



Original Paper

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Anticonvulsive effects of the hydroethanolic extract of the leaf of *Kigelia africana*

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ABSTRACT

Kigelia africana is traditionally used in Togo to control epileptic seizures. We undertook this study in order to evaluate its anticonvulsive properties. We pretreated Wistar albino rats of both sexes with the hydroethanolic extract (v: v) of the leaf of *K. africana* at 250 mg/kg and 500 mg/kg of body weight. This pretreatment was done one hour prior to the administration of the convulsive drugs. We induced convulsions by administration (i.p) of strychnine, picrotoxin (PTX) or pentylenetetrazol (PTZ) respectively at 3 mg/kg, 6 mg/kg and 75 mg/kg. In the pentylenetetrazol's model, the above protocol was conducted both in male and in female groups of rats. We registered the latency and duration of generalized tonic-clonic convulsions in all models. The Incidence rates of the generalized convulsions in all models were decreased. Furthermore, the extract increased the survival rates of the rats in all model used in this study. The extract either at 250 mg/kg or 500 mg/kg significantly increased the latency of the onset of generalized tonic-clonic convulsions. Duration of the convulsions was significantly decreased in all models except for the picrotoxin-induced seizure's one. In the PTZ model, the extract was more active in female rat. The extract decreased the incidence rate, prolonged the mean latency and shortened the mean duration of the generalized convulsions induced with PTX, strychnine and PTZ. The leaf of *K. africana* possesses anticonvulsive properties. This partially explains its traditional use in epileptic conditions.

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Keywords: Extract, Convulsions, Pentylenetetrazol, Strychnine, Picrotoxin

INTRODUCTION

Epilepsy constitutes a heavy burden for patients as it is highly associated to poor quality of life (Strine et al., 2005). For example; epilepsy constitutes a significant source of stigmatization (McCagh et al., 2009). The poor quality of life is also explained by the high incidence of psychological troubles in them. Studies have

shown that incidence of depression, anxiety, panic and mood disorders (Thomson and Brennenstuhl, 2009) in people with epilepsy is very high. This explains the great risk of suicide in them (Bell et al., 2009). Epilepsy is also associated to reproductive dysfunctions. Luef (2009) found a low fertility rate in people with epilepsy. Children from epileptic mothers have a greater risk of teratogenicity

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than those born from healthy mothers (Thomas et al., 2009). Finally, mortality is very increased in relation to seizure activity among epileptic patients (Lathoo and Sander, 2007). The first approach of treatment in epileptic condition is based on the use of AEDs. Unfortunately, about 30% to 40% of patients treated with AEDs continue to manifest seizures (Kokaia, 2009). Furthermore, usage of these drugs in diverse cases is associated to adverse effects. Studies have shown an increased risk of adverse pregnancy outcome with exposure to antiepileptic drugs such as phenobarbital, phenytoin, carbamazepine and valproate (Tomson and Battino, 2005). Use of antiepileptic drugs (AEDs) is also associated with reproductive (Isojarvi, 2008) and other dysfunctions. Therefore, it is very important to continue the studies in order to find new AEDs which are more efficient. In traditional medicine systems worldwide, plants are commonly used in epileptic conditions. *Kigelia africana* is a plant which belongs to the Bignoniaceae family. Its fruit, leaf and stem bark are traditionally used as remedies for epileptic seizures in Togo. There is no study which has evaluated the antiepileptic potential of *K. africana*. We undertook this study in order to evaluate the anticonvulsive effect of the hydroethanolic extract of the leaf of *K. africana*.

MATERIALS AND METHODS

Materials

Plant material

We used the leaves of *K. africana* (Lam) Benth in this study. We collected it in Lomé (Capital of Togo) in April 2007. It was identified at the Department of Botany and Plant Ecology of the Faculty of Sciences (University of Lomé, Togo). We deposited a voucher specimen of the leaf in the herbarium of this Department. Its number is 126772. The leaves collected were washed and shade-dried.

Animals

We used in this study, Wistar albino rats of both sexes, weighing 150 g to 200 g. We obtained them from the research animal house of the Laboratory of Pharmacology of the Faculty of Sciences (University of Lomé). They had a 12 hours light/dark cycle (Light from 06 h 00 to 18 h 00). Humidity and temperature conditions were the environmental ones. Six hours before experimentations, the animals were deprived of food but not water. Except this period, they had free access to pelleted diet and tap water. All experimental procedures were approved by the national scientific ethical committee.

Methods

Preparation of the hydroethanolic extract of K. Africana leaves

We soaked in 1L of solvent, one hundred grams of dried leaves of *K. africana*. We used a mixture of distilled water and ethanol as solvent for the extraction. It was composed by 50% of distilled water and 50% of ethanol at 95°. We soaked the leaves in the solvent for 72 hours. During this period, we shook manually and intermittently the content. We filtered under vacuum with a Wattman paper the solution obtained. The filtrate was evaporated under vacuum with a rotary evaporator (Buchi) at 50 °C. We obtained a black-green pasty extract.

Pentylenetetrazol-induced seizures

We studied in this section, sex- related effects of the extract. Thus, we constituted four (4) groups of seven (7) female rats and four (4) groups of seven (7) male rats. Experimental procedures were the same either for female or male rats. Groups I were the control groups; we pretreated (i.g) rats in these groups with normal saline solution. Rats in groups II and III were respectively pretreated (i.g) by the extract at 250 mg/kg and 500 mg/kg of body weight. Rats in groups IV were pretreated (i.g) with Diazepam (Sigma) at 0.5 mg/kg. The extract and Diazepam were dissolved in normal saline solution before they were administered. One

hour after the rats were treated with the extract, normal saline solution or Diazepam, it was administered PTZ (Sigma) at 75 mg/kg (i.p) (Erbayat-Altay et al., 2008). Immediately after, we placed them inside an aired transparent plastic box and observed it. The rats were observed till one hour for seizures. We noted the latency and duration of the convulsions.

Strychnine- induced seizures

We used in this section, four groups of 7 rats of both sexes. Rats of group I were pretreated (i.g) with normal saline solution. We respectively pretreated rats of groups II and III with the extract at 250 mg/kg and 500 mg/kg. Diazepam at 0.5 mg/kg was administered (i.g) to rats. One hour later, we injected (i.p) to the animal, strychnine (Sigma) at 3 mg/kg. It was immediately placed under an aired transparent plastic box and observed. Parameters registered were latency and duration of the convulsions.

Picrotoxin-induced seizures

We used four groups of seven (7) rats of both sexes. Rats of group I were pretreated with normal saline solution. We pretreated rats in groups II and III with the extract respectively at 250 mg/kg and 500 mg/kg. Animals in group IV were pretreated with Diazepam (Sigma) at 0.5 mg/kg of body weight. One hour later, picrotoxin (Sigma) was administered (i.p) at 6 mg/kg (Perrazo et al., 2003) to the rat. Immediately after this, the animal was placed under an airing transparent plastic box and observed. We noted the latency and duration of convulsions.

Data analysis

We express durations and latencies of the convulsions as the mean \pm SEM of the data obtained. We compared the latencies and durations of different groups with one-way analysis of variance ANOVA, followed by the Fischer Least Significant Difference (LSD) test. We used two-way analysis of variance ANOVA, followed by Fischer's Least Significant Differences (LSD) test to compare matched latencies and durations of female and male rats in the PTZ model. We considered

the differences significant for $p < 0.05$. We used Systat 12.0 to compare groups. Data were graphed by Graph Pad Prism version 5.2a. Incidence of convulsions, protection of animals from seizures and survival rates were expressed as percentages.

RESULTS

Effects of the extract on seizures induced by PTZ

Latency and duration of the convulsions in female rat.

All female control rats showed generalized tonic-clonic convulsions. Latency of convulsions was defined as the time elapsed between administration of PTZ and the beginning of the convulsions. Control rats presented a mean latency of 1.67 ± 0.49 minutes. Treatment with the extract at 250 mg/kg and 500 mg/kg had significantly and respectively increased it to 13.83 ± 2.83 minutes ($p < 0.01$) and 11.50 ± 2.14 minutes ($p < 0.05$) (Figure 1). The mean duration of convulsions in the control rats was 13.50 ± 1.93 minutes. It was 3.50 ± 1.38 minutes ($p < 0.01$) at 250 mg/kg and $.75 \pm 1.30$ minutes ($p < 0.01$) (Figure 2).

Latency and duration of the convulsions in male rat

All control rats showed generalized tonic-clonic convulsions. Mean latency in them was 6.83 ± 0.40 minutes. It was significantly increased to 12.17 ± 1.05 minutes ($p < 0.05$) and to 15.83 ± 1.86 minutes ($p < 0.001$) respectively at 250 mg/kg and 500 mg/kg (Figure 3). The mean duration of the convulsions in rat treated with the extract at 500 mg/kg was significantly shortened ($p < 0.01$) (Figure 4).

Sex-related effects of the extract on seizures induced by PTZ

Mean latency in male control rats was significantly higher than the female's one. Values were 1.67 ± 0.49 for female 6.83 ± 0.40 minutes for male ($p < 0.05$). When rats were treated with the extract both at 250 mg/kg and 500 mg/kg, there was no more difference in the mean latency ($p > 0.05$)

(Figure 5). Mean duration of the convulsions was significantly higher in the female control group. Values were 13.50 ± 1.93 minutes for female and 3.83 ± 0.83 minutes for male control rats ($p < 0.01$). No significant difference was observed when rats were treated with the extract both at 250 mg/Kg and 500 mg/kg ($p > 0.05$) (Figure 6).

Effects of the extract on seizures induced by PTX

All control rats presented generalized tonic-clonic convulsions. Average latency in control rats was 12.00 ± 0.32 minutes. Rats pretreated with the extract at 500 mg/kg presented a mean latency of 17.40 ± 02.60 minutes ($p < 0.05$) (Figure 7). No significant variation of the duration of the seizures was obtained when rats were treated with the

extract both at 250 mg/kg and 500 mg/kg (Figure 8).

Effects of the extract on seizures induced by strychnine

All control animals showed generalized tonic-clonic convulsions. Latency of onset of the convulsions in control rats was 5.20 ± 0.86 minutes. It was significantly increased in rats treated with the extract at both doses. It was 13.17 ± 0.79 minutes at 250 mg/kg ($p < 0.01$) and 15.67 ± 0.88 minutes at 500 mg/kg ($p < 0.01$) (Figure 9). In control rats, the mean duration was 12.20 ± 0.80 minutes. Treated with the extract at 250 mg/kg, the mean duration became 4.33 ± 0.61 minutes ($p < 0.01$) and 4.83 ± 0.61 at 500 mg/kg ($p < 0.01$) (Figure 10).

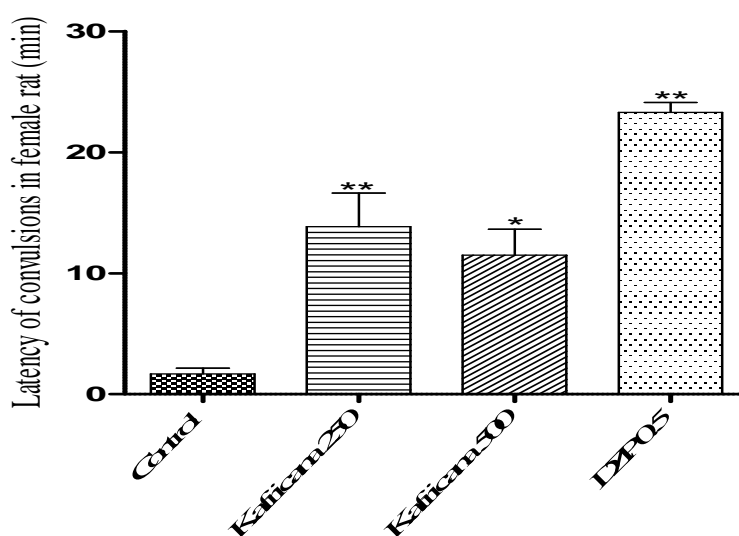


Figure 1: Effect of the hydroethanolic extract of *K. africana* on the latency of generalized tonic clonic convulsions induced by PTZ in female rat.

Values are the mean \pm SEM of 7 female rats. * $P < 0.05$, ** $P < 0.01$ compared to control. Different groups were compared with one-way analysis of variance ANOVA, followed by the Least Significant Difference test (LSD). *K. africana* 250 and 500 mean extract of *K. africana* at 250 mg/kg and 500 mg/kg. DZP 0.5 means Diazepam at 0.5mg/kg.

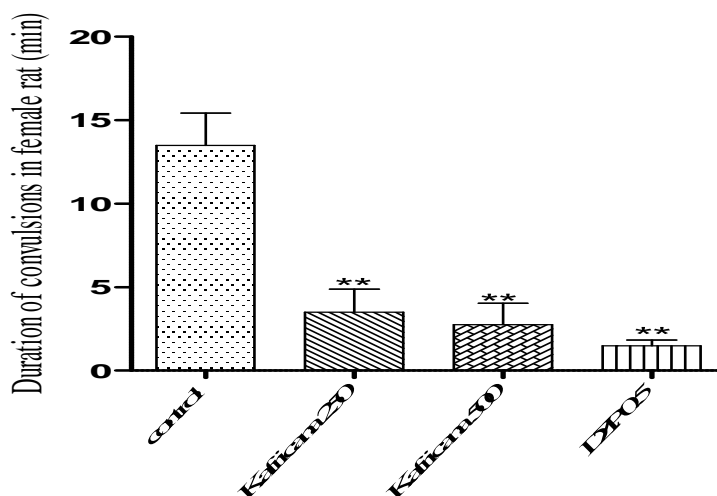


Figure 2: Effect of the hydroethanolic extract of *K. africana* on the duration of generalized tonic clonic convulsions induced by PTZ in female rat.

Values are expressed as the mean \pm SEM of 7 female rats. ** $P < 0.01$ compared to control. Data are compared with one-way analysis of variance, ANOVA followed by Least Significant Difference test (LSD). *K. africana* 250 and 500 mean extract of *K. africana* at 250 mg/kg and 500 mg/kg. DZP 0.5 means Diazepam at 0.5 mg/kg.

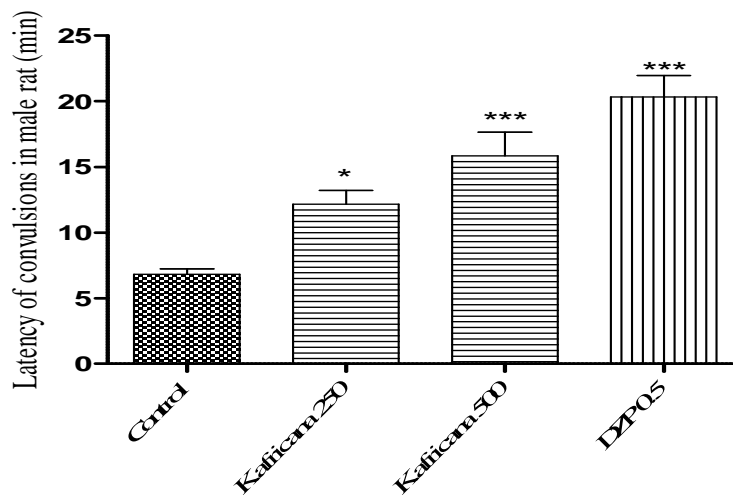


Figure 3: Effect of the hydroethanolic extract of *K. africana* on the latency of the generalized tonic clonic convulsions induced with PTZ in male rat.

Values are means \pm SEM of 7 male rats. * $P < 0.05$, *** $p < 0.001$ compared to control. Values are compared with one-way analysis of variance, ANOVA followed by the Least Significant Difference (LSD) test. *K. africana* 250 and 500 mean extract of *K. africana* at 250 mg/kg and 500 mg/kg. DZP 0.5 means Diazepam at 0.5 mg/kg.

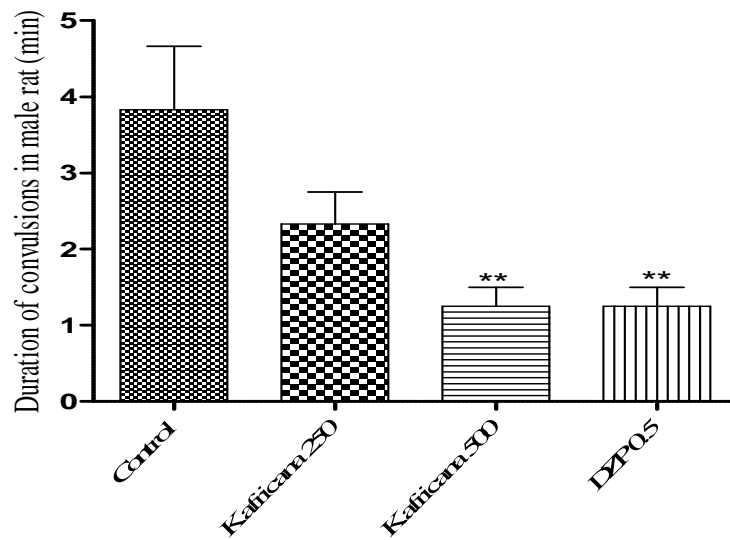


Figure 4: Effect of the hydroethanolic extract of *K. africana* on the duration of generalized tonic clonic convulsions induced with PTZ in male rat.

Values are the mean \pm SEM of 7 male rats. ** $p < 0.01$ compared to control. Data are compared with one-way analysis of variance, ANOVA followed by the Least Significant Difference test (LSD). *K. africana* 250 and 500 mean extract of *K. africana* at 250 mg/kg and 500 mg/kg. DZP 0.5 means Diazepam at 0.5 mg/kg.

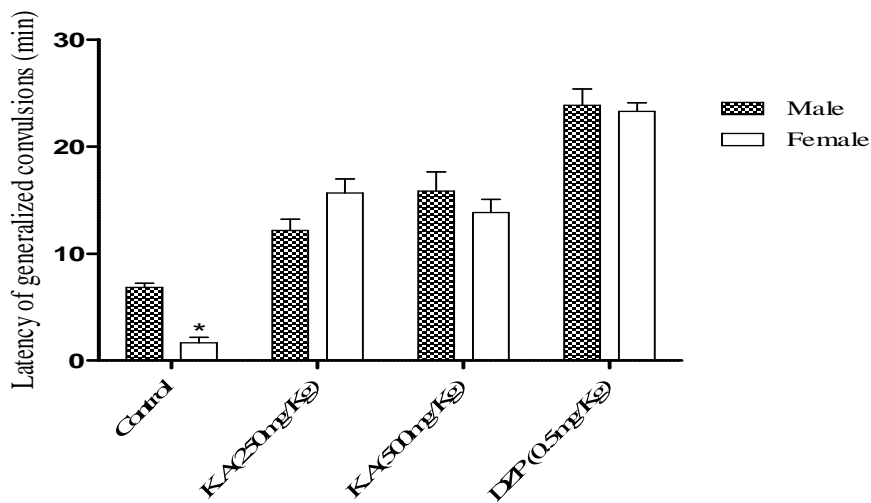


Figure 5: Effects of the hydroethanolic extract of *K. africana* on the latency of generalized tonic clonic convulsions induced by PTZ in male and female rats.

Values are expressed as the mean \pm SEM of 7 rats. * $P < 0.05$ compared to male control rats. Data are compared with two-way analysis of variance, ANOVA followed by the Least Significant Difference (LSD) test. K.A means extract of *K. africana*, DZP means Diazepam.

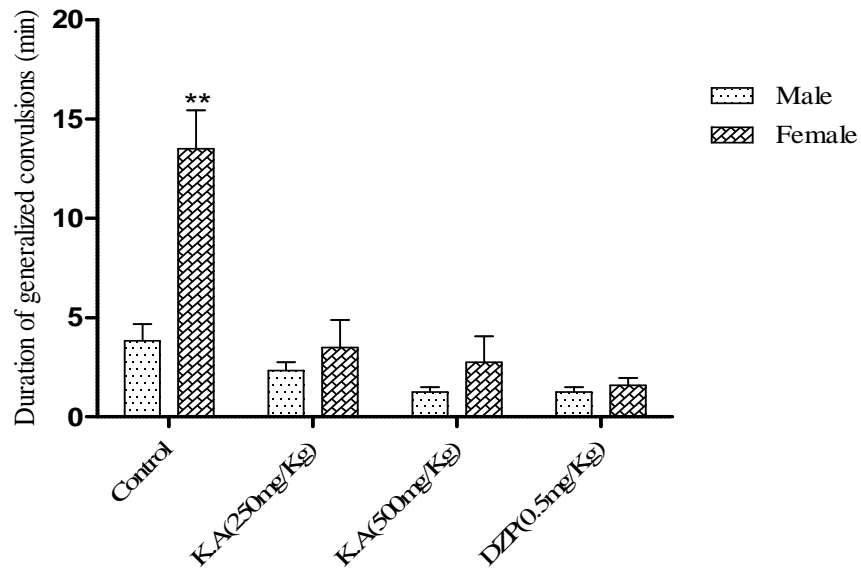


Figure 6: Effects of the extract of *Kigelia africana* on the duration of the generalized tonic-clonic convulsions induced with PTZ in male and female rats.

Values are mean \pm SEM of 7 rats. ** P < 0.01 compared to male control rat. Data are compared with two-way analysis of variance, ANOVA followed by the Least Significant Difference (LSD). K.A: Extract of *K. africana*, DZP: Diazepam.

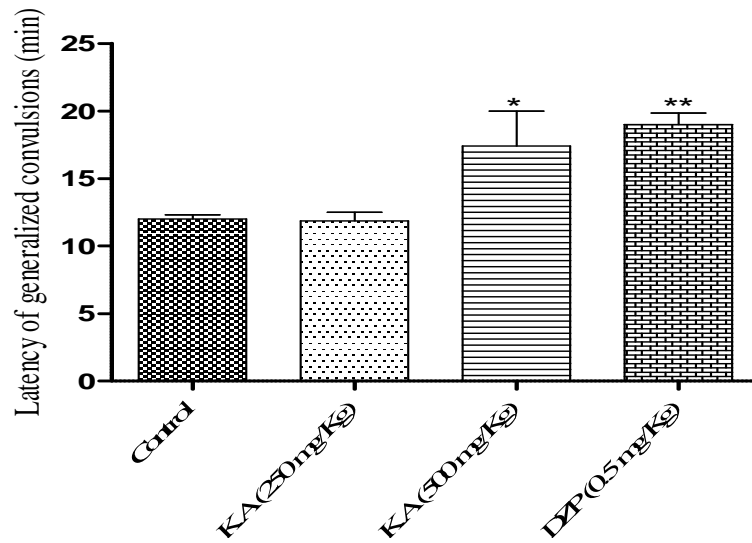


Figure 7: Effect of the extract of *K. africana* on the latency of generalized tonic-clonic convulsions induced in rat by PTX.

Values are mean \pm SEM of 7 rats. *p < 0.05, **p < 0.01 compared to control. Data are compared with one-way analysis of variance, ANOVA followed by the Least Significant Difference test (LSD). K.A means extract of *K. africana*.

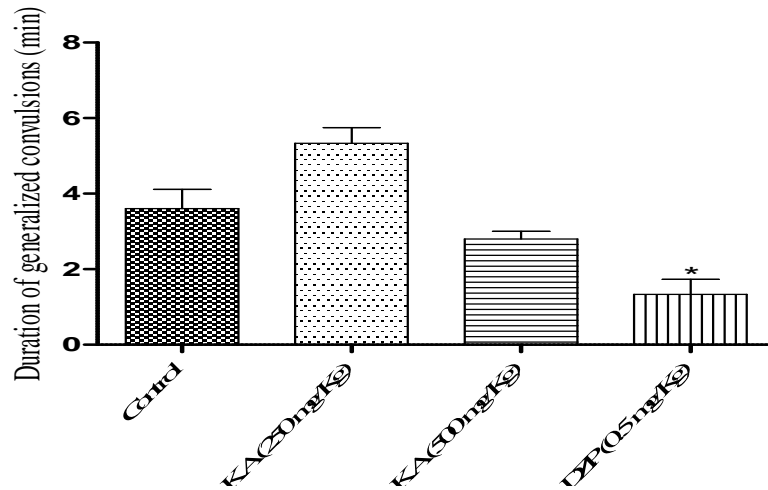


Figure 8: Effect of the hydroethanolic extract of *K. africana* on the duration of generalized tonic-clonic convulsions induced in rat with PTX.

Values are expressed as mean \pm SEM of 7 rats of both sexes. * $P < 0.05$ compared to control. Data are compared with one-way analysis of variance ANOVA, followed by the Least Significant Difference test (LSD). K.A means extract of *K. africana*. DZP means Diazepam.

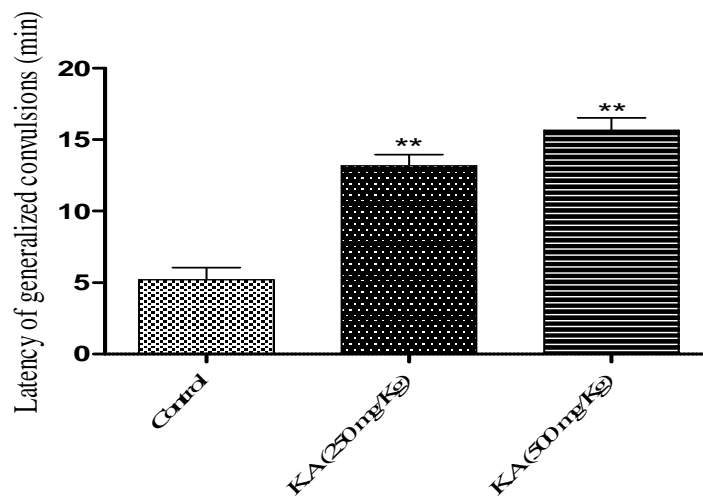


Figure 9: Effect of the hydroethanolic extract of *K. africana* on the latency of generalized tonic-clonic convulsions induced by strychnine in rat.

Values are expressed as mean \pm SEM of 7 rats of both sexes. ** $P < 0.01$ compared to control. Data are compared with one-way analysis of variance, ANOVA followed by Least Significant Difference (LSD) test. K.A means extract of *K. africana*.

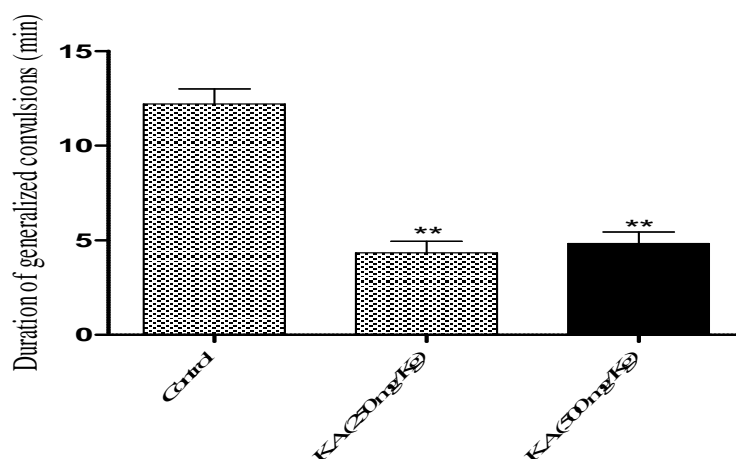


Figure 10: Effect of the hydroethanolic extract of *K. africana* on the duration of generalized tonic clonic convulsions induced with strychnine in rat.

Values are mean \pm SEM of 7 rats of both sexes. **p < 0.01 compared to control. Data are compared with one-way analysis of variance ANOVA, followed by the Least Significant Difference test (LSD). K.A: extract of *K. africana*.

DISCUSSION

Convulsions constitute the motor symptoms associated to epileptic conditions. They are associated both to partial and generalized epilepsies. The fruit, leaf and stem bark of *Kigelia africana* are traditionally used to treat epilepsy in Togo. We undertook this work in order to study its anticonvulsive properties. Convulsions occur when the central nervous system (CNS) is led to hyperexcitability. This state is due to the disturbance between the excitation and inhibitory transmissions in the CNS. Thus, factors that decrease the inhibitory or exacerbate the excitatory transmissions produce convulsions. In the brain, GABA is the major inhibitory neurotransmitter. The GABA_A receptor is a ligand-gated chloride channel. Fixation of GABA on its receptor induces influx of chloride ion and thus hyperpolarization of the neuron. Factors that inhibit the GABAergic transmission or the chloride current are experimentally used to produce convulsions. We produced convulsions with PTZ. PTZ is known to block the chloride ion channel coupled to GABA_A receptor and therefore decreases the

chloride current (Rebrov et al., 2004). The extract significantly delayed the latency of onset and increased the duration of the seizures. It is possible that the extract antagonized the GABA_A chloride ion channel blockade induced by PTZ both in male and in female rats. Glutamate is the major excitatory neurotransmitter in the brain. Feng et al. (2005) have observed an increase of the glutamate level in the rat's brain after administration of PTZ at 60 mg/kg. Increase of the glutamate level activates the NMDA receptors (NMDARs) (Roal et al., 2001). Studies have also shown an increase of NMDARs in the hippocampus and cortex after the intraperitoneal administration of PTZ (Zhu et al., 2004). NMDARs have been demonstrated to be involved in epileptogenesis and spread of epileptic neuronal process (Manjarrez et al., 2001). Then, incidence of convulsions in our study was also due to an increase of glutamate and NMDARs levels in the brain. The anticonvulsive effects displayed by the extract could be due to its action both on the GABAergic and glutaminergic transmissions. We have studied sex-related effects of the

extract in this model. Our results showed a dimorphism in the latency and duration of the generalized tonic-clonic convulsions in control rats. Latency in female control rats was significantly lower. The female rat is then more prone to convulse. Additionally, the duration of the convulsions in female rat is significantly higher. Sexual dimorphism in epileptic seizures is well established. It is known that seizure's frequency and intensity is menstrual cycle stage-dependent in women with epilepsy (Kariyawassam et al., 2009). This is due to the effects of steroids female hormones on the seizure's activity (Thomas et al., 2009). Estrogens are known to have convulsive and progesterone anticonvulsive properties (Scharfman and MacLusky, 2006). Our results show no significant differences in the latencies and durations of the convulsions when rats were treated with the extract. We can conclude that the extract is more active in the female rat.

We also used strychnine to produce convulsions. It abolishes the inhibitory system (Ghavanini et al., 2005) both in the spinal cord and brainstem. Strychnine blocks the glycine receptor (Apprison et al., 1996). Our results showed that the latencies of the seizures were significantly prolonged and the durations significantly decreased in this model. We can conclude that the extract had actions on the glycinergic transmission. PTX was also used in this study to produce convulsions. We obtained significant decrease of the duration of the convulsions in this model. Furthermore latencies of the convulsions in this model were significantly delayed when rats were treated with the extract. Picrotoxin is a blocker of the chloride channel coupled to the GABA_A receptor as PTZ. The difference in the mechanisms of actions of the two drugs remains on their sites of fixation (Huang et al., 2001). The effects of the extract in this model reinforce the idea that, the extract could modulate the GABA_A receptor chloride channel's activity in the brain. Additionally, Schmieden et al. (1989) have shown that PTX also inhibits the

chloride flux of the glycine receptor. Then, the extract could also modulate the chloride channel of the glycine receptor. The extract protected animals against death in all models used in this study. Mortality during or after seizures is frequent in epileptic patients (Kokaia, 2009).

Conclusion

We aimed to evaluate the anticonvulsive effects of the hydroethanolic extract of the leaf of *K. africana*. In PZT-induced seizure's model, the extract at 250 mg/kg and 500 mg/kg significantly increased the latencies of generalized tonic-clonic convulsions both in male and in female rats. Durations of the convulsions were significantly decreased both in male and in female rats. The extract was more active in female rat. The same trends were found in picrotoxin and strychnine-induced seizure's models. The leaf of *K. africana* possesses anticonvulsive properties. This explains partially why the plant is traditionally used to treat epileptic seizures.

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