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Anticonvulsant and Sedative Activity of Leaves of *Senna spectabilis* in Mice

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Abstract: *Senna spectabilis* DC. is a small tree, 3 to 5 m, found in tropical areas in Africa, Asia, Australia, Latino and South America. It is used in traditional medicine in Cameroon to treat many diseases (constipation, insomnia, epilepsy, anxiety, etc.). Therefore, the aim of this study was to look scientifically for the anticonvulsant and sedative properties of *S. spectabilis*. *In vivo* animal models of epilepsy (Maximal Electroshock (MES), N-Methyl-D-Aspartate (NMDA), Pentylenetetrazol (PTZ) and Strychnine (STR) induced convulsions or turning behavior) and insomnia (diazepam-induced sleep) were used. Mice were divided in six groups: one negative control group, one positive control group and four groups treated with the plant extract, (except for diazepam-induced sleep test). Four doses of the ethanolic extract were used: 100, 200, 500 and 1000 mg kg^{-1}. The ethanolic extract of the leaves of *Senna spectabilis* strongly increased the total sleep time induced by diazepam ($p<0.001$). It also protected mice against Maximal Electroshock (MES) ($p<0.01$), pentylenetetrazol ($p<0.001$), picrotoxin ($p<0.01$) strychnine ($p<0.01$) and n-methyl-d-aspartate ($p<0.001$)-induced seizures and turning behavior and increased the latency to the onset of seizure in Isonicotinic Hydrazide Acid (INH) test ($p<0.01$). The results lead to the conclusion that the extract of *Senna spectabilis* possesses anticonvulsant and sedative properties in mice and could explain its used in traditional medicine in Africa, in the treatment of insomnia and epilepsy.

Key words: Traditional medicine, plant, extract seizures, *Senna spectabilis*

INTRODUCTION

More than 200,000 over 300,000 plants species known in the whole word grow in tropical areas in Africa and others continents (Sofowora, 1996). And Africa is a continent endowed with an enormous wealth of plant resources. Over 5000 distinct species are known to occur in the forest region alone and most of them have been used for several centuries in traditional medicine for the prevention and treatment of diseases (Iwu, 1993). According to the WHO report, more than 80% of the populations in Africa are using traditional medicine for their health problems. Thus, in Africa, phytotherapy still play an important role in the management of diseases, mainly among populations with very low income (Adjanohoun, 1988). *Senna spectabilis* DC. (Ceasalpiniaceae) (*S. spectabilis*) grows in Tropical areas in Africa, Asia, Australia, South and Latina America. In Africa, it is found in Angola, Burundi, Cameroon, Thad, Kenya, Nigeria, Tanzania, Togo, South Africa, etc. (Nsonde-Ntandou *et al*., 2005; Thirakul, 1990). *Senna spectabilis* is one of the medicinal plants used in Cameroon by traditional Healers. Personal communications with some Cameroonian Healers showed that the infusion of its leaves is used to treat various types of diseases: constipation, insomnia and anxiety. The decoction is used in the treatment of epilepsy. The plant is also used to treat malaria, dysenteries and head aches (Nsonde-Ntandou *et al*., 2005). Some pharmacological studies showed that *S. spectabilis* was fully efficacious in reverting scopolamine-induced amnesia in mice (Viegas *et al*., 2005). The plant also possesses antimalarial activity (Nsonde-Ntandou *et al*., 2005), antimicrobial activity (Chukeatirote *et al*., 2007) and cytotoxic activity on brine shrimp (Sriphong *et al*., 2003). Some chemical studies showed that *S. spectabilis* possess alkaloids (Sriphong *et al*., 2003; Viegas *et al*., 2005). The present study was conducted in order to look for the anticonvulsant and sedative properties of *S. spectabilis* used in traditional medicine in Cameroon to treat insomnia and epilepsy.

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MATERIALS AND METHODS

Animals: This study was conducted at the University of Ngaoundéré in Cameroon in March, 2006. 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 5% Tween 80 in distilled water), one positive control group (received appropriate substance) and four groups receiving the ethanolic extract. Drugs were administered intraperitoneally, in a volume of 10 mL kg^{-1} of body weight, except NMDA (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 No. 85-23, revised in 1985).

Plant material: The plant specimens of *Senna spectabilis* used were collected in Cameroon in the vicinity of Ngaoundéré in the raining season (March, 2006). A voucher specimen of the plant (7847/HNC) was authentified at the National Herbarium of Cameroon in Yaoundé.

Preparation of the extracts

Decoction: The dried leaves of *S. spectabilis* were ground. The powder (10 g) was put for maceration in 50 mL of distilled water for 1 h. The mixture was boiled for 20 min. After cooling, the supernatant (decoction) was collected and filtered with Wattman paper No. 1. The stock solution obtained (40 mL) corresponds to a concentration of 0.2 g mL^{-1}, that is 10 g of leaves in 50 mL distilled water, representing a 4.1% yield. The decoction, administered intraperitoneally (i.p.) 1 h before the test was used only in pentylene tetrazol test (Ngo-Bum *et al*., 2001).

Ethanolic extract: The dried leaves of *S. spectabilis* were ground. The powder (250.17 g) was macerated for 4 days in 1.1 l ethanol. The mixture was filtrated and the filtrate was evaporated using a Rota vapor. The quantity of extract obtained after evaporation was 54.33 g of ethanolic extract that represent a 21.7% yield. The ethanolic extract was used in each test. Extracts were administered 1 h before the test. The following doses were used: 100, 200, 500 and 1000 mg kg^{-1}.

Chemicals: Clonazepam, D-2-amino-7-phosphonoheptanoate (D-AP7), diazepam, Isonicotinic Acid Hydrazide (INH), N-Methyl D-Aspartate (NMDA), penthylenetetrazole (PTZ) and Strychnine (STR) are from Sigma Chemical, USA.

Pharmacological tests

Maximal electroshock (MES) test: Tonic convulsions of the hind extremities of mice were induced by passing alternating electrical current (50 Hz, 30 mA, 0.2 sec) through eyes electrodes (Ngo-Bum *et al*., 2001, 2004). For each experiment one group served as a negative control and received 5% Tween 80 in distilled water. One group served as a positive control and received diazepam, 5 mg kg^{-1} i.p.). The tested mice received 100, 200, 500 and 1000 mg kg^{-1}. 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}, 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 characterized by two consecutive 360° cycles fulfilled by the same animal (Ngo-Bum *et al*., 2009a, b). There were two control groups: one with placebo (5% Tween 80 in distilled water) and a positive control group receiving 33 ηmol kg^{-1} of D-AP7 (Ngo-Bum *et al*., 2009a).

Strychnine (STR) test: The STR convulsions followed by death were induced in male mice by the i.p., injection of 2.5 mg kg^{-1} STR nitrate. A protective effect of the extract given i.p. 1 h prior to STR was recorded and compared to the one of 3 mg kg^{-1} clonazepam (Ngo-Bum *et al*., 2001, 2009b). Animals that survived more than 10 min were qualified protected.

Picrotoxine (PIC) test: Clonic seizures were induced in male mice by the i.p., injection of 7.5 mg kg^{-1} PIC. (Ngo-Bum *et al*., 2005, 2009b). A protective effect of the extract against PIC-induced clonic seizures was recorded. A dose of 0.4 mg kg^{-1} of clonazepam was used as positive control.

Pentylenetetrazol (PTZ) test: Clonic seizures were induced in male mice by the i.p., injection of 70 mg kg^{-1} PTZ (Ngo-Bum *et al*., 2001, 2009a-c). The protective effect of the plant was recorded in the mice treated 1 h before with the extracts (ethanolic extract and decoction). There were two control groups, a negative control group receiving placebo and a positive control group receiving 0.1 mg kg^{-1} of clonazepam.

Isonicotinic hydrazide acid (INH) test: Animals were injected i.p., with INH 250 mg kg^{-1} (Ngo-Bum *et al*., 2009b) 1 h after the administration of the ethanolic extract and the time to onset of clonic or tonic seizures was recorded. Data of the control group (treated with placebo) were compared to data of the group treated with the ethanolic extract. The positive control group received diazepam, 10 mg kg^{-1} (per os).

Diazepam-induced sleep in mice: A method already described was used (Rakotonirina *et al*., 2001; Ngo-Bum *et al*., 2009a-c). Sleep potentiating effects of the plant was studied in the mice that received diazepam at a dose of 50 mg kg^{-1}, 1 h after the extract and placebo administration. The time between the loss of the straightening reflex and the regain of this reflex measured the sleeping time.

Statistical analysis: For INH and diazepam-induced sleep tests, the Analysis of Variance (ANOVA) followed by Dunnet (HSD) were done. For other anticonvulsant tests (MES, PIC, PTZ, STR and NMDA), the Fisher exact test (two-tail) was used to compare the percentage of protected mice. A value of $p<0.05$ was considered significant.

Chemicals: Clonazepam, D-2-amino 7-phosphonoheptanoate, diazepam, Isonicotinic acid hydrazide, N-methyl D-aspartate, penthylene tetrazole and strychnine are from Sigma Chemical, USA.

RESULTS

Effect of *S. spectabilis* on MES-induced seizures: The anticonvulsant compound diazepam completely protected mice against MES-induced seizures ($p<0.001$). The effect of the ethanolic extract of *S. spectabilis* was dose-dependent. The dose 1000 mg kg^{-1} protected 82.5% of mice ($p<0.01$) (Fig. 1).

Effect of *S. spectabilis* on PTZ-induced seizures: The ethanolic extract of *S. spectabilis* and clonazepam prevented mice against PTZ-induced seizures. The effect of the ethanolic extract of *S. spectabilis* was dose-dependent. The dose 1000 mg kg^{-1} protected 87.5% of mice ($p<0.001$) (Fig. 1).

Effect of *S. spectabilis* on NMDA-induced turning behavior: The extract of *S. spectabilis* dose dependently and significantly antagonized NMDA-induced turning behavior in mice. The extract of *S. spectabilis* at the dose of 1000 mg kg^{-1} protected 87.5% of mice ($p<0.001$)

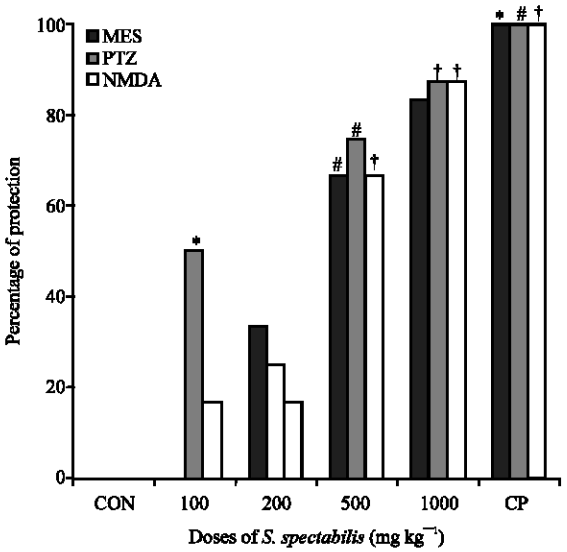

Fig. 1: Effect of *S. spectabilis* on MES, PTZ and NMDA -induced seizures and turning behavior in mice. Histograms represent the percentage of protected animals. N = 6 or 8 per dose, *$p<0.05$, #$p<0.01$, †$p<0.001$. CON: 5% Tween 80 in distilled water. CP: Diazepam 5 mg kg^{-1} for MES, clonazepam 0.1 mg kg^{-1} for PTZ and D-2-amino7-phosphonoheptanoate 33 ηM kg^{-1}

(Fig. 1). Mice treated with D-AP7 were totally protected against turning behavior induced by NMDA ($p<0.001$).

Effect of *S. spectabilis* on PIC-induced clonic seizures: *Senna spectabilis* extract significantly protected mice against PIC-induced seizures in mice. The dose 500 mg kg^{-1} protected 75% of mice ($p<0.01$). As expected, Clonazepam, showed total protection against PIC-induced seizures ($p<0.001$) (Fig. 2).

Effect of *S. spectabilis* 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, *S. spectabilis* extract significantly increased the number of mice protected against STR-induced seizures and exitus. The dose 500 mg kg^{-1} protected 66.7% of mice ($p<0.01$) (Fig. 2).

Effect of *S. spectabilis* on INH-induced seizures: The ethanolic extract of *S. spectabilis* dose-dependently and significantly increased the time to the onset of seizures (Fig. 2).

Effect of *S. spectabilis* on diazepam-induced sleep: Animals injected with diazepam (50 mg kg^{-1}, i.p.) showed

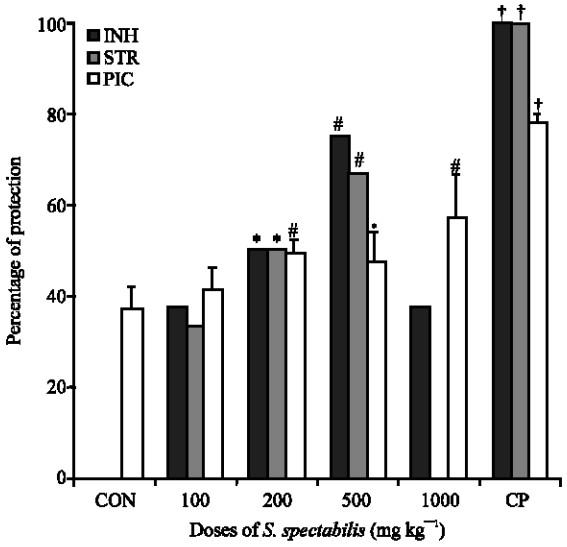

Fig. 2: Effect of *S. spectabilis* on PIC, STR and INH-induced seizures in mice. Histograms show the percentage of protected animals in PIC and STR tests and Mean±SEM in INH test. n = 6 or 8 per dose, *p<0.05, #p<0.01, †p<0.001. CON: 5% Tween 80 in distilled water or distilled water. CP: clonazepam 0.4 mg kg^{-1} for PIC, clonazepam 3 mg kg^{-1} for STR and diazepam 10 mg kg^{-1} for INH

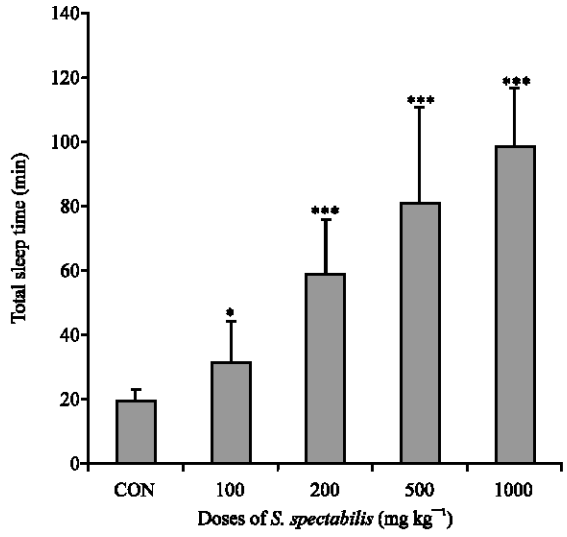

Fig. 3: Effect of *S. spectabilis* on diazepam-induced sleep in mice. Histograms represent the total sleep time (min) induced by diazepam in the presence of different doses of the extract in mice. Data are expressed as Mean±SEM, n = 8 per dose, *p<0.05, ***p<0.001. CON: 5% Tween 80 in distilled water

loss of the straightening reflex within 2 to 5 min of administration. The extract of *S. spectabilis* strongly potentiated in a dose-dependent manner the sleeping time induced by diazepam (3 to 5 times the sleeping time of the control group) (p<0.001) (Fig. 3).

DISCUSSION

The ethanolic extract of *S. spectabilis* completely antagonized NMDA-induced turning behavior. Given the involvement of the NMDA receptor complex in epileptic and epileptiform activity *in vivo* (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 subtypes and their respective modulatory sites have been shown to possess anticonvulsant and antiepileptic properties in several animal models of epilepsy (Davies *et al*., 1986; Löscher and Schmidt, 1988; Ngo-Bum *et al*., 1996) it can be suggested that the ethanolic extract of *S. spectabilis* possesses anticonvulsant properties. *Senna spectabilis* also significantly protected mice against PIC-, PTZ- and STR-induced seizures in mice. The inhibition by the extract of *S. spectabilis* of STR-induced seizures suggests the presence of anticonvulsant properties (Park *et al*., 2007; Salih and Mustafa, 2008) and the involvement of glycine receptors (Findlay *et al*., 2002). As PTZ and PIC have been shown to interact with the GABA neurotransmition (Salih and Mustafa, 2008; Pérez-Saad and Buznego, 2008), the antagonism of PTZ- and PIC-induced seizures suggests the interaction of the extracts of *S. spectabilis* with the GABA-ergic neurotransmission. The extract of *S. spectabilis* completely antagonized MES-induced seizures by probably prolonging the inactivation of sodium channels (Holmes, 2007). The MES and PTZ tests are of predictive relevance considering the clinical spectrum of activity of experimental compounds (Kupferberg and Schmutz, 1997). They are assumed to identify anticonvulsant drugs effective against generalized tonic-clonic/partial seizures and generalized clonic seizures, respectively (Holmes, 2007; Kupferberg and Schmutz, 1997; Ngo-Bum *et al*., 2009a-c). The effect of the extract in these tests could therefore, suggest anticonvulsant efficacy against the above mentioned seizures types in man.

In addition, the ethanolic extract of *S. spectabilis* strongly increased the total sleep time induced by diazepam. The potentiation of the sleep time suggests the presence of sedative properties in the extract of *S. spectabilis* (Rakotonorina *et al*., 2001; Ngo-Bum *et al*., 2004, 2009a, b). The sedative properties of *S. spectabilis*

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, *S. spectabilis* possesses sedative and anticonvulsant properties in mice. These properties could explain the use of this plant in traditional medicine in Africa, particularly in Cameroon in the treatment of insomnia and epilepsy. Research is ongoing in our laboratory in order to find the mechanisms of action of this extract.

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