Effect of the decoction of rhizomes of *Cyperus articulatus* on bicuculline-, N-methyl-D-aspartate- and strychnine-induced behavioural excitation and convulsions in mice

Elisabeth Ngo BUM[1]*, Célestin GWA[1], Fidèle NTCHAPDA[1], Nyemb NYUNAÏ[1], Sélestin SOKENG[1], Vincent Silvère RAKOTONIRINA[2], Alice RAKOTONIRINA[3]

[1]*Department of Biological Sciences, Faculty of Sciences, B.P. 565 University of Ngaoundéré, Cameroon*
[2]*Laboratory of Animal Physiology, Faculty of Sciences, University of Yaoundé I, B.P. 812 Yaoundé, Cameroon*
[3]*Laboratory of Cellular Electrophysiology, Ecole Normale Supérieure, University of Yaoundé I, Yaoundé, Cameroon*

ABSTRACT

The decoction of the rhizomes of *Cyperus articulatus* is empirically used in several African countries in the treatment of a wide variety of human diseases. Some of the diseases treated with the decoction of rhizomes of *Cyperus articulatus* affect the nervous system (e.g. migraine, headaches, and epilepsy). Studies conducted in mice showed that the decoction of rhizomes of *Cyperus articulatus* possesses central activities. It antagonised strychnine-induced convulsions and death. 70% of mice treated with the decoction *of Cyperus articulatus* were protected against strychnine-induced convulsions and death, while the protection provided by phenobarbital was 100%. Mice treated with the decoction were also protected against N-methyl-D-aspartate- and bicuculline-induced behavioural stimulation. The antagonism of the decoction of *Cyperus articulatus* on strychnine, N-methyl-D-aspartate- and bicuculline-induced behavioural stimulation and convulsions suggests the presence of anticonvulsant properties in this extract. These anticonvulsant properties explain at least part of the therapeutic efficiency claimed for this plant in traditional medicine.

Keywords: *Cyperus articulatus*; Behavioural stimulation, decoction

RESUMÉ

La decoction des rhizomes de *Cyperus articulatus* est empiriquement utilisée dans plusieurs pays d'Afrique pour le traitement d'une grande variété de maladies chez les hommes. Certaines de ces maladies concernent le système nerveux central (migraine, maux de tête, epilepsie). Les études faites chez les souris ont montré que la décoction des rhizomes de *Cyperus articulatus* possède des effets centraux. Cette décoction antagonise les convulsions et la mort induites par la strychnine. 70% des souris traitées par la décoction de *Cyperus articulatus* sont protégées contre les effets de la strychnine pendant que le phénobarbital protège 100% des souris. Les souris traitées par la décoction sont aussi protégées contre la stimulation comportementale induite par le N-methyl-D-aspartate et la bicuculline. L'antagonisme de la décoction de *Cyperus articulatus* sur la stimulation comportementale et les convulsions induites par la strychnine, le N-methyl-D-aspartate et la bicuculline suggere la presence des propriétés anticonvulsivantes dans cet extrait. Ces propriétés anticonvulsivantes expliquent, au moins en partie, l'utilisation therapeutique de cette plante en médicine traditionnelle.

Mot clés: cyperus articulatus, stimulation comportementale, décoction.

*Corresponding author Tel.: +237 251139
Fax: +237 251139 or +237 252767
E-mail address: boum@cna-assur.com (E. Ngo Bum) and eli_bum@yahoo.fr

1. Introduction

Rhizomes of *Cyperus articulatus* L. (Cyperaceae) are used in many countries in Africa (Cameroon, Central African Republic, Gabon, and Senegal) and in Amazonia as a traditional medicine for the treatment of headaches, migraines, fever, stomach-aches, malaria, etc (Dalziel, 1937; Bouquet, 1969; Adjanohoun et al., 1984; Burkill, 1985; Schultes and Raffaud, 1990). As some of the diseases treated with *Cyperus articulatus* affect the nervous system, some pharmacological works have been done to define its interaction with this system. These studies showed that the decoction of rhizomes of *Cyperus articulatus* reduce spontaneous motor activity in mice. The decoction also showed some sedative properties as it facilitated and prolonged diazepam- or sodium thiopental-induced sleep in mice. In the same studies, this decoction did not show any muscle relaxant or analgesic activity. Phytochemical characterisation of these rhizomes showed the presence of flavonoids, saponins, sugars, triterpenes, polyuronides (Rakotonirina et al., 2001). Other studies showed that the decoction of the rhizomes of *Cyperus articulatus* could inhibit the spontaneous epileptiform discharges of the rat cortical wegde initiated in the free magnesium artificial cerebro-spinal fluid (aCSF-Mg^{2+}). This decoction inhibited N-Mehtyl-D-Aspartate (NMDA) but not α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA)-induced depolarisations in the same rat cortical wedge preparation (Ngo Bum et al., 1996). According to a personal communication, the rhizomes *of Cyperus articulatus* are used to treat epilepsy. Since NMDA receptor antagonists have anticonvulsant properties (Schmutz et al., 1990; Croucher and Bradford, 1991; Löscher et al., 1993), and given the fact that the methanolic decoction of *Cyperus articulatus* possessed anticonvulsant properties (Ngo Bum et al., 2001), it was decided to test the purported antiepileptic effects of the decoction of the rhizomes of *Cyperus articulatus* in NMDA-, strychnine- and bicuculline-induced behavioral stimulation or convulsions in mice.

2. Experimental

2.1. Plant material

The rhizomes of *Cyperus articulatus* used in this study were collected in the dry season (June 1996) in the vicinity of Douala, Cameroon. A voucher specimen (Rakotonirina 002, reference 1256 / HNC) was deposited in the National Herbarium of Cameroon. The rhizomes were washed, dried at room temperature (about 25 °C) in the laboratory for about 30 days and then ground in a mill to a grain size of < 1 mm.

2.2. Preparation of the total extract

Ten grams of the powdered plant material were added to 100 ml of distilled water. After 18 h of maceration at room temperature, the mixture was boiled for 10 min and centrifuged (1160 × g, 15 min) after cooling. The supernatant (dry residue: 0.1 g/ml, corresponding to an 8 % yield) was the decoction used in this work.

2.3. Animals and treatments

Swiss mice weighing 23 ± 3 g were used. They were housed in standardised environmental conditions and fed with standard food for rodents (Laboratoire National Vétérinaire, Garoua, Cameroon) and water ad libitum. Treatments were administered intraperitoneally (exempt NMDA subcutaneously) in a volume of 20 ml/kg. For the decoction the dose (2 g/kg) that had shown good effect in previous tests was used (Rakotonorina et al., 2001).

2.3.1. N-methyl-D-aspartate (NMDA)-induced "turning behaviour"

Mice were injected subcutaneously (s.c.) with NMDA, 75 mg/kg, 30 min after administration of the extract. They were observed for 30 min. Animals that did not present "turning behaviour" within the 30-min observation period were declared protected. Turning behaviour was characterised by two consecutive 360° cycles fulfilled by the same animal. For the non-protected animals, the number of the "turns" was counted. There were two control groups: one with placebo and a "positive control group" receiving 0.33 nmol/kg D-2-amino-7-phosphonoheptanoic acid (D-AP7) as a NMDA antagonist (Schmutz et al., 1990).

2.3.2. Strychnine-induced convulsions

The method has been described previously (Lehmann et al., 1988). In brief, strychnine convulsions followed by death were induced in mice by the i.p. injection of 2.5 mg/kg of strychnine nitrate. A protective effect of the decoction given i.p. 30 min prior to strychnine was recorded and compared to the generated by 3.75 mg/kg of phenobarbitone (Löscher and Schmidt, 1988) and to the control. The number of animals that survived more than 10 min served as criterion of protection. The time to onset of death was recorded in non-protected mice.

2.3.3. Bicuculline-induced behavioural excitation

Mice were injected (i.p.) with bicuculline 2 mg/kg (Goth, 1984) 30 min after administration of the extract. They were observed for 1 h. Locomotion, rearing, sniffing, immobility and sedation times were recorded. Data of mice treated with 2 g//kg of the decoction were compared to data of control mice treated with placebo.

2.4. Statistical analysis

Data were analysed using Student's *t*-test. *P* values ≤

0.05 were considered as statistically significant.

3. Results

3.1. Effect on NMDA-induced behavioural excitation (turning behaviour) in mice.

90% of mice treated with 75 mg/kg NMDA (s.c.) presented the turning behaviour within 30 min after its administration. The decoction of *Cyperus articulatus* rhizome antagonised this behaviour. The decoction protected 60% of mice from the excitation induced by NMDA. D-AP7, a selective NMDA antagonist provided the same protection. In non-protected animals, the decoction reduced the turning behaviour by 88.8% (Fig 1).

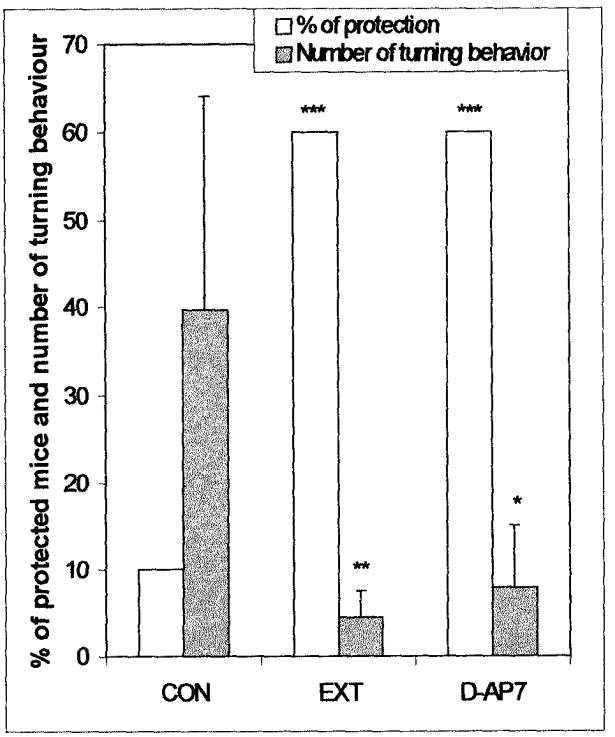

Fig. 1: Effect of the decoction of *Cyperus articulatus* rhizomes on NMDA-induced turning behaviour in mice: percentage of protected mice and the frequency of the turning behaviour. For the frequency of the turning behaviour, values are mean ± S.D. ($n = 10$); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control (Con), (Fisher exact test, two tail and Correction for multiple *t*-test by Bonferroni method).

3.2. Effect on strychnine *-induced convulsions and death in mice.*

STR nitrate, at a dose of 2.5 mg/kg, induced convulsions and death in 100% of the animals. The decoction of *Cyperus articulatus* rhizome at a dose of 2 g/kg (i.p) protected 70% of mice against death induced by strychnine while phenobarbital, an anticonvulsant and antiepileptic drug protected 100% of mice. The decoction had no effect on the time of the onset of convulsions and death Fig 2).

3.3. Effect on bicuculline *-induced behavioural excitation in mice*

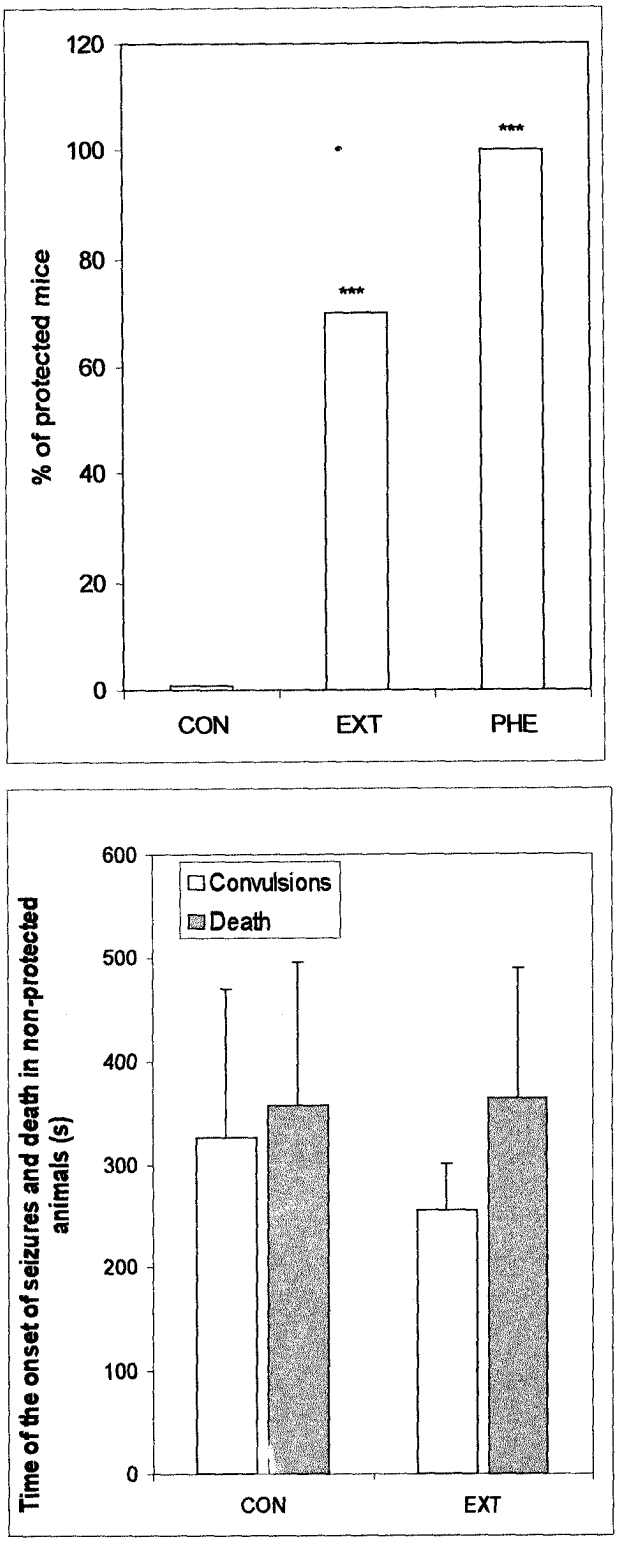

Fig. 2. Effect of the decoction of *Cyperus articulatus* rhizomes on strychnine-induced tonic seizures and death in mice: a) percentage of protected mice; b) time of the onset of convulsions and death (s). Values for (b) are mean ± S.D. ($n = 10$, $n = 3$ for the treated group); *** $P < 0.001$ vs. control (Con), (Fisher exact test, two tail).

Table 1: Inhibition of BIC-induced hyperactivity by the aqueous decoction of *Cyperus articulatus* rhizomes in mice [a]

Treatment / Behavioural aspects	Bicuculline 2 mg/kg	*C. articulatus* 2 g/kg	% inhibition
Locomotion (cm)	72.3 ± 22	19.2 ± 13.2	73.4***
Rearing (s)	2460 ± 684	1272 ± 766	48.3***
Grooming (s)	79.5 ± 11.2	25.9 ± 11.8	67.4***
			% of increase
Immobility (s)	450 ± 383	1452 ± 610	323***
Sedation (s)	48 ± 112	91 ± 74	190

[a] Values are mean ± S.D. (n = 10), ***P <0.001 vs. control.

Mice treated with 2 mg/kg bicuculline (i.p) presented hyperactivity when compared to the controls. Behavioural aspects like locomotion, rearing, grooming were increased by bicuculline. The decoction of *Cyperus articulatus* at a dose of 2 g/kg inhibited the hyperactivity induced by bicuculline. The inhibition of those behavioural aspects was 73, 48 and 67% for locomotion, rearing and grooming, respectively. Mice treated with the decoction were more immobile than control mice. The time of immobility was increased by 323%. In some cases, this immobility was accompanied by sedation as the mice presented palpebral ptosis. (Table 1).

4. Discussion

The results showed that the decoction of *Cyperus articulatus* rhizomes prevented NMDA-induced turning behaviour in mice, suggesting that the decoction possesses NMDA antagonistic properties in vivo. These results are in accordance with the results already obtained where the decoction of the rhizome of *Cyperus articulatus* selectively antagonised NMDA- but not AMPA-induced depolarisations in the rat cortical wedge in vitro (Ngo Bum et al., 1996). NMDA antagonists are known to possess anticonvulsant and antiepileptic properties (Meldrum, 1992; Rogawski, 1992). The results obtained here confirm other results where the methanolic extract of the *Cyperus articulatus* was found to possess anticonvulsant properties in mice (Ngo Bum et al. 2001). Anticonvulsant properties through the NMDA receptor explain at least part of the purported antiepileptic properties of the decoction of *Cyperus articulatus*. The decoction of *Cyperus articulatus* also antagonised strychnine-induced convulsions and death. The antagonism of strychnine-induced convulsions and death lend further support for the anticonvulsant properties of this extract (Fisher, 1989). The result also showed that the decoction of *Cyperus articulatus* inhibited bicuculline-induced hyperactivity. Inhibiting the excitation-induced by bicuculline is a sign of the presence of the central depressant properties of the decoction (Rakotonorina et al., 2001; Goth, 1984).

In conclusion, our results show that the total aqueous decoction of the rhizome of *Cyperus articulatus*, administered intraperitoneally, antagonised NMDA-, strychnine - and bicuculline -induced behavioural stimulation and convulsions. The inhibition of behavioural stimulation suggests the presence of anticonvulsant properties in the decoction of *Cyperus articulatus* rhizomes. These anticonvulsant properties of the *Cyperus articulatus* extract explain the therapeutic effect of the decoction when used in traditional medicine to treat diseases like epilepsy.

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