



Anticonvulsant and narcotic properties of stems of *Cissus quadrangularis* in mice

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Abstract

Cissus quadrangularis Linn grows in Savannah areas in Africa (Cameroon, Mali, Mauritania, Senegal, etc). In traditional medicine, the plant is used to treat anorexia, asthma, sickle cells, colds, pains, malaria, asthma and as an analgesic. In vivo animal models of epilepsy (maximal electroshock, n-methyl -d-aspartate, pentylenetetrazol, isonicotinic hydrazid acid and strychnine -induced convulsions or turning behavior) and insomnia (diazepam -induced sleep) were used. The aqueous extract of the stems of *C. quadrangularis* strongly increased the total sleep time induced by diazepam (50 mg/kg i.p.). It also protected mice against maximal electroshock, pentylenetetrazol, strychnine and n-methyl-d-aspartate-induced seizures or turning behavior and delayed the onset time of seizures induced by isonicotinic hydrazid acid. The results lead to the conclusion that the extract of *C. quadrangularis* possesses anticonvulsant and sedative properties in mice and could explain its use in traditional medicine in Africa, in the treatment of insomnia and epilepsy.

Keywords: Traditional medicine, plant, extract, seizures, *Cissus quadrangularis*

INTRODUCTION

Traditional medicine in many areas of the world relies on the use of a wide variety of plant species. In Africa, phytotherapy still plays an important role in the management of diseases, mainly among populations with very low income (Geoffrey and Kirby, 1996). *Cissus quadrangularis* Linn (Vitaceae) (*C. quadrangularis*) originated from India and Malaysia grows in Savannah areas in Africa (Cameroon, Mali, Mauritania, Senegal, Somalia and Chad) (Arbonier, 2000; Dumas-Champion, 1997). In traditional medicine, the plant is used to treat haemorrhoids, anorexia, indigestion, and asthma, (Rajpal, 2002)

In sahelian areas particularly, *C. quadrangularis* is used in the treatment of sickle cells, syphilis, gonorrhoea, fractures, colds, pains, malaria, abscess, asthma and as an analgesic (Arbonier, 2000). The plant is also used in Cameroon to treat epilepsy (personal communications). Chemical studies showed the presence of sterols, steroids, tannins, flavonoïds, carotenes, ascorbic acid, linoleic acid in *C. quadrangularis* (Murthy et al., 2003; Saburi, 1999; Sen, 1966). Pharmacological studies of fresh leaves and roots showed that *C. quadrangularis* possesses antioxidants, antibacterial, analgesic and neurosedative activities (Amos 2001, Murthy et al., 2003; Viswanatha et al., 2006). Subchronic toxicity study for three months suggested that *C. quadrangularis* administered per os at a dose of 3 g/kg is not toxic (Attawish et al., 2002) . Studies on some other plants claimed to cure insomnia and epilepsy showed that *Cyperus articulatus*, *Mimosa pudica*, *Passiflora edulis*, *Sporospermum febrifugum*, *Balanites aegyptiaca* possess sedative and anticonvulsant properties (Bum et al., 2001; 2003; 2004a;

Abbreviations: D-2-amino-7-phosphonoheptanoate (D-AP7), Maximal Electroshock (MES), Isonicotinic hydrazide acid (INH) N-Mmethyl-D-Aspartate (NMDA), *Cissus quadrangularis* (*C. quadrangularis*), Strychnine (STR), Pentylenetetrazol (PTZ).

2004b; 2005a; 2005; 2006; 2007; Rakotonirina et al., 2001). The aim of the present study was to look for the anticonvulsant and sedative properties of *C. quadrangularis* used in traditional medicine in Cameroon to treat epilepsy.

MATERIALS AND METHODS

Animals

Adult male mice (*Mus musculus* Swiss, 22 ± 2 g, 6 per group) were used for this study. The animals were housed in standard cages, at 25°C, on a 12/12 h light-dark cycle. They were supplied with food and water ad libitum. Mice were divided in 6 groups of 6 mice: One negative control group received distilled water; one positive control group received appropriate substance and four groups received the plant extract. Drugs were administered intraperitoneally, in a volume of 10 ml/kg of body weight, except n-methyl-d-aspartate (subcutaneous injection) and diazepam in the isonicotinic hydrazide acid test (per os). The study was conducted in accordance with the nationally and internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication #85-23, revised in 1985).

Plant material

The plant specimens of *C. quadrangularis* used were collected in Cameroon in the vicinity of Ngaoundéré in the dry season (November 2006). A voucher specimen of the plant was authenticated at the National Herbarium of Cameroon in Yaoundé by a number 36966 HNC.

Preparation of the aqueous extract

The dried stems of *C. quadrangularis* were ground. The powder (250 g) was macerated for 3 days in 2.5 l of distilled water at room temperature. The mixture was filtrated with a Watman n°1 filter paper and the filtrate was evaporated using a Rota vapor at a temperature of 70°C. The quantity of extract obtained after evaporation was 16 g that represent a 6.4% yield.

Extracts were administered per os 1 h before the test. The following doses were used: 100, 200, 500, 1000 and 2000 mg/kg.

Preparation of some solutions

75 mg of n-methyl-d-aspartate were dissolved in 10 ml of distilled water. The solution was used to induce turning behaviour in mice. 2.5 mg of strychnine dissolved in 10 ml of distilled water were used to induce convulsions. 70 mg of pentylene tetrazole and 250 mg of isonicotinic hydrazide acid were put in 10 and 10 ml of distilled water respectively and were use to induce convulsions in each case.

Pharmacological tests

Anticonvulsant tests

Maximal electroshock (MES) test: With a Rodent Shocker from HARVARD, tonic convulsions of the hind extremities of mice were induced by passing alternating electrical current (50 Hz, 30 mA, 0.2 s) through eyes electrodes (Ngo Bum et al., 2001, 2004a). For each experiment one group served as a negative control (distilled water) and one group as a positive control (diazepam, 5 mg/kg i.p.). The

number of animals protected from tonic hind limb extension was determined in each dose group.

N-methyl-D-aspartate (NMDA) test

Mice were injected subcutaneously (s.c.) with NMDA, 75 mg/kg, 1 h after administration of the extract. They were observed for 30 min. Animals that did not exhibit turning behaviour within the 30 min of observation period were declared protected. Turning behaviour was characterised by two consecutive 360° cycles fulfilled by the same animal (Schmutz et al., 1990). The positive control group received 33 mol/kg of D-AP7 (Croucher et al., 1982; Ngo Bum et al., 2004b).

Strychnine (STR) test

STR convulsions followed by death were induced in male mice by the i.p. injection of 2.5 mg/kg STR nitrate. A protective effect of the extract given i.p. 1 h prior to STR was recorded and compared to the groups treated with the extract (Ngo Bum et al., 2001; Ngo Bum et al., 2002). Animals that survived more than 10 min were qualified protected.

Pentylene tetrazol (PTZ) test

Clonic seizures were induced in male mice by the i.p. injection of 70 mg/kg PTZ (Ngo Bum et al., 2001). The protective effect of the plant was recorded in the mice treated 1 h before with the extract. The positive control group received 0.1 mg/kg of clonazepam.

Isonicotinic hydrazide acid (INH) test

Animals were injected i.p. with INH 250 mg/kg (Bernasconi et al., 1988) 1 h after the administration of the extract and the time to onset of clonic or tonic seizures was recorded. Data of the control group were compared to data of the groups treated with the extract. The positive control group received diazepam, 10 mg/kg (per os).

Diazepam-induced sleep in mice

The method described by Beretz et al. (1978) and modified by Rakotonirina et al. (2001) was used. Sleep potentiating effects of the plant was studied in the mice that received diazepam at a dose of (50 mg/kg) 1 hour after the extract and distilled water administration. The time between the loss of the straightening reflex and the regain of this reflex measured the sleeping time. The loss or the regain of the straightening reflex was measured by stimulating the external ear. When the mouse anterior paw does not move after stimulation with horsehair, the animal is sleeping. When the mouse is awaked, it moves its paw.

Statistical analysis

For the latency of the onset of sleep and the sleeping time, the mean values of the control groups were compared to the mean values of the groups treated with the decoction using the correction for multiple t-test by Bonferroni method. The Fisher exact test (two-tail) was used to compare percentage of protected mice in each case. $P < 0.05$ was considered significant.

Chemicals

Clonazepam, D- 2-amino-7-phosphonoheptanoate, diazepam, Isonicotinic acid hydrazide, N- methyl D- aspartate, pentylene te-

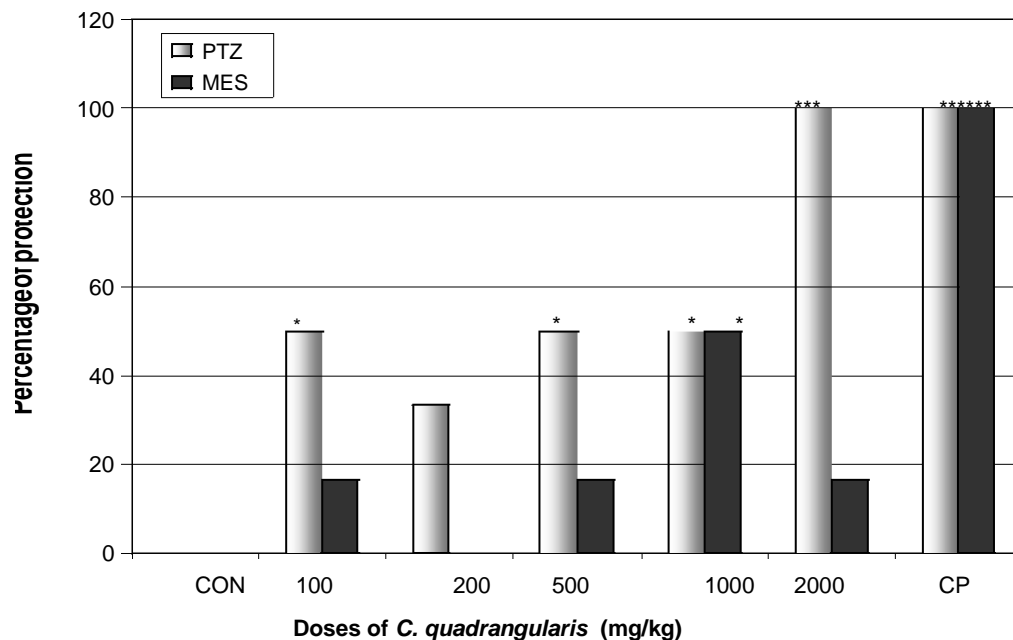


Figure 1. Effect of *C. quadrangularis* on PTZ- and MES- induced seizures in mice. Histograms represent the percentage of protected animals. The percentage of protection of CON was compared to the percentage of protection of the groups treated with the extract and CP. N = 6 per dose, * = $p < 0.05$, *** = $p < 0.001$ (Fisher exact test: two tail). CON = distilled water. CP = clonazepam 0.1 mg/kg in PTZ test and diazepam 5 mg/kg in MES test.

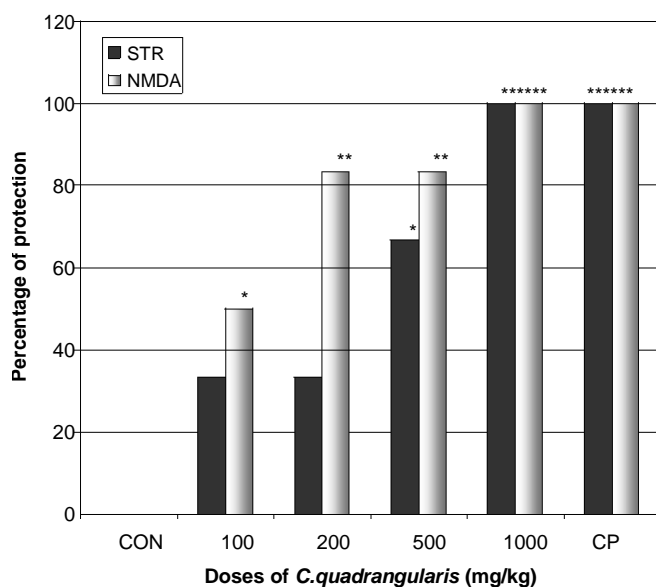


Figure 2. Effect of *C. quadrangularis* on STR- and NMDA-induced seizures and turning behavior in mice. Figure 2 shows the percentage of protected animals. The percentage of protection of CON was compared to the percentage of protection of the groups treated with the extract and CP. N = 6 per dose, * = $p < 0.05$, ** = $p < 0.01$, **** = $p < 0.001$ (Fisher exact test: two tail). CON = distilled water. CP = clonazepam 3 mg/kg in STR test and D-2-amino7-phosphonoheptanoate 33 M/kg in NMDA test

RESULTS

Effect of *C. quadrangularis* on PTZ-induced seizures

The extract of *C. quadrangularis* protected 50% of mice at doses of 100, 500 and 1000 mg/kg. Dose 2000 mg/kg of the extract of *C. quadrangularis* totally prevented mice against PTZ- induced seizures ($p \leq 0.001$). Clonazepam, a known anticonvulsant compound also protected 100% of mice against PTZ- induced seizures ($p \leq 0.001$) (Figure 1).

Effect of *C. quadrangularis* on MES- induced seizures

The anticonvulsant compound diazepam completely protected mice against MES-induced seizures ($p < 0.001$). The extract of *C. quadrangularis* had a moderated effect by protecting 50% of mice at a dose of 1000 mg/kg (Figure 1).

Effect of *C. quadrangularis* on NMDA-induced turning behavior

The extract of *C. quadrangularis* dose dependently and significantly antagonized NMDA- induced turning behavior in mice. 50, 83.3 and 83.3% of mice were protected at the doses of 100, 200 and 500 mg/kg respectively. Animals were completely protected both by the extract and by D-AP7 (dose 1000 mg/kg and D-AP7, $p < 0.001$), (Figure 2).

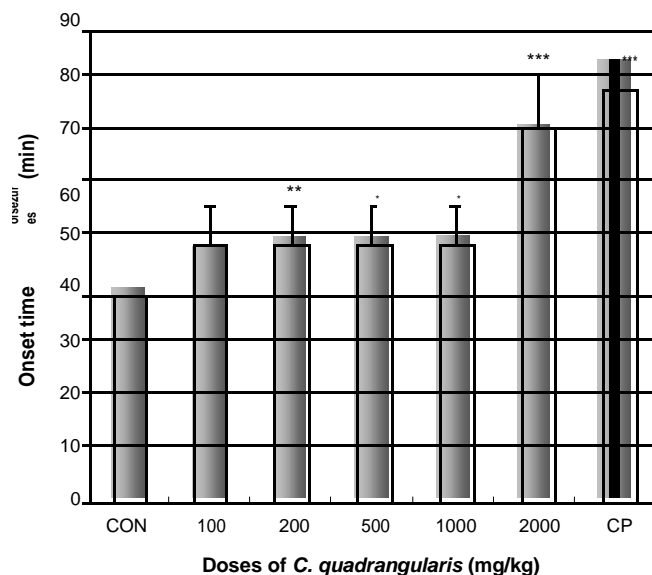


Figure 3. Effect of *C. quadrangularis* on INH-induced seizures in mice. The figure shows the latency time (min) of seizures induced by INH in the presence of different doses of the extract in mice. Histograms are expressed as mean + S.E.M. The mean value of CON was compared to the mean values of the groups treated with the extract and CP. N = 6 per dose, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ (Correction for multiple t-test by Bonferroni method). CON = distilled water, CP = diazepam 10 mg/kg.

Effect of *C. quadrangularis* on STR-induced seizures and exitus

Clonazepam, an anticonvulsant compound showed total protection against STR-induced seizures and exitus ($p < 0.001$). In the same way, *C. quadrangularis* extract at a dose of 1000 mg/kg totally protected mice against STR-induced seizures and exitus ($p < 0.001$). The doses 200 and 500 mg/kg protected 33 and 66% of mice (Figure 2).

Effect of *C. quadrangularis* on INH-induced seizures

The time to the onset of seizures was significantly increased in the presence of the extract from 39 ± 2 min in the control group to 70 ± 11 min in the group tested with the extract at a dose of 2000 mg/kg. In the presence of diazepam, seizures appeared after 76 ± 5 min. (Figure 3).

Effect of *C. quadrangularis* on diazepam-induced sleep

The extract of *C. quadrangularis* strongly potentiated in a dose-dependent manner the sleeping time induced by diazepam: from 21 ± 8 min in the control group to 309 ± 59 min in the group treated with the dose 1000 mg/kg of the extract (5 to 10 times the sleeping time of the control

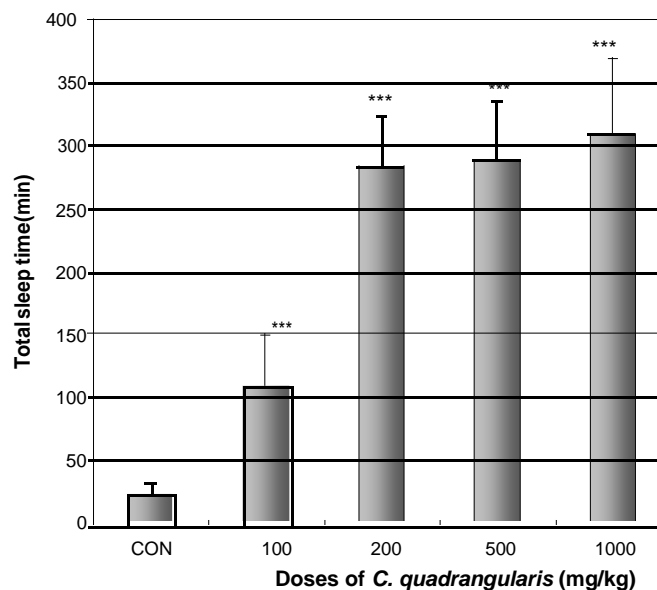


Figure 4. Effect of *C. quadrangularis* on diazepam-induced sleep in mice: Total sleep time. Figure 4 shows the total sleep time (min) induced by diazepam in the presence of different doses of the extract in mice. Histograms are expressed as mean + S.E.M. The mean value of CON was compared to the mean values of the groups treated with the extract. N = 6 per dose, *** = $p < 0.001$ (Correction for multiple t-test by Bonferroni method). CON = distilled water

group) ($p < 0.001$ from dose 100 mg/kg) (Figure 4). The extract also decreased the latency time to sleep from 4.7 ± 0.8 min in the control group to 1.8 ± 0.5 min in the group of mice treated with 1000 mg/kg of the extract (Figure 5).

DISCUSSION AND CONCLUSIONS

The extract of *C. quadrangularis* completely antagonized both NMDA-induced turning behavior and STR-induced seizures in mice. Given the involvement of the NMDA receptor complex in epileptic and epileptiform activity in vivo (Löscher and Hönack, 1991; De Sarro and De Sarro, 1993; Ngo Bum et al., 1996) and since excitatory amino acid antagonists acting at the NMDA or non-NMDA receptor have been shown to possess anticonvulsant and antiepileptic properties in several animal models of epilepsy (Davies et al., 1986; Croucher and Bradford, 1991; Meldrum, 1992; Rogawski, 1992; Löscher, 1993), it can be suggested that the extract of *C. quadrangularis* possesses anticonvulsant properties. The inhibition by the extract of *C. quadrangularis* of STR-induced seizures suggests the presence of anticonvulsant properties (Fisher, 1989; Rogawski, 1992) and the involvement of glycine receptors (Findlay et al., 2002; Löscher, 1993). *C. quadrangularis* also significantly protected mice against PTZ-induced seizures in mice. Since PTZ has been shown to interact with the GABA neurotransmission

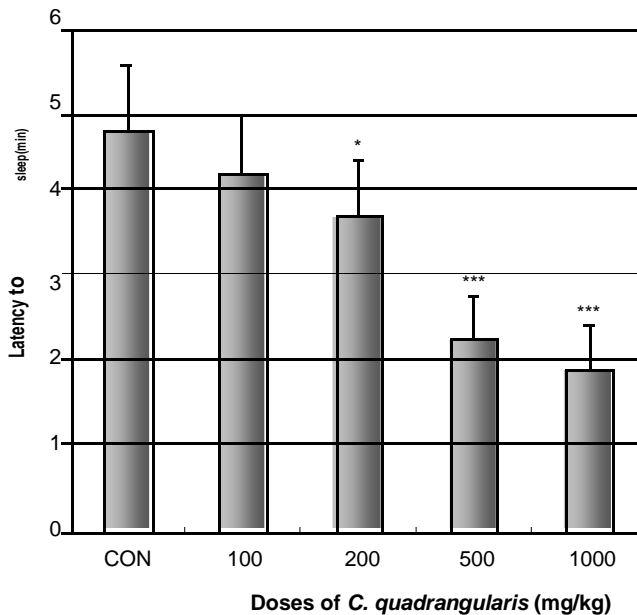


Figure 5. Effect of *C. quadrangularis* on diazepam-induced sleep in mice: Onset to sleep. Figure 5 shows the onset time of sleep (min) induced by diazepam in the presence of different doses of the extract in mice. Histograms are expressed as mean + S.E.M. The mean value of CON was compared to the mean values of the groups treated with the extract. N = 6 per dose, * = $p < 0.05$, *** = $p < 0.001$ (Correction for multiple t-test by Bonferroni method). CON = distilled water.

(Löscher and Schmidt, 1988; De Deyn et al., 1992), the antagonism of PTZ- induced seizures suggests the interaction of the extracts of *C. quadrangularis* with the GABA-ergic neurotransmission. The extract of *C. quadrangularis* possesses moderate effect against MES-induced seizures. The PTZ test is assumed to identify anticonvulsant drugs effective against generalized clonic seizures (Löscher and Schmidt, 1988; De Deyn et al., 1992; Kupferberg and Schmutz, 1998). The effect of the extract in the PTZ test could therefore suggest anticonvulsant efficacy against the above mentioned seizures types in man.

The increase of the latency time to the onset of seizures in INH test suggests the presence of anticonvulsant properties of this plant. In addition, extract of *C. quadrangularis* strongly increased the total sleep time induced by diazepam and precipitated the sleep onset. The potentiation of the sleep time suggests the presence of sedative properties in the extract of *C. quadrangularis* (Rakotonirina et al., 2001; Ngo Bum et al., 2004a; 2007). The sedative properties of *C. quadrangularis* could be related to the presence of some components in the extract activating the benzodiazepine and/or GABA receptors in the GABA receptor complex (Rang et al., 1999). In conclusion *C. quadrangularis* possesses sedative and anticonvulsant properties in mice. These properties could be mediated by several compounds present in

the extract and could explain the use of this plant in traditional medicine in Cameroon in the treatment of insomnia and epilepsy.

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