Molecular & Clinical Pharmacology

Inforesights Publishing

Anticonvulsant effects of the Stem bark extract of *Annona senegalensis* Pers.

Konate Almamy[1]; Sawadogo Wamtinga Richard[4]; Dubruc Franck[2,3] Caillard Olivier[2,3] et Guissou Innocent Pierre [1,4]

[1]Laboratoire de Pharmacologie-Toxicologie, UFR/SDS Université de Ouagadougou 03 B.P. 7021, Ouagadougou 03/ Burkina Faso.
[2]Institut National de la Santé Et de la Recherche Médicale (Inserm), Unité Mixte de Recherche en Santé (UMR-1072, 13015, Marseille, France
[3]Aix-Marseille Université, Unité de Neurobiologie des canaux Ioniques et de la Synapse (UNIS), 13015, Marseille, France
[4]Institut de Recherche en Science de la Santé (IRSS), Département Médecine-Pharmacopée Traditionnelle et Pharmacie (MEPHATRA/PH),.03 BP 7192 Ouagadougou 03/ Burkina Faso.

[*]Corresponding author: konatealmamy@gmail.com; Tel: 0022670241568

Received: 12 July 2012, **Revised**: 30 July 2012, **Accepted**: 31 July 2012

Abstract

Annona senegalensis Pers. is claimed in traditional medical practice, to be useful in the treatment of epilepsy in some parts of Burkina Faso. In the present work, the anticonvulsant property of methanolic extract, n-hexane fraction, ethyl acetate fraction and aqueous fraction were investigated one seizures induced by pentylenetetrazole (70 mg/kg) or pilocarpine (240 mg/kg). Extracts were administered *intraperitoneally* and *per os,* at the pre-treatment time of 30 minutes at 200 and 400 mg/kg and mice and rats. Extracts and fractions had no significant increased the latency to the first convulsion induced by pentylenelenetetrazole or pilocarpine. The treatment with methanolic extract and aqueous fraction (400 mg/kg) significantly ($p< 0.05$) protected against pentylenetetrazole or pilocarpine induced seizures. The result obtained in this study suggests that the stem-barks of this plant may possess anti-convulsant property in mice and rats.

Keywords: Anticonvulsant; pentylenetetrazole; pilocarpine; scopolamine

Introduction

Epilepsy is one of the most common neurological disorders and is characterized by seizures, which are of various types and result from episodic neuronal discharges (Gaustaut, 1973). Of the 50 million people with epilepsy worldwide, 10 million live in Africa alone (Senanayake & Roman, 1993). In Burkina Faso, it's estimated to 10.6 ‰ in the general population (Millogo *et al.*, 2004; Ngounou *et al.*, 2007). In the developed countries, where drugs are easily available, epilepsy responds to treatment in up to 70% of the patients. However, for ec-

onomic and social reasons, 3/4 of people with epilepsy in Africa have no access to healthcare provisions and are not appropriately treated.(Shorvon, 1996). Despite the development of new molecules indicated for the treatment of the patient epileptics, several problems remain supported. The proportion of drug-resistant epilepsy was little changed by the appearance of these new therapies (Heinemann *et al.*, 1994; Raza *et al.*, 2001). Moreover, the available drugs are active as anti-epileptics i.e, to minimize the risk of occurrence of seizures, but do not prevent alterations secondary to repeated seizures. Currently, there is no substance to the real potential antiepileptogenic (Heinemann *et al.*, 1994; Mattson, 1995). Consequently there is still a real to develop new antiepileptic drugs with more selective anticonvulsant, easily accessible and with fewer side effects. Facing to the inaccessibility of certain news molecules, beliefs in supernatural cause, epilepsy still remains a public health problem, with socio-cultural, economical and medical impacts in Africa. The recourse to the traditional resource of medicine and pharmacopeia to face the public health problems is a secular practice (Millogo *et al.*, 2004). In Burkina Faso, the research tasks on the medicinal herbs strongly contribute to a better use of these medicinal herbs and to reduce certain risks of intoxications relating to the consumption of certain natural resources (Ouedraogo, 1996; Millogo *et al.*, 2004). This revalorization is a contribution as well to the public health as with the creation of a climate of trust around traditional medicine and the traditional healing practices. Facing to the toxicity relative of certain news molecules anti-epileptics and to their inaccessibility, the relevance of the contribution of traditional medicine is done more pressing we were interested in their traditional treatments. Our work thus is the object of a sustained effort of revalorization and promotion of African traditional medicine in order to contribute to the assumption of responsibility of the epilepsy. *Annona senegalensis* Pers. is widely distributed in West African, different parts of this plant are used traditionally in the treatment of many diseases such as fever, intestinal troubles, stomach ache, gonorrhea, syphilis, rheumatism and central disorders (Oliver-Bever, 1982; Okoli, 2010; Okoye *et al.*, 2010). It has been reported that hydroalcoholic of leaf and root barks extracts of *Annona senegalensis* Pers. had an anticonvulsive effect on Maximal electroshock induced seizures and Pentylenetetrazole induced seizures to the orally administration (Okoli, 2010; Okoye *et al.*, 2010). The objectives of the present work were to investigate the anticonvulsant effect of methanolic extracts (MEAS), n-hexane extracts (HEAS), ethyl acetate fractions (EAAS) and aqueous residual fraction (AFAS) from the stem-bark of *Annona senegalensis* Pers. (*A. senegalensis*) extract on two animal's model of convulsion: pilocarpine (pilo) and Pentylenetetrazole (PTZ).

Materials and methods

Collection and identification of plan material

Air dried leaves and stem barks of *Annona senegalensis* Pers. were procured in the month of August at Ouagadougou, Burkina Faso. The plant was authenticated by Professor Jeanne R. Millogo (Botany Department, University of Ouagadougou). A voucher herbarium specimen with number HNB8713 was deposited in the National Herbarium of Burkina.

Extraction

The stem-bark of *A. senegalensis* was cleaned, and then was air-dried at room temperature and pulverized using mechanical grinder. The powder (1500 mg/kg) was submitted to

maceration with hydroalcoholic solution (methanol /water, 7:3, v/v) at room temperature during 24 h. This procedure was repeated three times with the same powder. The hydroalcoholic solution was filtered and the filtrate was concentrated to dryness to give 158 g of methanolic extract of *Annona senegalensis* (MEAS). 115 g of MEAS were suspended in dilled water and successively extract with n-hexane and ethyl acetate. This procedure was repeated three times with each solvent. Organic extracts were concentrated using rotary evaporator while aqueous residue was evaporated in an oven at 50°C. The following fractions were obtained: n-hexane (16 g); ethyl acetate (18 g) and aqueous residue (14 g).

Animals and Experimental Procedures

Male mice (25–30 g) and male rats (200–250 g) were used. The study animals were kept and maintained under laboratory conditions of temperature, humidity, and 12-h light dark cycle; and were allowed free access to food (standard pellet diet) and water *ad libitum*. All animals were fasted for 16 h, but still allowed free access to tap water, before the beginning of experiments. All experiments were performed between 09:00 am and 4:00 pm in a quiet small laboratory maintained at a temperature of 20–25 ◦C. All experimental procedures were carried out according to the animal care guideline of the National institute for health (NIH) Guide. More specifically, experiments described were reviewed and approved by the Research Institute in Health Sciences of Ouagadougou (Burkina Faso) and conformed to the guidelines issued by the International Association for the Study of Pain for animal pain experimentation (Zimmermann, 1983).

Drug administration

Diazepam (2 mg/kg; *i.p*, as positive control) was administrated 20 min before the induction of seizures in both pilocarpine and pentylenetetrazole models. The solution of ethyl acetate (EAAS) and n-hexane (HEAS) fraction were prepared in 3% of DMSO and the aqueous residue (AFAS) was prepared in the distilled water, and then administered *intraperitoneally* 45 min or *per os* 1h before the induction of seizures in both Pilocarpine and pentylenetetrazole models.

Experimental design

Pentylenetetrazole (PTZ) induced convulsions

Absence seizures were induced by single dose administration of PTZ (70 mg/kg; *Sc*) to the mice. This dose was then given to 11 groups of 6 mice each pretreated *i.p* or *per os* (200 and 400 mg/kg) with the aqueous methanolic (MEAS) extract, n-hexane (HEAS) extract, ethyl acetate (EAAS) fraction and aqueous (AFAS) fraction. Mice were placed in a clear plexiglass chamber and observed for 45 min. Latency for the development of clonic seizure and mortality protection were determined of each group. Mice that did not convulse 30 min after injection of the PTZ were considered protected (Krall *et al.*, 1978; Wang *et al.*, 2000).

Pilocarpine (Pilo) induced seizures

Seizures were induced by single dose administration of pilocarpine (240 mg/kg; *i.p*) to the rats. This dose was then given to 13 groups of 6 rats each pretreated *i.p* or *per os* (200 and 400 mg/kg) with the MEAS, HEAS, EAAS and AFAS. The cholinergic antagonist methyl-scopolamine (1 mg/kg, *i.p.)* was injected to animals, 45 min before pilocarpine to prevent peripheral muscarinic stimulation. During the 2 h period following the pilocarpine injection, the animals were continuously observed. Animals were placed in individual plexiglass cages to observe: the latency to SE and number of animals that died after pilocarpine administration. For each animal, manifestations of the seizures were rated on a 6-point scale according to the Racine's scale (1972) that is widely used in studies on animal models of epilepsy (Turski *et al.*, 1983; Setkowicz *et al.*, 2003):

(1) Light seizures (rated as 0.5 or 1.0):

0.5: immobility, piloerection, salivation, narrowing of eyes, face and vibrissae twitching, ear rubbing with forepaws; **1.0**: head nodding and chewing movements;

(2) Intermediate seizures (rated as 1.5 or 2.0):

1.5: clonic movements of forelimbs, and mild whole body convulsions, exophthalmia, aggressive behaviour; **2.0**: rearing and running with stronger tonic–clonic motions including hind limbs, tail hypertension, lock jaw;

(3) Heavy seizures (rated as 2.5 or 3.0):

2.5: rearing and falling, eye congestion; **3.0**: loss of postural tone with general body rigidity.

Protocol 1 (intraperitoneally administration)

The first group received vehicle control (vehicle) whereas Group-II received standard drug (Diazepam, 2mg/kg), Group-III received aqueous methanolic (MEAS) extract (200 and 400 mg/kg/body weight), Group-IV n-hexane (HAAS) extract (200 and 400 mg/kg/body weight), Group-V received ethyl acetate (EAAS) extract (200 and 400 mg/kg/body weight) and Group-VI received aqueous fraction (AFAS) (200 and 400 mg/kg/body weight)

Protocol 2 (per os administration)

The first group received vehicle control (vehicle) whereas, Group-II received MEAS (200 and 400 mg/kg/body weight), Group-III receive HEAS (200 and 400 mg/kg/body weight), Group-IV received EAAS (200 and 400 mg/kg/body weight) and Group-V received AFAS (200 and 400 mg/kg/body weight).

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the groups was assessed using one way and multiple way analysis of vari-

ance (ANOVA). The test followed by Dunnet's test P values than 0.05 were considered as significance.

PTZ-induced convulsions test

We examined the anticonvulsant effects of the different fraction of stem-bark of *A. senegalensis* Pers. The different fractions (200 and 400 mg/kg; *p.o*) did not significantly increased the latency to the first convulsion (table 1). The different fractions (200 and 400 mg/kg; *i.p*) also did not significantly increased the latency to the first convulsion (table 1). However, Diazepam (2 mg/kg; *i.p*) increased significantly *(p< 0.0001)* the latency to the first convulsion (table 1). Whereas MEAS, HEAS, EAAS, AFAS (200 mg/kg; *i.p*) respectively have shown 33 %, 16.01 %, 21 % and 33 % protection against PTZ induced seizures (figure 1).

We also find that the pretreatment with MEAS, HEAS, EAAS, AFAS (400 mg/kg; *i.p*) respectively have shown 55.33 %, 16 %, 21 % and 49.66 % protection against PTZ induced seizures (figure 1). The treatment with MEAS (400 mg/kg; *i.p*) significantly (*p< 0.05*) protected against PTZ induced seizures (figure 1). The treatment with (200 and 400 mg/kg; *i.p*) significantly (p< *0.05*) protected against PTZ induced seizures (figure 1). Diazepam (2 mg/kg; *i.p*) treated animals have shown 94.33 % protection, it's significantly (p< *0.0001*) protected against PTZ induced seizures (figure 1). MEAS, HEAS, EAAS, AFAS (200 mg/kg;

Table1: Effects of stem-barks extracts of *A. senegalensis* in the latency of seizures after administration on PTZ (70 mg/kg) in mice.

Groups	Dose (mg/kg)	Latency of the 1st convulsion (min)
Oral Route		
Control 1		0.74 +/- 0.03
MEAS	200 p.o	0.75+/- 0.05
MEAS	400 p.o	0.76.0.04
HEAS	200 p.o	0.74 +/-0.04
HEAS	400 p.o	0.74 +/- 0.03
EAAS	200 p.o	0.74 +/- 0.02
EAAS	400 p.o	0.75 +/- 0.04
AFAS	200 p.o	0.76 +/- 0.05
AFAS	400 p.o	0.076 +/- 0.02
Intraperitoneal Route		
Control 2		0.74 +/- 0.03
MEAS	200 *i.p*	0.77 +/- 0.02
MEAS	400 *i.p*	0.88 +/- 0.04
HEAS	200 *i.p*	0.74 +/- 0.02
HEAS	400 *i.p*	0.76 +/- 0.03
EAAS	200 *i.p*	0.75 +/- 0.04
EAAS	400 *i.p*	0.76 +/- 0.07
AFAS	200 *i.p*	0.78 +/- 0.03
AFAS	400 *i.p*	0.87 +/- 0.06
Diazepam	2 *i.p*	1200 +/- 80 ***

Results are expressed as means ± SEM. *P < 0.05 compared with the wild-type mice by ANOVA one-way followed by Dunnet's test.

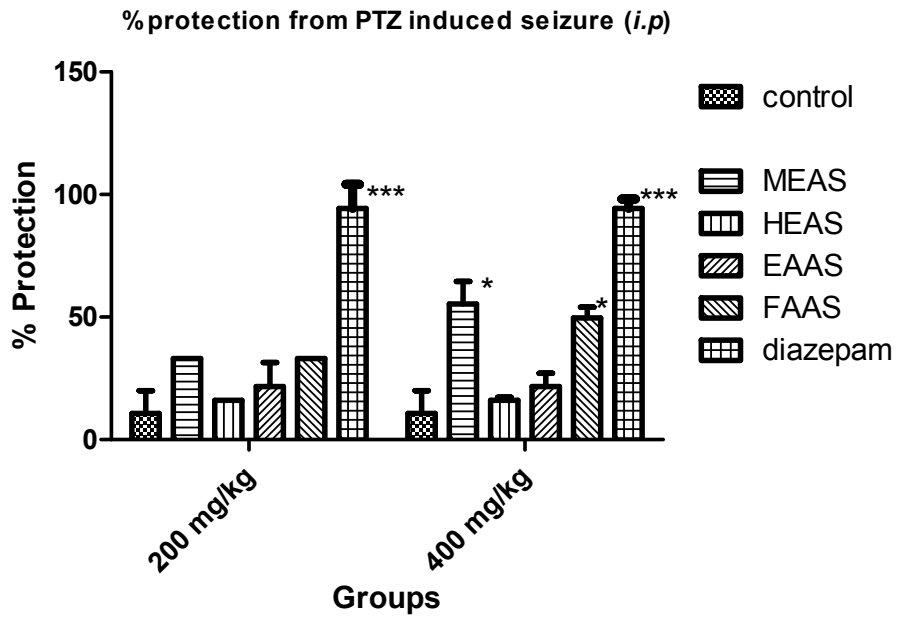

Figure 1: Effects of stem-bark extract of *A. senegalensis* (*intraperitoneally* administration) on seizures PTZ induced seizures. Results are expressed as means ± SEM *P < 0.05 compared with the wild-type mice by ANOVA one-way followed by Dunnet's test.

Table 2: Effects of stem-barks extracts of *A. senegalensis* in the latency of the latency after injection of pilocarpine (240 mg/kg) in rats.

Groups	Dose (mg/kg)	Latency of the 1st convulsion (min)
Oral Route		
Control 1		15.50 +/- 2.28
MEAS	200 *p.o*	16.43 +/- 2.32 **
MEAS	400 *p.o*	16.06 +/- 2.06 **
HEAS	200 *p.o*	15.44 +/- 2.02
HEAS	400 *p.o*	15.56 +/- 1.88
EAAS	200 *p.o*	15.49 +/- 2.11
EAAS	400 *p.o*	15.52 +/- 2.60
AFAS	200 *p.o*	16.07+/- 3.87 **
AFAS	400 *p.o*	16.57 +/- 1.34 **
Intraperitoneal Route		
Control 2		15.50 +/- 2.28
MEAS	200 *i.p*	16.03 +/- 1.22 **
MEAS	400 *i.p*	16.44 +/- 2.36 **
HEAS	200 *i.p*	15.44 +/- 2.19
HEAS	400 *i.p*	15.56 +/- 2.28
EAAS	200 *i.p*	15.50 +/- 2.31
EAAS	400 *i.p*	16.44 +/- 1.30
AFAS	200 *i.p*	16.56 +/- 2.22 **
AFAS	400 *i.p*	16.61 +/- 1.34 **
Diazepam	2 *i.p*	1200 +/- 80 ***

Results are expressed as means ± SEM. *P < 0.05 compared with the wild-type mice by ANOVA one-way followed by Dunnet's test.

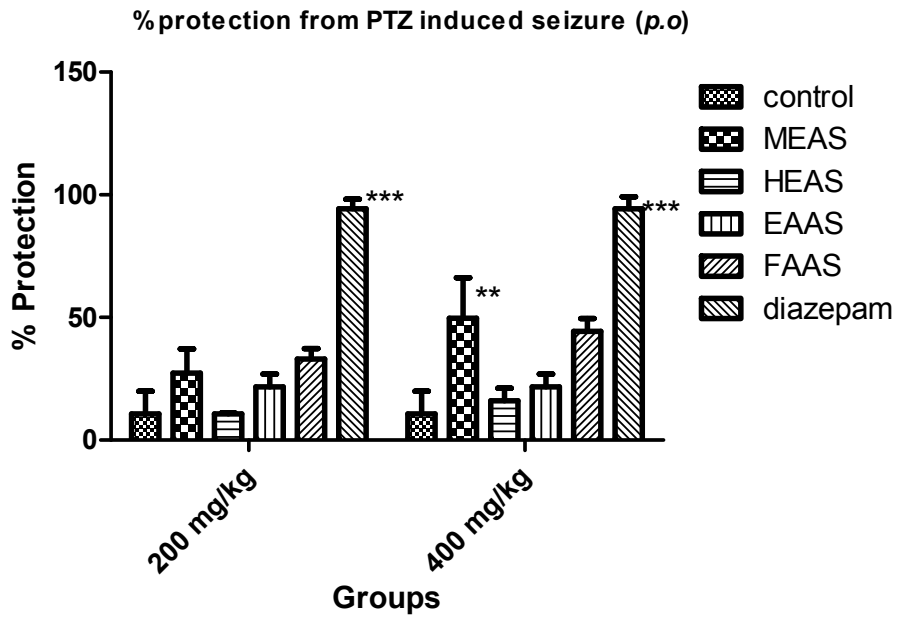

Figure 2: Effects of stem-bark extract of *A. senegalensis* (*per os* administration) on seizures PTZ induced seizures. Results are expressed as means ± SEM. *P < 0.05 compared with the wild-type mice by ANOVA one-way followed by Dunnet's test.

p.o) have shown 27.33 %, 10.66 %, 21 % and 33 % respectively (figure 2). The dose (400 mg/kg; *p.o*) have protect at 49.66 %, 21.66 %, 21.66 % and 44.33 % respectively for MEAS, HEAS, EAAS, AFAS (figure 2). The treatment with MEAS (400 mg/kg; *p.o*) significantly (*p< 0.05*) protected against PTZ induced seizures (figure 2)

Pilocarpine-induced convulsions test

The different fractions of HEAS and EAAS (200 and 400 mg/kg; *p.o*) did not significantly increased the latency to the first convulsion (table 2). HEAS and EAAS fraction (200 and 400 mg/kg; *i.p*) did not significantly increased the latency to the first convulsion (table 2). However, MEAS and AFAS fractions (200 and 400 mg/kg; *p.o*) increased significantly *(p< 0.01)* the latency to the first convulsion (table 2). MEAS and AFAS fractions (200 and 400 mg/kg; *i.p*) increased significantly *(p< 0.01)* the latency to the first convulsion (table 2). Diazepam (2 mg/kg; *i.p*) increased significantly *(p< 0.0001)* the latency to the first convulsion (table 2).

We also find that pretreatment with MEAS, HEAS, EAAS, AFAS (200 mg/kg; *p.o*) respectively have shown 67.45 %, 64.66 %, 65.65 % and 69.05 % protection against Pilocarpine induced seizures (table 3). The pretreatment with MEAS, HEAS, EAAS, AFAS (400 mg/kg; *p.o*) respectively have shown 69.84 %, 63.05 %, 66.69 % and 69.58 % protection against Pilocarpine induced seizures (table 3).The treatment with MEAS (200 and 400 mg/kg; *p.o*) significantly (*p< 0.05*) protected against Pilocarpine induced seizures (table 3).The treatment with AFAS (200 and 400 mg/kg; *p.o*) significantly (*p< 0.01*) protected against Pilocarpine induced seizures (table 3). However, the treatment with MEAS (200 and 400 mg/kg; *i.p*) significantly (*p< 0.01*) protected against Pilocarpine induced seizures (table 3). The treatment with AFAS (200 and 400 mg/kg; *i.p*) significantly (*p< 0.05*) protected against Pilocarpine induced seizures (table 3).

Table 3. Effects of stem-barks extracts of *A. senegalensis* on pilocarpine-induced seizures and lethality in the rats.

Groups	dose (mg/kg)	Percentage seizure	Percentage survival (%)
Oral Route			
Control 1		80	63,78
MEAS	200 *p.o*	65	67,45 *
MEAS	400 *p.o*	60	69,84 *
HEAS	200 *p.o*	77	64,66
HEAS	400 *p.o*	78	63,05
EAAS	200 *p.o*	74	65,65
EAAS	400 *p.o*	74	66,69
AFAS	200 *p.o*	68	69,05 **
AFAS	400 *p.o*	67	69,58 **
Intraperitoneal Route			
Control 2		80	63,78
MEAS	200 *i.p*	65	70,45**
MEAS	400 *i.p*	60	79,04**
HEAS	200 *i.p*	78	64,16
HEAS	400 *i.p*	78	64,2
EAAS	200 *i.p*	76	67,02
EAAS	400 i.p	74	68,25
AFAS	200 *i.p*	66	67,35*
AFAS	400 *i.p*	66	70,66*

Results are expressed as means ± SEM. *P < 0.05 compared with the wild-type mice by ANOVA one-way followed by Dunnet's test.

Discussion

Animal model are widely used screening model for testing anticonvulsive compounds. The PTZ test was used because this is one of the first assays developed to identify anticonvulsant drugs effective against generalized tonico-clonic, partial seizures and genralised clonic seizures (Löscher & Schmidt, 1988). Although, the mechanism by which pentylenete-trazole elicits its action has not been completely understood. However, two mechanisms have been proposed for the mode of PTZ-induced seizures. This type of seizures can also be prevented by drugs that enhance gamma amino butyric acid type A ($GABA_A$) receptor mediated inhibitory neurotransmission (Corda *et al.*, 1990; Mac Donald & Kelly K.M., 1994; White, 1997), or by increasing the central noradrenergic activity (De Potter *et al.*, 1980).

The potent anticonvulsant of a drug against PTZ seizure indicates its probable effectiveness against absence seizures (McNamara, 1989). The standard antiepileptic drug (diazepam) used in this study, exerts its antiepileptic might antagonize PTZ induced seizures by enhancing $GABA_A$ receptor-mediated inhibitory neurotransmission (Meldrum, 1997; Nils Ole, 2003).

Data from this study show that the treatment with the different fractions did not significantly increase the latency to the convulsion (table 1) by *per os* and *intraperitoneally* administration. The treatment with MEAS (400 mg/kg; *i.p*) significantly ($p< 0.05$) protected against PTZ induced seizures (figure 1). The treatment with AFAS (200 and 400 mg/kg;*i.p*)

significantly (*p<0.01*) protected against PTZ induced seizures (figure 1). Diazepam (2 mg/kg; *i.p*) treated animals have shown 94.33% protection, it's significantly (p< *0.0001*) protected against PTZ induced seizures (figure 1). The treatment with MEAS (400 mg/kg; *p.o*) significantly (*p< 0.05*) protected against PTZ induced seizures (figure 2). Potent anticonvulsant effects MEAS and AFAS in PTZ test suggest the presence of bioactive compounds effective in this plant. The mice treated with MEAS and AFAS were partially protected from seizures induced by PTZ. Based on this partial reduction of the mortality, it is difficult to report MEAS and AFAS as having anticonvulsant effect against PTZ seizure that could be interfering with GABA neurotransmission (Löscher & Schmidt, 1988). However, this partial effectiveness of this plant to reduce mortality after convulsion might suggest some potential usefulness of a drug against neurotoxicity which causes the death following extensive seizures (Westmoreland *et al.*, 1994).

In the pilocarpine model, our study showed that MEAS and AFAS fractions (200 and 400 mg/kg; *p.o* and *i.p*) increased significantly *(p< 0.01)* the latency to the first convulsion (table 2). Diazepam (2 mg/kg; *i.p*) increased significantly *(p< 0.0001)* the latency to the first convulsion (table 2).

The treatment with MEAS (200 and 400 mg/kg; *p.o*) significantly (*p< 0.05*) protected against Pilocarpine induced seizures (table 3).The treatment with AFAS (200 and 400 mg/kg; *p.o*) significantly (*p< 0.01*) protected against Pilocarpine induced seizures (table 3). However, the treatment with MEAS (200 and 400 mg/kg; *i.p*) significantly (*p< 0.01*) protected against Pilocarpine induced seizures (table 3).The treatment with AFAS (200 and 400 mg/kg; *i.p*) significantly (*p< 0.05*) protected against Pilocarpine induced seizures (table 3).

The lethal time of the Pilocarpine induced seizures in a dose-dependent manner test in the animals comparing with controls. The animal model of temporal lobe epilepsy induced by pilocarpine represents one of the most successful and worldwide used models to test the efficacy of new antiepileptic agents. Essentially, the effectiveness of a drug against Pilocarpine seizure indicates its probable effectiveness against absence seizures (McNamara, 1989). Although animal models is widely used screening model for testing anticonvulsive compounds, the mechanism may exert their anticonvulsant activity by a potentiation of inhibitory and/or inhibition of excitatory neurotransmission by many mechanisms, pre- and post-synaptically (Khongsombat *et al.*, 2008). An imbalance between excitatory and inhibitory neurotrans-mission of the brain, which could be produced by a decrease in GABAergic and/or an increase in glutamatergic transmission, is involved in the generation of epilepsy in patients with epilepsy as well as in animal models including pilocarpine-induced seizures in rodents (Peña & Tapia, 2000). Thus, receptors for these two neurotransmitters are regarded as important targets for antiepileptic drugs. The inhibitory action of GABA consists in the opening of chloride channels to allow hyperpolarizing the membrane (Gottesmann, 2002).

The finding of the present study justifies folkloric uses of *A. senegalensis* in the treatment of epilepsy. Further studies will be necessary to determine the anticonvulsant mechanisms of *A. senegalensis* and to isolate the molecular compounds responsible for these cli-nical properties.

Acknowledgement

Our acknowledgement goes towards the embassy of France to the Burkina Faso, which granted us an education grant and to EGIDE which was the structure in charge of management of the education grant.

Conflict of interest

There is no conflict of competing interest associated with the authors of this paper.

References

Corda, M.G., Giorgi, O., Longoni, B., Orlandi, M. & Biggio, G. (1990) Decrease in the Function of the γ-Aminobutyric Acid-Coupled Chloride Channel Produced by the Repeated Administration of Pentylenetetrazol to Rats. *J Neurochem* 55, 1216-1221.

De Potter, W.P., De Potter, R.W., De Smet, F.H. & De Schaepdryver, A.F. (1980) The effect of drugs on the concentration of dopamine β-hydroxylase in the cerebrospinal fluid of rabbits. *Neuroscience* 5, 1969-1977.

Gaustaut, H. (1973) Dictionary of Epilepsy. I. Definitions. *World Health Organisation*, Geneva, pp. p. 75.

Gottesmann, C. (2002) GABA mechanisms and sleep. *Neuroscience* 111, 231-239.

Heinemann, U., Draguhn, A., Ficker, E., Stabel, J. & Zhang, C.L. (1994) Strategies for the Development of Drugs for Pharmacoresistant Epilepsies. *Epilepsia* 35, S10-S21.

Khongsombat, O., Watanabe, H., Tantisira, B., Patarapanich, C. & Tantisira, M.H. (2008) Acute effects of N-(2-propylpentanoyl)urea on hippocampal amino acid neurotransmitters in pilocarpine-induced seizure in rats. *Epilepsy Res.* 79, 151-157.

Krall, R.L., Penry, J.K., White, B.G., Kupferberg, H.J. & Swinyard, E.A. (1978) Antiepileptic Drug Development: II. Anticonvulsant Drug Screening. *Epilepsia* 19, 409-428.

Löscher, W. & Schmidt, D. (1988) Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. *Epilepsy Res*, 2, 145-181.

Mac Donald, R.L. & Kelly K.M. (1994) *Mechanisms of action of new anticonvulsant drugs. In John Wiley & Sons* Chichester

Mattson, R.H. (1995) Efficacy and adverse effects of established and new antiepileptic drugs. *Epilepsia* 36, S13–S26.

McNamara, J.O. (1989) Development of New Pharmacological Agents for Epilepsy: Lessons from the Kindling Model. *Epilepsia* 30, S13-S18.

Meldrum, B.S. (1997) Identification and Preclinical Testing of Novel Antiepileptic Compounds. *Epilepsia* 38, S7-S15.

Millogo, A., Ratsimbazafy, V., Nubukpo, P., Barro, S., Zongo, I. & Preux, P.M. (2004) Epilepsy and traditional medicine in Bobo-Dioulasso (Burkina Faso). *Acta Neurol Scand*, 109, 250-254.

Ngounou, E.B.Q., F.; Dubreuil, C.M.; Marin, B.; Houinato, D.; Nubukpo, P.; Dalmay, F.;, Millogo, A.N., G.; Kouna-Ndouongo, P.; Diagana, M.; Ratsimbazafy, V.; & Druet-Cabanac, M.P., P.M. (2007) Épidémiologie de l'épilepsie en Afrique subsaharienne *Cahiers d'études et de recherche francophones/Santé* 16, 225-238.

Nils Ole, D. (2003) Inhibition of γ-aminobutyric acid uptake: anatomy, physiology and effects against epileptic seizures. *Eur J Pharmacol*, 479, 127-137.

Okoli, C.O., Onyeto, C. A., Akpa, B. P., Ezike, A. C.*, Akah, P. A. and Okoye, T. C. (2010) Neuropharmacological evaluation of Annona senegalensis leaves. *Afr J Biotechnol* 9, 8435-8444.

Okoye, T.C., Akah, P.A. & Omeke, C.P. (2010) Evaluation of the anticonvulsant and muscle relaxant effects of the methanol root bark extracts of Annona senegalensis. *Asian Pac J Trop Med.*, 3, 25-28.

Oliver-Bever, B. (1982) Medicinal plants in tropical West Africa I. Plants acting on the cardiovascular system. *J Ethnopharmacol* 5 1-72.

Ouedraogo, O.G. (1996) Plantes médicinales et Pratiques médicales Traditionnelles au BURKINA FASO: cas du plateau central *Thèse Doct. d'Etat ès Sciences Nat. Université de Ouagadougou* T1&T2, 242 et 285 pages.

Peña, F. & Tapia, R. (2000) Seizures and neurodegeneration induced by 4-aminopyridine in rat hippocampus in vivo: role of glutamate- and GABA-mediated neurotransmission and of ion channels. *Neuroscience* 101, 547-561.

Raza, M.F., Shaheen, M.I., Choudhary A, Suria, A.U., Rahman S., Sombati & Delorenzo, R.J. (2001) Anticonvulsant activities of the FS-1 Sub-fraction isolated from roots of Delphinium denudatum. *Phytother Res* 15, 426-430.

Senanayake, N. & Roman, G.C. (1993) Epidemiology of epilepsy in developing countries. *Bull. W.H.O.*, 71, 247–258.

Setkowicz, Z., Klak K. & K., J. (2003) Long-term changes in postnatal susceptibility to pilocarpine induced seizures in rats exposed to gamma radiation at different stages of prenatal development. *Epilepia* 44, 1267-1273.

Shorvon, S.D. (1996) The Epidemiology and Treatment of Chronic and Refractory Epilepsy. *Epilepsia* 37, S1-S3.

Turski, W.A., Cavalheiro, E.A., Schwarz, M., Czuczwar, S.J., Kleinrok, Z. & Turski, L. (1983) Limbic seizures produced by pilocarpine in rats: Behavioural, electroencephalographic and neuropathological study. *Behav Brain Res* 9, 315-335.

Wang, H.-H., Liao, J.-F. & Chen, C.-F. (2000) Anticonvulsant effect of water extract of Scutellariae radix in mice. *J Ethnopharmacol* 73, 185-190.

Westmoreland, B.F., Benarroch, E.E., Dube, J.R., Regan, T.J., & Sandok, B.A. (1994) *Medical Neurosciences*.

White, H.S. (1997) Clinical Significance of Animal Seizure Models and Mechanism of Action Studies of Potential Antiepileptic Drugs. *Epilepsia* 38, S9-S17.

Zimmermann, M. (1983) Ethical guidelines for investigations of experimental pain in conscious animals.. *Pain* 16, 109-110.

