British Journal of Pharmacology and Toxicology 3(5): 245-250, 2012
ISSN: 2044-2459; E-ISSN: 2044-2467

Submitted: August 09, 2012 Accepted: September 03, 2012 Published: October 25, 2012

Phytochemical and Anticonvulsant Properties of *Annona senegalensis* Pers. (Annonaceae), Plant Used in Burkina Folk Medicine to Treat Epilepsy and Convulsions

[1,2,4]A. Konate, [4]W.R. Sawadogo, [2,3]F. Dubruc, [2,3]O. Caillard, [1]M. Ouedraogo and I.P. [1,4]Guissou
[1]Laboratory of Pharmacology and Toxicology, UFR/SDS University of Ouagadougou, (French), 03 BP 7021 Ouagadougou 03/Burkina Faso
[2]Inserm, UMR_S 1072, 13015, Marseille, France
[3]Aix-Marseille Université, UNIS, 13015, Marseille, France
[4]Research Institute in Health Sciences (IRSS) Department Medicine-Pharmacy and Traditional Pharmacopoeia (MEPHATRA/PH) (French), .03 BP 7192 Ouagadougou 03/Burkina Faso

Abstract: *Annona senegalensis* Pers. (Annonaceae) is claimed in traditional medicine in Burkina Faso to be useful in the treatment of epilepsy. The present study aimed to investigate the phytochemical and pharmacological properties of the aqueous extracts of its root bark. The phytochemical analysis was performed using standard tests according to the Ciulei method. The acute toxicity was carried out in rats and the effect of aqueous extract on the pilocarpine and picrotoxin induced seizure in mice and rats were investigated by curative and preventive modes. The phytochemical screening showed the presence of alkaloids, terpinoids and saponins in the aqueous extract of *Annona senegalensis* root bark. The intraperitoneal median lethal dose (LD50) was found to be more than 3000 mg/kg in rats. The curative treatment induced a significant decrease of the intensity of the seizure caused by peritoneal injection of pilocarpine in the treated mice when compared to the negative controls. Time put to inhibit 50 % of the seizures by the plant extract was 16 min at the dose of 150 mg/kg against 14 min for standard diazepam (2 mg/kg). In preventive mode, the plant extract prolonged the latency of convulsions. These results suggest that the root bark of *Annona senegalensis* may possess anticonvulsant properties in rodents.

Keywords: Anticonvulsant, picrotoxin, pilocarpine, preventive, scopolamine, treatment

INTRODUCTION

Epilepsy is the second most common neurological disorder after stroke characterized by recurrent seizures (Gaustaut, 1973; Sander and Shorvon, 1996). In Africa, epilepsy has always been poorly understood by society and has frequently been associated with numerous myths and beliefs (Matuja and Rwiza, 1994; Millogo *et al.*, 2004). Among the 50 million people with epilepsy in the world, approximately 80% of them live in developing countries of which 10 million people in Africa alone (Ngounou *et al.*, 2007; Senanayake and Roman, 1993). Despite an increasing number and variety of anti-epileptic drugs, many refractory cases of epilepsy still remain highly resistant to AED treatment (Heinemann *et al.*, 1994; Shorvon, 1996). Moreover, the current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, including chronic toxicity and adverse effects on cognition (Devinsky, 1995; Mattson, 1995). Due to the limited availability of affordable conventional pharmaceutical medicines in many tropical countries, about 80% of the African rural population is using the complementary and alternative medicine; this is often the only treatment available (Millogo *et al.*, 2004; Preux and Druet-Cabanac, 2005). The frequency of epilepsy, coupled with inaccessibility of some antiepileptic, makes it a major public health problem in developing countries (Ngounou *et al.*, 2007; Senanayake and Roman, 1993). In Burkina Faso, beliefs in supernatural causes and traditional treatment of epilepsy contribute to the underutilization of the medical health services (Millogo *et al.*, 2004; Nitiéma *et al.*, 2012). In this context, it thus becomes important to identify and evaluate commonly available traditional treatment of epilepsy, which are not completely free from adverse effects (Ngounou *et al.*, 2007; Njamnshi *et al.*, 2010; Pradeep *et al.*, 2009).Our study is thus the subject of an effort to upgrade and promote the African traditional medicine in order to contribute to the management of epilepsy. Central effects were already demonstrated in several species belonging to the Annonaceae such as *Annona muricata*, *Annona diversifolia*, *Annona senegalensis* (Ezugwu and Odoh, 2003; Leboeuf *et al.*, 1980; Ma Eva *et al.*, 2006; N'gouemo *et al.*, 1997; Okoli *et al.*, 2010; Pradeep

Corresponding Author: A. Konate, Laboratory of Pharmacology and Toxicology, UFR/SDS University of Ouagadougou, (French), 03 BP 7021 Ouagadougou 03/Burkina Faso, Tel. 0022670241568.

et al., 2009). *Annona senegalensis* Pers. is widely distributed in West African, different parts of this plant are reputed to be useful in the treatment such as fever, intestinal troubles, stomach ache, gonorrhea, syphilis, rheumatism and central disorders (Okoli *et al*., 2010; Oliver-Bever, 1982; Ouedraogo, 1996). This study is a contribution to the study of the anticonvulsant effect of macerated aqueous bark of roots of *Annona senegalensis,* plant used in traditional treatment of epilepsy and convulsions in Burkina Faso. In this study, we investigated the ethno medical use of *Annona senegalensis* as an antiepileptic agent, by testing the anticonvulsant effect of the aqueous extract of the plant's root bark using two standard experimental models: pilocarpine and picrotoxin induced seizures. The acute toxicity potential (LD_{50}) and phytochemical constituents of the extract were also tested.

MATERIALS AND METHODS

Collection and identification of plant material: *Annona senegalensis* Pers. (Annonaceae) was collected in August 2010 at Ouagadougou, Burkina Faso and was authenticated by Millogo *et al*. (2004) (Botany Department, University of Ouagadougou). A voucher herbarium specimen with number HNB8713 was deposited in the National Herbarium of Burkina.

Preparation of *Annona senegalensis* root barks aqueous extract: After collection, the root-barks were washed with distilled water, dried in a ventilated room For 2 weeks and afterwards ground into fine powder. 450 g of the powder was macerated for 24 h in water (1.5 L) and filtered. The resultant filtrate was freeze-dried, to obtain the dried plant extract which was kept in a desiccators. Working solutions were freshly prepared on each day of the experiment.

EXPERIMENTAL ANIMALS

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).

Phytochemical screening: Phytochemical tests were performed according to Ciulei (1982). Bontrager's reaction was used to characterize antraquinones; FeCl3 was used for tannins test; Dragendorff reaction for alkaloids; UV light of alkalinized extracts for coumarins; frothing test for saponins and the Liebermann-Buchard reaction for triterpenoids and steroids.

Acute toxicity test: Determination of acute toxicity: The median lethal dose (LD_{50}) of *Annona senegalensis* (*A. senegalensis*) root bark aqueous extract was determined in rats according to a modified method of Lorke (1983). The extracts were administered at the doses of 100, 500, 1000, 2000 and 3000 mg/kg i.p., to 5 groups of rats. Another group was given normal saline to serve as the control. They were all kept under same conditions and mortality was recorded in each group within 24 h. Symptoms and signs of toxicity were noted and the LD_{50} estimated from as the square of the lowest lethal dose and the highest nonlethal dose from the second stage of dosing.

PHARMACOLOGICAL TESTS

Pilocarpine induced seizures: Seizures were induced according to the method of Setkowicz *et al.* (2003) with slight modification. A single dose of pilocarpine (240 mg/kg, i.p) was injected. 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 Plexiglas cages and observed. 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 (Setkowicz *et al*., 2003; Turski *et al*., 1983):

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

Intermediate seizures (rated as 1.5 or 2.0):

- **1.5:** Clonic movements of forelimbs and mild whole body convulsions, exophthalmia, aggressive behavior

- **2.0:** Rearing and running with stronger tonic-Clonic motions including hind limbs, tail hypertension, lock jaw

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

The curative treatment: The animals were randomly divided into 6 groups of 6 mice. Group (A 1) mice were treated with physiological saline and used as negative control animals. Group (A 2) mice received injections of diazepam (2 mg/kg i.p) and used as positive control animals. Groups (A 3, A 4 and A 5) were treated respectively with 50, 100 and 150 mg/kg, i.p 30 min before the maximum peak of the convulsions.

The preventive treatment: The animals were randomly divided into 3 broad experimental groups of 6 mice. Group (B 1) mice were treated with physiological saline and used as negative control animals. Group (B 2) mice received injections of diazepam (2 mg/kg i.p) and used as positive control animals. Group (B 3) was treated one dose of the extract (50 mg/kg, i.p) 30 min before the start of the convulsions. The time of the peak seizures was determined.

Therefore, during the 4h period following the pilocarpine injection, the animals were continuously observed. 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). Mice experiencing lethal convulsions were excluded from the study. The Percentages of Inhibition (P.I) of the convulsions were calculated according to the following formula:

$$P.I = [(A - B)/A] \times 100$$

A = score of the control group
B = score of the treated groups

Picrotoxin induced seizures: The anticonvulsant effect of *A. senegalensis* root bark aqueous extract was tested in the rats according to Vellucci method with some modifications (Vellucci and Webster, 1984). The standard convulsant agent, picrotoxin (PTX, 10 mg/kg i.p) was used to induce convulsions in the rats. Diazepam (DZP, 2 mg/kg i.p) was used as reference anticonvulsant drug for comparison. Rats of either sex were randomly divided into five groups of 10 rats (Mahomed and Ojewole, 2006). The positive control group of rats received diazepam (2 mg/kg, i.p) 20 min before picrotoxin (10 mg/kg, i.p) injection. The test groups were injected of (200, 300 and 400 mg/kg, i.p) doses of the extract, 30 min before picrotoxin (10 mg/kg, i.p) injection. Following induction of convulsions; the animals were observed for 30 min for signs of neurological deficits. Subsequent period of time for latency to first convulsion, time before onset of clonic convulsions and mortality percentage were recorded. Abolition of clonic convulsions during 30 min of observation was the criterion of anticonvulsant activity. Rats that did not convulse 30 min after injection of the picrotoxin were considered protected (Adeyemi *et al.*, 2010; Yemitan and Adeyemi, 2005).

Statistical analysis of data: The data are expressed as mean±S.E.M. The difference among means has been analyzed by using one-way ANOVA followed by Dennett's Multiple Comparison test. A value of $P<0.05$ was considered as statistically significant.

RESULTS

Phytochemical screening: Phytochemical analysis of the extract showed the presence of sterols and/or triterpenes, anthocyanes, glucids, coumarins, flavonoids and alkaloids.

Acute toxicity study: The intraperitoneal administration of *A. senegalensis* root bark aqueous extract in rats gave an LD_{50} value superior to 3000 mg/kg.

ANTICONVULSANT TESTS

Effect of the plant extract on pilocarpine-induced seizures: Animal reached their convulsion peak 2 h 30 min after the injection of pilocarpine (Fig. 1). Thus, the curative treatment was carried out 30 min before the maximum intensity. The anticonvulsant effect of *A. senegalensis* root bark aqueous extract is represented by Fig. 2 curative mode and 3 preventive modes. Times to inhibit 50% of the pilocarpine induced seizures were of 30; 22; 16 minutes for the respective doses of 50; 100 and 150 mg/kg for the plant extract and 14 min for Diazepam (2 mg/kg). Results showed that the plant extract (100; 150mg/kg i.p) has significant ($p<0.05$) curative effects on the convulsions induced by pilocarpine. The results showed that the plant extract (50 mg/kg i.p) has significant ($p<0.001$) preventive treatment effects on the convulsions induced by pilocarpine.

Table 1: Effects of *Annona senegalensis* root bark aqueous extract and Diazepam (DZP) on picrotoxin-induced convulsions in rats

Treatment dose (mg/kg i.p)			N°. convulsing / N° used	Animal not convulsing (%)	Latency of tonic convulsions (min.) Mean ±S .E.M.
PTX	A.senegalensis	Diazepam			
10	-	-	0/10	0	6, 40±3, 03
10	200	-	1/10	10	6, 89±2, 16
10	300	-	2/10	20	6, 63±2, 12
10	400	-	4/10	40	6, 67±1, 41*
10	-	2	8/10	80	12, 00±0, 58***

Values are expressed as mean ± SEM. *: p<0.05; *** : p<0.0001; compared with the control. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test

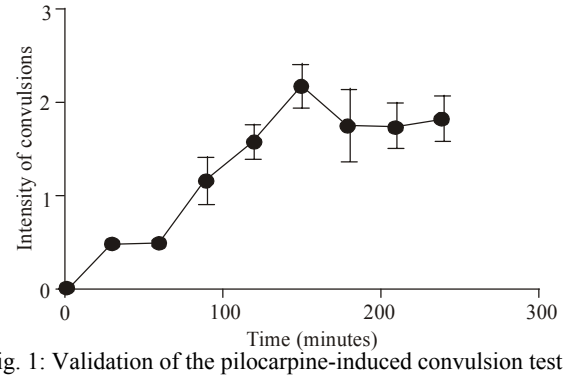

Fig. 1: Validation of the pilocarpine-induced convulsion test

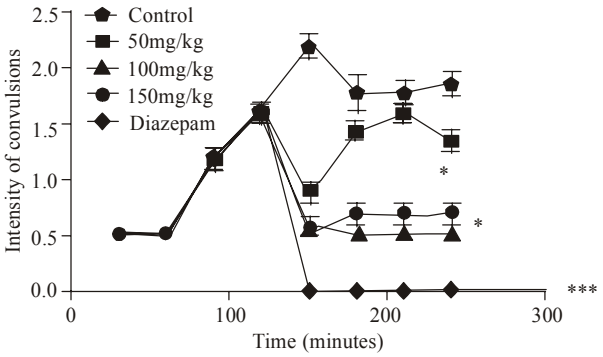

Fig. 2: Curative effect of the plant extract on the convulsions. Values are mean ±S.E.M. for six mice. *: p<0.05; ***: p<0.0001; significant from control (one way ANOVA analysis followed by Dennett's test)

Effect of the plant extract on picrotoxin (PTX)-induced seizures: Table 1 represents the survival probability of animals. Diazepam used as positive control showed a high significant (p<0.001) protection against the convulsion effects of picrotoxin injection. *A. senegalensis* root bark aqueous extract protects significantly (p<0.05) at 400 mg/kg.

DISCUSSION

The phytochemical analysis of the extract showed the presence of sterols and/or triterpenes, anthocyanes, glucids, coumarins and alkaloids; either one or combination of which may be responsible for the observed anti-convulsant effect of *A.* senegalensis. Acute toxicity test established a high LD_{50} which suggests that the aqueous extract of *A.senegalensis* may be generally regarded as safe with a remote risk of acute intoxication at high doses. This finding probably suggests that the plant aqueous extract is relatively safe in, or non-toxic to, rats.

In previous experimental studies, anticonvulsant effect has been shown for the hydro alcoholic fraction of the root bark extract and leaf of *A.senegalensis* in effect on Maximal Electroshock (MES) induced Seizures and Pentylenetetrazole (PTZ) induced seizures when administered orally (Okoli *et al*., 2010; Okoye *et al.*, 2010). According to Mahomed and Ojewole (2006), oral administration could lead to loss of some pharmacological activities of plant extracts (Mahomed and Ojewole, 2006). This study was undertaken to complete the pharmacological investigation of *A.senegalensis* root bark aqueous extract as an anticonvulsant. The model of temporal lobe epilepsy induced in rats by the muscarinic cholinergic agonist, pilocarpine reproduces most clinical, developmental and neuropathlogical features of human temporal lobe epilepsy (Turski *et al.*, 1983).

In the preventive test, the plant extract prolongs the latency of convulsions. Figure 3 indicates that it was preferable to consider the preventive treatment than the curative treatment at the dose of 50 mg/kg. *A.senegalensis* root bark aqueous extract significantly delayed the onset of seizures induced by pilocarpine. Picrotoxin has been reported to produce seizures by inhibiting Gamma-Aminobutyric Acid (GABA) neurotransmission by blocking the chloride channels linked to $GABA_A$-receptors (Coulter *et al.*, 1989; Nils Ole, 2003). The intraperitoneal administration of *A. senegalensis* root bark aqueous extract protects against picrotoxin-induced seizures in rats. The drug that possesses activity against the picrotoxin-induced seizures gives the probable idea about their mechanism of action (Raza *et al.*, 2010). This hypothesis could explain the observed *in vivo* antagonistic actions of diazepam against picrotoxin-induced seizures.

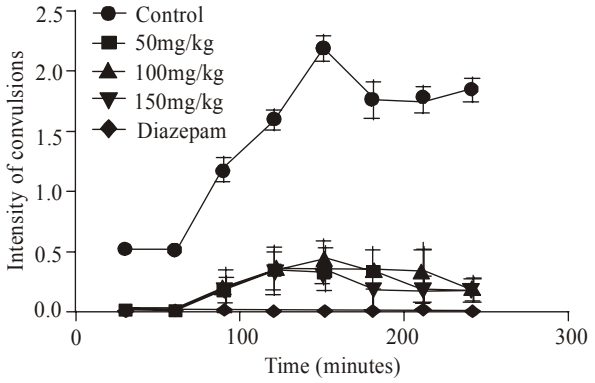

Fig. 3: Effect of plant extract on the convulsions in preventive treatment. ***: p<0.001; significant from control (one way ANOVA analysis followed by Dennett's test)

The observed anticonvulsant activity of *A. senegalensis* root bark aqueous extract may also be due by one or more of the known mechanisms of anticonvulsant action, such as alteration of Na^+/K^+ ATPase expression and inhibition of expression of inducible nitric oxide (Kang *et al*., 2004; Mahomed and Ojewole, 2006). These mechanisms could explain, at least in part, some of the anticonvulsant effects of *A. senegalensis* root bark aqueous extract. According to the literature one or more of the major chemical constituents of the plant, flavonoids, triterpenoids, may possibly account for the observed anticonvulsant activity of the plant extract, but there is, at present, insufficient evidence to justify this speculation (Pradeep *et al*., 2009).

CONCLUSION

The findings of the present laboratory animal study could justify folkloric uses of *A. senegalensis* root bark aqueous extract in the treatment of epilepsy. Further studies will be done to determine the mechanisms of the activity of the extracts and to isolate compounds which are responsible of this activity.

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