MUHIMBILI UNIVERSITY COLLEGE OF HEALTH SCIENCES

SCHOOL OF PHARMARCY

TITLE; INVESTIGATION OF SOME BIOLOGICAL ACTIVITIES OF THE EXTRACTS OF *DIOSPYROS FISCHERI* ROOTS

DISSERTATION SUBMITED AS PARTIAL FULFILMENT FOR THE AWARD OF THE DEGREE OF BACHELOR OF PHARMACY.

AUTHOR; Magelewanya, Edward, S
Reg No; 37321/T 02
B.PHARM IV
2005/2006

SUPERVISORS; Dr. M.J MOSHI and Dr. Z.H MBWAMBO,
Institute of Traditional Medicine.

TABLE OF CONTENTS

Table of content.....1
Acknowledgement.....2
Abstract.....3
Introduction.....4-5
Problem statement.....6

Objectives.....6
Methodology.....6-7
Results.....8-11
Discussion.....11
Conclusion and Recommendation.....11
References.....12

ACKNOWREDGEMENT

First, I appreciate the strong support from the Tanzania Government, especially the Ministry of Science Technology and Higher Education, for giving me a loan.
I also would like to express my sincere thanks to my supervisors, Dr. Mainen J. Moshi, Director Institute of Tradition Medicine (ITM), and Dr. Z.H. Mbwambo who really worked tirelessly with me to ensure my tremendous success in this project.
Thanks to Dr. Pax Jessey, Mr Ramadhani Nondo for their advice and help during this research study.
Thanks also to Mr.Nicodemus Michael, and Mama Asha who gave their help in one way or another.
It is my appreciation to my classmates who were giving me moral support.
Lastly to my late father and mother, my brother and sister for the support they always give to me in my education up to now.

ABSTRACT:

Diospyros fischeri (Ebenaceae) Gurke has been used for quiet a long time in Tanzanian societies for treatment of convulsions.

The dried plant material was extracted sequentially by using solvents of different polarities starting with dichloromethane, ethyl acetate, ethanol, and water

Another fresh sample was extracted using water alone.

Extracts of Dichloromethane, ethyl acetate and ethanol at the doses of 100,400 and 1600mg/kg body weight caused a dose-dependent delay of the latency to the occurrence of pentylenetetrazole-induced seizures but did not completely prevent the occurrence of the seizure. The root extracts of *D. fischeri* exhibited low toxicity against brine shrimps, suggesting low toxicity of the extracts.

INTRODUCTION

1.1. General introduction

The term epilepsy is used to refer to a group of seizure disorders that are associated with high frequency discharge of impulses by a group of neurons in the brain [2]. It is a neurological disorder that affects people in every country throughout the world. Epilepsy is also one of the oldest conditions known to mankind. The belief widely held in many countries is that a person with epilepsy is seized by a supernatural power. This ancient belief is reflected in the name of the disorder-the word "epilepsy" being derived from the Greek word "epilambanein" which means, "to seize or attack [1].

This disease (epilepsy) can be classified into partial and generalized seizures. Partial seizures are further sub-classified into simple and complex partial seizures. Simple partial seizures begin in a small-localized area of the brain, and do not lead to altered consciousness. Complex partial seizures begin in a small-localized area of the brain, leading to altered consciousness [7]. Generalised seizures involve the whole brain including the reticular system, thus producing abnormal electrical activity throughout all the hemispheres. Immediate loss of consciousness is the characteristic of this type of epilepsy.

There are two major types of generalised seizures; it can be tonic clonic seizures (grand mal) or absence seizures (Petit mal) [8]. Tonic clonic seizure consists of an initial strong contraction of whole musculature causing a rigid extension spasm, respiration stops, defecation, micturition and salivation. Absence seizures occur in children; they are much less dramatic and may occur much more frequently than tonic clonic seizures. The patient abruptly stops whatever was doing, sometimes stopping speaking in between sentences and phrases, vacantly, in few seconds with little or no motor disturbances

1.2. EPIDEMIOLOGY.

The prevalence of epilepsy is high in tropical countries particularly in Africa where it carries a prevalence of 10 to 55 per 1000 [3] with an estimated mean prevalence of 15 per 1000. The prevalence in Africa is 4-6times higher than in the developed world [4].

The incidence in developing countries suggests that this figure is at 100 per 100,000; while in developed countries are 50 per 100,000. The main reason for higher incidence of epilepsy in developing countries are conditions like neurocysticercosis, meningitis, malaria, pre-and perinatal complications and malnutrition which can lead to permanent brain damage. Apart from the above reasons, epilepsy can also be due to genetic factors, brain tumour, cerebrovascular disease, head injury, trauma, and brain infection, and febrile illnesses.

Epilepsy continues to be one of the major stigmatizing conditions in the world for all ages. It is third largest neurological disorder after dementia and stroke. 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 [5].

Persons with epilepsy are shunned and discriminated against in education, employment, and marriage in Africa, because epilepsy is seen as highly contagious and shameful disease in the eyes of public. The observation comes from many studies carried out in Africa including recent ones in Nigeria and Liberia

1.3. EPILEPSY IN TANZANIA:

Epileptic seizure disorder in Swahili is known as kifafa. In Tanzania the first published

report is from the work that was done in the early 1960s among the Wapogoro tribe of Mahenge, Morogoro region Tanzania [9]. 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 11.6 years. 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 [10].

A study carried among secondary school students in Dar es salaam about the knowledge, attitude and practices towards epilepsy showed that people thought epilepsy is inherited, others attributed it to brain injury, infection and others associated it to witch craft. Eighty percent (80%) considered epilepsy controllable. Over 90% believed epileptics are mentally abnormal and considered this to be the reason of not sending an epileptic child to school. Sixty percent (60%) considered epilepsy to be contagious through physical contacts, breath, and saliva [6]. The study revealed that most deaths are due to status epilepticus, drowning, burns, and some die in or after seizures [11].

1.4. *TREATMENT OF EPILEPSY;*

Epilepsy is treated using well established anti-epileptic agents, which are phenytoin; carbamazepines; sodium valproate; ethosuximide; benzodiazepines e.g. Clonazepam and diazepam; and phenobarbitone. Other newer agents are vigabatrine, gabapentine, lamotrigin, tiagabine, and topiramate [12].

Carbamazepine, phenytoin, sodium valproate, and benzodiazepines act by inhibiting fast Na+- gated channels. Ethosuximide acts by inhibiting T-type ca+ channels. GABA analogues act by increasing the concentration of GABA e.g. Gabapentin [6, 14]

1.5. *PLANTS AS A SOURCE OF NEW DRUGS;*

The potential of plants as a source of new drugs is still largely unexplored. Among the estimated 250000-500000 plant species on the earth, only a small percentage has been investigated phytochemically, biologically and pharmacologically [14]. The search for bioactive drugs from plants including antimalarial [16], anticonvulsant [18], anticancer [15] is guided by knowledge on the indigenous herbal remedies.

Plants continue to be a definite source of lead compounds for drug discovery. Among these compounds that have been reported to have anticonvulsant actions are flavonoids [22, 9]. Flavonoids such as chrysin (5,7-dihydroxy flavones) and 5,7-dihydroxy-6-methoxyflavore are known to interact with the benzodiazepine binding site of the $GABA_A$ receptor leading to sedation [22]. This may lead to inhibition of tonic clonic seizures induced by pentylenetetrazole.

PROBLEM STATEMENT

Epilepsy is treated mainly with drugs which are synthetic such as phenytoin, benzodiazepines, GABA analogues, barbiturates etc. Brain surgery may be used for severe cases. However these drugs are associated with chronic toxicities and side effects such as central nervous system damage, peripheral neuropathy, cardiac depression, megaloblastic anaemia and osteomalacia [20,21,].

In developing countries where drugs are not easily available many patients do not always have access to modern drugs; sometimes only a few are available.
A study performed in Dar es salaam, Tanzania showed that 21% of the patients consult traditional healers before going to public health facilities (Kilima et al., 1993).
In developing countries where drugs are not easily available epilepsy responds to treatment up to 70% of the patients. However about 75% of people with epilepsy do not receive the treatment they need (WHO information fact sheet, 2001, WHO press release, 2001).

OBJECTIVES

BROAD OBJECTIVE

Investigation of the efficacy of extracts of *D.fischeri* roots in the management of epilepsy.

SPECIFIC OBJECTIVES

-To test the effect of extracts of *D.fischeri* roots against pentylenetetrazole induced convulsions.
-To determine the extract with the best activity.
-To determine the toxicity of *D.fischeri* root extracts using Brine Shrimps Test.

METHODOLOGY:

A: Materials and equipment:
Dichloromethane, ethyl acetate, ethanol, water, pentelenetetrazole, syringes, stopwatch, column, bottles for storing chemicals, gauze, adsorbent, flasks, funnels, filter papers and experimental animals (mice).

B: Sequential extraction
Fresh powdered dried plant material was extracted sequentially with solvents of decreasing polarity. The solvents used and the percentage yields were as followed:
Dichloromethane 4gm (0.4%), ethyl acetate 5gm (0.5%), methanol 6gm (0.6%).
All the above were obtained after vaporization of the solvent followed by freeze drying of the extracts using rotor vapour.

C; *Experimental Animals*

Male and female Thrillers original albino white mice, weighing 20 to 33gms were used. The animals were starved for 16 to 20hrs but allowed to drink water only from the start of starvation up to the end of the experiment. The animals were weighed and grouped into two groups of 10 mice.

E; *Procedures:*

- The dried plant material was extracted sequentially with dichloromethane, ethyl acetate, ethanol, and water.
- Another fresh sample was extracted using water alone.
- The extracts were concentrated with rotor vapour, followed by freeze-drying.
- The powder obtained then was dissolved in specified solvent e.g. for aqueous extract was dissolved in distilled water and filtered prior to injection in the mice.
- Then the prepared solution was injected in mice 30minutes before convulsions were induced using pentylenetetrazole
- Finally the activity of *D. fischeri*_extract was observed whether can protect the mice from pentylenetetrazole-induced convulsions.

F; *Testing for anticonvulsant activity*

Animals were pre-dosed with plant extracts orally, 30 minutes before administering the convulsant, pentylenetetrazole. Pentylenetetrazole 20mg/ml was then administered interperitonially (i.p). The time of onset of convulsions was recorded from which the latency period was calculated by subtracting the time of onset of convulsions. The observation was done for a maximum period of 60 minutes.

BRINE SHRIMP LETHALITY TEST

Materials and reagent

-*Artemisia Salina* Leach (brine shrimp from pet shop)
-artificial sea salt
-small container to hatch shrimp
-syringes 5mls and 2mls
-lamp to attract larvae

The men results of brine shrimp mortality against the logarithms of concentrations were plotted using the Fig P computer program, which also gives the regression equations were used to calculate LC_{16}, LC_{50}, LC_{84} values.

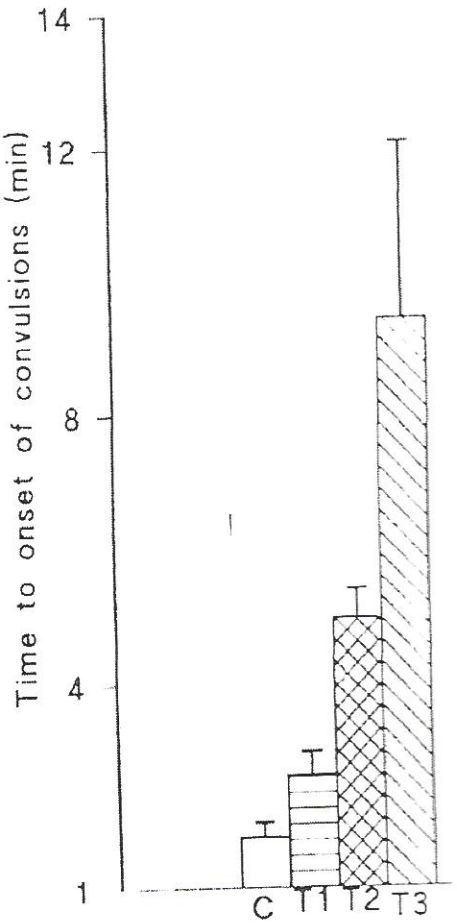
Confidence Intervals (95% CI),were calculated. From there; LC_{50}, LC_{84} and LC_{16} were calculated for the root extracts and tabulated as follows

RESULTS

The effect of Dichloromethane, Ethyl acetate and Methanolic extracts on pentylenetetrazole induced convulsions.

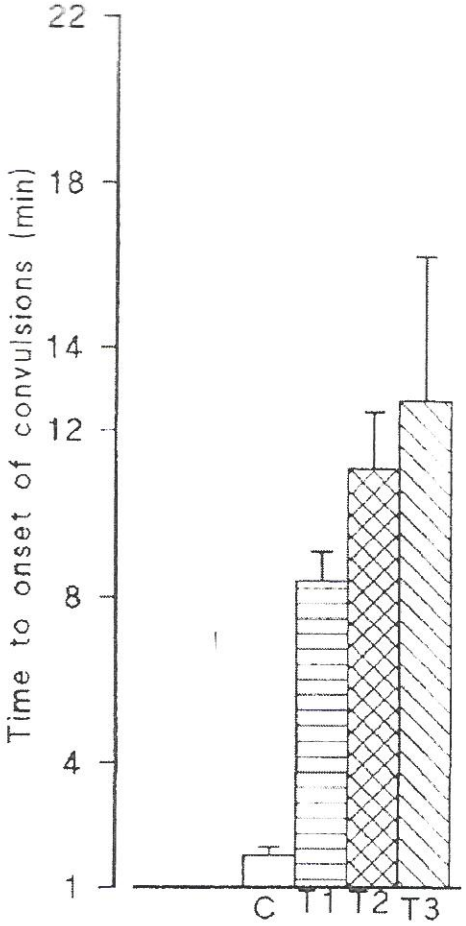
Three doses 100,400 and 1600mg/kg body weight were used. Figures 1-3 show that all the three extracts significantly increased the latency period to the onset of convulsions as compared to solvent treated mice. The effect was dose-dependent up to the dose of 1600 mg/kg body wt.

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



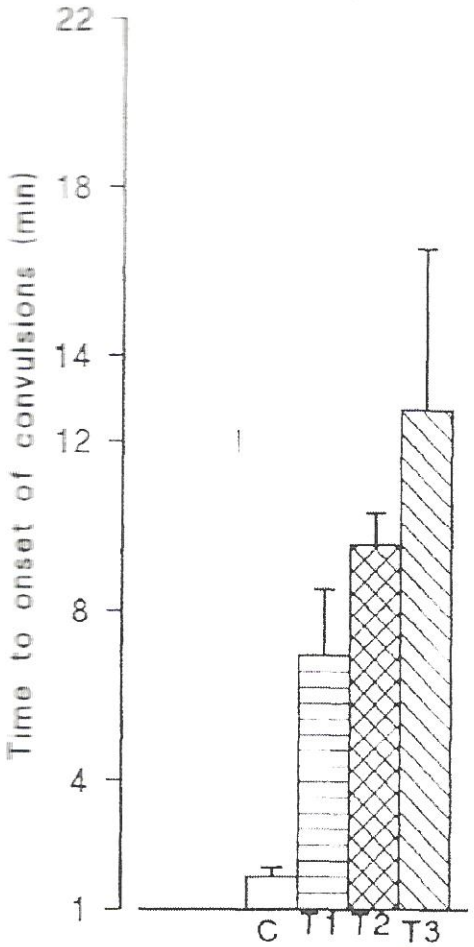
Each bar represents mean±SD (n=10). Key: C= 5 ml/kg body wt of 1% CMC; T1-T3 represent 400, 800 and 1600 mg/kg body wt of ethanol extract of *D. fischeri*.

Figure 2: **The effect of ethyl acetate extract of *D. fischeri* 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 ethanol 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: Shows the mean brine shrimp survival rate against concentration.

Concentration	DCM	EA	ET	AQ
240	1,2	0,0	3,4	10,10
120	3,3	1,2	4,4	10,10
80	5,6	4,5	6,7	10,10
40	6,7	6,6	8,8	10,10
24	8,8	8,8	10,10	10,10
16	8,9	10,10	10,10	10,10

Table 2: Brine shrimp lethality of *D. fischeri* root extracts. The results are presented as LC_{50} (µg/ml) and 95% confidence intervals

Extracts	LC50	95% CI	Regression formula
DCM	69.11	50.06-95.44	Y=61.4492(logx)-63.0366
EA	57.3	45.4-89.22	Y=84.9230(logx)-99.3070
ET	119.61	87.19-94.81	Y=62.5751(logx)-80.0162

The results show that all the extracts of the plants had low toxicity against brine shrimps. The LC_{50} for Cyclophosphamide used as standard was 16.5, So less than 20 the compound is so toxic.

DISCUSION

Extract from the root of *Diospyros fischeri* family *Ebenaceae* appear to contain an anticonvulsant principal in the manner that it appear to be dose dependent against latency period, and its latency period is longer than that of control, however does not offer protection against the Pentylenetetrazole induced convulsions may be because it is a crude drugs and some active ingredients lost due to heating effect during concentration of the drugs.

Also the plant does not contain cytotoxicity compounds.

Comparing with the standard drug Cyclophosphamide whose Lc_{50} 16.5 and 95% c.i was 14.5-25

The table above shows that all the extracts of the plants were not toxic to shrimps, The concentration killing 50% of the shrimps were more than that of cyclophosphamide.

CONCLUSION AND RECOMMENDATION

Extracts of *Diospyros fischeri* roots appear to contain an anticonvulsant principle. The effect was dose-dependent and it suggests receptor mediated effect.

The extracts could not offer complete protection against the Pentylenetetrazole induced convulsions.

Also the extracts did not significantly reduce mortality rate following administration of 100mg/kg body weight Pentylenetetrazole.

Using brine shrimps test it has been shown that the extracts may have low toxicity.

Further studies are suggested to explore this activity for possible clinical use.

REFERENCES:

1. Ashock, K. chauhan, Mahaveer, DP. and Bhuwan Joshi. A review of medicinal plants showing anticonvulsant activities, *Journal of Ethnopharmacology22(1988)11-2*
2. WHO press release, Fact sheet No.165. Epidemiology, aetiology and prognosis of epilepsy, February .2001.
3. Caroline Debrock ,Pierre-Marie Preux ,Dismand Houinato et al. estimation of prevalence in the Benin region of Zenvie using the capture -recapture method, International journal of epidemiology,2000,29:330-335
4. Hutten I, Ensimbo, HM. the falling disease: A study on the knowledge , altitude and practices towards epilepsy in Kaisho, Murongo Division in Tanzania *occasional paper41*
5. Peter A. Akah and John Inwaino Anticonvulsant activity of root and stem extract of Colliendra portoviensis,1987,journal of ethno pharmacology(1988):205-210
6. Matuja WB and Rwiza HT -Knowledge ,Altitude ,Practice towards epilepsy in secondary school students in Tanzania- central Africa journal 1994,40(1);13-8
7. Davenport and the relationship between starvation metabolic acidosis and convulsive seizures Journal of Ethnopharmacology,139-151;1995
8. Rang, HP, Dale, MM, Ritter, JM. Pharmacology book 5th edition.
9. Neuman RJ .Kwon JM, Jilek Aall, Rwiza et al. Genetic analysis of Kifafa , a complex seizures disorder ,Journal of human genetics 1995 ;4, 902-910
10. Rwiza HT ,Kilonzo GP, Haule J, Matuja WB ,G Mwag'ombola , R Mwaijande et al.- Prevalence and Incidence of epilepsy in Ulanga district ,a community based study epilepsia 1992;33 1056-6
11. Jilek- all, Rwiza HT -Prognosis of epilepsy in rural African community Tanzania Epilepsia 1992;33(4) 645-50
12. Mary J, Mycek , Richard A Harvey ,Pamela C champe pharmacology book 2nd edition 2000;143
13. Suffness M, and Dorous J current status on the NCI plant and animal products program journal of natural products 1982;45
14. Tripath KD Essentials of Medical Pharmacology 4th edition 2002;384-389
15. Wani M, C, Tylor H, L, Wall M, E et al Novel Potent antitumor agents,journal of A merican Chemical Society 1971,93,2325.
16. Hallock, Y.F. Manfrei,K.P. Blunt,J,W et al Novel antimalarial alkaloid from ancistroclaudus korupensis, Journal of organic chemistry 1994,59,6349.
17. Kazaura, C.K Makwaya et al, Lecture notes on biostatics 1997,61-67
18. UODEN,Onu some pharmacological properties of solenostemon monostachy us. J of herb and species and Medicinal plants vol.4. Nov 1996, 3-7.
19. Huen MS, Leung JW, Ng W,Lw WS, Chan MN, Wong It,Xue H.Biochem pharmacol 2003,66:125-32.
20. Dekker PB(2002),Epilepsy a mammal for medical and clinical officers in Africa WHO Geneva pp,554-55,1353.
21. Caroline JH, Paldini AC, Wolfman C, Levi de stain M ,Calvo D, Diac L, Pena C. Chrysin (5,7-dihydroxy flavone), a naturally, occurring ligand for benzodiazepine receptors, with anticonvulsant properties, Biochem Pharmacology 1990;40;2227-31.