

Anti-convulsivant and sedative like-effect of Abyssinone V-4' methyl ether Isolated from *Erythrina droogmansiana* (Leguminosae)

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ABSTRACT

Erythrina droogmansiana is used in traditional medicine in Cameroon to treat epilepsy, insomnia and headaches. From this plant, many secondary metabolites are extracted including alcaloids, tannins, flavonoids, etc. Amongst the flavonoid, abyssinone V-4' methyl ether is used in this manipulation. Animal models of epilepsy: pentylenetetrazol (PTZ), picrotoxine (PIC) and pilocarpine (PILO)-induced convulsions or turning behavior were used to evaluate anticonvulsant activity while diazepam-induced sleep test was used to evaluate sedative activity of the extract. Four doses of extracts were used for each group test (12.5, 25, 50 and 100 mg/kg). Abyssinone V-4' methyl ether protected 100% of mice against the convulsions induced by the pentylenetetrazole at the dose of 100 mg/kg. Abyssinone V-4' methyl ether protected 100% of mice at the doses of 25 and 100 mg/kg and 80% at the doses of 12.5 and 50 mg/kg against generalized convulsions induced by picrotoxine. This flavonoid protected 100% of mice at the doses 25 and 50 mg/kg and 80% at the doses of 12.5 mg/kg against generalized convulsions induced by pilocarpine. Moreover, for the test of induction of convulsions by pilocarpine, Abyssinone V-4' methyl ether protected 100% of mice at doses of 25 and 50 mg/kg and 71.43% at the doses of 12.5 and 100 mg/kg against death after 1h and 24h respectively. Abyssinone V-4' methyl ether has anticonvulsivant properties and not sedative properties. These results explain the use of *Erythrina droogmansiana* to treat epilepsy.

Abbreviations: PTZ: Pentylenetetrazol, PIC: Picrotoxin, PILO: Pilocarpine, ED: Distilled water, Ip: Intraperitoneal.

INTRODUCTION

Epilepsy is a disease that affects about 40 millions people worldwide (Njamshi *et al.*, 2010). In 1968, the prevalence of epilepsy in Africa was about 4.8 to 40 ‰. In 1996, Diop and collaborators reported in Senegal a prevalence of epilepsy of 21 ‰ (Diop *et al.*, 1996). In 2006, Ngounou and collaborators estimated the prevalence in sub-Saharan Africa to be two or three times higher than the rate in developed world (Ngounou *et al.*, 2007). In Cameroon, some epidemiological studies on epilepsy have shown that the prevalence of epilepsy is estimated to vary from 5-136/1000. Thus, epilepsy is one of the major public health problems in Cameroon. In Africa and in Cameroon particularly, phytotherapy in traditional medicine still plays an important role in the management of diseases, mostly amongst

populations with very low income (Geoffrey and Kirby, 1996). Phytotherapy relies on the use of a wide variety of plant species including *Erythrina droogmansiana*. Abyssinone V-4' methyl ether is a prenylated flavonoid isolated from plant *Erythrina droogmansiana*. 50 flavonoids have been obtained during the last three decades from about 15 species of *Erythrina* genus (Kamat *et al.*, 1981; Njamen *et al.*, 2003; Kebenei *et al.*, 2011; Mvondo *et al.*, 2012), with prenylated flavonone, isoflavones and pterocarpanes being the major non alkaloid secondary metabolites isolates so far (Mvondo *et al.*, 2012; Wandji *et al.*, 1994). Among these metabolites, there are abyssinones which are prenylated flavonoids isolated from *Erythrina abyssinica* (Kebenei *et al.*, 2011).

These molecules have gained attention since abyssinone II was reported to show aromatase inhibitory activity (Maiti *et al.*, 2007). The antioxidant and cytotoxic activities of abyssinone I, abyssinone II and related compounds have been reported (Rao GV *et al.*, 2009).

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The anti-inflammatory and antioxidant activities of prenylflavanones isolated from *Erythrina sigmoidea* have been reported (Njamen *et al.*, 2004). Some abyssinones especially abyssinone V have recently been reported to inhibit the activity of the protein tyrosine phosphatase-1B (PTP 1B), which is directly linked to type-2 diabetes and obesity therapy (Na MK *et al.*, 2006; Kone *et al.*, 2011) and to exhibit estrogenic properties (Mvondo *et al.*, 2012). The anticonvulsant and sedative property of abyssinone V-4'-methyl ether has not yet been evaluated. Therefore, the present study was undertaken to investigate the anticonvulsant and sedative effect of abyssinone V-4'-methyl ether, a prenylated flavonoid isolated from *Erythrina droogmansiana*.

MATERIALS AND METHODS

Plant material

The root bark of *E. droogmansiana* T. Durand was collected from Nkomkoui, Yaounde-Cameroon in August 2010. Identification and authentication of the plant material was done at the National Herbarium Yaoundé, Cameroon, where a voucher specimen N°4261/SRFK has been deposited.

Extraction and isolation of the compound

Air-dried and pulverized root bark of *E. droogmansiana* (1.2 Kg) was extracted successively with ethyl acetate and methanol. The extract was filtered and the solvent evaporated under reduced pressure, 150 g of residue was obtained. This extract was subjected to chromatography over silica gel packed in n-hexane. Gradient elution was done using n-hexane, ethyl acetate and methanol in increasing polarity to give 7 series of fractions mixed on the basis of TLC. Repeated column chromatography with hexane-EtAOc (90:10) yielded YG4 and other compounds. The structures have been elucidated using spectral methods (MS, NMR, and element analysis).

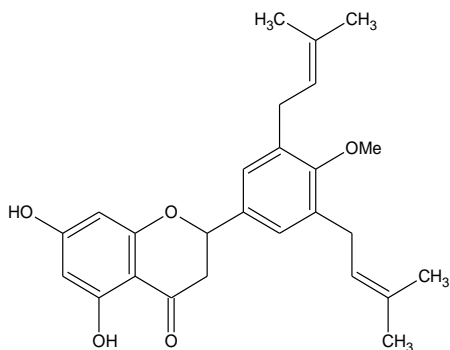


Fig. 1: Abyssinone V-4'-methyl ether.

The compound YD4 was obtained as a white powder (500 mg) and showed a $[M]^+$ at m/z 422.2094 corresponding to molecular formula ($C_{26}H_{30}O_5$). This compound was identified as abyssinone V-4'-methyl ether (Figure 1). The presence of a flavanone skeleton was evident from the 1H NMR spectra at 5.33 (1H, dd) and 2.76 (1H, 2q) corresponding to the H-2 and to

the H-3 proton of the C-ring of flavonones respectively. From the ^{13}C NMR spectra, the presence of signal at 79.3 and 42.5 respectively indicated the C-2 and C-3 of the C-ring of flavanone. The 1H and ^{13}C NMR spectra data of this compound were compared to those previously published (Yenesew *et al.*, 1998). Other compounds obtained were droogmascarpine, phaseollidin, stigmaterol and β -sitosterol. All extract were prepared according to traditional healers and chemists, and were administered 1 hour before the tests at the following doses 12.5, 25, 50 and 100 mg/kg.

Animals and drug administration

Adult male mice: *Mus musculus* Swiss 22 ± 3 g, 2 months old and obtained from the animal laboratory of our University were used for this study. Abyssinone V-4'-methyl ether and distilled water were given orally to experimental animals after suspending in a mixture of distilled water and 0.5% dimethylsulfoxide (DMSO). The positive control groups received the same experimental handling as those of the test groups.

Pharmacological tests

Diazepam-induced sleep test

Mice were divided into five groups of 5 mice and received different treatments. Group I (negative control) was treated with distilled water. Groups II to V (test groups) were treated with 4 doses of Abyssinone. The sleep potentiating effects of the plant extracts were studied in mice that had received diazepam (ip) at a dose of 50 mg/kg 1 hour after treatment. The time taken from the loss of the straightening reflex to its regain gave the sleeping time (Ngo Bum *et al.*, 2009a,b; Rakotonirina *et al.*, 2001).

Pentylenetetrazol (PTZ) test

Mice were divided into six groups of 5 mice each and received different treatments. Group I (negative control) was treated with distilled water. Groups II to V (test groups) were treated with 4 doses of the abyssinone. Group VI clonazepam, 0.1 mg/kg ip, was used as positive control. Clonic seizures were induced in mice by the ip injection of 70 mg/kg PTZ. The protective effect of the different treatments given 1 h before PTZ injection was recorded. Animals that did not convulse within the 10 min of observation were qualified protected (Ngo Bum *et al.*, 2010; Ngo Bum *et al.*, 2001; Wamil *et al.*, 1994.).

Picrotoxine (PIC) test

Six groups of 5 mice each were treated as above. However the positive control group received 0.4 mg/kg clonazepam ip. Clonic seizures were induced in mice by the ip injection of 7.5 mg/kg PIC. Mice were observed for 15 min. A protective effect of the different treatments given 1 h before PIC-induced clonic seizures was recorded. Animals that did not convulse within 15 min of observation were qualified protected (Ngo Bum *et al.*, 2005; Ngo Bum *et al.*, 2001; Bernasconi *et al.*, 1988; Lehmann *et al.*, 1988).

Pilocarpine (PILO) test

Mice were divided into six groups of 7 mice each and received different treatments. Group I (negative control) was treated with distilled water. Groups II to V (test groups) were treated with 4 doses of the abyssinone. Group VI Diazepam, 0.3 mg/kg ip, was used as positive control. Generalized convulsions were induced in mice by the ip injection of 375 mg/kg PILO. The scopolamine administered ip (1 mg/kg) 15 min after different treatments and 45 min before PILO injection was recorded. Animals that did not convulse and did not die within the following one to 24 hours of observation were qualified protected (Ethel *et al.* 2010; Henrik *et al.*, 2003).

Chemicals

Clonazepam (Rivotril[®], was from Roche Pharma, ReinachSchweiz); Diazepam: (Valium[®], was from Roche, Neuilly, France); scopolamine: (hyosinebutylbromide); pentylentetrazol, picrotoxine: (Sigma Aldrich Inc., St Louis, MO, USA) and pilocarpine (isotopilocarpine).

Statistical analysis

Three parameters were measured: the protection against convulsion induced by PTZ, PIC and PILO, the latency and the sleeping time. The percentages of protected animals were analyzed using the Fisher Exact Test (two-tail). The Fischer Exact test was used to compare the percentage of protected mice. Data were $p < 0.05$ were qualified significant. The ED50 were determined with Statgraphics Plus (confident limits at 95%).

RESULTS

Effect of Abyssinone on diazepam-induced sleep in mice.

Abyssinone V-4' methyl ether protected mice for convulsions induced by chemically substances. In diazepam-induced sleep test, the abyssinone not increased sleeping time of the control group (fig.2).

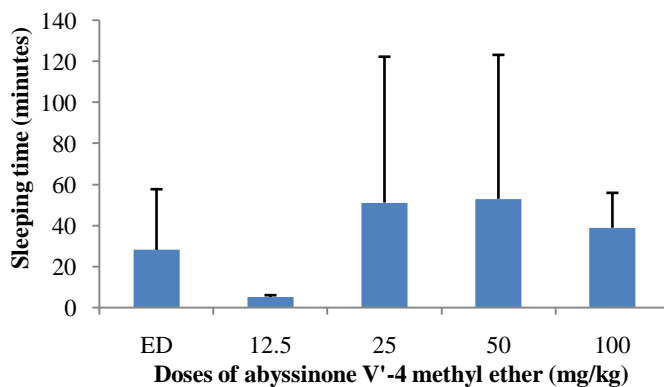


Fig. 2: Effect of Abyssinone on diazepam-induced on sleep time in mice.

Effect of Abyssinone V-4' methyl ether PTZ-induced convulsions in mice

Abyssinone at the dose of 100 mg/kg protected 100% of mice against PTZ-induced seizures ($p < 0.001$) (Fig.3).

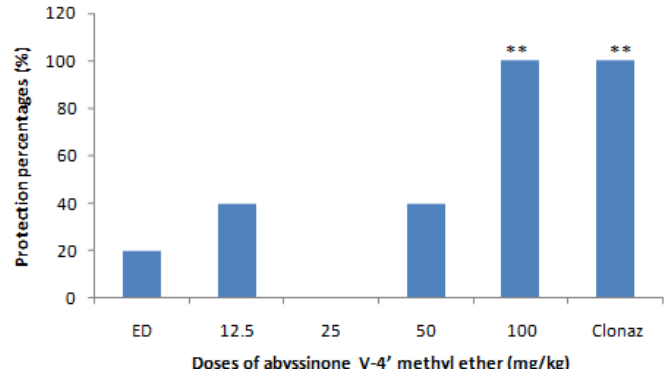


Figure 3: Effect of abyssinone V-4' methyl ether PTZ-induced on convulsions in mice.

Effect of Abyssinone V-4' methyl ether PIC-induced convulsions in mice.

The doses of 25 and 100 mg/kg provided all protection against PIC-induced seizures ($p < 0.001$) and at a doses 12.5 and 50 mg/kg the extract protected 80% of mice ($p < 0.001$) (fig.4)

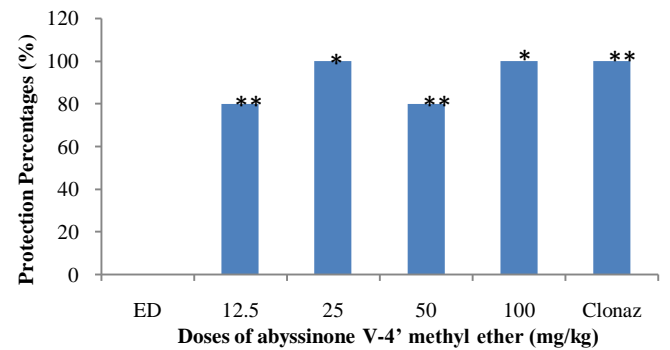


Fig. 4: Effect of abyssinone V-4' methyl ether PIC-induced on convulsions in mice.

Effect of abyssinone V-4' methyl ether PILO-induced convulsions in mice.

At doses of 25 and 50 mg/kg, Abyssinone completely protected mice against PILO-induced convulsions ($p < 0.001$) and ($p < 0.01$) at doses of 12.5 and 100 mg/kg, (Fig.5).

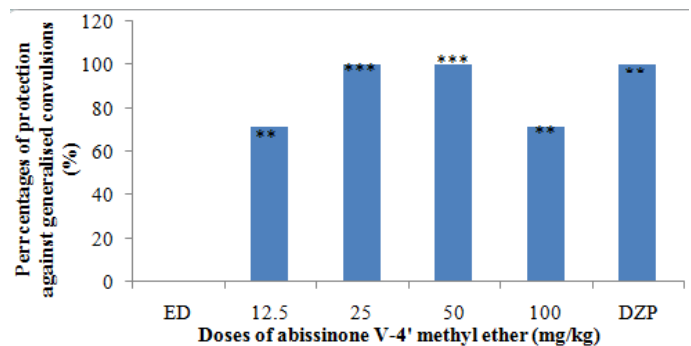


Fig. 5: Effect of abyssinone V-4' methyl ether PILO-induced on convulsions in mice.

Figure 6 presents the different stages reached by mice after administration of the pilocarpine. At doses of 25 and 50

mg/kg, Abyssinone protected the mice ($p < 0.001$) from dead one hour after the administration of PILO and protected the mice ($p < 0.001$) 24hours after the administering the dose 50 mg/kg of Abyssinone, that which is more that the anticonvulsant reference drug.(fig.7).

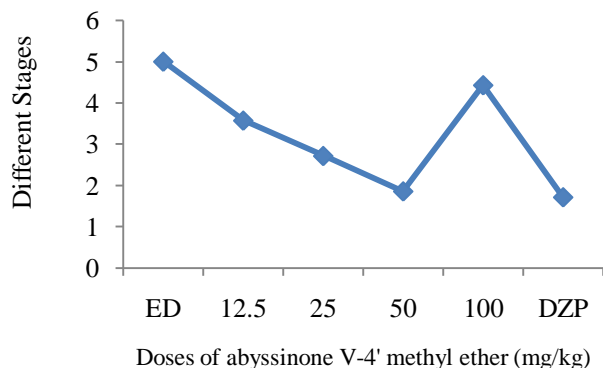


Fig. 6: Effect of abyssinone V-4' methyl ether PILO- induced on the different stages in mice.

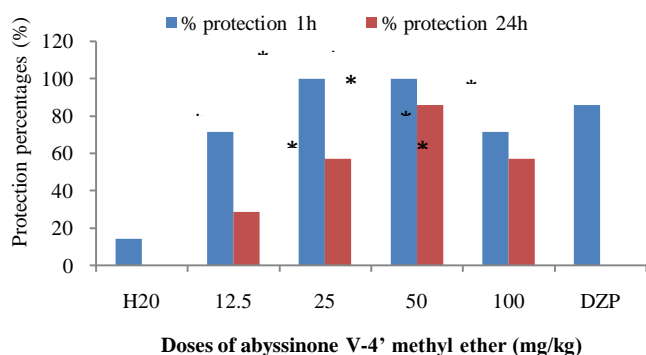


Fig. 7: Effect of abyssinone V-4' methyl ether PILO- induced on the death of mice.

DISCUSSION

Abyssinone did not potentialise the sleep time. The sedative properties are absent in this extracts (Ngo Bum *et al.*, 2009a; 2009b; Rakotonirina *et al.*, 2001). This result could be related to the absence of some components in the extracts activating the benzodiazepine, barbiturate and/or GABA receptors in the GABA_A receptor complex (Bonin and Orser, 2008; Oikola and Ahonen, 2008; Rang *et al.*, 1999). While sodium thiopental that act on the barbiturate binding site directly gate the chloride ion channel of the GABA_A receptor complex.

Abyssinone presents anticonvulsant properties. This extract antagonizes the PTZ, PIC-and PILO-induced seizures, this suggests the interaction of these extract with the GABA-ergic neurotransmission (Salihand Mustafa, 2008; Perez-Saad and Buznego, 2008). GABA is the main inhibitory neurotransmitter substance in the brain and is widely implicated in epilepsy. Inhibition of GABA-ergic neurotransmission or activity has been shown to promote and facilitate seizures, while enhancement of GABA-ergic neurotransmission is known to inhibit or attenuate seizures (Li-Ping *et al.*, 2008). Moreover, some studies showed

that PTZ diminishes the GABA-ergic tone (Ahmadiani, 2003), probably by a competitive antagonist action on the BZD receptors (Rehavi *et al.*, 1982). Drugs that enhance GABA_A-receptor neurotransmission, such as BZDs (Ahmadiani *et al.*, 2003; White, 1997) can block seizures induced by PTZ.

PIC is known to be a non competitive GABA antagonist exerting his effect by blocking the chloride channel in the GABA_A receptor complex. Isoniazide can enhance convulsions in patients with seizure disorders, and it is regarded as a GABA-synthesis inhibitor (Kale Shubhangi *et al.*, 2010).

The antagonism of PILO -induced convulsions suggests the presence of anticonvulsant effect through in the limbic system, particularly in the hippocampus, entorhinal cortex and amygdala (Bartolomei *et al.*, 2005). The subject of this review, the pilocarpine model, belongs to status Epilepticus models. This model appears to be highly isomorphic with the human disease, so it has been used in many laboratories since its first description a quarter of a century ago (Turski *et al.*, 1983a, b).

CONCLUSION

Abyssinone V-4' methyl ether did not show sedative properties but instead showed very good anticonvulsant activities against PTZ, PIC or PILO- induced seizures. However, *Erythrina*, medicinal plants could be used in the treatment of epilepsy.

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