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ANTICONVULSANT ACTIVITY OF *TACAZZEA APICULATA* OLIV. (PERIPLOCACEAE)

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ABSTRACT

The methanol extract of *Tacazzea apiculata* root-bark was subjected to successive fractionation with chloroform and n-butanol. The maximal electroshock test (MEST) in chicks and pentylenetetrazole-induced seizure were carried out on n-butanol fraction (TBF) to evaluate its anticonvulsant effects in chicks and mice respectively. Diazepam-induced sleep in mice was used to evaluate the sleep-potentiating activity of TBF. The fraction protected 80% of the chicks against MEST at doses of 10 and 20mg.kg^{-1}. The fraction did not protect the mice against pentylenetetrazole-induced seizure, However, it significantly ($P<0.05$) increased the latency of seizures in unprotected animals. The extract also significantly (*P<0.01*) and dose-dependently reduced the onset of diazepam-induced sleep and prolonged its duration in mice at the highest dose tested (20mg.kg^{-1}). This result suggests that TBF possesses anticonvulsant property against maximal electroshock seizure and lend pharmacological credence to the ethnomedical use of the plant in the management of convulsion and epilepsy.

Key words: *Tacazzea apiculata*, epilepsy, convulsion, sleep

INTRODUCTION

In developing countries remedies from plant play an important role in the health care of millions of people. Despite immense technological advancement in modern medicine, many people in developing countries still depend on traditional healing practices and medicinal plants for their daily health care needs (Ojewole, 2004). The unregulated destruction of the flora of tropical rain forest poses a threat to the medicinal plants. There is every virtue in intensifying research into medicinal flora, especially those claimed to have beneficial effects in serious disorders such as epilepsy.

Tacazzea apiculata Oliv. is woody climber indigenous to tropical Africa. It is popularly called Craw-craw vine. The Hausa of northern Nigeria refer to it as "Yaadiyar kada". The powdered leaves are used in snake bite and stings by venomous animals (Burkill, 1997). *T. apiculata* is also reported to be used in Hausa traditional medicine for the treatment of inflammation (Abubakar *et al.*2007). The decoction of the root is used for convulsion and epilepsy by the Hausa people of North-western Nigeria (Personal communication).
Acute toxicity (LD_{50}, 113mgkg^{-1}), analgesic activity and anticonvulsant activity of the methanol extract have been demonstrated (Ahmed *et al.,* 2007 Ahmed *et al.* 2006). In our laboratory, the search for compounds

effective in the treatment of epilepsy and sleep disorders is a priority. The study was therefore aimed at investigating the anticonvulsant and sedative activities of *T. apiculata* through bioassay-guided fractionation with the ultimate target of isolating the compound responsible for the activity.

MATERIALS ANS METHODS

Plant material

The plant material was collected in June 2004 from Sakaru village, along Zaria-Jos road, Kaduna state, Nigeria. It was authenticated by Mall. Musa Mohammed and Umar Gallah (the herbarium keepers) of the Department of Biological Sciences, Ahmadu Bello University, Nigeria by comparison with a voucher specimen (# 6975) already deposited.

Preparation of the extract

The root bark was dried under shade and commuted into fine powder with the aid of pestle and mortar. The powdered plant material (100 g) was macerated with 500 ml of methanol (98%) for 72 h with occasional shaking. The mixture was filtered and concentrated to dryness under reduced pressure at 45°C (yield: 4.5%). The dried methanol extract of *T. apiculata* was suspended between water and chloroform followed by n-butanol. The n-butanol fraction (TBF) was then concentrated *in vacuo.*

Animals

Adult Swiss albino mice weighing 20-25 g of both sexes were obtained from the animal house facility of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University Zaria, Nigeria. They were housed in a standard animal cage at 25± 2°C on a 12/12 light-dark cycle and fed with standard rodent feed and water *ad. libitum*. One-day-old white Ranger cockerels were obtained from National Animal Production Research Institute Shika Zaria, Nigeria.

Drugs and Chemicals

Pentyleneytetrazole (Sigma, UK), Phenytion (Sigma, UK), Diazpam (Roche, Pakistan), Valproic acid (Sigma, UK).
All intraperitoneal (*i.p.*) injections were administered in volumes not higher than 10 $ml.kg^{-1}$ of body weight of animals.

Maximal electroshock test in chicks

The method described by Swinyard and Kufferberg (1985) as modified by Sayyah *et. al.,* (2002) was employed in this study. 25 one-day-old white Ranger cockerels were randomly divided into five groups each containing five chicks. They were treated with the extract (5, 10 and 20 $mg.kg^{-1}$), phenytoin (20 $mg.kg^{-1}$) or vehicle (equivalent volume administered with extract). All administrations were by *i.p* route. Thirty minutes after pre-treatment, maximal electroshock was administered to induce seizure in the chicks using Ugobasile electroconvulsive machine (Model 7801) connected to Claude Lyons stabilizer with corneal electrodes placed on the upper eyelids of the chicks. The shock duration, frequency and pulse width were set and maintained at 0.80s, 200 pulse per second and 0.8ms respectively. A current of about 90 mA, which produced tonic seizures in 90% of the control chicks, was used throughout the study. Seizures were manifested as tonic hind-limb extension (THLE) (Swinyard, 1969). The ability to prevent this feature or prolong the latency and or onset of the THLE was considered as an indication of anticonvulsant activity (Swinyard, 1969; Sayyah *et al*., 2002).

Pentylenetetrazole-induced Seizure in mice

The method of Swinyard *et al.* (1989) was employed. Twenty-five mice were divided into five groups each containing five mice. They were treated with either the extract,

valproic acid (20 $mg.kg^{-1}$) or vehicle (equivalent to the volume administered with extract), *i.p*. Thirty minutes post-treatment, mice in all the groups received 85 mg pentylenetetrazole (PTZ) per kilogram *subcutaneous* (*s.c.*) and were observed over a period of 30 minutes. Absence of an episode of clonic spasm of at least 5 seconds duration indicated the sample's ability to abolish the effect of pentylenetetrazole on seizure threshold.

Diazepam-induced sleep in mice

The method described by Beretz *et al*. (1978) and modified by Rakotonirina *et al*. (2001) was adopted for this study. The sleep potentiating effect of Tacazzea butanol fraction (TBF) was studied in mice that received diazepam at a dose of 30 $mg.kg,^{-1}$ 30 minutes after receiving the extract (5, 10, 20 $mg.kg^{-1}$) or vehicle ($10ml.kg^{-1}$), *i.p*. Loss of rightening reflex was considered as the criterion for sleep (Rolland *et al*., 1991) while the interval between the loss and the recovery of reflex was regarded as the duration of sleep (Fujimori, 1965). Five mice were used in each group.

Statistical Analysis

The results were analyzed for statistical significance using Student's t-test. A *P*-value *<0.05* was considered significant.

RESULTS AND DISCUSSION

Tacazzea butanolic fraction (TBF) significantly ($P<0.05$) reduced the onset of diazepam-induced sleep and prolonged the duration of sleep by 300% (148.3 mins) compared with the control (49.3 mins.) as shown in Table 1. The ability of the fraction to potentiate the diazepam-induced sleep suggests that the fraction possess sleep inducing properties (Rakotonirina *et al*., 2001). Sedative-hypnotic agents such as the benzodiazepine act to increase γ-aminobutyric acid (GABA)-mediated synaptic inhibition either by directly activating GABAA receptors or, more usually, by enhancing the action of GABA on GABAA receptors (Johnston, 2005). It is therefore, plausible to suggest that the fraction may possibly act by interacting with GABA-mediated synaptic transmission.

Table 1: Effect of n-butanol fraction of the root of *T. apiculata* on diazepam-induced sleep in mice

Treatment	Dose	Onset of sleep (mins.) ±SEM	Duration of sleep (mins.) ±SEM
NS	$10\ mg.ml^{-1}$	5.0 ± 0.2	49.3 ± 3.2
TBF	$5\ mg.kg^{-1}$	4.8 ± 1.2	50.3 ± 3.5
TBF	$10\ mg.kg^{-1}$	3.8 ± 0.9*	55.3 ± 4.1
TBF	$20\ mg.kg^{-1}$	2.8 ± 0.8**	148.3 ± 8.4***

NS= Normal Saline, TBF = Tacazzea butanol fraction n=5; *p<0.05;**p<0.01;***p<0.001 Compared to control (Student's *t*-test)

Table 2: Effect of n-butanol fraction of *T. apiculata* on maximal electroshock test in chicks

Treatment	Dose	Quantal protection	Mean onset of seizure (sec.) ±SEM	Mean recovery time (min.)±SEM	Percentage protection (%)	Percentage mortality (%)
NS	10 ml.kg^{-1}	0/5	6.2 ± 1.3	5.6 ± 1.8	0.0	0.0
TBF	5 mg.kg^{-1}	2/5	6.8 ± 1.2 $^{\Psi}$	4.5 ± 0.4 $^{\Psi}$	40	0.0
TBF	10 mg.kg^{-1}	4/5	7.0 ± 0.0	4.2 ± 0.0	80	0.0
TBF	20 mg.kg^{-1}	4/5	1.3 ± 0.0	3.6 ± 0.0	80	0.0
Phenytoin sodium	20 mg.kg^{-1}	5/5	-	-	100	0.0

NS = Normal saline, TBF = Tacazzea butanol fraction Ψ = statistically not significant vs control (Student's *t*-test) n = 5

Table 3: Effect of n-butanol fraction of the root of *T. apiculata* on pentylenetetrazole-induced seizure in mice

Treatment	Dose	Quantal protection	Mean onset of seizure in unprotected animals (mins.)±SEM	Percentage protection (%)	Percentage mortality (%)
NS	10ml.kg^{-1}	0/5	4.2 ± 0.6	0.0	0.0
TBF	5 mg.kg^{-1}	0/5	9.3 ± 1.2**	0.0	0.0
TBF	10 mg.kg^{-1}	0/5	8.3 ± 0.9**	0.0	0.0
TBF	20 mg.kg^{-1}	0/5	10.3± 1.1***	0.0	0.0
Sodium valproate	200 mg.kg^{-1}	5/5	-	100	0.0

NS= Normal saline, TBF= Tacazzea butanol fraction n=5; *p<0.05;** p<0.01;*** p<0.001 compared to control

The fraction exhibited 40%, 80% and 80% protection against the maximal electroshock test (MEST) at 5, 10 and 20mg.kg^{-1} doses respectively (Table 2). There is no false negative in MEST and all currently available antiepileptic drugs that are clinically effective in the management generalized tonic-clonic and partial seizures such as phenytoin, carbamazepine and lamotrigine all suppress THLE in MEST (Browning, 1992; Rho and Sankar, 1999). Ability of the extract to inhibit the THLE suggests anticonvulsant activity of

T. apiculata for the treatment of generalized tonic-clonic and partial seizures. The fraction did not protect the animals against PTZ-induced seizure. However, it significantly prolonged the onset of seizure. Anticonvulsant activity in PTZ test identifies compounds that can raise seizure threshold in the brain (White *et al.*, 1998). PTZ has been shown to interact with GABA neurotransmitters and the GABA receptor complex (De Deyn *et al.*, 1992). Antagonism of PTZ-induced seizures suggests effects on GABA-ergic neurotransmission.
Moderate anticonvulsant activity of the extract against PTZ-induced seizures (Table 3) (as shown by the increase in the onset of seizure) suggests that the fraction may be effective in the therapy of absence or myoclonic seizures. From the result of this study, it is concluded that TBF contains bioactive constituents that are sedative in nature and possess anticonvulsant activities against maximal electroshock test and not PTZ-induced seizure. The findings lend pharmacological credence to the ethnomedical use of the plant in the management of generalized tonic-clonic seizure eg. grand-mal and partial seizure. It is however not effective against absence seizure eg. Petit-mal seizure. Further works are currently going on in our laboratory to elucidate the possible mechanism of action of the fraction and to isolate the compound(s) responsible for the observed effects.

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