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RESEARCH ARTICLE

# Neuropharmacological Profile of the Aqueous Leaf Extract of *Croton zambesicus* (Euphorbiaceae) in some Laboratory Animals

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## ABSTRACT

To determine the neuropharmacological activity of the leaf extract of *Croton zambesicus* in mice and chicks. The effect of aqueous leaf extract of *Croton zambesicus* on thiopental sodium-induced sleeping time in mice, on gross locomotor activity (GLA) in 2-day old chicks and effect of extract on apomorphine-induced stereotyped behaviour in chicks were evaluated. The aqueous extract (1000 and 1500 mg/kg) administered *per os* (p.o) prolonged the thiopental sodium-induced sleeping time in mice. Extract (20-40 mg/kg i.p) produced a significant $p<0.05$ decrease in GLA in 2-day old chicks in a dose dependent manner. 40-60 mg/kg administered intraperitoneally (i.p) produced sedation and sleep with a significant $p<0.05$ decrease in onset and an increase in duration of sleep. The extract administered subcutaneously (s.c.) had an insignificant $p>0.05$ effect on apomorphine-induced stereotyped behaviour in chicks. These results suggest that leaf extract of *Croton zambesicus* possesses CNS depressant, sedative, and hypnotic activity.

**Keywords:** *Croton zambesicus, central nervous system, depressant, sedative, hypnotic*

Ethnopharmacological approach to drug discovery is of great value in developing countries, since scientific validation of a local remedy may encourage its use and introduction into therapy in its original habitat [1]. Efficacy and safety are evaluated and potential harm is minimized through a selected method of preparation of the plant materials.

*Croton zambesicus* muell Arg. (Euphorbiaceae) syn *C. amabilis* muell. Arg., syn. *C. gratissumus* Burch, is an ornamental tree grown in villages and towns of Nigeria. It is a Guineo-Congolese species widely spread in tropical Africa [2]. The leaf decoction is used in Benin Republic, as antihypertensive and antimicrobial (urinary infections) [3]. The Ibibios in Urunan area of Akwa Ibom state of Nigeria use the leaf traditionally as a remedy for malaria[2]. Antidiabetic activity of the ethanolic leave extract has also been reported [4].

The ent-trachyloban-3β-ol, a trachylobane diterpene isolated from dichloro-methane extract has activity on Hela cells [5]. The alkaloidal fractions of the leaf have been reported to possess weak antimicrobial activity [6]. While the essential oil found in the leaves contain p-cymene, linalool and beta-caryophyllene [7]. The constituents of the essential oil found in the flowering tops include; pinene, limonene linalool, menthol, carvone, thymol, alpha-humulene and ceisnerolidol [8]. In Jos, Plateau State of Nigeria, the decoction of the leaves is used in the prevention and treatment of epileptic seizures (Traditional herbalist, Dr. Azija, personal Communication). This study aims to evaluate the effect of the aqueous leaf extract of *Croton zambesicus* on the central nervous system.

## MATERIALS AND METHOD

### *Plant Material*

The leaves of *Croton zambesicus* linn were collected from Bauchi Road, in Jos, Plateau State, Nigeria in July 2006. The plant was identified by Mr. I.A. Kareem, at the Federal College of Forestry, Jos and confirmed at Forestry Research Institute of Nigeria (FRIN), Ibadan.

The fresh leaves of the plant were shade dried for 8 days and then powdered using mortar and pestle. Fifty grams (50 g) portion of the powdered leaves was extracted by macerating with distilled water for 24 hours and then boiled for 15 minutes, allowed to cool and filtered. The extract was evaporated to dryness at a tem-

Table 1. Effect of aqueous leaf extract of *Croton zambesicus* on gross locomotor activity (GLA) in 2

| Treatment | Dose (mg/kg) | Count per hour (ph) | | | | |
|---|---|---|---|---|---|---|
| | | Movement | Escape Episode | Jumping | Pecking | GLA |
| Distilled water | | 101.2 ± 9.31 | 1.6 ± 0.40 | 3.2 ± 0.73 | 204.0 ± 14.78 | 310.0 + 16.97 |
| Extract | 10 | 46.0 ± 4.30* | 2.0 ± 0.63 | 1.0 ± 0.32* | 102.0 ± 5.15* | 151.0 ± 8.60* |
| | 20 | 12.2 ± 2.25* | 0.8 ± 0.49 | 0.8 ± 0.49* | 18.8 ± 10.98* | 32.6 ± 6.23* |
| | 40 | 3.8 ± 0.58* | 0.8 ± 0.49 | 1.8 ± 0.92* | 0.6 ± 1.34* | 6.0 ± 1.05* |
| | 60 | 2.2 ± 0.66* | 0.0 ± 0.00 | 0.0 ± 0.00* | 0.0 ± 0.00* | 2.2 ± 0.66* |

Results are expressed as mean ± S.E.M. (n=5) *$p$<0.05 compared with control. Mann-Whitney U test.

perature of 40-45°C. A yield of 3.90 g was obtained and kept at 4°C prior to use.

### *Animals*

Albino mice (20-25g) of either sex were obtained from animal house of the Department of Pharmacology, University of Jos, Nigeria and day-old chicks (25-35 g) obtained from National Poultry Farm Complex Barkin-Ladi Plateau State, Nigeria. The Chicks were collected on the day they were hatched and kept warm in cages for another 24 hours to acclimatize to the laboratory conditions. Animals were provided with feed (Vital Feeds) and water *ad libitum*.

### *Drugs*

The drugs used were supplied from the stock of Department of Pharmacology laboratory, University of Jos, Nigeria and include; apomorphine (Sigma) and thiopental sodium (Rotex). Each drug was dissolved in distilled water just before use.

### *Effect of extract on barbiturate-induced sleeping time in mice*

The method employed in this study was as described by Vogel [9]. Five (05) groups of five (05) mice were used and treated as follows;

Group I: thiopental sodium (50 mg/kg i.p)

Group II-IV: graded doses of the aqueous extract (500, 1000, 1500 mg/kg p.o), 60 minutes prior to the i.p. administration of thiopental sodium. The animals were placed on their back and the reappearance of the righting reflex was recorded as sleeping time.

### *Effect of extract on behavioural activities in 2-day old chicks*

A total of twenty-five chicks were used with five chicks per group. Two chicks were observed at a time. The experiment was performed in a noiseless environment and chicks were placed in open boxes. The extract (10, 20, 40, and 60 mg/kg i.p) was administered to the test groups respectively and distilled water (0.2 ml) was administered to the control group. Using the method of Wannang *et al* [10] behavioural activities including; locomotion (ability to move from one square compartment drawn on the floor of cage to another, pecking habits, jumping and escape episode were observed and recorded. The summation of all these was described as gross locomotor activity, while sleep described as both eyes closed, with either crouching with the beak on the floor or leaning beak against the wall of the cage. Sedation was evaluated as described by Fugner and Hoefks [11] as remained quiet, immobile with or without closure of eyes for over five minutes and with or without drooping head.

### *Effect of aqueous extract on apormorphine-induced stereotyped behaviour in chicks*

Apomorphine and sodium metabisulphite (antioxidant) were dissolved in distilled water prior to use. Two-day old chicks were divided into five groups of five chicks each and treated as follow; Group I: distilled water 0.2 ml; Group II: apomorphine (0.3 mg/kg s.c.) Group III: extract (20 mg/kg i.p.) then apomorphine (0.3 mg/kg s.c.); Group IV: extract (40 mg/kg i.p.) then apomorphine (0.3 mg/kg s.c.); Group V: extract (60 mg/kg i.p.) then apomorphine (0.3 mg/kg i.p.). Stereotyped behaviour including; pecking at each other, pecking at food, pecking at non food and jumping were observed for 90 minutes after each administration.

### *Statistical Analysis*

The data are expressed as mean ± S.E.M. The data were statistically analyzed using Mann-Whitney U Test to determine significance of differences with respect to the control group. Values of $p$<0.05 were considered as significant.

## RESULTS

### *Effect of extract on barbiturate-induced sleeping time in mice*

The extract (500 mg/kg p.o.) produced no significant $p$>0.05 increase in thiopental sodium–induced sleep. (1000 and 1500 mg/kg p.o) produced a significant $p$<0.05 increase in thiopental sodium-induced sleep in mice compared with the control group (Fig.1).

### *Effect of extract on behavioural activities in 2-day old chicks*

The aqueous extract (10-60 mg/kg i.p) produced a significant $p$<0.05 decrease in gross locomotor activity (GLA) when compared with control group. The decrease in GLA was dose dependent (Table 1). At (10-60 mg/kg) there was a significant $p$<0.05 decrease in

Table 2. Effect of extract on apormorphine-induced stereotyped behaviour in chicks

| Treatment | Dose (mg/kg) | Count per hour (cph) Pecking at each other | Pecking at food | Pecking at non-food | Jumping |
|---|---|---|---|---|---|
| Distilled water | | 0.8 ± 0.37 | 184.0 ±17.56 | 10.2 ± 1.66 | 3.2 ± 0.58 |
| Apomorphine | 0.3 | 117.6± 3.76* | 50.0 ± 3.85* | 202.0± 15.62* | 11.6 ± 1.29 |
| Extract + Apomorphine | 20 0.3 | 106.6 ± 4.23* | 50.2 ± 4.21* | 206.4 ± 6.82* | 12.8 ± 1.91 |
| Extract + Apomorphine | 40 0.3 | 116.6 ± 6.65* | 24.0 ± 1.14* | 182.8 ± 14.83* | 10.8 ± 1.56 |
| Extract + Apomorphine | 60 0.3 | 110.8 ± 6.72* | 23.2 ± 1.85* | 206.8 ± 3.74* | 11.0 ± 2.00 |

Results are expressed as mean ± S.E.M. (n=5) *$p$<0.05 compared with control. Mann-Whitney U test.

movement, pecking, escape episode, and jumping compared with control group. There was abolition of escape episode, jumping and pecking at (60 mg/kg). The extract (10-60 mg/kg i.p.) produced sedation (Fig. 3) and a significant $p$<0.05 decrease in onset and an increase in duration of sleep (Fig. 2).

### *Effect of aqueous extract on apormorphine-induced stereotyped behaviour in chicks*

Apomorphine produced a significant $p$<0.05 increase in pecking at each other, pecking at non-food and jumping compared with control group.

The extract (20-60 mg/kg s.c.) co-administered with apomorphine produced no significant $p$>0.05 decrease in pecking at each other, pecking at non food and jumping. (40-60 mg/kg) of the extract produced a significant $p$<0.05 decrease in pecking at food (Table 2).

## DISCUSSION

The aqueous leaf of *Croton zambesicus* extract was assessed for neuropharmacological activity, and found to prolong the thiopental sodium– induced sleeping time in mice. A depressant effect of an extract on the CNS is indicated by a prolongation of the barbiturate-induced sleeping time [12]. At doses of (1000 and 1500 mg/kg i.p) there was a significant increase in sleeping time compared to the control, though this increase was not

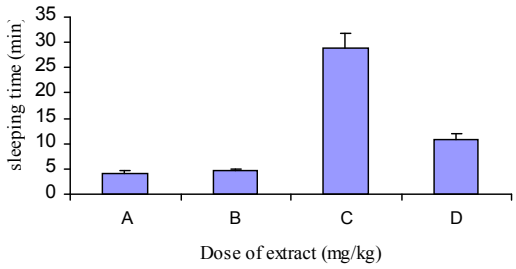


Fig 1. Effect of *Croton zambesicus* Leaf Extract on Thiopental Sodium–Induced Sleep in Mice
(A) Control; (B) Extract (500 mg/kg); (C) Extract (1,000 mg/kg); (D) Extract (1,500 mg/kg)
Results are expressed as mean ± S.E.M. (n=5) *$p$<0.05 compared with control.. Mann-Whitney U test.

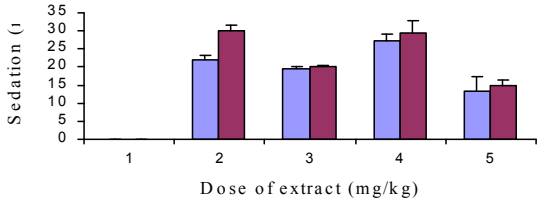


Fig 2. Effect of *Croton zambesicus* Leaf Extract on Sedation in 2-Day Old Chicks
Onset Duration
(1) Control; (2) Extract (10 mg/kg); (3) Extract (20 mg/kg); (4) Extract (40 mg/kg); (5) Extract (60 mg/kg).
Results are expressed as mean ± S.E.M. (n=5) *$P$<0.05 compared with control. Mann-Whitney U test.

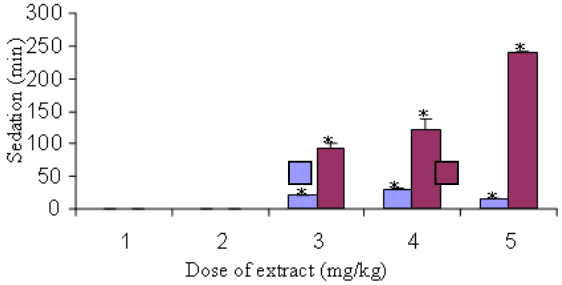


Fig 3. Effect of *Croton zambesicus* Leaf Extract on Sleep
Onset Duration
(1) Control; (2) Extract (10 mg/kg); (3) Extract (20 mg/kg); (4) Extract (40 mg/kg); (5) Extract (60 mg/kg).
Results are expressed as mean ± S.E.M. (n=5) *$P$<0.05 compared with control. Mann-Whitney U test.

dose dependent. This might be attributed to low dose stimulation and high dose inhibition of response, observed in drugs that do not exhibit the classic dose-response relationship [13]. The extract (10-60 mg/kg i.p) produced a significant $p<0.05$ decrease in movement and GLA in a dose dependent manner in 2-day old chicks (Table 1). A change in behavioural output is generally believed to be as a result of alteration in CNS activity [10]. Spooner and Whiters [14] in a study on neuropharmacological profile of the young chicks found out that the CNS of the chick at birth permits behavioural pharmacological investigation since they lacked a functional blood brain barrier. The chicks were sedated and slept, with a decrease in onset and increase in duration of sleep. The sedative effects of the crude extract may be attributed in part to linalool (linalool is a constituent of the essential oil found in the leaves of *Croton zambesicus*[6]). Psychopharmacological evaluation of linalool in mice revealed that this compound has dose-dependent marked sedative effects at the CNS; including protection against PTZ, picrotoxin, quinolic acid and electroshock induced convulsions [15]. The extract had no significant effect on apormorphine-induced stereotyped behaviour in chicks indicating the extract has no antidopaminergic activity.

These results revealed that the leaf extract of *Croton zambesicus* prolonged barbiturate sleeping time in mice, produced sedation, hypnosis and significant inhibition of motor activity in chicks and thus possess CNS depressant and sedative and hypnotic activity. There is plan to isolate and identify the active principle in this plant in due course.

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## REFERENCE

1. Houghton PJ. *In*: Traditional plant medicines as a source of new drugs. Trease and Evans pharmacognosy 15th ed. London Bailliere Tindal; 2002. p. 125-134.
2. Okokon JE, Ofodum KC, Ajibesin KK, Danladi B, Gamaniel KS. Pharmacolgical screening and evaluation of antiplasmodial activity of Croton zambesicus against Plasmodium berghei berghei infection in mice. Indian Journal of Pharmacology, 2005; 37:243-246.
3. Okokon JE, Bassey AL, Obot J. Antidiabetic activity of ethanolic leaf extract of Croton zambesicus muell.(Thunder plant) in Alloxan diabetic rats. African Journal of Traditional, Complementary and Alternative Medicines, 2006; 3 (2):21-26.
4. Adjanohaun EJ, Adjakidje V, de Souza S. Contribution to ethnobotanical and floristic studies in Benin Republic (1989). Vol. 1, Agency for cultural and technical cooperation.
5. Block S, Stevigny C, de Pauw-Gillet MC, de Hoffman E, Llabres G, Ajakidje V. Ent-trachyloban-3β-ol, a New Cytotoxic Diterpene from Croton zambesicus. Planta Medica, 2002; 68:647-8.
6. Abo KA, Ogunleye JS. Antimicrobial potential of Spondias mombin, Croton zambesicus, and Zygotritoma crocea. Phytotherapy Research, 1991; 13:494-497.
7. Menut C, Lamaty G, Bessiere JM, Suleiman AM, Fendero P,Maidou E. Aromatic Plants of Tropical Central Africa. XXII. Volatile Constituents of Croton aubrevillei, J. Leonard and C. zambesicus Muell. Arg J. Essen Oil Researh, 1995; 7: 419-22.
8. Mekkawi AG. The essential oil of Croton zambesicus. fitoterepia, 1985; 56:181-183.
9. Vogel HG, Vogel WH. (eds) Drug Discovery and Evaluation, Pharmacological Assays. Springer-Verlag, Berlin Heidelberg; 1997. p. 267-269.
10. Wannang, N.N., Anuka, J.A., Kwanashie, H.O. and Bichi, L.A. (2004). Effects of Solanum nigrum L. Aqueous Extract on the Behavioural Activities in Chicks. Biological and Environmental Sciences Journal for the Tropics 1:139-142.
11. Fugner A, Hoefks A. A sleep – like state in chicks caused by biogenic amines and other compounds. Quantitative evaluation. Arzeimittes/Forsch, 1977; 21:1243-1252.
12. Williamson EM, Okpako DT, Evans FJ. Pharmacological Methods in Phytot herapy Research Vol. I, selection, preparation and pharmacological evaluation of plant material, 1996; p. 183-187.
13. Calabrese,EJ., and Baldwin, L.A. Hormesis: The dose-response revolution. Annual Review Pharmacology and Toxicology. 2003 43:175-197.
14. Spooner CE, Whiters WO. Neuropharmacological profile of the young chicks. International Journal of Neuropharmacology, 1966; 5:217-236.
15. Elisabetsky E, Silva Brum LF, Souza DO. Anticonvulsant properties of linalool on glutamate related seizure models. Phytomedicine, 1999; 6:113-119.

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