writhing indicated a peripheral effect, which was more potent than aspirin. Tail flick analgesic testing is usually considered suitable for centrally acting analgesic though clear cut dose response relationship was observed. The efficacy of the most herbal remedies is attributed to various active principles in combination. The extract was found to produced alteration in general behavior pattern, significant reduction of spontaneous motor motility, exploratory behavior pattern, motor coordination and potentiation of pentobarbitone induced sleeping time in a dose-dependent fashion. The present findings suggest that CQ possesses CNS-depressant action. The extract significantly reduced spontaneous motor activity. The activity is a measure of the level of excitability of the CNS (Mansur, et al, 1971) and this decrease may be closely related to sedation resulting from depression of the central nervous system (Ozturk, et al, 1996). The CQ root extract possessed central nervous system depressant activity as indicated by the decrease in exploratory behavior [Adzu, 2002] in mice as demonstrated by the reduction of the head-dip test. It also showed a marked sedative effect as indicated by the reduction in gross behavior and potentiation of pentobarbitone induced sleeping time. Earlier studies have related prolongation of barbital hypnosis to pentobarbital metabolic inhibition or action on the CNS involved in the regulation of sleep (Kaul and Kulkarni, 1978).

It is generally accepted that the sedative effect of drugs can be evaluated by measurement of spontaneous motor activity and pentobarbitone induced sleeping time in laboratory animal model (Ming-chin, 1998). These results corroborate those of (Fujimori 1995) who proposed that the enhancement of barbital hypnosis is a good index of CNS depressant activity. Results of the exploratory behavior test (table) further support the neurosedative activity and its possible application in anxiety condition (Amos 2001). Present findings of analgesic activity are similar to those reported for pentozocin and aspirin (Distasi et al, 1988). It has been reported that the saponins show a
potent sedative activity when tested in similar models and also inhibit spontaneous motor activity in mice (Dubois, et al, 1986). Therefore, the saponin content of this extract might be contributing in part to the experimental pharmacological effects. Further studies are planned to establish mechanism of CNS-depressant action of CQ by using various agonists and antagonists.

REFERENCES

Rajpal, V. (2002): Standardization of Botanicals. 1; 77-81 Easter Publishers, New Delhi, India.