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ANTICONVULSANT ACTIVITY OF *DIOSPYROS FISCHERI* ROOT EXTRACTS

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Abstract

Diospyros fischeri Gurke (Ebenaceae) is used in traditional medicine for the treatment of epilepsy. Dichloromethane, ethylacetate, and ethanol extracts of the roots, at doses between 100 and 1600 mg/kg BW, inhibited convulsions induced by the γ-aminobutyric acid type A ($GABA_A$) receptor antagonist, pentylenetetrazole (PTZ), in a dose dependent manner. The extracts also exhibited low toxicity against brine shrimps giving LC_{50} values between 45 .4 and 95.4 μg/ml. These results provide evidence for the potential of *D. fischeri* extracts to treat absence seizures, especially given their seemingly innocuous nature.

Keywords: *Diospyros fischeri*; Pentylenetetrazole; Anticonvulsant activity; Brine shrimp toxicity

Introduction

Epilepsy continues to be one of major stigmatizing conditions in the world for all age groups. It has a high psychosocial impact because those suffering from the seizures are vulnerable to be discriminated upon and rejected and their ability to live life to the fullest can dramatically be affected (Hutten, 1994; Matuja and Rwiza, 1994)).

In Tanzania the first published report on epilepsy is from the work that was done in the early 1960s among the Wapogoro tribe of Mahenge, Morogoro region (Jilek-Aall and Rwiza, 1992). A 1990 epidemiological survey estimated the prevalence in Tanzania to be in the range of 19/1000- 36/1000, with the mean age at onset of 11.6 years (Rwiza et al., 1992). The prevalence among villagers at Ulanga district in Tanzania was found to range from 5.1 to 37.1 in 1000 (age adjusted 5.8- 37.0) with an annual incidence of 73.3 in 100,000 (Rwiza et al., 1992).

The Tanzanian community, urban and rural alike, still depends on traditional medicines for their day to day health care (Kilima et al., 1993), including the management of epilepsy (Moshi et al., 2005). It is on this premise that efforts are being made to evaluate the plants that are being used. In a previous communication it was reported that *D. fischeri* is one of plants that are used, in Tanzania, for the treatment of epilepsy and that extracts of the stem bark inhibited picrotoxin-induced convulsions in mice (Moshi et al., In Press). This study has expanded the scope of the work by testing root extracts, and using PTZ, which is more predictive than picrotoxin of the potential to treat absence seizures (Rang et al., 2003).

Materials and Methods

Materials

Petroleum ether, dimethylsulfoxide (DMSO) and methanol were purchased from Fisher Scientific UK. Ltd (Bishop Meadow Road, Loughborough, Leicestershire, LE 11 5RG, UK), while carboxymethyl cellulose (CMC) and pentylenetetrazole (PTZ) were purchased from Sigma Chemical Company Ltd (Poole, Dorset, UK). Phenobarbitone Sodium injection (manufactured by Laboratory and Allied Ltd, Nairobi Kenya) was purchased from J.D Pharmacy Ltd (Dar es Salaam, Tanzania).

Collection and preparation 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. The dried bark was ground into fine particles using a milling machine.

Sequential extraction

Fresh powdered dried roots of *D. fischeri* (1.0 kg) were extracted sequentially with solvents of decreasing polarity. The solvents used and the corresponding extract yields were; dichloromethane, 4 g (0.4%), ethyl acetate, 5 g (0.5%), ethanol, 6 g (0.6%). All the dry extracts were obtained after drying the extract solutions using a rotary evaporator, followed by freeze drying.

Experimental animals

Male and female Theiller's original albino white mice, weighing 20-33 g were used. The animals were starved for 16-20 h but allowed free access to drinking water. The animals were weighed and grouped into 2 groups of 10 mice each for the treated and control group. This work was done according to College guidelines for handling animals and was done under the supervision of a qualified veterinary surgeon that has permission to handle animal work (Dr. Pax J. Masimba is a qualified, licensed veterinary surgeon).

Preparation and dosing with extracts

The dichloromethane extract was suspended in a mixture of DMSO and 1% CMC (3:7) while the other extracts were suspended in 1% CMC.

Testing for anticonvulsant activity

Animals were pre-dosed with plant extracts orally, 30 minutes before administering the convulsant, PTZ, at a dose of 100 mg/kg BW intraperitoneally (i.p). The time of onset of convulsions was recorded and the latency period was noted as the interval between PTZ administration and onset of convulsions. from which the latency period was calculated by subtracting the time of administering pentylenetetrazole from time of onset of convulsions. The observation was done for a maximum period of 60 min.

Data analysis

Results are expressed as a mean ± S.D. The data was analyzed by using one way analysis of

variance, and the means compared using the Neuman Keul's range test. The differences between the mean latencies were considered significant at $P \leq 0.05$.

The brine shrimp lethality test

The brine shrimp lethality test (BST) was used to predict the presence, in the extracts, of cytotoxic activity (Meyer et al., 1982). Assay procedures and analysis of results were done as reported earlier (Moshi and Mbwambo, 2005).

Results

Anticonvulsant activity

Figures 1-3 show that ethanol, ethyl acetate, and dichloromethane extracts of *D. fischeri* roots inhibited pentylenetetrazole-induced convulsions in mice. All the three extracts significantly ($P \leq 0.05$), and dose-dependently, increased the latency period before the onset of convulsions as compared to the control group. Whereas control mice convulsed within 2 minutes of administration of PTZ, the extracts delayed the convulsions to between 6.9±4.9 and12.7±3.8 min. None of the extract doses tested completely suppressed the convulsions. Most of the mice given 50 mg/kg BW phenobarbitone resisted the convulsions beyond the 60 min. observation time. Only a few convulsed with an overall mean latency of about 58 minutes.

Brine shrimp lethality

The results, in Table 1, show that all the extracts of *D. fischeri* had low toxicity against brine shrimps. The LC_{50} for Cyclophosphamide used as standard was 16.5µg/ml, which was lower than the lowest value of 45.4 µg/ml for the ethyl acetate extract.

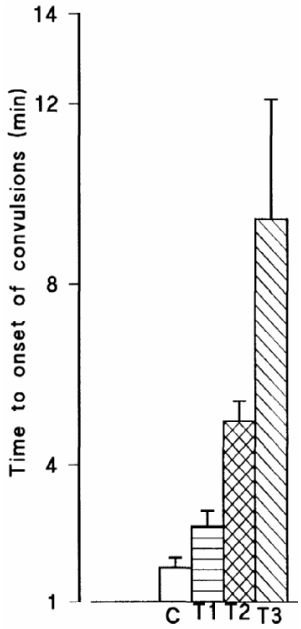
Discussion

D. fischeri is a traditional remedy for epilepsy in Iringa, which lies within the Southern Highlands of Tanzania. In a previous communication it was reported that its stem bark extracts inhibited picrotoxin-induced convulsions in a dose-dependent manner, thus suggesting the possibility that the extracts may contain one or more compounds that act on $GABA_A$ receptors. This paper examines the possibility that the anticonvulsant activity also resides in the roots. Indeed, the roots have also shown activity against PTZ-induced convulsions. PTZ, like picrotoxin, acts by inhibiting the $GABA_A$ receptors. The effect was dose-dependent up to 1600 mg/kg BW, thus strongly suggesting a receptor-mediated effect.

Pentylenetetrazole (PTZ) is a central nervous system convulsant that is thought, based on binding studies, to act at the picrotoxin (PTX) site of the γ-aminobutyric acid type A ($GABA_A$) receptor (Huang et al., 2001). PTZ and picrotoxin interact with overlapping but distinct domains of the $GABA_A$ receptor (Huang et al., 2001). It has been established that drugs which inhibit PTZ-induced convulsions and raise the threshold for the production of electrically-induced seizures are generally effective against absence seizures, whereas those that reduce the duration and spread of electrically-induced convulsions are effective in focal types of epilepsy, such as tonic-clonic seizure (Rang et al., 2003).

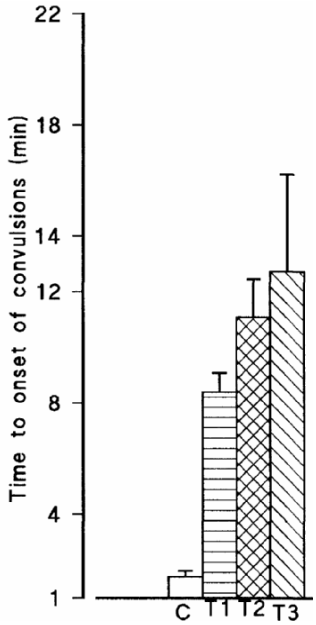
In addition to the anticonvulsant activity, preliminary results have been obtained from brine shrimps, which suggest that the extracts may not be out rightly toxic. The LC_{50} values obtained do not rule out the presence of other biological activities, such as anticancer activity, but they are a good

Figure 1: The effect of ethanol extract of *D. fischeri* roots on pentylenetetrazole-induced convulsions in mice.



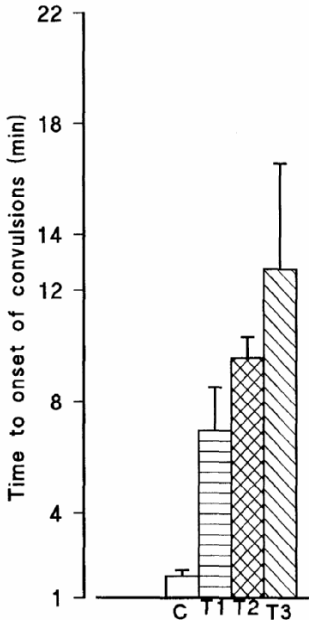
Each bar represents the mean ± SD (n=10). Key: C= 5 ml/kg body wt of 1% CMC; T1-T3 represent 400, 800 and 1600 mg/kg body wt of ethanol extract of *D. fischeri* roots.

Figure 2: The effect of ethyl acetate extract of *D. fischeri* pentylenetetrazole-induced convulsions in mice**.**



Each bar represents a mean ± SD (n=10). Key: C= 5 ml/kg body wt of 1% CMC; T1-T3 represent 400, 800 and 1600 mg/kg body wt of ethyl acetate extract of *D. fischeri*.

Figure 3: The effect of dichloromethane extract of *D. fischeri* roots on pentylenetetrazole-induced convulsions in mice.



Each bar represents mean±SD (n=10). Key: C= 5 ml/kg body wt of 1% CMC; T1-T3 represent 400, 800 and 1600 mg/kg body wt of dichloromethane extract of *D. fischeri.*

Table 1: Brine shrimp lethality of *D. fischeri* root extracts.

Extracts	LC_{50}µg/ml	95% CI
Dichloromethane extract	69.11	50.06-95.44
Ethyl acetate extract	57.3	45.4-89.22
Ethanol	119.61	87.19-94.81
Cyclophosphamide	16.3	10.6-25.2

The results are presented as LC_{50} (µg/ml) and 95% confidence intervals.

indicator of low level acute toxicity. These results agree with the previous ones on stem bark extracts which were shown to be innocuous in an acute toxicity study in mice (Moshi et al., In Press).

In conclusion it has been shown that the extracts of *D. fischeri* roots contain anticonvulsant activity, in support of traditional uses, and present a real potential for possible clinical application. However, more detailed studies are needed before the clinical potential of the extracts can be known. Thus a new ground has been opened to identify the specific compound(s) involved for evaluation of its real clinical potential.

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