

Anticonvulsant and sedative effects assessment of aqueous extract of *Crossopteryx febrifuga* seeds in mice.

D. J. Bassoueka^{1,2}, E.C. Loembet Mavethy¹, A.B.E. Loufoua^{1,2}, A.A. Abena¹

¹Laboratory of Pharmacodynamics and Experimental Physiopathology (L2PE), Faculty of Science and technology, University Marien Ngouabi, BP: 69 Brazzaville-Congo,

²Biochemistry and Pharmacology Laboratory, Faculty of Health Sciences, Marien Ngouabi University, BP : 69 Brazzaville- Congo

Abstract

The objective of this study was to evaluate the anticonvulsant and sedative effects of seeds of *Crossopteryx febrifuga*, a Congolese medicinal plant belonging to the rubiaceae family. Two pharmacological test were carried out to evaluate each of its properties, respectively: Picotoxine induced convulsions test (PIC 7.5 mg / kg) and Strychnine induced convulsions test (STR 2.5 mg / kg); the motricity test and the barbiturate sleep potentiation test induced by phenobarbital 50 mg / kg in mice. The results obtained showed that the aqueous extract of the seeds of *C. febrifuga* at a dose of 200 mg / kg caused a significant increase in the time to onset of the seizures induced by PIC and a non-significant decrease in the duration of seizures by compared to the control at doses of 100 and 200 mg / kg. However, the aqueous extract of *C. febrifuga* seeds at doses of 100 and 200 mg / kg resulted in an increase and a non-significant decrease in the time to onset and duration of seizures, respectively, compared to the control.

Keywords: *Crossopteryx febrifuga*, anticonvulsant, sedative, mouse, seed

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I. Introduction

Medicinal plants are an important reservoir of natural substances, attributing to them a capital importance for their use in the treatment of certain ailments (Thurre, 2007; Chaudhary et al, 2010; Singh et al, 2014). Finding a herbal remedy for epilepsy and anxiety would benefit thousands of lives. This study focuses on the seeds of *Crossopteryx febrifuga*, a plant belonging to the Rubiaceae family, traditionally used to treat several pathologies such as bacterial and parasitic infections (Chouna et al, 2015), stomach aches (Salawu et al. , 2011), cough (Sutovskaa et al, 2009), malaria (Flufoye et al, 2004). In addition, in the Republic of Congo, an ethnobotanical survey identified 13 plants suspected of anticonvulsants, including *Crossopteryx febrifuga* (Bassoueka et al, 2015). Thus, this work is based on the evaluation of anticonvulsant and sedative effects of aqueous extract of *C. febrifuga* seeds in mice.

II. Material and methods

2.1. Plant material

The seeds of *Crossopteryx febrifuga* collected in Kinkala (pool sub-prefecture) of Congo in March 2019.

2.2. Preparation of the extract

50 g of seed of *Crossopteryx febrifuga* powder was boiled in 500 mL of distilled water for 30 minutes at 75 ° C. After cooling, the decoction is filtered. The filtrate thus collected was then evaporated at reduced temperature (50-60 ° C). The dry extracts obtained were used to prepare the test solutions.

2.2. Animal material

Male and female Swiss mice albinos strain (weight: 20 - 35g), reared under standard conditions with free access to food and drinking water.

2.4. Evaluation of the effect of seeds of *C. febrifuga* on PicROTOXINE and Strychnine-induced convulsions (Lehmann 1988)

2.4.1. PicROTOXINE-induced convulsions test

Clonic convulsions are induced in mice one hour after all treatments by intrapéritonéal injection of PicROTOXINE 7.5 mg/kg. Mice were observed for 15 min, the time to onset as well and the duration of convulsion in each batch are determined (Ngo Bum et al. 2004 ; Bassoueka et al., 2016). The groups compound by 4 mice each one were formed: The negative control group received distilled water 0,5 ml/100g p.o, the positive control group

received clonazepam 3mg/kg, the test group are treated with aqueous extract of the seed of *C. febrifuga* at doses 100 and 200 mg/kg respectively

2.4.2. Strychnine-induced convulsions test

Tonic convulsions are induced in mice within 10 minutes by intraperitoneal administration of strychnine 2.5 mg / kg, one hour after all treatments. The animals are then observed. The time to onset as well as the duration of the convulsions in each batch are determined (Porte et al., 2001, Ngo Bum et al., 2004, Bassoueka et al., 2016). The groups compound by 4 mice each one were formed : the negative control received distilled water 0.5 mL / 100 g, p.o; the positive control group are treated with the reference molecule: diazepam 10 mg / kg, p.o, the test group received the aqueous extract of the seeds of *Crossopteryx febrifuga* at doses 100 and 200 mg / kg respectively

Preliminary study on the mechanism of aqueous extract of the seeds of *C. febrifuga*

2.4.3. Effects of aqueous extract of the seeds of *C. febrifuga* on motor activity

The test consists of appreciating, using a grid board cage comprising 16 squares of 40 × 40 cm, the number of squares traveled by a mice in five (5) minutes. The groups compound by 5 mice each one were formed: The negative control group received distilled water 0.5 mL / 100 g; the positive control group was treated with the diazepam 10 mg / kg (reference molecule), the test groups received the aqueous extract of the seeds of *Crossopteryx febrifuga* at doses of 100 and 200 mg / kg respectively. One hour after administration of the products, the animals are placed in turn in a grid cage, and the number of squares crossed by them after five (5) minutes is noted.

2.5.2. Phénobarbital induced-sleeping test

Intraperitoneal administration of phénobarbital induce spleep in mice. The groups compound by 5 mice each one were formed. The negative control group received distilled water 0,5 mL/100 g, p.o., the positive control group was treated with the diazépam 10 mg/kg (référence molécule) p.o., the test groups received the aqueous extract of the seed of *Crossopteryx febrifuga* respectively at doses of 100 et 200 mg/kg, p.o. One hour after administration of the products, the sleeping is induced by intraperitoneal administration of phénobarbital 50 mg/kg. the time to onset and duration of sleep in mice (Ngo Bum et al., 2001, Rakotonina et al., 2001, Bassoueka et al., 2015).

III. Statistical analysis

Statistical analysis was performed using Excel software (Office 2010) and the results expressed as mean ± ESM were subjected to one-way analysis of variance followed by Student-Fischer's t test ($p < 0, 05$, $p < 0.01$, $p < 0.001$). (Schwartz D. E., 1963).

IV. Results

4.1 Effect of aqueous extract of the seeds of *C. febrifuga* against picrotoxin-induced convulsions

The figures 1 and 2 show the effects of the aqueous extract of the seeds of *C. febrifuga* on the time to onset and duration of seizures in mice, respectively. These results suggest that the aqueous extract of the seed of *C. febrifuga* at dose of 200 mg / kg caused a significant increase (** $p < 0.01$ and * $p < 0.05$) of the time to onset of convulsions in mice (Figure 1). Regarding the duration of the convulsions, the Figure 2 show that the aqueous extract of the seeds of *C. febrifuga* at doses 100 and 200 mg / kg caused a non-significant decrease in the duration of the convulsions in mice.

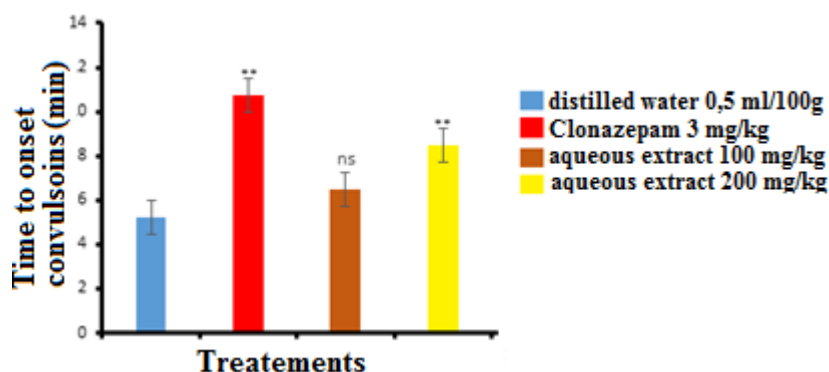


Figure 1: Effect of aqueous extract of the seeds of *C. febrifuga* on time to onset of seizures. The values are expressed as mean ± ESM; n = 4; ** $p < 0.01$; ns = not significant compared to the control.

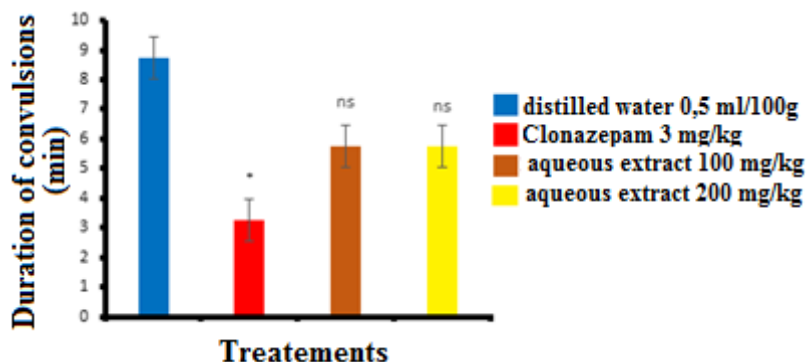


Figure 2: Effect of aqueous extract of seeds of *C. febrifuga* on duration of seizures. The values are expressed as mean \pm ESM; n = 4; * p < 0.05; ** p < 0.01; ns = not significant compared to the control.

4.2. Effect of aqueous extract of the seeds of *C. febrifuga* against strychnine-induced seizures (STR)

The figures 3 and 4 show the effect of the aqueous extract of the seeds of *C. febrifuga* on the time to onset and duration of seizures in mice, respectively. These results show that the aqueous extract of the seeds of *C. febrifuga* at doses of 100 and 200 mg / kg increased and decreased in a non-significant manner, respectively, the time and duration of onset of seizures compared to the control.

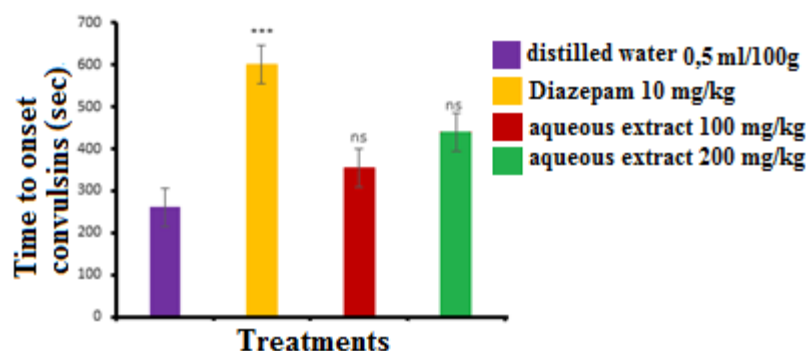


Figure 3: Effect of aqueous extract of the seeds of *C. febrifuga* on time to onset of seizures induced by strychnine; The values are expressed as mean \pm ESM; n = 4; *** p < 0.001; ns: not significant compared to control.

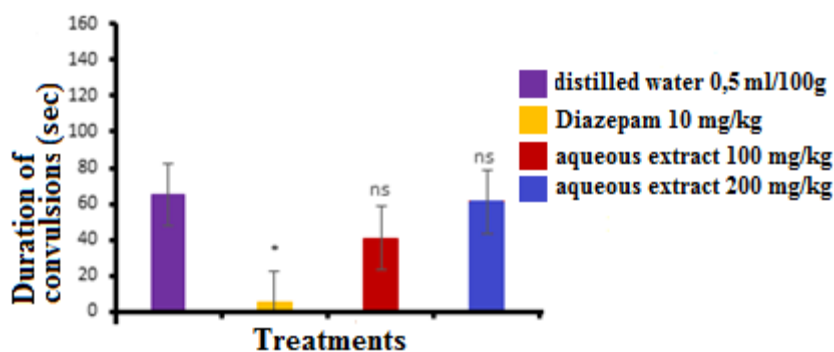


Figure 4: Effect of the aqueous extract of *C. febrifuga* on the duration of strychnine-induced seizures; The values are expressed as mean \pm ESM; n = 4; ns: not significant compared to control.

4.3. Effect of aqueous extract of the seeds of *C. febrifuga* on motor activity and barbituric sleep

4.3.1. Effect on motor activity

The results of motor activity are shown in the Table I. it was found that the aqueous extract of the seeds of *C. febrifuga* at a dose of 100 mg / kg caused an insignificant decrease in motor activity in mice compared to control.

Table I: Effect of aqueous extract on motor activity

Treatments	Doses (mg/kg)	Number of squares traveled during 5 minutes
Distilled water	0,5 (a)	236,2 ± 20,14
Diazepam	10	52,4 ± 3,04 *
Seed aqueous extract	100	167,8 ± 9,55 ^{ns}
Seed aqueous extract	200	225,6 ± 14,52 ^{ns}

(a) : mL/100g ; values are expressed in average ± ESM ; n=5 ; *p < 0,05 ; ns : non significative according to control

4.2.2. Effect of aqueous extract of *C. febrifuga* on barbituric sleep

The figures 5 and 6 show the effect of the aqueous extract of *C. febrifuga* on time to onset and duration of sleep in mice, respectively. These results suggest that the aqueous extract of the seeds of *C. febrifuga* increases significantly (* p <0.05; ** p <0.01) the time to onset sleep at a dose of 200 mg / kg (figure 5). *C. febrifuga* extract did not have an effect on sleep duration like the reference molecule (Figure 6).

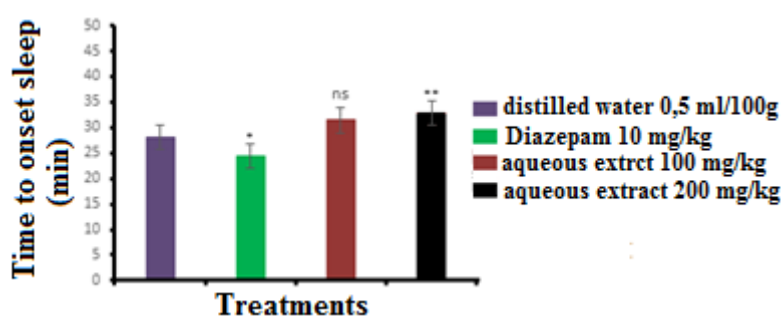


Figure 5: Effect of aqueous extract of *C. febrifuga* seeds on time to sleep onset; The values are expressed as mean ± ESM; n = 5; * p <0.05; ** p <0.01; ns: not significant compared to control;

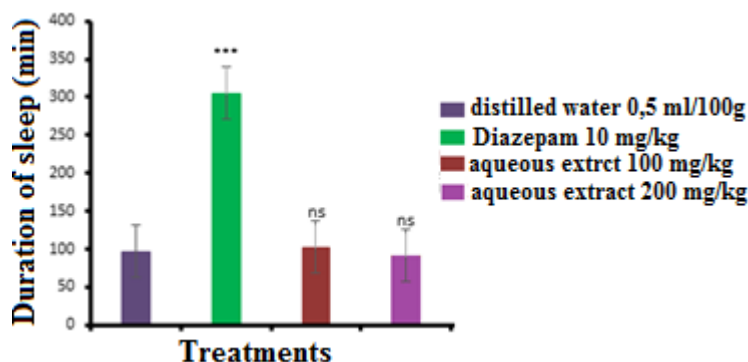


Figure 6: Effect of aqueous extract of *C.f* seeds on sleep duration; The values are expressed as mean ± ESM; n = 5; *** p <0.001; ns: not significant compared to the control.

V. Discussion

The aim of the present study was to evaluate the anticonvulsant and sedative properties of the aqueous extract of the seeds. of *C. febrifuga* Two pharmacological tests were used to evaluate each of its properties, respectively: Picrotoxine and Strychnine-induced convulsion test; the motor test and the barbituric sleeping potentiation test in mice. The results obtained showed that the aqueous extract of the seeds of *C. febrifuga* at a dose of 200 mg / kg causes a significant increase of the time to onset of convulsions induced by PIC, like clonazepam. At doses of 100 and 200 mg / kg the extract resulted a non-significant reduction in the duration of the convulsions compared to the control. This assumes that *C. febrifuga* would have a dose dependent anticonvulsant effect. (Oudugbemi, 2006; Mustafa et al., 2008; Ngo Bum et al., 2008; Bassoueka et al., 2016) because PIC is an antagonist of the GABAA receptor, the chloride channels of which it blocks (Rang et al., 2007) . Concerning the convulsions induced by STR, the results obtained show that the aqueous extract of the seeds of *C. febrifuga* at doses of 100 and 200 mg / kg respectively caused an increase and a non-significant decrease in the time to onset and the duration of the seizures. convulsions relative to the witness. These results suggest that the aqueous extract of the seeds of *C. febrifuga* would have an anticonvulsant effect at high doses and would act at the level of the glycine-dependent receptor, to inhibit the opening of the chloride channel.

(Bassoueka et al 2016). The aqueous extract of the seeds of *C. febrifuga* at a dose of 100 mg / kg caused an insignificant decrease in motor activity as well as barbiturate sleep in mice.

The results previously found with aqueous and methanolic extracts of the leaves of *C. febrifuga* were more interesting (Bassoueka et al 2016). This allows us to say that the anticonvulsant and sedative activity of *C. febrifuga* is more concentrated in the leaves than in the seeds.

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