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ANTICONVULSANT ACTIVITY OF EXTRACTS OF *DIOSPYROS FISCHERI* STEM BARK

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Abstract

Evaluation of extracts of *Diospyros fischeri* Gurke (Ebenaceae), which is used traditionally for the treatment of epilepsy shows that the aqueous extract of the tem bark has no effect against picrotoxin induced convulsions in mice. However, an 80% ethanol extract of the bark caused dose-dependent suppression of convulsions induced by 10 mg/kg body wt picrotoxin, , at doses between 100-3200 mg/kg body wt. Petroleum ether, 1:1 dichloromethane:methanol, and methanol extracts also suppressed picrotoxin-induced convulsions, but had a slightly lower inhibitory effect. The petroleum ether extract was the most active, but all were less active than the ethanol extract. Unlike phenobarbitone, which at 50 mg/kg body wt completely suppressed convulsions induced by 10 mg/kg body wt picrotoxin, none of the plant extracts completely suppressed convulsions in the mice. These results support the traditional uses of *D.fischeri* for the treatment of epilepsy. Given the seemingly innocuous nature of the extracts more work is suggested to ascertain their clinical application.

Key words: *Diospyros fischeri*; Picrotoxin; Anti-convulsant activity

Introduction

Epilepsy affects a large number of people, both in developed and underdeveloped parts of the world. The incidence in developing countries is twice that in developed countries, with an estimated mean prevalence of 15 per 1000 (Debrock et al., 2000). The prevalence in Africa is 4-6 times higher than in the developed countries, mainly due to meningitis, malaria, neurocysticercosis, perinatal complications, and malnutrition which can lead to permanent brain damage (Neuman et al., 1995). An epidemiological survey which was conducted in 1990 estimated the overall prevalence in Tanzania to be 19-36 per 1000 (Rwiza et al., 1992).

A number of anticonvulsants are available for the treatment of epilepsy, including phenytoin, valproate, carbamazepine, benzodiazepines, barbiturates and the newly discovered drugs like

vigabatrine, lamotrigine and tiagabine. Despite the availability of all these options an estimated 30–40% of epileptic patients, in developed countries, are not sufficiently controlled with the currently available antiepileptic drugs (AEDs) or the control is for a period of time, after which it fails (Caroline, 1987; Dekker, 2002). In Africa, and in Tanzania, the majority of the people in remote settings can only get access to a few options like phenytoin and phenobarbitone, sometimes none at all. Some of the easily available options are indeed traditional medicines, which have previously been reported to be used even in urban settings like Dar es Salaam (Moshi et al., 2005).

This study reports the effect of extracts of *Diospyros fischeri* Gurke (Ebenaceae) on picrotoxin-induced convulsions, a model based on the enhancement of the $GABA_A$-receptor inhibitory effects.

Materials and Methods

Materials

Petroleum ether, Dimethylsulfoxide (DMSO) and methanol were purchased from Fisher Scientific UK. Ltd (Bishop Meadow Road, Lough borough, Leicestershire, LE 11 5RG, UK), while carboxymethyl cellulose (CMC) and picrotoxin were purchased from Sigma Chemical Company Ltd (Poole, Dorset, UK). Phenobarbitone Sodium was purchased from Laboratory and Allied Ltd, Nairobi Kenya.

Collection of plant materials

The stem bark was collected by Mr. Selemani of the Department of Botany, University of Dar es Salaam, from the Ruaha National Park, Iringa Region. The specimen (voucher no. MB 22-2005) is kept at the Herbarium of the Institute of Traditional Medicine, Muhimbili University College of Health Sciences.

Preparation and extraction of plant material

The dried bark was ground into fine particles using a milling machine. The powdered plant material (1.0kg) was extracted with 80% ethanol (2.0 l) resulting in a yield of 400 gm of extract (40%) after drying *in vacuo* followed by freeze drying. Another portion of the plant material (1.0 kg) was extracted sequentially with solvents of increasing polarity, starting with petroleum ether, dichloromethane:methanol (1:1), and methanol. The extracts were dried using a rotary evaporator followed by freeze drying to remove residual water. The respective extract yields were petroleum ether-5gm (0.5%), dichloromethane-4gm (0.4), dichloromethane:methanol (1:1)-50gm (5%) and methanol-60gm (6%). An aqueous extract of the bark was made by boiling with water, followed by freeze drying.

Experimental animals

Male and female Theiller's original albino white mice, weighing 20-36 g were used. The animals were starved for 16-20 h but allowed free access to drinking water. The animals were weighed and allocated into 2 groups of 10 mice each, for the treated and control group, respectively.

Preparation of extracts

The aqueous extract was dissolved in distilled water while the petroleum ether extract was suspended in a mixture of DMSO and 1% CMC (3:7). The other extracts were suspended in 1%CMC.

Testing for anticonvulsant activity

Animals were pre-dosed with plant extracts orally, 30 min before administering the convulsant, picrotoxin, at a dose of 10 mg/kg body wt intraperitoneally (i.p). The latency to convulsions was calculated by subtracting the time of administering picrotoxin from time of onset of convulsions. Observation was done for a maximum period of 60 min.

Data analysis

Results are expressed as mean ± S.D. The data was analyzed using one way analysis of variance, and the means compared by the Neuman Keul's range test. Differences between the mean latencies were considered significant at $P \leq 0.05$.

Results

The effect of *D. fischeri* extracts on picrotoxin induced convulsions.

The aqueous extract, at 50-3200 mg/kg body wt, was inactive against picrotoxin-induced convulsions (Figure 1), but within the same dose range the 80% ethanol extract suppressed convulsions in a dose-dependent manner (Figure 2). Significant suppression of the convulsions was observed at 200 mg/kg body wt and above ($P \leq 0.05$), and the effect increased gradually until it peaked at 1600 mg/kg body wt. Increasing the dose of the extract to 3200 mg/kg body wt did not significantly increase the latency to convulsions ($P > 0.05$).

The effect of sequential extracts on picrotoxin-induced convulsions.

The extracts made with three solvents of varying polarity also caused dose-dependent suppression of picrotoxin-induced convulsions (Figures 3-5). It appears that the petroleum ether extract was slightly more potent than the other two, although none of them superseded the activity of the 80% ethanol extract.

Discussion

Extracts of the stem bark of *D. fischeri* delayed convulsions induced by the $GABA_A$ receptor antagonist, picrotoxin, in a dose-dependent manner. All the extracts tested, with the exception of the aqueous extract, exhibited anticonvulsant activity. The extracts could not completely prevent convulsions in most of the mice and did not significantly reduce mortality rate following administration of 10 mg/kg body wt picrotoxin. The highest dose of the ethanol extract of 3200 mg/kg body wt was unable to completely protect mice from picrotoxin, although the delay of onset was up to 22 min for some of the mice. In a separate experiment it was shown that at 3200 mg/kg body wt the extract was not toxic to mice and none of them died during a 24 h observation period.

Phenobarbitone, at 50 mg/kg body wt, offered full protection from convulsions in most but a few of the mice within the one hour observation period. Comparatively, a 60 fold dose of the plant extract only managed to delay the onset of convulsions by average 16 min, and a maximum of 22 min in a few of the mice..

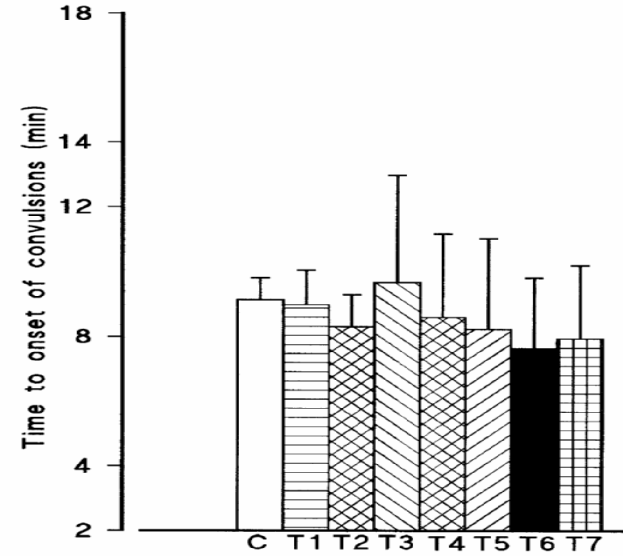

Figure 1: The effect of aqueous extract of *D. fischer*i on picrotoxin induced convulsions. Each bar represents mean±SD (n=10) of time taken for mice to convulse. Key: C=5ml/kg body wt distilled water; T1-T7 represent 50, 100, 200, 400, 800, 1600 and 3200 mg/kg body wt of aqueous *D. fischeri* stem bark extract, respectively.

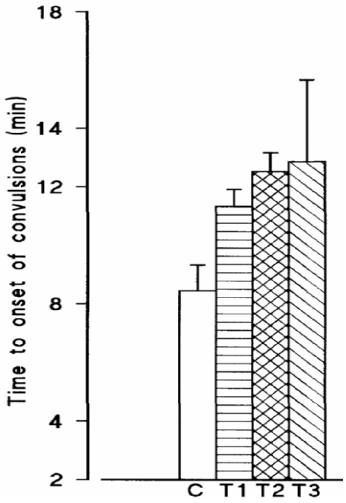

Figure 3: The effect of the petroleum ether extract of *D.fischeri* on picrotoxin induced convulsions.Each bar represents mean±SD (n=10) of time taken for mice to convulse. Key: C=5ml/kg body wt of 30% DMSO in 1% CMC; T1-T3 represent 100, 400, and 800 mg/kg body wt of petroleum ether extract of *D. fischeri* stem bark, respectively.

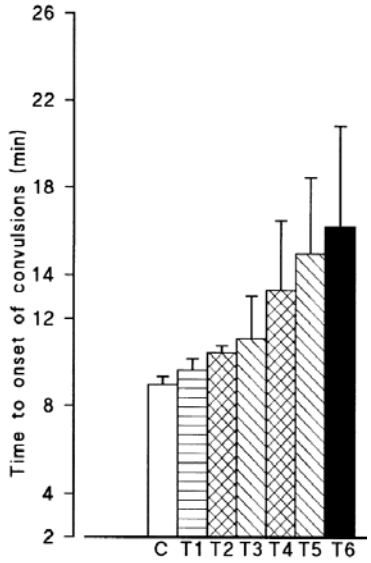

Figure 2: The effect of 80% ethanol extract of *D.fischeri* on picrotoxin induced convulsions. Each bar represents mean±SD (n=10) of time taken for mice to convulse. Key: C= 5ml/kg body wt 1% CMC; T1-T6 represent 100, 200, 400, 800, 1600 and 3200 mg/kg body wt of aqueous extract of *D. fischeri* stem bark, respectively.

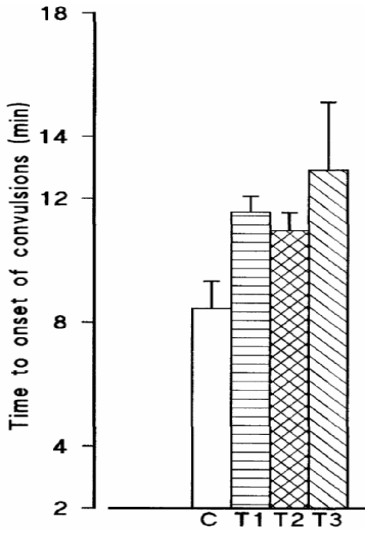

Figure 4: The effect of methanol extract of *D.fischeri* on picrotoxin induced convulsions. Each bar represents mean±SD (n=10) of time taken for mice to convulse. Key: C=5ml/kg body wt of 1% CMC; T1-T3 represent 100, 400, and 800 mg/kg body wt of methanol extract of *D. fischeri* stem bark, respectively.

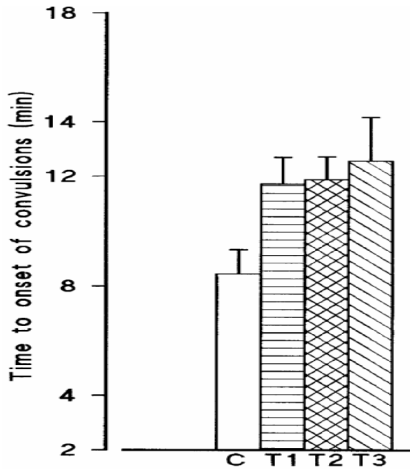

Figure 5: The effect of 1:1 dichloromethane:methanol on picrotoxin-induced convulsions. Each bar represents mean±SD (n=10) of time taken for mice to convulse. Key: C=5ml/kg body wt of 1% CMC; T1-T3 represent 100, 400, and 800 mg/kg body wt of dichloromethane:methanol (1:1) extract of *D. fischeri* stem bark, respectively.

The effect was dose-dependent, thus suggesting that possibly the inhibitory effect of the extracts is mediated by action on the $GABA_A$ receptor. All the organic extracts showed activity, suggesting that more than one compound may be involved. It also appears that the petroleum ether extract may contain the most active compound/s.

The present results support traditional uses of preparations of *D. fischeri* stem bark for treatment of epilepsy, and suggest the need for more detailed research to explore the clinical utility of this seemingly innocuous traditional remedy.

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