Crossopteryx febrifuga Benth (Rubiaceae) effect of GABA concentration on brain in rats

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Abstract

C. febrifuga known by the common name (Mvala) is a medicinal plant of the Rubiaceae family widely used by Congolese traditional healers to treat many diseases. For this study two extracts of aqueous and methanol were taken away with the yield of 8.5% and 11% respectively. The convulsions latency time in rats treated with different extracts was greater than 15 minutes. Among five rats one presented convulsions compared to the control group. *C. febrifuga* protected rats against convulsions induced by PIC and STR. Gaba dosage in rats brain homogenizing showed that *C. febrifuga* increased Gaba rate in rats brain (4.10 ± 0.39) compared to the control group (2.04 ± 0.03) significantly. Consequently, histamine rate decreased insignificantly.

Keywords: *Crossopteryx Febrifuga*; Anticonvulsant; Rat; Histamine; Gaba

1. Introduction

The butyric amino gamma acid (GABA) is a chemical messenger very responded in the brain. Its natural function is to reduce neurons nervous activity on which it is fixed. Some researchers raise that the Gaba is used among others for controlling fear and anxiety manifested by neuronal excitation. Gaba receptor is probably the most expanded in the mammalian nervous system where it is estimated that nearly 40% of nervous system synapses would work with the Gaba and therefore imply its receptor that is a channel receptor. That is to say when the Gaba is fixed it changes shapes lightly allowing the chlorine ions negatively charge to cross its central channel which has the effect of decreasing the neuron excitability. Epileptic discharge occurrence presumes a constitutional or acquired hyper excitability coexistence and the neurons group hyper synchrony. These basic electrophysiological disturbances result from an imbalance between the exciting neurotransmission system which neuromediators are amino acids (glutamate and aspartate) and the inhibitor system mediated by gamma amino butyric acid (GABA) [1]. Inquiry conducted nearby Congolese traditional healers showed that *C. febrifuga* shrub from 3 to 8 meters of height with a smooth bark and greyish, shrewdly flaky and brittle with oval leaves abruptly ending in thin and rounded tip to base (adonahum) is used to treat several diseases including the epilepsy [2, 3, 4]. Epilepsy is a chronic and often progressive seizures characterized by periodic and unpredictable occurrence due to abnormal electrical discharge of neurons in the central nervous system [5]. Earlier works with *C. febrifuga* have also shown that *C. febrifuga* reduces the aggressive factor in the gastro-duodenal mucosa and has anti-ulcerative activity [6, 7]. *C. febrifuga* is used traditionally for symptomatic relief of dry cough and for treatment of respiratory infections, fever, dysentery and pain. It has potential hypoglycemic and hypolipidemic activities.

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It is also used to treat trypanosomias and staphylococcus infections [8, 10]. The aim of this study is to assess the anticonvulsant activity for *C. febrifuga* against convulsions induced by picrotoxin and strychnine as well as determine the action mechanism of methanol and aqueous extracts of *C. febrifuga* by butyric amino gamma acid.

2. Material and methods

2.1. Plant materials

Crossopteryx febrifuga leaves were collected from Boko-Congo in the month of December (2008). *Crossopteryx febrifuga* botanical identification was done by Dr. Moutsambote being a botanist at the National School of Agronomy and Forestry of Marien Ngouabi University. A plant specimen was deposited in the herbarium at the Centre for Study of Plant Resources of Brazzaville (Congo) and this was labelled as number 8012. Aqueous extract of this plant was prepared daily where 10 g of plant powder was set in 50 ml of boiling water for 20 minutes at 100°C, then filtered decoct and evaporated had a yield of 10.5%. For methanolic extract, 10 g of leaves powder were left macerated daily in 100 ml of methanol under magnetic stirring for 72 hours, so filtered and evaporated macerated had a yield of 11%.

2.2. Experimental animals

Male albino rats of Wister strain weighing from 150 to 250 g were used for this study. These animals were kept in a right side with an ambient temperature of about 25°C and under a cycle of 12 hours of light and 12 hours of darkness with free access to food and water.

2.3. STR-induced convulsions test

The test consists of inducing tonic convulsions and death in rats within 10 minutes by injecting (IP) 3 mg/kg of strychnine [11]. The groups of five rats were formed and treated as follows: Group 1 received 10 ml/kg of distilled water, the group 2 was treated with 3 mg/kg of STR (IP). The test groups where two groups were treated with aqueous extract of 120 mg/kg and two others groups were treated with methanolic extract. One hour after treatments each group received a STR injection of 3 mg/kg. The latency to onset of seizures was determined and animals of all groups were subsequently sacrificed by cervical decapitation, so brains were subsequently removed and weighed for determining GABA and histamine dosage.

2.4. PIC-induced convulsions test

The test consists of inducing tonic convulsions for 15 minutes by 7.5 mg/kg of picrotoxin injection (IP) [11]. Each group of five rats were formed and treated as follows: the group 1 received 10 ml/kg of distilled water, the group 2 was treated with 7.5 mg/kg of PIC (IP). For groups tests two groups were treated with 120 mg/kg of aqueous extract and two others groups with methanolic extract. One hour after all treatments each group received 7.5 mg/kg of PIC injection (IP). The latency to onset of seizures was determined and animals of all groups were subsequently sacrificed by cervical decapitation, so the brains were subsequently taken away and weighed for determining the GABA and histamine dosage.

2.5. GABA concentration assessment in the central nervous tissue

GABA cerebral concentration was determined by spectrophotometric dosage technique of rat's brain homogenizing and the absorbance was read at 569 nm. Thereafter animals were sacrificed by decapitation to remove the cerebral brain. 600 mg of brain previously removed and weighed are introduced in 3 ml of methanol for homogenization and centrifugation at 3500 rpm/min for 10 minutes and their agent work consisted of a mixture of 1 g of ninhydrin and 100 ml of methanol. A brain homogenizing sample of 500 µl was taken away and added to 1 ml of ninhydrin at 9%. The mixture is incubated in a water bath for 5 minutes at 40 °C. 100 µg/ml standard of GABA solution as 0, 5, 2.5, 1, 0 µg/ml was prepared in parallel from different masses of GABA [12].

2.6. Determination of histamine concentration by the Colorimetric method

Histamine concentration was determined by spectrophotometric dosage technique of rat brain homogenizing and the absorbance was read at 530 nm. 600 mg of brain were introduced in 3 ml of NaCl for homogenization and centrifugation at 3500 rpm/min for 10 minutes and a solution of 0.5 ml of brain homogenizing was taken away and mixed with 0.1 ml of sulfanilic acid at 1% and 0.1 ml of sodium nitrite at 5% simultaneously. The mixture was incubated at ambient temperature. 10 minutes later 1.3 ml of the aqueous solution of 5% of sodium carbonate was added there and the ethanol at 75°C also two minutes later. A standard solution of histamine of 100 µg/ml at 50, 40, 30, 20, 10, 5 g/ml was prepared in parallel.

2.7. Statistical analysis

Results were expressed as mean $\pm$ SD. Data were analyzed automatically by one-way ANOVA Followed by turkey pair wise comparison (HSD). The difference was considered significant where $p < 0.05$.

3. Results and discussion

3.1. Latency onset seizures induced by PIC and STR

C. febrifuga anticonvulsant activity was assessed against convulsions induced by PIC and STR. It follows that *C. febrifuga* has delayed the latency onset of seizures to a delay greater than 10 minutes. Only one in five rats treated experienced seizures and these results suggest that *C. febrifuga* would own anticonvulsant activity that would thwart convulsions induced by STR and PIC and act at receptors of strychnine sensitive glycine. Antagonism by *C. febrifuga* convulsions induced by picrotoxin in rats could be explained by the presence of molecules used in extracts which have anti-convulsant properties, presumably by interaction with GABAergic neurotransmission. In did picrotoxin is known as a GABA receptor antagonist that blocks chloride channels [13].

Table 1 *C. febrifuga* effect by latency onset seizures induced by PIC and STR at rat

Treatments	latency (min)
DW	-
STR	7.6 $\pm$ 2.57
PIC	8.4 $\pm$ 2.41
EAQ + STR	> 15
EAQ + PIC	18
EMeOH + STR	15
EMeOH + PIC	11

DW: Distilled water, STR: Strychnine; PIC: Picrotoxin, MeOH: extrait Methanolique.

Extracts were administered at a dose of 20 mg/kg one hour before convulsions induction. Results are expressed as Mean $\pm$ Standard Deviation (n = 5).

3.2. *C. febrifuga* effect on GABA cerebral concentration

Table 2 Effect of aqueous and methanolic extracts of *C. febrifuga* on histamine cerebral concentration in rats

Treatments	Concentration of histamine in Central nervous tissue (μ g/ mg)
Control	0.11 $\pm$ 0.00
Picrotoxin (PIC)	0.07 $\pm$ 0.00 NS
Strychnine (STR)	0.07 $\pm$ 0.00 NS
Aqueous Extract + STR	0.07 $\pm$ 0.00 NS
Aqueous Extract + PIC	0.07 $\pm$ 0.07 NS
MeOH Extract + STR	0.08 $\pm$ 0.00 NS
MeOH Extract + PIC	0.06 $\pm$ 0.01 NS
Aqueous Extract	0.13 $\pm$ 0.04 NS
MeOH Extract	0.08 $\pm$ 0.04 NS

STR: Strychnine; PIC: Picrotoxin, MeOH: Methanol extract. Extracts were administered at 120 mg/kg one hour before seizure induction. Data are mean $\pm$ SD (n = 5), NS: difference no significantly using ANOVA followed by Turkey pairwise comparison.

Results of *C. febrifuga* effect on cerebral concentration of histamine are shown in Table 2. The extracts of *C. febrifuga* decreased not significantly the histamine rate in rats. This rate is increased from 0.11 ± 0.00 in the control group treated with distilled water to 0.06 ± 0.0 in rats. These results confirmed the *C. febrifuga* anticonvulsant activity because the neurocentral histaminergic system inhibits seizures provoked by histamine H1 for activating presynaptic receptors [15].

3.3. *C. febrifuga* Effect on histamine cerebral concentration

Results of *C. febrifuga* effect on cerebral concentration of histamine are shown in Table 3. The extracts of *C. febrifuga* decreased not significantly the histamine rate in rats. This rate is increased from 0.11 ± 0.00 in the control group treated with distilled water to 0.06 ± 0.0 in rats. These results confirmed the *C. febrifuga* anticonvulsant activity because the neurocentral histaminergic system inhibits seizures provoked by histamine H1 for activating presynaptic receptors [15].

Table 3 Effect of aqueous and methanolic extracts of *C. febrifuga* on histamine cerebral concentration in rats

Treatments	Concentration of histamine in Central nervous tissue ($\mu\text{g}/\text{mg}$)
Control	0.11 ± 0.00
Picrotoxin (PIC)	0.07 ± 0.00 NS
Strychnine (STR)	0.07 ± 0.00 NS
Aqueous Extract + STR	0.07 ± 0.00 NS
Aqueous Extract + PIC	0.07 ± 0.07 NS
MeOH Extract + STR	0.08 ± 0.00 NS
MeOH Extract + PIC	0.06 ± 0.01 NS
Aqueous Extract	0.13 ± 0.04 NS
MeOH Extract	0.08 ± 0.04 NS

STR: Strychnine; PIC: Picrotoxin, MeOH: Methanol extract. Extracts were administered at 120 mg/kg one hour before seizure induction. Data are mean $\pm$ SD (n = 5), NS: difference no significantly using ANOVA followed by Turkey pairwise comparison.

4. Discussion

C. febrifuga leaves are used in traditional medicine to treat several diseases including headaches, migraines, pain and tooth decay. Since some of these diseases concern the central nervous system we have found it useful to study the effects of this plant on the nervous system. *C. febrifuga* anticonvulsant activity has been evaluated against seizures induced by picrotoxin and strychnine. *C. febrifuga* delayed the latency time for onset of seizures to more than 10 minutes where only one in five rats treated with 120 mg/kg of *C. febrifuga* decoction had seizures. These results suggest that *C. febrifuga* would possess an anticonvulsant activity which would oppose the convulsions induced by STR and PIC and would act at sensitive glycine strychnine receptors [16]. Antagonism by *C. febrifuga* of convulsions induced by the picrotoxin in rats is explained by molecules existence in extracts used which would have anticonvulsant properties probably by interaction with the GABAergic neurotransmission. In effect the picrotoxin is known to be an antagonist of the GABAA receptor in which it blocks the chloride channels [17]. In order to confirm the *C. febrifuga* anticonvulsant effect we had to assess action mechanisms by measuring the cerebral concentration of gamma amino butyric acid (GABA) and histamine. It seems that the cerebral concentration of GABA increased from 2.04 ± 0.03 in the control group treated with distilled water to 4.10 ± 0.39 in animals treated with aqueous extract of *C. febrifuga* then the methanolic extract also increased and the cerebral concentration of GABA at 2.97 ± 0.20 . Animals which received inducers alone had shown a very significant slump in the cerebral concentration of GABA where this suggests that the STR would have interacted with the sensitive strychnine receptor in the spinal medulla and the brain system to inhibit the chlorine channel [18]. However PIC acts in GABAergic neurons preventing the GABA production. On the other hand in the animals treated with *C. febrifuga* extracts and having received the inducers one hour later it is noted that extracts used increased GABA rate in the brain. These results also suggest that *C. febrifuga* would have anticonvulsant properties and would act in the GABAergic neurons by stimulating the cerebral production of GABA which is the main inhibitory neurotransmitter of the CNS. *C. febrifuga* extracts decreased the histamine rate in rats insignificantly. This rate decreased from 0.11 ± 0.00 in the control group treated with distilled water to 0.06 ± 0.01 in rats. These results confirm the *C. febrifuga* anticonvulsant

activity. However the neurocentral histaminergic system inhibits epileptic seizures caused by histamine and activation of presynaptic H1 receptors [19, 20 and 21].

5. Conclusion

Results obtained showed that *C. febrifuga* protected animals against seizures induced by PIC and STR and also increased GABA cerebral concentration in rats. Gaba is a major inhibitor neurotransmitter in the central nervous system and this significant increase in the concentration of Gaba in rats treated with methanol and *C. febrifuga* aqueous extract confirm GABAergic action mechanisms of this plant.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest among all the authors.

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