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Full Length Research Article

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

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ABSTRACT

In this study, some neuropharmacological effects of methnolic 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, flovonols, 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, Pentobarbital sleeping time*

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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, pallidol, 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 Univercity, Dharwad, India. Root extracts were prepared by using methanol in a sohxlet 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, flovonols, 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 LD_{50} 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.

% 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

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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).

Statistical analysis

All the data obtained were expressed as mean ± standard error. Differences in means were estimated by means of ANOVA followed by Dunnet's post hoc test. Results were considered significant at $P < 0.05$.

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.

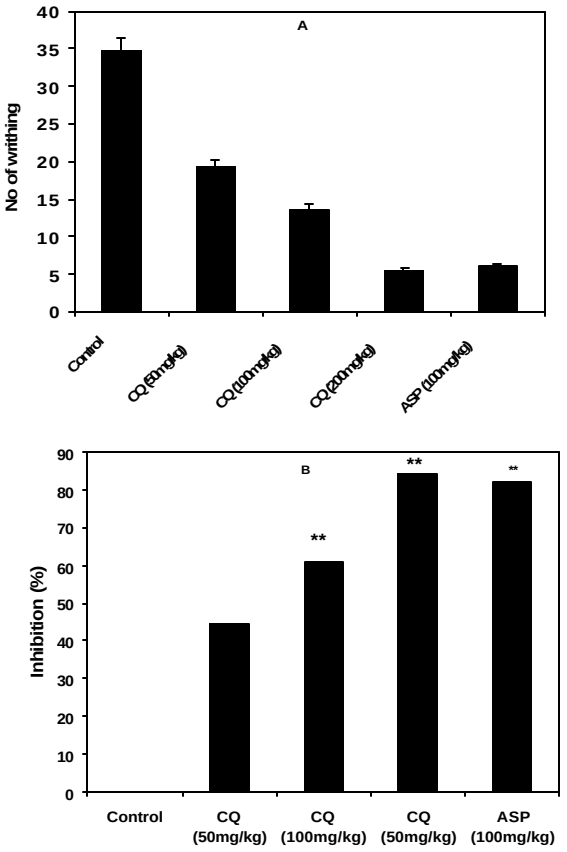

Fig. 1

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 penotobarbitone 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

Drug	Dose mg/Kg	Mean reaction time (sec)	Reaction time in sec.		
			15 min	30 min	60 min
Control Vehicle	0.2ml	3.82 ± 0.70	3.85±0.68	4.16±0.30	3.93±0.82
Extract	50	3.68 ± 0.27	5.11±0.16*	5.76±0.25	8.43±0.28**
	100	4.82 ± 0.14	5.25±0.14*	8.15±0.11**	9.10±0.65**
	200	4.85 ± 0.12	5.60±0.20**	9.03±0.26**	9.66±0.71**
Pentazocine	10	4.90 ± 0.20	6.21±0.13**	9.52±0.86**	9.83±0.94**
	F=	2..910 (4,25)	6.672 (4,25)	26.847 (4,25)	11.657 (4,25)
	P	0.0418	0.0008	0.0001	0.0001

Values are mean ± SME; n=6 in each group. [1]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

Treatment	Drug (mg/kg)	Experimental mean time (min)				
		0	**30**	**60**	**90**	**120**
Control	Saline	412.50±8.61	382.53**±5.42	168.63±6.17	196.16±7.16	246.25±8.53
CQ	50	410.00±8.23	96.30**±8.52	78.12**±9.36	81.20**±5.56	73.32**±5.16
	100	428.73±6.03	66.53**±4.81	46.52**±8.92	32.29**±3.93	58.23**±3.16
	200	438.35±7.58	38.23**±3.75	22.00**±3.63	18.19**±2.36	17.50**±3.22
Diazepam	1	418.54±5.24	32.20**±3.56	19.96**±6.73	16.63**±8.57	9.50**±6.33
F=		2.730(4,25)	713.65(4,25)	71.619(4,25)	162.69(4,25)	289.65(4,25)
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

Treatment(i.p.)	**Drug(mg/kg)**	**Mean head-dips in 3 min**		
		Pre-dose	**30 min**	**60 min**
Control	Saline 0.1ml/100g	69.16±2.18	67.00±2.89	66.50±2.94
CQ	50	64.50±2.26ns	42.06±2.83**	28.16±3.61**
	100	72.22±3.68ns	36.52±4.50**	18.33±5.42**
	200	66.63±6.32ns	24.32±5.68**	13.30±7.42**
Diazepam	1	63.24±9.90ns	18.66±4.32**	6.21±0.56**
F=		0.4054 (4,25)	20.284 (4,25)	26.492 (4,25)
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

Treatment (i.p.)	**dose (mg/kg)**	**Experimental mean time (min)**				
		0	30	60	90	120
Control	Saline	195.83±5.32	197.13±6.33	196.45±6.43	197.42±5.46	197.84±7.35
CQ	50	197.83±4.23ns	78.08±4.31**	82.50±2.40**	125.16±3.72**	148.82±2.94**
	100	196.00±3.96ns	66.62±3.01**	70.54±4.28**	119.18±5.42**	132.30±6.21**
	200	196.92±5.22ns	48.05±3.72**	64.16±4.16**	118.93±6.83**	116.68±8.59**
Diazepam	1	198.28±4.52ns	36.26±5.42**	49.73±5.82**	96.32±4.40**	94.36±6.33**
F=		0.05361 (4,25)	188.19 (4,25)	148.54 (4,25)	53.341 (4,25)	35.389 (4,25)
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

Treatment	Drug(mg/kg)	Onset of sleep(min)	Duration of sleep[1] (min)
Pentobarbitone	40	3.47 ± 0.36	39.12 ± 0.33
CQ	50	2.50 ± 0.22*	67.66 ± 0.47**
	100	2.16 ± 0.16**	70.66 ± 0.49**
	200	2.06 ± 0.12**	87.83 ± 0.55**
	1	2.00 ±0.25**	93.33 ±0.66**
Diazepam			
F=		6.599 (4,25)	1726.8 (4,25)
P		0.0009	0.0001

values are mean ± SME; n=6 in each group; [1]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.

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