

Anticonvulsant and Sedative Properties of Leaves of *Neoboutonia velutina* (Euphorbiaceae) Prain in Mice

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Abstract: *Neoboutonia velutina* Prain is a small tree of 6-12 m, found in tropical areas of Africa (Cameroon, Zimbabwe, Malawi, Nigeria, Angola and Southern Sudan). This plant is used in Cameroonian traditional medicine for the treatment of central nervous system diseases like epilepsy and for the treatment of hepatitis. In our knowledge, there are no published studies on biological activity of *N. velutina*. In the present study, *in vivo* animal models of epilepsy (strychnine, pentylenetetrazol and Picrotoxin-induced convulsions) and insomnia (diazepam -induced sleep) were used to evaluate the anticonvulsant and sedative properties of *N. velutina*. The aqueous extract of the leaves of *N. velutina* protected mice against strychnine ($p < 0.05$), pentylenetetrazol ($p < 0.01$), and picrotoxin ($p < 0.001$)-induced seizures. The extract strongly increased the total sleep time induced by diazepam (50 mg/kg *i.p.*) but did not significantly precipitate the onset of sleep. The results lead to the conclusion that the extract of *N. velutina* possesses anticonvulsant and sedative properties in mice and could explain its use in traditional medicine for the treatment of epilepsy and insomnia.

Keywords: *Neoboutonia velutina*, Aqueous Extract, Anticonvulsant, Sedative

1. Introduction

Pathologies of the nervous system are known as the most incapacitating diseases. Epilepsy and insomnia are two of them and are very widespread in the world. Epilepsy is characterised by the repetition of crisis which result from the excessive activity of neurons in brain [1]. According to the WHO (World Health Organisation) report figure of 2013 [2], about 50 million of persons in the world suffer of epilepsy. In Cameroon, his prevalence was about 5.8% in 2002 [3]. Insomnia is a neuropsychiatric disease characterized by the deterioration of the quality and/or sleeping time. It affects approximately 9% to 12% of the world population [4]. Modern treatments for these diseases are mostly based on the use of anti-epileptics and sleeping tablets. These drugs are efficient but present many side-effects such as dependence, drowsiness, nausea etc. Therefore, phytotherapy is an alternative to the modern treatments and plays an important role in the management of diseases mainly among peoples with very low incomes [5]. Over 300,000 plants species known in the world grow in tropical areas of Africa and other

continents. About 5000 species of these plants are used for centuries in traditional medicine to prevent and treat diseases [6]. Ethnobotanical enquiries have revealed that the infusion of leaves of *N. velutina* is used to treat epilepsy. The present study explores the anticonvulsant property of *N. velutina* by investigating its suppression of seizures induced by chemicals, pentylenetetrazol, strychnine and picrotoxin which enhance excitatory responses in the central nervous system by inhibiting inhibitory responses to glycine and gamma amino butyric acid respectively [7]. We also evaluated the sedative properties of *N. velutina* using *in vivo* mice models of insomnia.

2. Material and Methods

2.1. Animals

Adult male and female mice *Mus musculus* Swiss weighing 20-26 g between 8 and 12 weeks old were used for this study. The animals were housed in an environmentally controlled room (temperature 25° C; humidity 50-80 %; 12

hours light-dark cycle). They were provided tap water *ad libitum* and fed with a standard mice diet. The study was conducted in accordance with nationally and internationally accepted principles for laboratory animal use and care.

2.2. Plant Material

N. velutina Prain (Euphorbiaceae) grows in Africa precisely in Malawi, Mozambique, Zambia, Nigeria, Angola, Southern Sudan, Cameroon etc. It is a medium to large tree which can grow up to 6-12 m in height, and can be found in forest margins and woodland, usually in swampy ground beside streams, also in evergreen swamps forest. The plant specimen of *N. velutina* used was collected in Mbouda in the western region of Cameroon. A voucher specimen of the plant were authenticated (N° 51811/NHC) at the National Herbarium of Cameroun.

2.3. Chemicals

Diazepam and Clonazepam were purchased from Roche (Fontenay-sous-bois, France). Strychnine (STR) nitrate, Pentylene-tetrazol (PTZ) and Picrotoxin (PIC) were obtained from Sigma Chemical, (Saint louis, USA)

2.4. Preparation of the Aqueous Extract

The aqueous extract was prepared following the recommendations of the traditional healer. Dried leaves of *N. velutina* were ground. 600 g of the obtained powder were infused in 10 L boiled water for 1 hour. After cooling, the mixture was filtered with a Watman No4 filter paper and the filtrate lyophilized resulting to 58 g dried extract representing a 9.66 % yield.

2.5. Animal Experiments

For the anticonvulsant tests, the animals were randomly divided into five groups of five animals each except for the diazepam-induced sleep test, where four groups were used. As negative control, one group received vehicle (distilled water), another one, the positive control group, was given clonazepam as a reference, and three test groups received different doses of the plant extract. The aqueous extract was administrated (given) *per os* at the doses of 14; 77 and 140 mg/kg body weight (BW) respectively. Clonazepam, Diazepam, Strychnine nitrate, Pentylene-tetrazol and Picrotoxin were administrated intraperitoneally in a volume of 10 ml/kg BW. The study was conducted in accordance with nationally and internationally accepted principles for laboratory animal use and care.

2.5.1. Strychnine (STR) Test

Convulsions followed by death were induced in male mice by the *i.p.* injection of 2.5 mg/kg STR nitrate. Different treatments were given 1 hour before STR injection. Animals which survived more than 10 minutes after the injection of

strychnine were qualified protected. The positive control group received 3 mg/kg BW clonazepam [8].

2.5.2. Pentylene-tetrazol (PTZ) Test

Clonic seizures were induced in male mice by *i.p.* injection of 70 mg/kg BW PTZ [9]. The protective effect of *N. velutina* was recorded in mice treated 1 hour after the aqueous extract. The positive control group received *i.p.* 0.1 mg/kg BW of clonazepam. Animals were then individually placed in trays and observed immediately after injection of PTZ for a period of 10 minutes. The latency and duration of seizures were recorded [10].

2.5.3. Picrotoxin (PIC) Test

Seizures were induced in male mice by *i.p.* injection of 7.5 mg/kg BW PIC [8] 1 hour after different treatments. A protective effect of the aqueous extract against picrotoxin-induced seizures was recorded. The animals which did not convulse after 15 minutes of observation were qualified protected. The positive control received 0.4 mg/kg BW of clonazepam.

2.5.4. Diazepam-Induced Sleep in Mice

A well described method was used [11]. Mice were divided into four groups of five animals each: one negative control group received vehicle and three groups treated with three doses (14, 77 and 140 mg/kg BW) of *N. velutina* extract. The latency of onset of sleep and the sleeping time was recorded. The sleep-potentiating effects of plant extract were studied in mice that received diazepam, 50 mg/kg BW, and 1 h after the administration of the extract or distilled water. The time between the loss of the straightening reflex and its recovery was taken as sleep time. The straightening reflex (defined as the movement of the forehead which is on the same side of the stimulated ear) was elicited on a wake mice by stimulating the external ear with feather of a bird.

2.6. Statistical Analysis

The fisher exact (two tail) was used to compare percentage of protected mice in each anticonvulsant test. For the latency of onset of sleep and sleeping time, the mean values of the control group were compared with the mean value of the groups treated with the extract using analysis of variance (one way-ANOVA) followed by Dunnett's test. A value of $p < 0.05$ was considered significant.

3. Results

3.1. Effect of *N. velutina* on Strychnine-Induced Seizures

Strychnine induced seizures and death of all mice in the negative control group. Clonazepam completely protected mice against STR-induced seizures while the aqueous extract of *N. velutina* at the dose of 77 mg/kg BW, protected 60 % of mice against STR-induced seizures ($p < 0.05$) (figure 1).

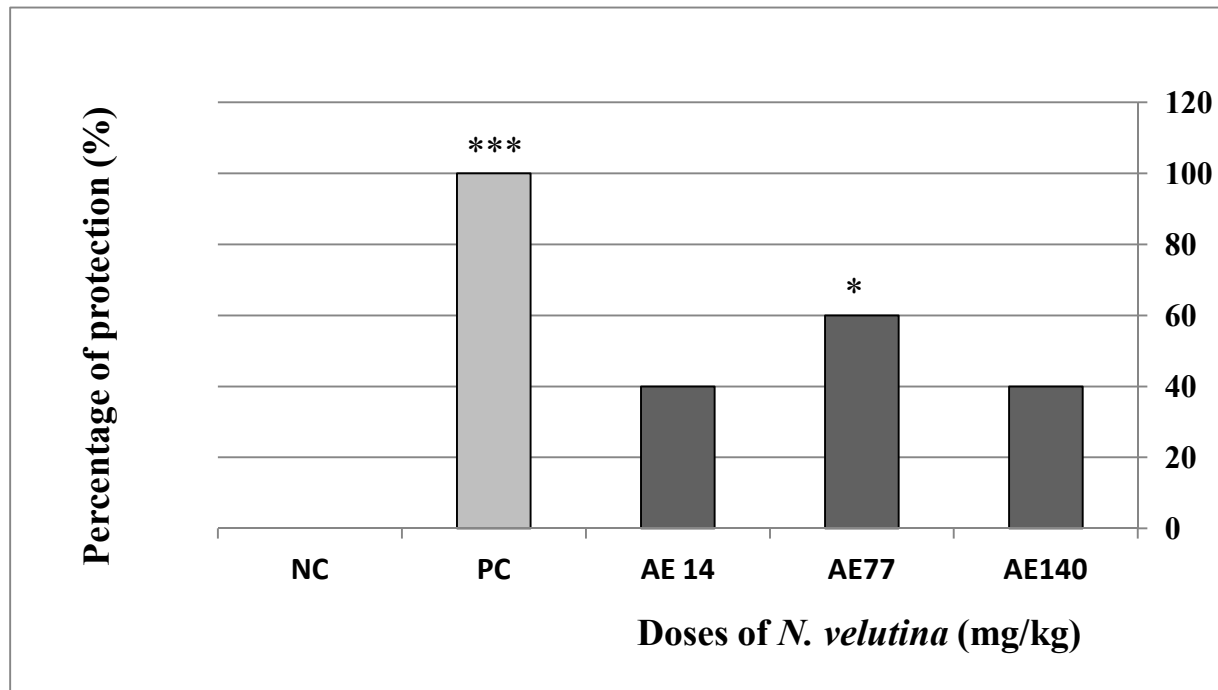


Figure 1. Effect of *N. velutina* on strychnine-induced seizures in mice. Histograms represent the percentages of protected animals. Each group consisted of 5 animals. * indicates a significant difference as compared to the vehicle-treated animals using Fisher's exact test; * $p < 0.05$, *** $p < 0.001$, NC, negative control: distilled water. PC, positive control: clonazepam 0.3 mg/kg. AE, aqueous extract.

3.2. Effect of *N. velutina* on Pentylènetétrazol -Induced Seizures

Compared to Clonazepam which completely protected mice against PTZ-induced seizures ($p < 0.001$), two doses (77

and 140 mg/kg BW) of the aqueous extract of *N. velutina* induced 80 % of protection of mice against PTZ-induced seizures ($p < 0.01$) (figure 2).

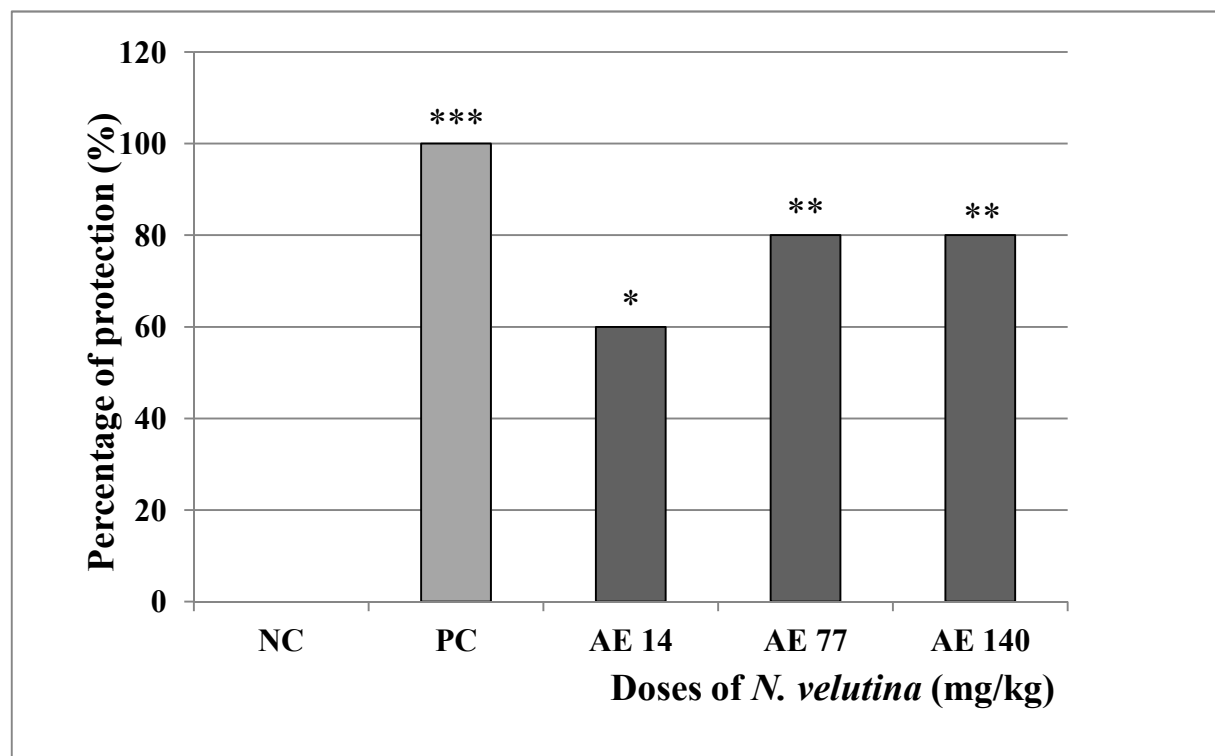


Figure 2. Effect of *N. velutina* on pentylènetétrazol-induced seizures in mice. Histograms represent the percentages of protected animals. Each group consisted of 5 animals. * indicates a significant difference as compared to the vehicle-treated animals using Fisher's exact test * $p < 0.05$, *** $p < 0.001$, NC, negative control: distilled water. PC, positive control: clonazepam 0.1 mg/kg. AE, aqueous extract.

3.3. Effect of *N. velutina* on Picrotoxin-Induced Seizures

N. velutina's extract significantly protected mice against PIC-induced seizures at all the tested doses (14 mg/kg BW

($p < 0.001$), 77 and 140 mg/kg BW ($p < 0.01$). As expected, Clonazepam completely protected mice against PIC induced seizures (Figure 3).

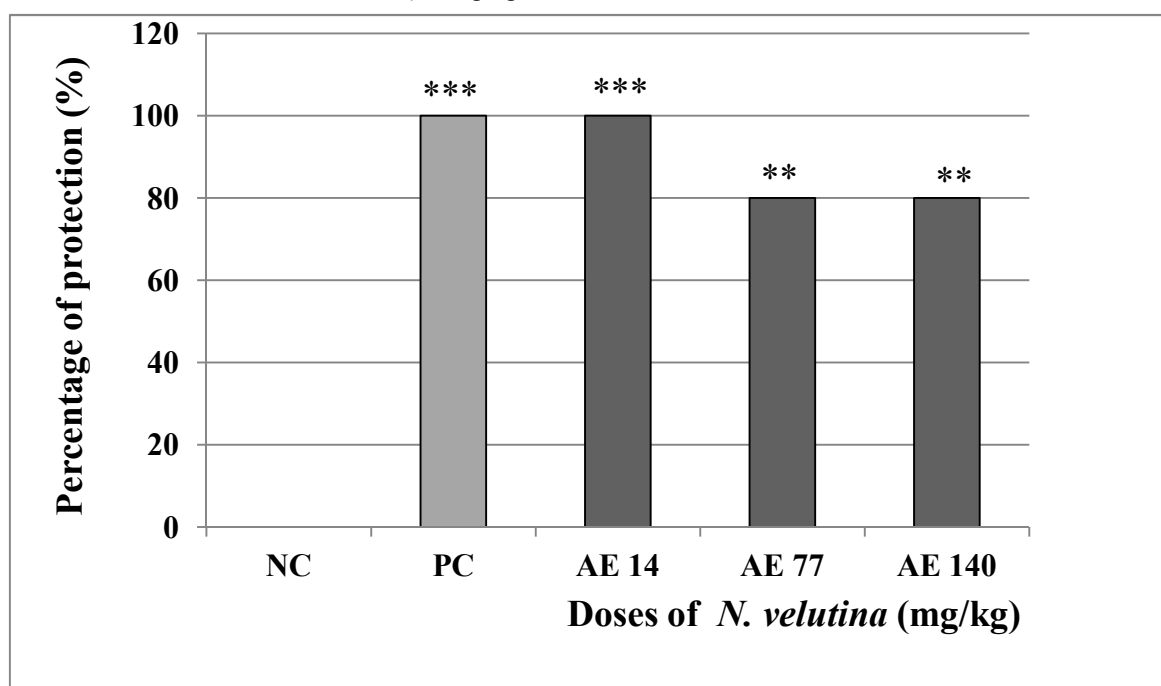


Figure 3. Effects of *N. velutina* on picrotoxin-induced seizures in mice. Histograms represent the percentages of protected animals. Each group consisted of 5 animals. * indicates a significant difference as compared to the vehicle-treated animals using Fisher's exact test; * $p < 0.05$, *** $p < 0.001$. NC, negative control: distilled water; PC, positive control: clonazepam 0.04 mg/kg. AE, Aqueous Extract.

3.4. Effect of *N. velutina* on the Latency of Onset of Sleep

The aqueous extract of *N. velutina* has no significant effect

on the latency of onset on sleep at all the tested doses (Figure 4).

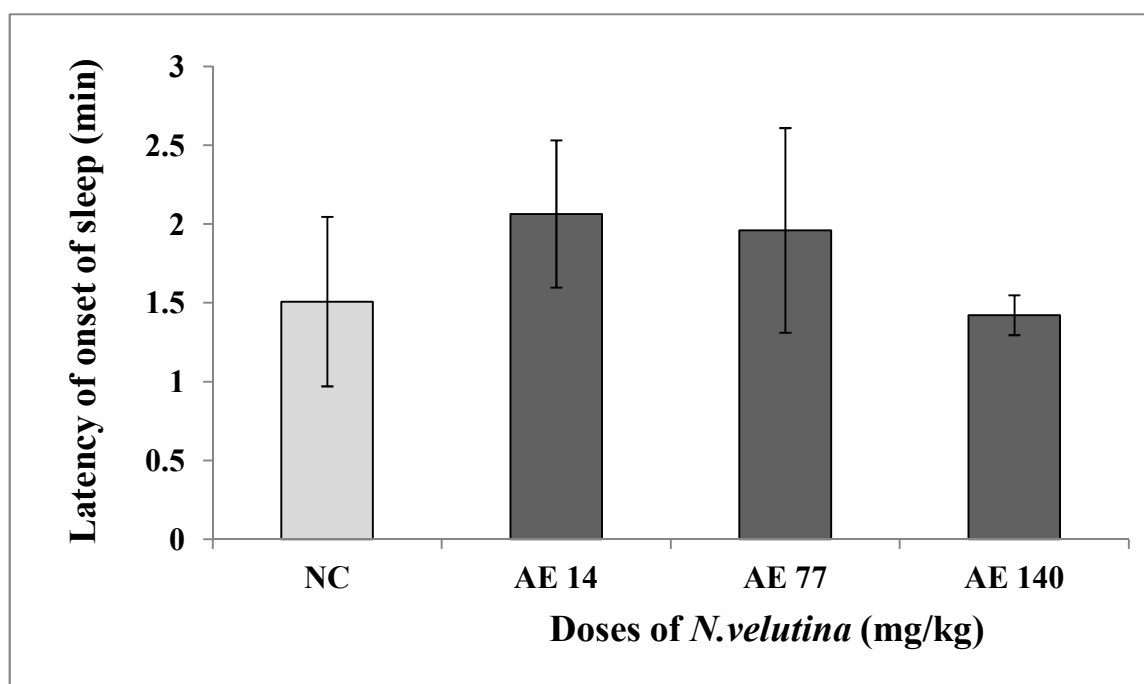


Figure 4. Effects of *N. velutina* on diazepam-induced sleep. Histograms show latency of onset of sleep induced by diazepam in the presence of doses of the extract in mice. Each group consisted of 5 animals. Data represent means \pm SEM. * indicates a significant difference as compared to the vehicle-treated animals (one-way ANOVA followed by Dunnett's test). NC, negative control: distilled water; AE, aqueous extract.

3.5. Effect on *N. velutina* on Total Sleep Time

The extract of *N. velutina* strongly potentiated ($p < 0.01$; $p < 0.001$) the sleeping time induced by diazepam (50 mg/kg BW): from 55.25 ± 8.25 min in the control group to $90 \pm$

15.1 min and to 144.66 ± 20.43 min in the groups treated with the doses 77 mg/kg and 140 mg/kg of the extract respectively (Figure 5).

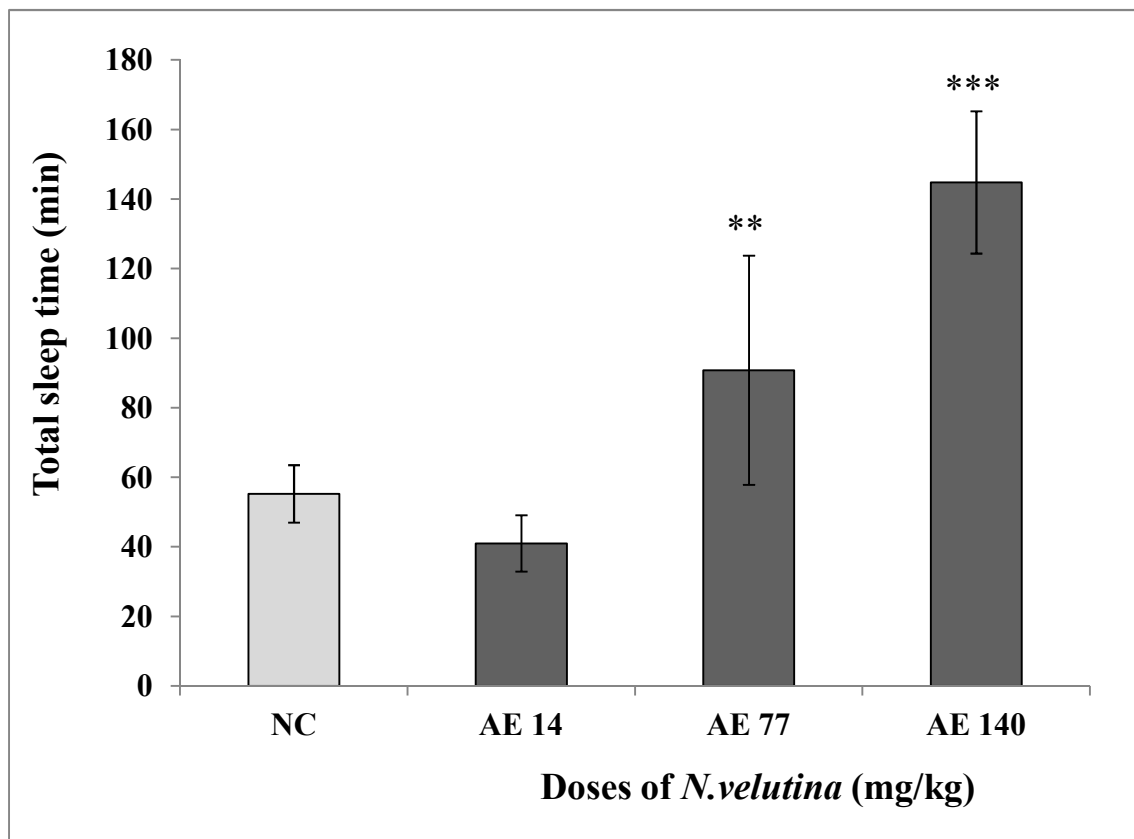


Figure 5. effects of *N. velutina* on diazepam-induced sleep in mice. Histograms represent the total sleep time (min) induced by diazepam in the presence of the extract in mice. Data represent means \pm SEM. * indicates a significant difference as compared to the vehicle-treated animals; ** $p < 0.01$, *** $p < 0.001$ (one-way ANOVA followed by Dunnett's test). NC, negative control: distilled water; AE, aqueous extract.

4. Discussion

Epileptic seizures arise from an excessive synchronous and sustained discharge of a group of neurons due to the alterations of synaptic transmission and/or intrinsic properties of neurons [12]. It could also be related to imbalance between elements that excite and those which depress the nervous system. In any case, anti-epileptic drugs (AEDs) could be able to reestablish the equilibrium. The aqueous extract of *N. velutina* (77 mg/kg BW) showed anticonvulsant effects in the strychnine induced seizure model. The aqueous extract also antagonized the seizures induced by pentylenetetrazol and picrotoxin. These observations suggest that the extract has considerable glycinergic and GABAergic potentiating mechanisms. Glycine and gamma amino butyric acid (GABA) are amino acids which act as inhibitory neurotransmitters in the central nervous system, the inhibition of which has been implicated in convulsions. Strychnine, a toxic alkaloid causes hyper-excitability and hyper-reactivity of neuron by his fixation on glycine receptors. It is a potent spinal cord convulsant that blocks

glycine receptors selectively to induce excitatory response in the central nervous system. The inhibition by the aqueous extract of STR-induced seizures suggests the presence of anticonvulsant properties [13, 14] and the involvement of glycine receptors [15]. This antagonism could be attributed to bioactive compounds present in the extract, capable to hinder the fixation or thwart the effect of STR as suggested by Ngo Bum *et al.* [8] in the study of anticonvulsant properties of *Senna spectabilis*.

The antagonism of pentylenetetrazol and picrotoxin-induced seizures suggests the interaction of the extract of *N. velutina* with GABA-ergic neurotransmission and contradicts the assertion that most drugs with an anticonvulsant activity do not counteract pentylenetetrazol seizures, but only retard them [16]. Pentylenetetrazol and picrotoxin both influence the GABA-ergic transmission by hindering the opening of the chlorine channels associated to GABA receptors [17, 18]. It is known that PTZ test is designed to identify anticonvulsant drugs effective against generalized clonic seizures [19, 20]. This is why it can be suggested that *N. velutina* possesses anticonvulsant efficacy against the above

mentioned seizures type in mice. Picrotoxine blocks GABA_A receptors and is widely used as a model of chemically-induced convulsion and produces a generalized clonic-tonic seizure [21]. The antagonism of PIC-induced seizures obtained in our study, confirmed the anticonvulsant effect of the aqueous extract of *N. velutina*. This corroborates the results of Karunakar *et al.* [22] who observed an inhibition by extracts of *Carissa carandas* of seizures elicited by pentylenetetrazol and picrotoxin in mice, and suggested that the obtained effects might be due to the activation of the GABA transmission precisely by opening the chorine channel associated to GABA receptors. The anticonvulsant action of the extract was probably due to its inhibition of the effects of strychnine and picrotoxin at glycine and GABA_A receptor sites respectively.

The aqueous extract of *N. velutina* has not precipitated the onset of sleep but significantly increase the total sleep time. This result suggests that the extract possess sedative effects [23, 24]. The sedative properties of *N. velutina* could be related to the presence of compounds in the extract activating the benzodiazepine and/or GABA sites in the GABA receptor complex [25]. Tannins could be those active components of *N. velutina* as they are known to possess anticonvulsant and sedatives properties [26].

In conclusion, this study suggests that *N. velutina* possess anticonvulsant and sedative properties. These properties could explain the use of this plant in traditional medicine in Africa, particularly in Cameroon in the treatment of epilepsy and insomnia.

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