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Anticonvulsant Activity of the Decoction of *Crossopteryx febrifuga* in Mice

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Abstract: ***The decoction of Crossopteryx febrifuga is evaluated for anticonvulsant activity in mice using strychnine (STR), picrotoxin (PIC), pentylenetetrazole (PTZ), maximal electroshock (MES) and isonicotinic hydrazid (INH) induced convulsions. A decoction of C. febrifuga at doses of 80 and 100 mg/kg protects 83.33% of mice against STR-induced convulsions. At the dose of 120 mg / kg, it protects 100% of mice against PIC-induced convulsions. On PTZ-induced convulsions, C. febrifuga decoction protects 66.67% and 100% of mice, respectively at doses of 120 and 100 mg / kg. While 66.67% and 83.33% of mice are protected against MES-induced convulsions respectively at the doses of 100 and 120 mg / kg. Moreover, it increased the latency time of convulsions induced by INH at the doses of 80, 100 and 120 mg / kg.***

Keywords: Epilepsy, anticonvulsant, *Crossopteryx febrifuga*, mice, decoction

1. Introduction

Belonging to Rubiaceae familly, *C. febrifuga* (Benth) is a species of origin Zambezian Sudano-GC-Z, it is a twisted tree with conspicuous tubular flowers, which is distributed throughouth the savanna region and forests of west and tropical Africa [1,3,4]. In Africa, *C. febrifuga* is used in traditional medicine to treat many diseases [1,3]. This plant is used by traditional healers to treat much affection. The roots juice diluted in water is used as bath against stomach pain; as nasal drop to calm headache, and as eye drops acting on the connective tissue of eye. All informants recognize that the contact of the juice with the mucosa is extremely painful. The decoction of the roots is also prescribed as a mouthwash to treat various oral diseases and tooth decay, in this last case, the treatment is followed by the application of the root powder on the decay tooth. Some fetishists use the plant to treat gonorrhea, epilepsy, heart aches and sores [3]. Many studies have been conducted on this plant; *C. febrifuga* has showed effect against peptic ulcer, acting by reducing the aggressive factor on the gastro-duodenal mucosa or by increasing mucosal resistance against it [10]. *C. fabrifuga* decoction possess anti-ulcerogenic activity [22]. It is used traditionally for symptomatic relief of dry cough and for treatment of respiratory infections, fever, dysentery and pain [19]. It possesses potential hypoglycemic and hypolipidemic activies [19]. Infectious diseases, It is used to treat trypanosomias, staph aureus infections [25,8]. In northem Nigeria *C. febrifuga* is widely used in the therapeutic management of trypanosomiasis, malaria and painful inflammatory disorders [23]. This plant is also used in Congo-Brazzaville to treat headaches, migraine, bacterial infections and epilepsy [4]. Epilepsy is a disease that affects 0.5% to 1% of the population and whose cause is unknown in 80% of cases. The occurrence of epileptic discharge supposes coexistence of constitutional or acquired hyperexcitability and a hypersynchrony of a group of neurons. These basic electrophysiological disturbances result an imbalance in the neurotransmission system excitatory neurotransmitters which are amino acids (glutamate and aspartate) and inhibitor-mediated system by gamma-aminobutyric acid (GABA) [1]. The etiologies for their part are discovered. They may be traumatic, tumor or infectious. Epilepsy is the most common disabling neurological condition. In Africa, its incidence is 73 ‰ inhabitants and a prevalence of 1 and 50 %. In Congo-Brazzaville, the prevalence of epilepsy is still unsolved. The literature reports no study on the anticonvulsant activity of this plant. The aim of the present study is to assess the anticonvulsant effect of *C. febrifuga* decoction in mice.

2. Material and Methods

2.1 Plant Material

Leaves of *C. febrifuga* was authenticated by Pr. MOUTSAMBOTE, botanist at the National School of Agronomy and Forestry (SCSTA) of University Marien NGOUABI-CONGO. A voucher specimen (reference 8012) was deposited in the national herbarium of the Centre for Study of Plant Resources (CERVE) of CONGO-Brazzaville. These leaves were dried at room temperature (25°) in the laboratory for approximately 20 days. The decoction of the plant is prepared daily. 10 g of the plant powder was set in 50 ml of boiling water for 20 min at 100 ° C. After cooling, the decoction is filtered. The Filtrate volume measured for each test specimen was 10 ml. Another solution was prepared in the same manner and concentrated to oven. After concentration, the yield was 14.4%.

2.2. Biochemicals

Diazepam, Clonazepam, Isonicotinic hydrazide acid (INH), Picrotoxin (PIC), Strychnine (STR) and Pentylenetetraol (PTZ) were used.

2.3. Animals

Male and female swiss mice (20-30g) were used in this research. Animals were obtained from laboratory of the Faculty of Science, University of Ngaoundere (Cameroon).

They were maintained under standard condition of light cycle (light/dark 12/12h), at the temperature of 25°C, with free access to food and water.

2.4. Strychnine-inducted convulsions test

Six groups compound by six mice each one were formed and treated as follows: the negative control group received distilled water at a dose of 10 ml/kg, the positive control group received clonazepam 3mg/kg intraperitoneally, and the test groups are treated with decoction at doses 60, 80, 100 and 120 mg/kg. Treatment was given 1h prior to STR. Convulsions flowed by death were induced in mice by intraperitoneal injection of strychnine 2.5 mg/kg. Then the mortality of animal was recorded. Animals that survived more than 10 min were qualified as protected [11, 15, 16].

2.5. Picrotoxin-inducted convulsions test

Six groups compound by six mice each one were formed, and treated as follows, the negative control group received distilled water at a dose of 10 ml/kg , the positive control group was treated with clonazepam 0.4 mg/kg intraperitoneally, the test group are treated with decoction of *C. febrifuga* at doses 60, 80, 100, and 120 mg/kg. An hour later, the animals received picrotoxin by intraperitoneal injection of 7.5 mg/kg. Mice were observed for 15 min, the animals that survived more than 15 min were qualified as protected [14, 16].

2.6. Pentylenetetrazol-inducted convulsions test

Six groups compound by six mice each one were formed and treated as follows: The negative control group received distilled water 10 ml/kg p.o, the positive control group received clonazepam 0.1 mg/kg intraperitoneally. Convulsing crises were induced in mice by intraperitoneal injection of pentylenetetrazol 70 mg/kg, the protected of the different treatments given 1 h before PTZ injection was valued. Animals that did not convulse within the 10 min were qualified as protect [11,14, 15].

2.7. Maximal electroshock- induced seizures

Mice were divided in Six groups compound by six mice each one, and Treated as follows, group 1 (negative control) received distilled water 10 ml/kg body weight, group II (positive control) was Treated with diazepam 50 mg/kg intraperitoneal, the four others groups (test groups) were treated with four different doses (60, 80, 100 and 120 mg/kg) of the plant decoction. Tonic convulsions of the hind extremities of mice were induced by passing alternating electrical current (50 Hz, 20 mA, 0.2 s) via two electrodes initially immersed in a solution of 9 % of Nacl and them applied to mice eye [14,17].The number of animals protected from tonic hindlinb extension was determined in each group.

2.8. Isonicotinic hydrazide acide test

Six groups compound by six mice each one were formed and treated as follows, the control group received the distilled water 10 ml/kg, the reference group was treated with diazepam 10 mg/kg orally, the four test groups were orally treated with different doses of *C. febrifuga* decoction (60, 80, 100 and 120 mg/kg). Then, animals were injected intraperitoneally with isonicotinic hydrazide acide (250 mg/kg), 1 h after the different treatments, and latency times of clonic convulsants were recorded [14].

2.9. Statistical Analysis

Two parameters were measured: the latency time of seizures for INH test and the percentage of protection in mice against MES and chemically products induced convulsions. The averages of these parameters are compared with those obtained in mice of the negative control group. The results are expressed as mean ± SEM. ANOVA followed by Dunnett test was used for Percentages of protection. The test "t" student was used to analyze latency time of convulsions.

3. Results

3.1. Percentage of mice protection against STR- induced seizures

The decoction of *C. febrifuga* protected 50% of mice at doses of 60 and 120 mg/kg, and 83.33% of mice ($p \leq 0.001$) at doses of 80 and 100 mg/kg against STR-induced seizures (Fig. 1)

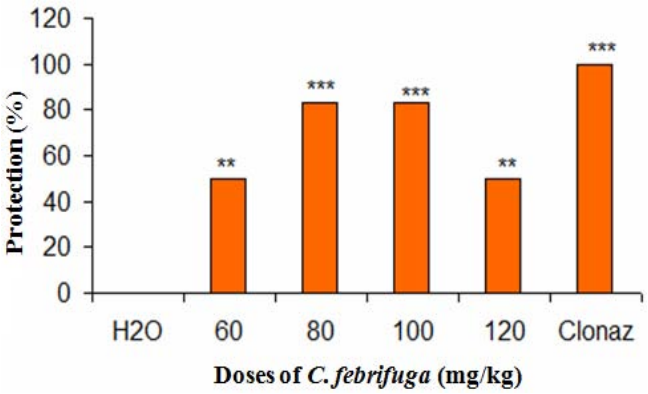

Figure 1: Effect of *C. febrifuga* on the percentage of mice protected against STR-induced seizures. Data are presented as mean ± SEM. n = 6 per dose, ** $P \leq 0.01$; *** $P \leq 0.001$, significant difference from the negative control group. H2O = Distilled water (negative control), Clonaz = Clonazepam 3 mg/kg (positive control)

3.2. Percentage of mice protection against PIC- induced seizures

The decoction of *C. febrifuga* at doses 80, 100 and 120 mg/kg protected receptively 50% ($p \leq 0.01$), 83.33 % and 100% ($p \leq 0.001$) of mice against PIC-induced seizures (Fig. 2)

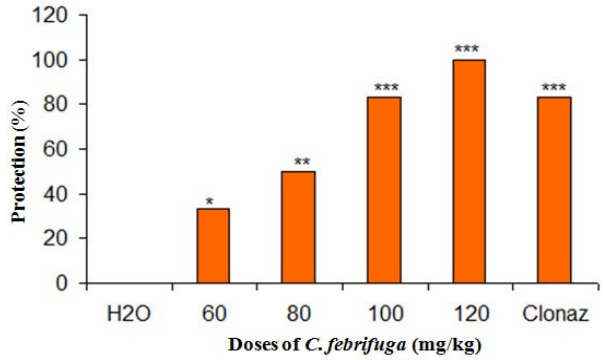

Figure 2: Effect of *C. febrifuga* on the percentage of mice protected against PIC-induced seizures. Data are presented as mean ± SEM. n = 6 per dose, * P ≤ 0.05 ** P ≤ 0.01; *** P ≤ 0.001, significant difference from the negative control group. H2O = Distilled water (negative control), Clonaz = Clonazepam 0,4 mg/kg (positive control)

3.3. Percentage of mice protection against PTZ- induced seizures

At doses 80 and 120 mg/kg, the decoction protected 66.67 % (p <0.01) of mice, 100% of mice at the dose 100 mg/kg against PTZ-induced seizures (Fig. 3)

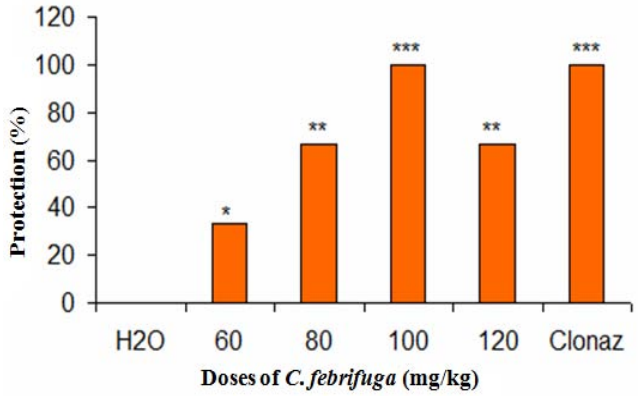

Figure 3: Effect of *C. febrifuga* on the percentage of mice protected against PTZ-induced seizures. Data are presented as mean ± SEM. n = 6 per dose, * P ≤ 0.05 ** P ≤ 0.01; *** P ≤ 0.001, significant difference from the negative control group. H2O = Distilled water (negative control), Clonaz = Clonazepam 0,1 mg/kg (positive control)

3.4. Percentage of mice protection against MES- induced seizures

The fig 4 shows the percentage of mice protection against MES-induced seizures, it appears, at doses of 60, 80 and 120 mg/kg the decoction protected receptively 66.67% of mice, and 83.33% of the mice (p ≤ 0.001) at a dose of 100 mg/kg.

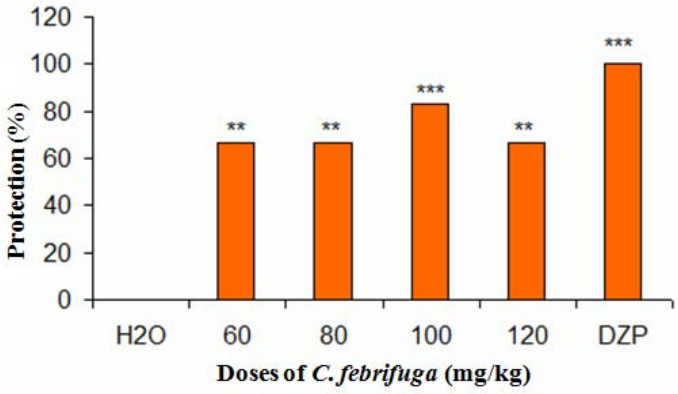

Figure 4: Effect of *C. febrifuga* on the percentage of mice protected against MES-induced seizures. Data are presented as mean ± SEM. n = 6 per dose, * P ≤ 0.05 ** P ≤ 0.01; *** P ≤ 0.001, significant difference from the negative control group. H2O = Distilled water (negative control), Diaz = DZP 50 mg/kg (positive control)

3.5. Latency to onset of seizures by INH- induced seizures

The decoction of *C. febrifuga* increased significantly the time to onset of seizures in the INH test (p ≤ 0.001) (Fig.5)

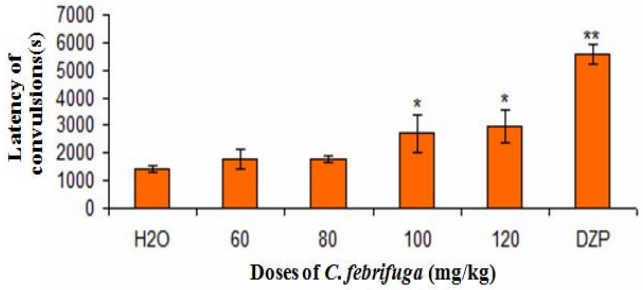

Figure 5: Effect of *C. febrifuga* on the convulsions induced in mice by INH, illustrated here are the time to onset of seizures against INH-induced convulsions. Data are presented as mean ± SEM. n = 6 per dose, * P ≤ 0.05 ** P ≤ 0.01; *** P ≤ 0.001, significant difference from the negative control group. H2O = Distilled water (negative control), DZP = Diazepam 10 mg/kg (positive control)

3.6. Discussion

C. febrifuga, Sudano-Zambezian species is widespread in all savanna and african tropical forests. The literature research conducted on this plant revealed that it has anti-convulsant potential [1,4], which have not been subject to scientific studies. The anticonvulsant activity was studied with a decoction of *C. febrifuga*. Diazepam and clonazepam were used as reference molecules. Two parameters were evaluated, the latency time of convulsions and the percentage of protection of the mice. At doses 80 and 100 mg/kg, the decoction protected 83.33% (p ≤ 0.001) against STR-induced convulsions. These results suggested that the *C. febrifuga* decoction has anticonvulsant activity and acts on glycine strynine-sensitive receptors [9]. On PIC- induced convulsions *C. febrifuga* decoction protected 83.33% of mice at a dose of 100 mg/kg and 100% of the mice at a dose of 120 mg/kg. Antagonism of *C. febrifuga* against PTZ-induced convulsion, at a dose 70 mg/kg, can be explain by

the presence of compounds in the decoction that have anticonvulsant effect probably due to interaction with GABAergic neurotransmission [13, 18, 21]. It appears that at a dose of 120 mg/kg the decoction protected 66.67% of mice ($p < 0.01$) and 100% of mice at a dose of 100 mg/kg very significantly. These results suggest that the decoction of *C. febrifuga* would be able to inhibit the PTZ effects. In addition, substances that block PTZ- induced convulsions would do by improving GABA neurotransmission receptors [18]. This extract may have fore there effects on GABAergic distribution. The decoction at doses 60, 80 and 120 mg/kg protected 66.67%, 83.33% and 100 % of mice against MES-induced convulsions respectivly. These results suggested that the decoction of *C. febrifuga* have anticonvulsant properties and act by inactivation of voltage-dependent sodium channels involved in the genesis of clonic convulsions [7,18]. Moreover, the decoction of *C. febrifuga* at all doses delayed the latency time of INH-induced convulsions. This suggested that *C. febrifuga* have anticonvulsant effect through interaction with GABAergic neurotransmission [2,5,6,7,12,23].

4. Conclusion

The results from our experimental study revealed that decoction of *C. febrifuga* administered per-os in mice, caused a protection of 50 % of animals. At doses of 100 and 120 mg/kg the decoction of *C. febrifuga* protected 100% of animals against PTZ-induced convulsion and PIC-induced convulsion respectively as the reference molecule of diazepam. These results prooved that the plant possesses properties anticonvulsant and it can be continued to use by traditional healers for epilepsy treatment.

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