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EVALUATION OF ANTI-CONVULSANT ACTIVITY OF *AFZELIA AFRICANA* LEAVES AQUEOUS EXTRACT ON WISTAR RAT

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ABSTRACT: *Afzelia africana* (Smith) is medicinal plant of traditional pharmacopoeia, belong to Leguminosae-Caesalpinoideae family, used for neurological diseases. This study aimed to evaluate the anticonvulsant activity of *Afzelia africana* leaves' aqueous extract on an animal model. Beforehand phytochemical screening and acute oral toxicity have been carried out. LD_{50} was determined in Wistar rats, as well as biochemical parameters (urea, creatinin, transaminases) and haematological's (Complete Blood Count) after administration of a single dose of 2000 mg/Kg body weight. Results have shown the presence of flavons, leuco-anthocyanins, anthocyanins, quinone derivatives, alkaloids, tannins, mucilages, reducing compounds and anthracenics (O-glycosides and C-glycosides) in *Afzelia africana* leaves' aqueous extract. This aqueous extract has been shown to be non-toxic. On a pilocarpine-induced epilepsy model (360 mg/kg body weight) developed in Wistar rat, the administration of aqueous extract of *Afzelia africana* leaves at 100 mg/kg body weight has significantly delayed the onset of convulsion and reduced significantly its intensity. It also protected rats from precipitated death due to violent seizures ($p < 0.05$). These results constitute a pharmacological arguments in favor of traditional use of *Afzelia africana* plant for epilepsy treatment.

KEYWORDS: *Afzelia africana*, extract, toxicity, convulsions, biological parameters

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1. INTRODUCTION

Epilepsy is a neurological condition that affects about 1% of population [1]. Epileptic disease, or epilepsy in a broad sense, is characterized by spontaneous epileptic seizures repetition called convulsions in the same subject [1]. It is manifested by occurrence of seizures or acute attacks during an anormal synchronization of brain neurons [1]. After headache, epilepsy is the more neurological pathology frequently met in the world [2]. The World Health Organization (2012) estimates that epileptics number worldwide is around 50 million and 80% of them found under the Tropical latitudes [2]. Under these latitudes, epilepsy is a major problem of public health by medical, social, cultural and economic consequences for antiepileptic patients but also for his society [2]. Epidemiology of epilepsy has been particularly studied in industrialized countries where its prevalence has been estimated about 5 cases per 1000 inhabitants [3]. In the tropic countries, this prevalence is higher on average (15‰) [4]. In Benin, some studies have shown that epilepsy has a strong rural dominance. Thus, the prevalence of this disease in Beninese population was: 24.5 ‰ in Agbogbome in 1995, 15.2 ‰ Savalou in 1996 and 33.5 ‰ Zinvié in 2000 [5]. Conventional drugs of first choice used in epilepsy treatment such as valproïque acid are expensive and not easily accessible for developing countries people [6]. These drugs called antiepileptic drugs are not devoid side effects. Also therapeutic management has limitations because some patients don't respond to currently available molecules. To overcome this state, population has recourse to traditional medicine to cure this disease. This is explained not only by the fact that they don't have the financial means to buy pharmaceutical drugs, but also, in some cases, because they are rural people who don't have dispensaries or pharmacies at reasonable distance or traditionally, some treatments are surrounded by " magic" significant importance [7]. Despite this wide spread use of plants for treatment of diseases in Benin, there are few published data on ethno-pharmacological evaluation of plants used against epilepsy and convulsions. The main problem of traditional treatments, especially herbal treatments, is the lack of scientific knowledge regarding efficacy, mode of action, active components, and doses to be used, indications, safety and efficacy quality control. Nearly 80% of natural substances used in biomedical come from tropical plants [8]. Ethnobotanical study conducted by Adjanohoun et *al.* (1989) has shown that therapeutic recipes based on herbal remedies were used to treat convulsion, migraine,

mental disorders, insanity and epilepsy [9]. Among these plants, was *Afzelia africana* which is a tree 33 m high savannahs, rainforests, edges, dry forests and hills; found throughout tropical Africa [10]. Treatment consists of using the decoction of *Afzelia africana* leaves as a drink [9].That is why, in this present work, we envisage the evaluation of anti-convulsive activity of *Afzelia africana* leaves' aqueous extract.

2. MATERIALS AND METHODS

The experimental protocols have been approved by Benin Institute of Applied Biomedical Sciences Ethical Committee.

Plant material

The leaves of *Afzelia africana* were used. They were harvested in Bassila (North Benin). This plant has been identified and certified, a specimen has been deposited in the National Herbarium of Benin under the number AA6514 / HNB.

Animal material

The animal material is composed of young rats both sexes of Wistar strain, 190-230 g weight. They were acclimated to the conditions of the Human Biology Unit's Laboratory, Faculty of Health Sciences, University of Abomey-Calavi, Benin Institute of Applied Biomedical Sciences. They were housed in groups of 06 in standard cages steel tray floor, at 25 – 30 °C temperature, 70 to 80% for relative humidity and 12 h/12 h for light/dark duration. They were fed diet consisted of 53% crushed maize, 19% fish meal, 20% wheat bran, 5% groundnut oil, 1.5% vitamin complex (Olivitasol), and 1.5% NaCl. The chemical analysis of the diet showed that they contained 16.1% crude protein, 12.9% crude fiber and 2.6% crude fat. They have food and water *ad libitum*. The daily time of animals' treatment (extract administrations) was before 10 am.

Preparation of the aqueous extract of *Afzelia africana*

Harvested leaves were dried at laboratory temperature and then ground into fine powder with the mill (MK 1861 AP). Five hundred (500) g of this powder was put in 5 L of distilled water and boiled at 100 ^{0}C for 30 min. The decoction was filtered on hydrophilic cotton and Whatman filter paper and freeze-dried. The method yielded 90 g lyophilizate which was then stored in a refrigerator at 4 ^{o} C until use.

Phytochemical study

Phytochemical screening was performed on the extract following the method of Houghton and Amala (1998)[11].

Acute oral toxicity

The acute oral toxicity test is carried out on non-pregnant female rats, according to the recommendations of Directive 423, code 17 of the Organization for Economic Cooperation and Development (OECD) originally adopted in March 1996, but revised December 17, 2001 for the testing of chemicals.[12] The aqueous extract is administered to the rats (lots of 10 rats) by

gavage. The assessment of the toxicity of the plant is made according to the presence or absence of signs of visible toxicity (death, behavioral disorders, and neurological disorders), blood count and urea, creatinin, transaminase. The samples are taken on the first day Jo before the administration of the extract and the 14th day after the test.

Blood sample collection

The rats were anesthetized with ether and the blood was taken from the retro-orbital sinus using a micropipette, then distributed in a dry tube and a tube containing the anticoagulant ethylene diamine tetra acetic acid (EDTA).

Heamatological and biochemical analyzes

From the blood contained in the EDTA tube, the blood count (NFS) was performed on the Automaton (Brand Ext 2000 i). The blood collected in the dry tubes was centrifuged at 4000 rpm in a refrigerated centrifuge for 10 min. The biochemical parameters were determined on the serum obtained by the automaton (Humanlyser 2000). The oxaloacetic and pyruvic glutamic transaminases (TGO TGP) were determined according to the kinetic method [13] while urea and creatinine [14] were determined by the colorimetric method.

Evaluation of anti-convulsive activity

Experimental convulsion model

In our study, we chose to develop a convulsion model in the Wistar rat by induction with pilocarpine. The pilocarpine model is one of the most used models among experimental epilepsy models and particularly for temporal lobe epilepsy (ELT). This model produces lesions in animals, which is similar to the electrographic and pathological aspects of those occurring in humans [15]. Thus, male and female rats of about 12 weeks old (adult stage) were used ; adult rats being the most sensitive to pilocarpine. The epileptic model was performed according to the method described by Setkowicz and Mazur (2006) [16]. It therefore consisted of injecting pilocarpine at a dose of 360 mg/kg instead of 380 mg/kg intra-peritoneally (ip) to Wistar rats. It should be noted that 30 minutes before the administration of pilocarpine, a dose of cholinergic and parasympatholytic antagonist, the methyl nitrate scopolamine was administered to the animal at the dose of 1 mg/kg *ip* in order to limit the peripheral effects of the Pilocarpine (acceleration of intestinal transit, increased salivary secretions, mucus of the respiratory tract and drainage of ocular aqueous humor, contraction of striated muscles)[17], and consequently reduced mortality of animals.

Criteria for evaluating the activity

The epileptic model developed in the adult Wistar rat, has three distinct phases: an acute phase of status epilepticus, a silent phase of normalization of the electroencephalogram and behavior and a chronic phase characterized by spontaneous and recurrent seizures [18]. In this study, we focused on the acute phase, which according some studies would last at most 6 hours [16]. Thus during this

phase, the clinical signs mimicking the convulsive behavior of the rats are continuously observed. This observation made it possible to study the following parameters:

- the intensity of seizures is evaluated according to escale six scores [19].

- Score 0 : no answer.
- Score 1 : convulsions including hypoactivity and automatism of the mouth and face.
- Score 2 : convulsions including nodding and chewing.
- Score 3 : convulsions including clonic movements of the forelegs without rising.
- Score 4 : convulsions including clonic movements of the forelegs and climbing.
- Score 5 : to rise and fall with loss of postural tone; general rigidity of the body (tonic-clonic crisis).

- The time of appearance of the first motor sign is the latency time. The latter is defined as the lapse of time separating the administration of pilocarpine from the appearance of the first episode of convulsion (clonic movements of the forelegs);
- the time at which the status epilepticus appears (status epilepticus), it is defined as an episode of uninterrupted convulsion of score 5 for at least 30 min [20];
- rat mortality due to violent convulsion.

Investigation of anticonvulsant activity

It involved the administration of the extract to different groups of Wistar rats and at doses of 100, 200, 300 and 400 mg/kg of body weight orally; 30 min after scopolamin was administered intra-peritoneally (*ip*). Then 30 minutes after administering pilocarpine (*ip*). Finally we observe the rats for 3 hours. We made six (6) groups of six rats: Four groups receive the extract at different doses and one group (control) receives and the last group (reference) receives the Diazepam at 4 mg/kg**.** Diazepam is the reference medicine chosen as a positive control. It is a molecule of the benzodiazepine class with anti-convulsive, myo-relaxant, sedative and anxiolytic properties in first-line epileptic seizure and status epilepticus for its action. The effect of plant extracts is evaluated on the basis of the inhibition or not of convulsions taking into account the following parameters: the variation of the intensity of the convulsions, the latency, the delay of appearance of the epilepticus status, as well as the mortality of the rats. Convulsion inhibitory percentages are calculated according to the formula described by Konate [21].

PI = [(S- s) / S] × 100

S = score of the control animal

s = score of the treated animal

Any animal that has not experienced seizures at a score greater than or equal to 2 during the observation period (3 hours) is considered protected [20].

Statistical analysis

The values were presented in the form of the M ± ESM (Standard Error on Mean). The exploitation of the results was carried out by the test "t" Student with Tukey-Karmer's post-hoc test using the Statistica 7.0 software. The difference was considered statistically significant for a probability level p less than 0.05.

3. RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical screening of *Afzelia africana* leaves' aqueous extract revealed the presence of alkaloids, tannins, flavons, anthocyanins, leucoanthocyanins, quinone derivatives, mucilages, reducing compound, O-glycosides and C-glycosides (Table 1).

Table 1: Phytochemical screening of *Afzelia Africana* leaves' aqueous extract

Chemical groups	Results
Alkaloids	+
Catechism and gallic tannins	+
Flavonoids	+ **(flavon)**
Anthocyanins	+
Leuco-anthocyanins	+
Quinone derivatives	+
Saponosides	-
Triterpenoids	-
Steroids	-
Cardenolides	-
Mucilage	+
Reducing compound	+
O-glycosides	+
C-glycosides	+
Cyanogenicderivatives	-

(+) positive test; (-) negative test

Acute oral toxicity test

The acute oral toxicity test performed on the aqueous extract of *Afzelia africana* leaves at a single oral dose of 2000 mg/kg revealed no evidence of toxicity (death, behavioral disturbances, neurological disorders). On all Wistar rats submitted to the experiment, we note an increase of the body mass (Figure 1). D L50 is greater than 2000 mg/kg and according to the OECD (2009) [22] Globally Harmonized Classification System, our aqueous extract may be classified in Category 5 and considered a non-toxic oral substance but must be

consumed in moderation. The toxicological safety of the aqueous extract of *Afzelia africana* was assessed through the study of certain biochemical parameters (urea, creatinin and transaminases) and haematological parameters (hemogram).

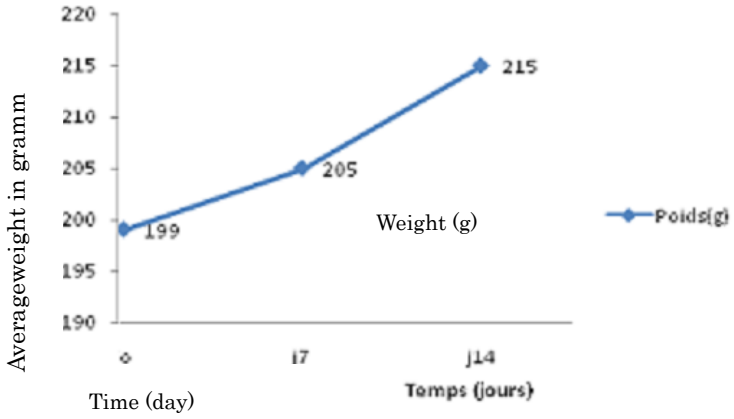


Figure 1: Body Weight Evolution Curve of Wistar Rats in the Acute Oral Toxicity Test

Effect of the extract on biochemical parameters

Biochemical parameters such as creatinin and transaminases (ASAT, ALAT) did not change significantly ($p > 0.05$). In contrast, there was a significant ($p < 0.05$) decrease in serum urea concentrations in the lot treated with the single 2000 mg/kg dose given to the control (Table 2).

Table 2: Determination of Biochemical Parameters in Rats Following the Acute Oral Toxicity Acute oral Test

Settings	J_0 (Witness)	J_{14} (Extract 2000 mg/Kg)	p-value
Urea (g/L)	0.65 ± 0.04	0.45 ± 0.08 *	**0.02**
Creatinine (mg/L)	7.67 ± 2.08	8.00 ± 1.00	ns
Transaminases ASAT (UI/L)	198.7 ± 3.5	184.00 ± 3.26	ns
Transaminases ASAT (UI/L)	70.3 ± 0.70	77.80 ± 2.90	ns

$p < 0.05$: * significant difference ns : not significant

Effect of the extract on haematological parameters

The results of the haematological parameters obtained are shown in Table 3. Almost all the average values of these parameters agree with the reference values [23]. The analysis of the hemogram showed no cytomorphological particularity. However, there was a significant decrease ($p = 0.03$) of the mean hemoglobin corpuscular concentration (MCHC) in the batch treated with the single dose of 2000 mg/kg on day J_{14} compared to the control (J_0).

Table 3: Determination of Hematological Parameters in Rats Following the Acute Oral Toxicity Test

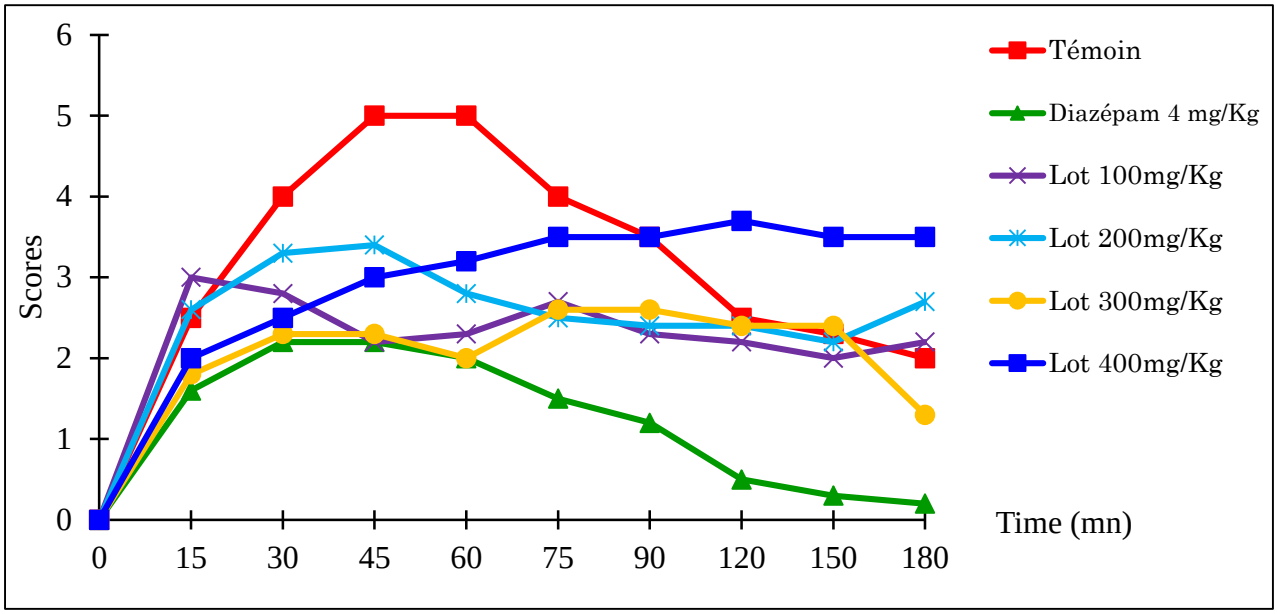
Settings	J_0 (Witness)	J_{14} (Extract 2000 mg/Kg)	p-value
Red blood cells (T / L)	7.46 ± 0.54	6.94 ± 0.25	ns
Hemoglobin (g / dL)	13.47 ± 0.50	13.28 ± 0.40	ns
Hematocrit (%)	41.90 ± 1.80	43.63 ± 2.33	ns
Meancell volume (fL)	56.33 ± 2.80	62.33 ± 4.62	ns
Mean corpuscular hemoglobin content (pg)	18.07 ± 0.86	19.00 ± 0.96	ns
Mean corpuscular concentration in hemoglobin (%)	32.17 ± 0.25	30.38 ± 0.73 *	**0.03**
White blood cells (G / L)	10.23 ± 1.72	4.13 ± 3.86	ns
Lymphocyte (%)	61.33 ± 0.72	59.00 ± 10.53	ns
Monocyte (%)	7.00 ± 1.73	12.33 ± 4.93	0.15
Eosinophilic (%)	6.33 ± 2.08	4.00 ± 1.00	0.16
Neutrophile (%)	25.33 ± 5.13	23.33 ± 11.01	0.79
Platelets (G / L)	530.67 ± 26.69	909.0 ± 76.59 **	**0,001**

$p < 0.05$: * significant difference ; ** highly significant difference ; ns : not significant

Anti-convulsive activity

Intensity of seizures

Regarding the results of the evaluation of anti-convulsive activity. Figure 2 shows the evolution curves of the scores at the different doses of the treatment, compared to that of the positive control batch. Figure 2 shows that the administration of the aqueous extract of *Afzelia africana* to rats, has led to a decrease in the intensity of convulsions. This effect is statistically significant for doses of 100 mg/kg and 300 mg/kg body weight for 30, 45, 60, 75, 90, 120, 150 min and 15, 30, 45, 60, 75, 90, then 180 min, compared to the witness. Treatment with diazepam resulted in a statistically significant decrease in seizure intensity, at all times of measurement, compared with control. The effect of diazepam is more marked than that of the extract. The doses of 100 mg/kg and 300 mg/kg appear as the doses of the extract leading to a better decrease in the intensity of the convulsions. The effect of the extract at 300 mg/kg is comparable to that of diazepam for the first 60 minutes



$p < 0.05$ (statistically significant difference)

Figure 2: Curves of evolution of the scores at the different doses of the treatment, compared to that of the positive control group

Table 4 shows the percent inhibition of convulsions at different doses of the extract and diazepam at given time intervals for 3 hours of effect. A comparative study of the values of the inhibition percentages of the extract at doses of 100 and 200 mg / kg shows that the effect is inversely proportional to the dose. The same comparison made at doses of 300 and 400 mg / kg shows that the effect is inversely proportional to the dose at all the measurement times. Diazepam showed higher inhibition percentages than the extract regardless of the dose.

Table 4: Percentage (%) of Inhibition as a function of time in min

Doses	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**120**	**150**	**180**
100 mg/Kg	0	-20	30	56	54	32.5	34.28	12	13,04	-10
200 mg/Kg	0	-4	17.5	32	44	37.5	31.42	4	4.34	-35
300 mg/Kg	0	28	42.5	54	60	35	25.71	4	-4.34	35
400 mg/Kg	0	20	37.5	40	36	12.5	0	-48	-52.17	-75
Diazepam 4 mg/Kg	0	36	45	56	60	62.5	65.71	80	86.95	90

Latency

Figure 3 shows in histogram form the lag times of onset of clonic movements in Wistar rats in the different treatment groups, compared to that of the control group. This figure shows that administration of the Wistar rat extract delayed the onset of clonic movements of the forelegs. This effect is statistically significant at doses of 100 and 300 mg/Kg with mean latencies of 42.74 and 40.37 min respectively compared to the control group with a mean latency time of 11.31 min.

The 100 mg/kg dose appears as the dose of the extract, which delays the onset of convulsions. Treatment with diazepam did not result in convulsions with a score greater than or equal to 3 in rats (Figure 3). This indicates that none of the rats that received Diazepam showed clonic movements of the forelegs and therefore did not have latency. We can conclude that Diazepam inhibits the appearance of clonic movements induced by pilocarpine in the rat.

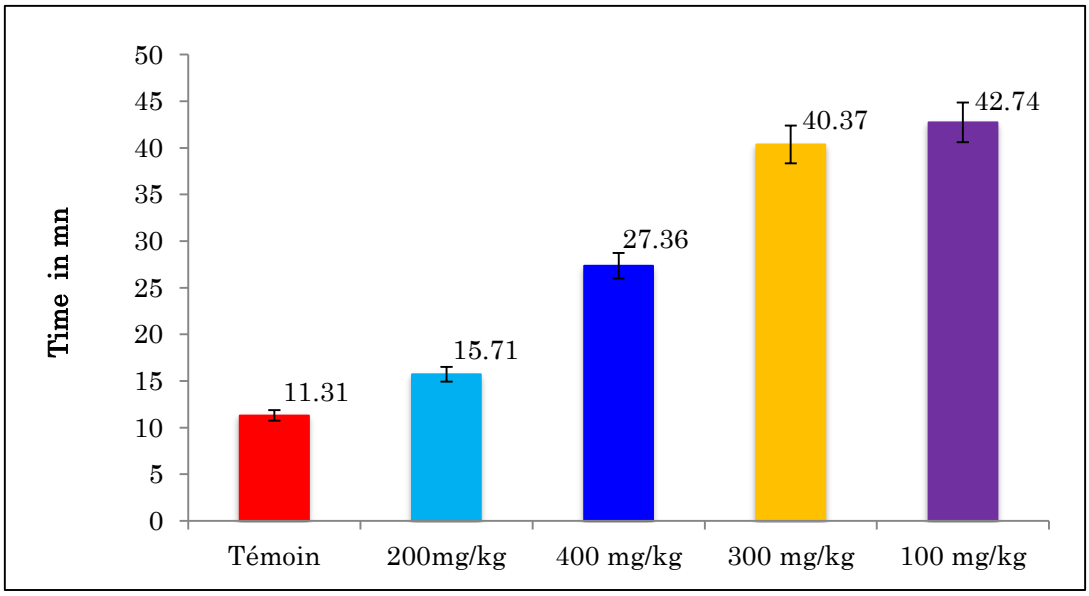


Figure 3: Average latency of appearance of clonic movements in Wistar rats in the different treatment groups, compared to that of the control group

Maximum Scores reached

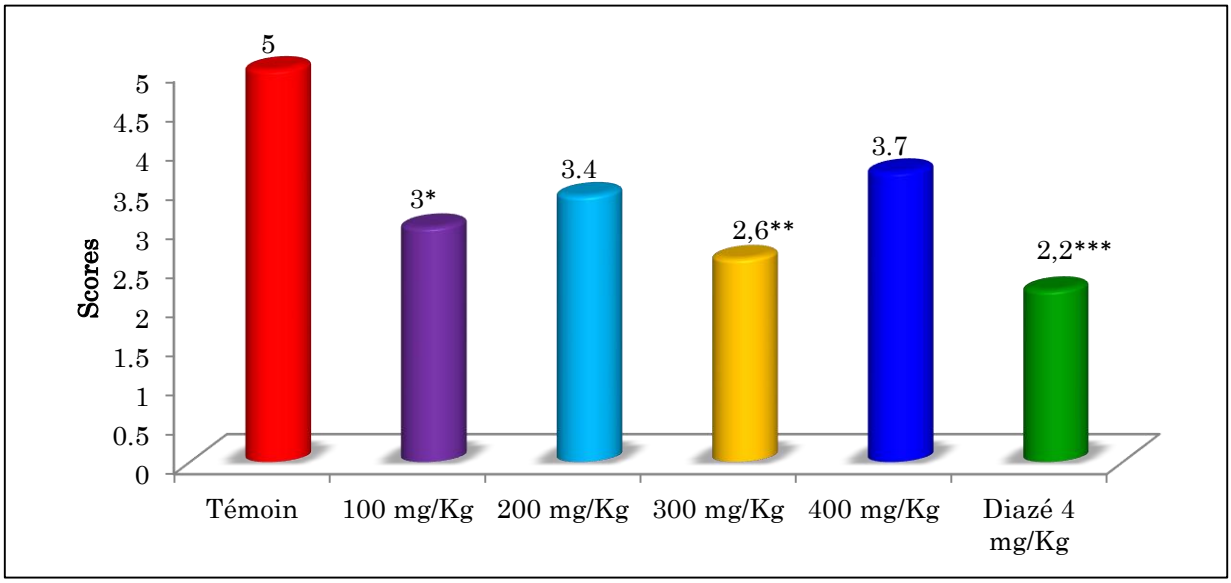


Figure 4: Maximum scores at different doses of the treatment, compared to that of the control group

* p <0.05 (statistically significant difference); ** p <0.01 (highly significant difference)

*** p <0.001 (very highly significant difference)

Figure 4 presents as a histogram the maximum scores at the different doses of the treatment, compared to that of the control group. Treatment of rats with aqueous extract of *Afzelia africana*, at doses of 100 and 300 mg / kg, induced a statistically significant, dose-dependent reduction in the mean maximum score from 5 to 3 and 2.6, respectively. The effect of the reference drug, Diazepam, is greater than that of the extract (regardless of dose) with a statistically significant reduction in the mean maximum score from 5 to 2.2. The 300 mg / kg dose appears to be the dose of the extract resulting in a better reduction of the mean maximum score. Two out of six (2/6) rats , during treatment with the 300 mg / kg extract and the 400 mg / kg dose developed epilepticus respectively from 22 min and 36 min. We did not notice development of statusepilepticus with doses of 100 and 200 mg / kg of extract, and with Diazepam at 4 mg / kg. These results indicate that doses of 100 and 200 mg / kg of extract, together with Diazepam 4 mg / kg, inhibited the development of epilepticus in Wistar rats.

Mortality rate

Table 5 shows the mortality rates for each batch treated with the 100, 200, 300, 400 mg / kg body weight doses of the extract and the 4 mg / kg Diazepam dose, respectively. Table 5 shows that treatment with the aqueous extract *of Afzeliaafricana* leaves at doses of 300 and 400 mg / kg did not protect the rats from death. The extract at a dose of 200 mg / kg reduced the rat mortality rate by 66.77% compared to the control group. The extract at a dose of 100 mg / kg and 4 mg / kg Diazepam completely protected the rats from death.

Table 5: Mortality of rate after treatment

Groups /Rats (n = 6)	Number of deaths	Mortality rate
Witness Lot	6	100%
Lot 100 mg/kg	0	0%
Lot 200 mg/kg	2	33.33%
Lot 300 mg/kg	6	100%
Lot 400 mg/kg	6	100%
Lot Diazepam 4 mg/kg	0	0%

DISCUSSION

Afzelia africana is a medicinal plant known for its therapeutic virtues in the traditional treatment of many diseases. In general, the most used traditional preparation is herbal tea. So, in order to stay in the same pattern as the traditional use that is made of the plant, we have chosen to perform an extraction based on the principle of the decoction that joins the principle of preparation of the herbal tea. The phytochemical study carried out, according to the method of Houghton and Amala (1998)[11], in our study on the leaves of *Afzelia africana* revealed the presence of alkaloids, tannins, flavonoids, anthocyanins, leuco-anthocyanins, quinone derivatives, mucilages, reducing

compounds, O-glycosides and C-glycosides . The results of the phytochemical study of the methanolic extract the bark of the trunk of *Afzelia africana* conducted in Nigeria, during the evaluation of its anti-helminthic properties by Simon et *al.* , **(**2013**)** [24] revealed the presence of alkaloids, steroids, tannins, flavonoids, saponins, cardiac glycosides and carbohydrates. There is therefore a difference between our results and those of these authors at the level of the major chemical groups of *Afzelia africana.* Several factors could explain this difference. Indeed, according to Sofowora [25], the composition of a plant with secondary metabolites varies according to the geographical situation, the organ sampled, the period, the sampling time and the storage conditions. This difference could also be explained by the increased sensitivity of their phytochemical screening methods, using thin layer chromatography associated with tube characterization reactions compared to ours. In order to prove the safety of the aqueous extract, we have studied the study of acute oral toxicity. Aqueous extract of *Afzelia africana* leaves strengthened purifying effect of the kidneys by allowing the elimination of urea (decrease of the plasma level). In total, this aqueous extract at a dose of 2000 mg/kg has a beneficial effect on the renal functions and the liver and, to a lesser degree, the muscles have not been affected. The aqueous extract of *Afzelia africana* did not disrupt red cell constant, the number of white blood cells and WBC, but has led to a very significantly increased blood platelet levels (thrombocytosis) in Wistar rats. The aqueous extract of *Afzelia africana* leaves did not cause any changes in the erythrocyte and leukocyte lineages. This result is similar to that of Oyedemi et *al.,* (2011)[26]. Indeed, by studying the influence of the aqueous extract of the bark of the trunk of *Afzelia africana* on the haematological parameters and its anti-diabetic properties in the Wistar rat, these authors showed that the administration of the aqueous extract *Afzelia* bark *africana* to rats does not alter the levels of red blood cells, hemoglobin, hematocrit, white blood cells, lymphocytes, neutrophils and monocytes. In addition, our study found an increase in the thrombocyte lineage e after 2 weeks of treatment. This result is similar to that of Koné et al. (2009)[27]. In fact, by evaluating the toxicity of an aqueous extract of *Sacoglottis gabonensis* (Baille) Urban (Humiriaceae) in rodents, a plant used in the treatment of Buruli ulcer in Ivory Coast ; these authors showed that the administration of *Sacoglottis gabonensis* to rats induced an increase in the thrombocyte lineage after 2 weeks of treatment. On the other hand, this result is contrary to that of Oyedemi et *al.,* (2011)[26] which showed that the aqueous extract of the bark of the trunk of *Afzelia africana* improved the rate of thrombocyte. This difference in these two results could be explained by the type of plant organ used (leaves, bark of the trunk) and the doses of extract administered (200 and 2000 mg/kg). With regard to the results obtained, we can deduce that our extract proved non-toxic for the hematological parameters tested; and it has a beneficial effect on the functioning of vital organs like liver, muscle and the kidneys. This virtually shows the safety of *Afzelia africana,* and its use as in traditional recipes for the treatment of epilepsy and seizures in Benin. Our study is the first to

scientifically evaluate the anti-convulsive properties of the aqueous extract of *Afzelia africana* on an animal model of pathology. The results obtained from our study prove that aqueous extract of *Afzelia africana* leaves presents anti-convulsive properties. Indeed, the administration of the extract at different doses 100, 200, 300 and 400 mg/kg body weight did not prevent the onset of convulsions in the treated rats. Nevertheless, they showed statistically significant reductions in all parameters of the convulsive activity considered compared to the control rats. The extract showed a significant delay in the onset of convulsions. This effect is inversely proportional to the dose; with a major effect obtained at a dose of 100 mg/kg. A significant decrease in seizure intensity is obtained at doses of 100 and 300 mg/kg. In addition, the extract showed a significant, dose-dependent decrease in the maximum score achieved in rats with a major effect attributed to the 300 mg/kg dose. An inhibition of the development of the status epilepticus, with a major effect attributed to the dose of 100 mg/kg. We achieved 100% death at 300 mg/kg versus 0% at 100 mg/kg. In view of these results, the dose of the extract that would offer a major anti-convulsant effect, according to the parameters considered in our study is 100 mg/kg. Since it would at the same time significantly reduce the intensity of convulsions, delay the onset of convulsions and protect rats against death. These results are consistent with that of Sanogo (2010) [28]. In fact, during the study of the anti-oxidant and anti-convulsive activities of two (02) medicinal plants from Mali, Sanogo showed that *Pteleopsis suberosa* present an anti - significant convulsant to 100 mg/Kg ; convulsions caused by Penthylenetetrazol PTZ in mice. This data indicates that Diazepam is more active than aqueous extract *Afzelia africana* on convulsion in rats induced by pilocarpine Wistar. Our results proved that our properties were anti-convulsive in this plant. other molecules of its category. *Afzelia africana* could be converted into additive or synergistic against seizures: affinity for the fixation of Benzodiazepines or GABA A receptor; stabilizing effect of cell membranes by blocking channels dependent on sodium tension (phenytoin, carbamazepine, lamotrigine, topiramate); blocking calcium channels dependent on T-type voltage (ethosuximide); GABA A (Benzodiazepines, Topiramate), inhibition of degradation (Gamma-vinyl-GAGA) or inhibition of synaptic recapture (Tiagabine) of GABA; inhibition of excitatory amino acid release, glutamate and aspartate (lamotrigine); NMDA receptor blockade (Felbamate); kaenate/AMPA glutamate receptor blockade (Topiramate). The anticonvulsant effect of the aqueous extract of the leaves of *Afzelia africana* draw near chemical compounds detected in its leaves. Some chemical compounds detected as alkaloids have been treated with anti-convulsant *Afzelia africana*. According to Bruneton [29] and the work done by Gaignault et al., 1977 [30] on Iboga alkaloids, alkaloids are very interesting for their pharmacological activities which are carried out in more varied fields: to the central system; at the level of the autonomic nervous system where they can be sympathomimetic, sympatholytic, parasympathomimetic, anti-cholinergic and ganglioplegic. The anticonvulsant extracts have an effect of *A. africana*, could be partially justified by their flavonoid richness [31], and the presence

of polyphenolic substances, which is strongly anti-radical activity. Indeed, several studies have shown that antioxidants protect the body against seizures. In Spencer (2009)[32], flavonoids prevent neuro-degeneration associated with Parkinson's disease, Alzheimer's disease and epilepsy. It has also been found that flavonoids have anxiolytic, sedative and anticonvulsant activities. Their action in the central nervous system takes place through various interactions with receptors and in different signaling pathways. Studies showed the benzodiazepine binding site (BZD) affinity flavonoid sites on the GABA receptor A receptor [31]. Also flavonoids and their derivatives have an affinity for the BZD binding site on the GABA a receptor [33] and a number of them have shown in vivo activities [34]. In sum, the anti-convulsive activity of the aqueous extract of *Afzelia africana* leaves is due to the presence of alkaloids and polyphenols (tannins and flavons).

4. CONCLUSION

Our study provides the scientific justification for the traditional use of *Afzelia africana* leaves in the treatment of seizures and epileptics. The aqueous extract is not toxic to rats Wistar. The Phytochemical Screening of *Afzelia africana* leaves revealed the presence of convulsive muscle groups. This activity could be due either to a synergistic action or to an isolated action of the compound. These results reveal other studies focusing on the isolation of chemical compounds present in the leaves of the plant and which are responsible for its anticonvulsant activity. Also we will investigate the mechanisms of action and possibly test the other parts of the plant (bark, roots and fruits).

CONFLICT OF INTEREST

Authors have no any conflict of interest.

REFERENCES

1. Picot TM, Baldy-Moulinier M, JP Daures, Dujols P, Crespel A: Prevalence of drug-resistant epilepsy and epilepsy in adults: a study based on the population of a Western European country. Epilepsia, 2008, 49 (7): 123-128p.
2. World Health Organization (WHO): Epilepsy - Fact Sheet No. 999. Website: World Health Organization. Geneva, Switzerland); 2012 [seen on July 10, 2014].
3. Genton P, Rémy C: Epilepsy. Collection Living and Understanding Epilepsy Editions Ellipses, 2003, 221p.
4. Preux PM, Diagana M, Nsengiyumva G, Druet-Cabanac M: Epilepsy in tropical zone. Neurology, 2002, 5: 216-20p.
5. Druet-Cabanac M: Epidemiology and etiology of epilepsy in sub-Saharan Africa. Lancet Neurol, 2005. 4: 21-31p.
6. Diop AG, Ndiaye M, Thiam A: Antiepileptic care sector in Africa. Epilepsies, 1996, 10: 115-121p.

7. Baba-Moussa F, Akpagana K, Bouchet P: Antifungal activities of seven West Africans. Combretaceae used in traditional medicine. J Ethnopharmacol 1999. 66 (3): 335-338p.
8. Campa C: Candidature file for the diploma of aptitude to direct research. University Montpellier II, France, 2005, 120p.
9. Adjanohoun EJ, Adjakidje V, Ahy MRA, Ake L, Akoegninou AA, Almeida J, Apovo F, Boukef K, Chadare M, Gusset G, Dramane K, Gassita JN, Gbaguidi N, Goudoté E, Guinko S, Hougnon P, Keita ILA, Kinffo HV: Contribution to ethnobotanical and floristic studies in the People's Republic of Benin. Agency for Cultural and Technical Cooperation. (ACCT) Paris, 1989, 859p.
10. Akoègninou, Van der Burg and Van der Maesen: Analytical flora of Benin, Cotonou et Wageningen edition, 2006, p. 609-610.
11. Houghton PJ, Amala R: Laboratory Manual for Splitting Natural Extracts. 1 st edition, Chapman and Hall, 1998 244P.
12. Organization for Economic Co-operation and Development (OECD), Line 423, Code 17 Guidelines for Testing of Chemicals Purchased on December 17, 2001.
13. Gella FJ, Olivella T, Cruz Pastor M, Moreno R, Durban R, Gomez JA: A simple procedure for the routine determination of aspartate amino transferase and alanine aspartate amino transferase with pyridoxal phosphate. Clin Chim Acta, 1985. 153: 241-247.
14. Kroll MH, Roach NA, Poe B, Elin RJ: Interference mechanism with Jaffe's reaction to creatinine. Clin Chem, 1987, 33 pages 1129-1132.
15. Meldrum BS: First reading in memory of Alfred Meyer. Epileptic brain damage: a consequence and a cause of seizures. Neuropathol. Appl. Neurobiol, 1997, 23: 185-201p.
16. Setkowicz Z, Mazur A (2006) Physical training decreases susceptibility to subsequent pilocarpine-induced seizures in the rat. Epilepsy Res 71: 142-148. WA Turski, EA Cavalheiro, Schwarz M, Czuczwar SJ, Kleinrok Z.
17. Turski L: Limbic seizures produced by pilocarpine in rats: behavioral, electroencephalographic and neuropathological study. Behav Brain Res, 1983, 9, 315-335p.
18. Lahteinen S, Pitkanen A, Knuuttila J, Toranen P, Casren E: Signaling of neurotrophic factors derived from the brain of the expression of hippocampal genes during epileptogenesis in transgenic mice. EUR. J. Neurosciences, 2004, 19: 3245-3254.
19. Osamu I, Eriko S, Nobuyashi H, Noriko T, H Rie, Masatoshi Y, Kohji A, Antony G: Blocks of methyl ethyl ketone, lithium pilocarpine-induced epileptic status in the rat. British Journal Pharmacology, 2009. 158 (3): 872-878p.
20. N'Gouemo P: Amiloride delays the onset of pilocarpine-induced convulsions in rats. Brain Res, 2008, 1222: 230-232p.
21. Konaté AR, Sawadogo M, Ouedraogo Y, Potchoo, Guissou IP: Anti-convulsive activity of *Annona senegalensis* Pers. (Annonaceae), a plant used in traditional medicine for the treatment

of epilepsy in Burkina Faso. 15th Conference on African Traditional Pharmacopoeia and Medicine: 1 to 4 December 2008, Libreville. Book of Resumes, 2008, 31p.

22. Globally Harmonized Classification System and Labeling of Chemicals, established under the auspices of WHO, 2009.
23. Charles River Laboratories: Reference Values in Hematology and Clinical Chemistry for Charles River Wistar Rats. (CRL: (WI) BBR) by sex and age, Technical Bulletin, 1998.
24. Simon MK. And Jegede CO: Phytochemical Screening and Anthelmintic Assessments of *Afzelia Africana* 'SM' Bark Bark (Keay, 1989) against *Nippo strongylus barziliensis* in Wistar rats. Agro technol, 2013, 2: 111-119p.
25. Sofowora A: Medicinal Plants and Traditional Medicine of Africa. Ed Kartaland; 1996, 378p.
26. Oyedemi SO, Adewusi EA, Aiyegoro OLA, Akinpelu DA: Antidiabetic and hematologic effect of an aqueous extract of *Afzelia africana* stem bark (Smith) on diabetic Wistar rats induced by streptozotocin. Asia-Pacific Journal of Tropical Biomedicine, 2011, 1 (5): 353-358p.
27. Kone M, Bleyer NM, Yapo AP, Vangah MO, Ehile EE: Evaluation of the toxicity of an aqueous extract of *Sacoglottis gabonensis* (Baille) Urban (Humiriaceae) in rodents, a plant used in the treatment of Buruli ulcer in Côte d'Ivoire. Int. J. Biol. Chem. Sci. 2009. 3 (6): 1286-1296.
28. Sanogo Benjamin M: Study of antioxidant and anti-convulsive activities of two (02) medicinal plants *Pteleopsis suberosa* and *Flueggea virosa* from Mali. Thesis of pharmacy, Mali, 2010. 56p.
29. Bruneton J: Pharmacognosy: phytochemistry medicinal plants. TEC & DOC Edition, Paris; International Medical Edition 4th Edition, 2009, 799p.
30. Gaignault JC, Delourme Houdé J: The Alkaloids of Iboga, Tabernanthe iboga H. Bn. Fitoterapia, 1977, 48: 243-265.
31. Dekermendjian K, Kahnberg P, Witt M-R, Sterner O, Nielson M, Liljefors T: Structure-activity relationships and molecular modeling of flavonoids binding to the benzodiazepine site of the rat brain GABA A receptor complex. J Med Chem, 1999, 42: 4343-4350.
32. Spencer JPE: Flavonoids and brain health: multiple effects underpinned by common mechanisms. Genes Nutr, 2009, 4: 243-250p.
33. Jane R, Hanrahan TM, Graham AR, Johnston: Flavonoids modulation of GABAA receptors. Bristish Journal of Pharmacology 2011, 163 (2): 234-245p.
34. Ai Jinglu, Wang X, Meilson M: Honokiol and magnolol selectively interact with GABAA receptors subtypes in vitro. Pharmacology, 2001, 63: 34-41p.