A STUDY OF THE EFFECT OF EXTRACTS OF *CODIAEUM VARIEGATUM* (L.) A. JUSS ON PICROTOXIN-INDUCED CONVULSIONS IN MICE

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Summary

***Codiaeum variegatum* (L.) A. Juss (Euphorbiaceae) leaves are used in Kagera and Coast regions for treatment of epilepsy. To confirm this claim aqueous and methanol extracts were tested for ability to inhibit picrotoxin-induced convulsions in adult Theiller's white albino mice. The methanol extract showed a significant protection of the mice against picrotoxin-induced convulsions (P<0.05), using both the i.p and oral routes. The aqueous extract also showed significant protection when administered by the intraperitoneal but not the oral route. Inactivation of active compound/s in the aqueous extract by metabolism or other mechanism is speculated. Further studies to isolate active compounds following bioassay guided fractionation and the use of other complimentary models of convulsions is suggested.**

Key words: *Codiaeum variegatum*, Methanol, aqueous extracts, anticonvulsant activity, picrotoxin, mice

Introduction

Codiaeum variegatum(L.) A. Juss (Euphorbiaceae) is a shrub with narrow, elongated, yellow variegated leaves and growing to a height of about 1.5 metres**.** It is an ornamental plant commonly found in gardens in different parts of Tanzania. It is commonly known as "Maua vitenge" in Kiswahili. *Codiaeum variegatum* is reported to contain 4-hydroxybenzoic acid, chlorogenic acid, coumaric acid, ellargic acid, ferulic acid(1), choline, acetylcholine(2) and propionylcholine.(3) The plant is used as antiseptic, abortfacient(4), and contraceptive (5). Extracts of the leaves were shown to have antitumor(6), antimutagenic(7), antimycobacterial(8), Molluscicidal(9), and antifungal activity.(1)

A methanol extract of the leaves activated Epstein-Barr virus infections in two types of cell lines.(10) A decoction of the leaves is used by the Swahili and Haya people for the management of epilepsy, but its anticonvulsant activity has not been reported before. The aim of this study was, therefore, to test the extracts of the plant for anticonvulsant activity using the picrotoxin-induced convulsions model.

Materials and Methods

Materials

Chloroform, petroleum ether, dimethylsulfoxide (DMSO), and methanol were purchased from Fisher Scientific UK Ltd (Bishop Meadow Road, Loughborough, Leicestershire, LE 11 5RG, UK), while Carboxymethylcellulose (CMC), Phenobarbitone sodium and Picrotoxin were purchased from Sigma Chemical Company Ltd (Poole, Dorset, UK).

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Collection and processing of plant material

The leaves of *Codiaeum variegatum* were collected from the gardens of Muhimbili University College of Health Sciences (MUCHS) by one of the investigators. Identification of the plant was *done* at the Department of Botany, University of Dar es salaam, by Mr. F. Mbago, and the voucher specimens are kept in the Herbarium of the School of Pharmacy, MUCHS. The leaves were sun dried for four days, and any contaminants on the materials were removed. Mortar and pestle was used for the grinding of dry *Codiaeum variegatum* leaves.

Preparation of Aqueous extract

Dried leaves (800 gm) of the plant were boiled with 1500 ml of distilled water for one hour, cooled and filtered. The filtrate was freeze-dried and the dry powder was stored at –20 ^{O}C until the day of use. On the day of use the dry extract was reconstituted in distilled water to the required concentration.

Preparation of Methanol extract

The dried leaves (80 gm) of *the* plant were soaked with 450 ml of 99.5%methanol for 48 hours, in a percolator. The methanol extract was drained from the percolator and evaporated on a rotar vapour (30^{O}C), to dryness. The dry extract obtained was stored in a deep-freezer, at –20 ^{O}C, until used. On the day of use the extract was first dissolved in DMSO and then 1% CMC in distilled water was added to make a suspension containing 30% DMSO.

Testing for Anticonvulsant activity

Animals

White Theiller's original albino mice, of either sex, weighing 20 – 36 gm were used. The mice were kept in the animal house (in cages) at a temperature of 27±2°C, supplied with water and food ad libitum. Prior to the experiment the mice were starved for 12 –14 hr, but only allowed free access to drinking water. On the day of the experiment the mice were randomly allocated to control and treatment groups each consisting of 10 mice. All experiments were carried out between 08.00 and 16.00 hr.

Effect on picrotoxin-induced convulsions

Mice were pre-dosed intraperitonially (i.p) or orally with a vehicle, a standard anticonvulsant drug (Phenobarbitone) 50mg/kg body weight or a plant extract (50-800 mg/kg body wt), 30 min before they were

challenged with 10 mg/kg body wt picrotoxin, i.p . The number of mice convulsing, and the latency to full tonic-clonic convulsions were recorded for all the groups. Observation was done for a maximum of 1 hr beyond which mice which had not convulsed were considered protected.

Effect of the aqueous extract on picrotoxin-induced convulsions

Six groups of mice, each containing 10 mice, were used. The first group received 5ml/kg body wt distilled water, i.p, and served as the control group. The second group received 50 mg/kg body weight phenobarbitone, while the remaining 4 groups received 50, 100, 400, and 800 mg/kg body weight, respectively, of the aqueous extract of *Codiaeum variegatum* i.p and then challenged with picrotoxin as explained above. The procedure was repeated using the oral route with another set of animals.

Effect of Methanol extract on picrotoxin-induced convulsions

Six groups, each containing 10 mice, were used. The control group received a 5 ml/kg body wt of a 3:7 mixture of DMSO:1% CMC in distilled water , i.p. The second group received 50 mg/kg body weight phenobarbitone, while the remaining 4 groups received i.p, 50, 100, 400, and 800 mg/kg body wt, respectively, of a suspension of the methanol extract dissolved in a 3:7 mixture of DMSO and 1% CMC in distilled water, respectively, and then challenged with picrotoxin 30 minutes later as explained above. The procedure was repeated using the oral route with another set of animals.

Data analysis

The mice were observed for 60 min, beyond which those which had not convulsed were considered protected. The mean values of the latency (LP) to convulsions were compared using one way ANOVA. The difference between mean latencies were determined by the use of the Neuman Keul's range test. Differences were considered significant when $P \leq 0.05$

Results

Effect of the aqueous extract on picrotoxin-induced convulsions

Table1 shows that when the aqueous extract was administered by the ip route there was a significant increase in latency to convulsions as compared to the solvent treated group ($P<0.05$), at doses of 50 to 400 mg/kg body wt. The effect was dose dependent but leveled off at 400 mg/kg body wt, so there was no further increase of latency at 800 mg/kg body wt. When the extracts were administered by the oral route there was no suppression of picrotoxin-induced convulsions. There was no significant difference in latency between the solvent controls and mice treated with 50–800 mg/kg body wt of the aqueous extract ($P>0.05$).

Table1: Effects of *Codiaeum variegatum* aqueous extracts against picrotoxin-induced convulsions (n = 10; *Significant Vs Control $P \leq 0.05$)

Pre-treatment with	Oral LP (Mean ± SD) min	I.P LP (Mean ± SD) min
Distilled water 5 ml/kg body wt	7.84±1.41	7.84±1.83
50 mg/kg body wt	8.41±4.06	8.71±1.2 6
100 mg/kg body wt	6.10±0.88	10.75±1.92
400 mg/kg body wt	6.54±1.60	13.13±4.99
800 mg/kg body wt	7.38±2.22	9.14±2.44

Effect of methanol extract on picrotoxin-induced convulsions

Administration of the methanol extract orally at doses of 50,100, and 400 mg/kg body wt caused a dose dependent increase in the latency (LP) to convulsions as compared to solvent treated controls ($P<0.05$). Increasing the dose to 800 mg/kg body wt did not further increase the latency period (Table 2), indicating leveling off of the anticonvulsant effect at the 400 mg/kg body wt dose. Using the IP route there was significant increase of latency to the onset of convulsions at 50, 100 and 400 mg/kg body wt ($P<0.05$), and like with the oral route no further increase of latency was observed at 800 mg/kg body wt.

Phenobarbitone (50 mg/kg body wt), which was used as the positive control, completely suppressed the picrotoxin-induced convulsions well beyond the observation period of 60 min in most of the mice used.

Table 2: Effect of *Codiaeum variegatum* methanol extracts against picrotoxin- induced convulsions (n = 10; *Significant Vs Control $P \leq 0.05$)

Pre-treatment with	Oral LP (Mean ± SD) min	IP LP (Mean ± SD) min
30% DMSO in 1% CMC	7.45±1.72	7.72 ±1.46
50 mg/kg body wt	10.66±2.17	12.05±3.16
100 mg/kg body wt	12.09±4.06	10.19±2.30
400 mg/kg body wt	13.31±3.62	11.72±3.03
800 mg/kg body wt	8.66±2.62	8.54±2.37

Discussion

Aqueous and methanol extracts of *Codiaeum variegatum* suppressed convulsions induced by the $GABA_A$-receptor antagonist, picrotoxin. The effect was dose dependent, thus suggesting a possible ligand-receptor mediated effect. The suppression of the picrotoxin-induced convulsions supports the traditional uses of the plant for the treatment of epilepsy. Traditionally the aqueous extract of the leaves, made by boiling is used. These results do not support the use of the aqueous extract orally as the effect obtained was not significant. The aqueous extract was

effective when administered intraperitoneally. However, the methanol extract was active orally and intraperitoneally. Two speculations can be made, that the active ingredients are not very soluble in water and that they are possibly inactivated either in the gastrointestinal tract or undergo first pass metabolism as they pass through the liver. Extraction with methanol picks up a large amount of the active ingredients sufficient to express activity even in the presence of inactivation or first pass metabolism. This means that the amount extracted into water is small and most is lost by inactivation/metabolism,leaving amounts that are inadequate to show biological activity. The amounts extracted into methanol are large such that even with destruction/metabolism there is enough left to show activity and that is why there was no difference in latencies between the orally and intraperitoneally administered methanol extract.

It is difficult to make any speculations as regards the usefulness of the present results, especially since the extract was unable to completely inhibit the picrotoxin induced convulsions. The effect of the extracts was only dose-dependent up to 400 mg/kg body wt and then leveled off. This may suggest that the active compound/s has partial agonist properties, and therefore exhibits low efficacy. When the dose is increased beyond a certain level inhibition of the GABA receptors occurs and hence the reduced effect observed at 800 mg/kg body wt. This speculation agrees with the observation that the extract could not completely suppress the picrotoxin-induced convulsions. We know that the plant contains different classes of compounds including, 4-hydroxybenzoic acid, chlorogenic acid, coumaric acid, ellargic acid, ferulic acid [(1)], choline, acetylcholine[(2)], and propionylcholine.[(3)] Tannins, flavonoids, and alkaloids were indicated to be present in general chemical tests [(11,12)]. Flavonoids such as chrysin (5,7-dihydroxy flavone) and 5,7-dihydroxy-6-methoxyflavone are known to interact with the benzodiazepine binding site of the $GABA_A$-receptor leading to sedation.[(13, 14)] This may also lead to inhibition of tonic-clonic seizures induced by pentylenetertrazol.[(13)] It is suggested to fractionate the methanol extract to isolate the flavonoid fraction and confirm this claim. Other chemical convulsants such as pentenyltetrazol, and strychnine should also be tried to further characterize the observed activity. Pentenyltetrazol acts on the same GABA receptors but it is believed to be better in predicting compounds that are effective on partial seizures, while strychnine acts on a peripheral site, on the spinal cord.

It is being concluded that the leaves of *Codiaeum variegatum* exhibited a dose-dependent suppression of picrotoxin induced convulsions. The nature of the active compound/s and the potential usefulness of the current results are a subject for immediate future follow-up.

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