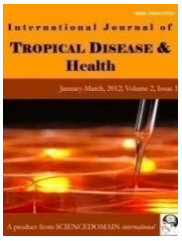

International Journal of TROPICAL DISEASE & Health
2(1): 33-41, 2012

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Anticonvulsant and Depressant Activity of Methanol Leaf Extract of *Croton zambesicus*

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Research Article

Received 24th December 2011
Accepted 22nd January 2012
Online Ready 6th March 2012

ABSTRACT

Aims: To study anticonvulsant and central nervous system depressant activity of methanol leaf extract of *Croton zambesicus* (MECZ) in Swiss albino mice and investigate the role of serotonin in these activities.
Methodology: Anticonvulsant activity of graded doses (200, 300 and 400 mg/kg p.o) of MECZ was assessed through seizures induced by picrotoxin and pentylenetetrazole (PTZ). Effects of the extract on pentobarbitone-induced sleep and amphetamine-induced stereotype behavior were also evaluated. Possible involvement of serotonergic pathways was studied using cyproheptadine (4mg/kg i.p), a non-selective serotonin antagonist (5-HT1/5HT2).
Results: In both picrotoxin and PTZ-induced seizures, the extract significantly delayed onset of seizure ($p<0.05$) in a dose-dependent manner and provided significant protection against death. There was a dose-dependent increase of pentobarbitone sleeping time and a significant reduction ($p<0.05$) in the sleep latency. The extract also produced a significant reduction in amphetamine-induced stereotype behavior. Pretreatment with cyproheptadine abolished the anticonvulsant effect of the extract. The inhibitory effect of the extract on amphetamine-induced hyperactivity and its potentiation of pentobarbitone-induced sleep were also reversed by cyproheptadine.
Conclusion: The results of this study showed that methanol extract of *Croton zambesicus* leaf possesses anticonvulsant activity and other CNS depressant activities and these activities are possibly mediated through interaction between serotonergic and GABAergic transmissions.

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Keywords: Seizure; serotonin; Croton zambesicus; cyproheptadine; neurotransmission.

1. INTRODUCTION

Epilepsy is one of the most common serious neurological disorders that affect about 30,000 people around the world every year (Swaraman and Muralidaran, 2010). This group of brain disorders is characterized by seizures. The seizures occur when recurrent episodes of brain dysfunction, with synchronized discharge of brain cells, lead to alterations in sensory, motor or other activity. Sometimes localized parts of the brain are affected leading to partial seizures with or without loss of consciousness. Generalized seizures can also occur when both sides of the brain are involved in the synchronous discharges. These include tonic-clonic (grand mal) seizures, subtle body jerks or loss of postural tone (Hart and Shorvon, 1995). Epilepsy has many causes but in most cases it is caused by brain damage resulting from infections, trauma, stroke, brain tumor or developmental abnormalities (Sharma *et al*., 2011). The impact of epilepsy on an individual is enormous. It is a combination of physical consequence of the seizures, the effect on the social position and the psychological outcome of these effects. In the majority of cases, epilepsy is treated with antiepileptic drugs (AEDs). These drugs abolish the agitated neuronal activity that occurs during a seizure by interacting with neurotransmitters such as gamma amino butyric (GABA), serotonin, noradrenalin and dopamine. These neurotransmitters are involved in the inhibitory and excitatory signals in the brain (Clinckers*etal*., 2005). However, many patients with seizures are not adequately managed by these established synthetic antiepileptic drugs. This is because there is high incidence of detestable adverse effects from the use of these drugs. The cost of treatment is also high which made it unaffordable to many patients especially those in economically poor nations (Harish *et al*., 2010). A good alternative is herbal remedy which has proved useful and indispensable for seizure management and for future development of new antiepileptic drugs. *Croton zambesicus* (Euphorbiaceae) is reported to possess many therapeutic benefits in traditional system of medicine. It is one of the medicinal plants employed by traditional healers in Nigeria to treat convulsions (Okokon and Nwafor, 2009). Different parts of the plant are used as herbal therapy around the continent of Africa. It is also used to treat diabetes and malaria in Nigeria (Okokon et al., 2006). In Sudan the root is used for menstrual pain (El-Hamidi, 1970). The root is also used to treat hypertension and urinary infections in Benin (Adjanohoun et al*.,* 1989). In this study, the anticonvulsant activity and CNS depressant effects of *Croton zambesicus* leaf extract was investigated to provide a basis for future development of new antiepileptic drugs.

2. MATERIAL AND METHODS

2.1 Collection and Extraction of Plant Materials

Croton zambesicus leaves were collected in April, 2011 from the premises of Polytechnic Ibadan, South-West Nigeria. The sample was identified and authenticated by a taxonomist in the Department of Botany, University of Ibadan, Nigeria and voucher specimen was deposited in the herbarium of the Department. The plant sample was thoroughly washed with clean water and air-dried in the laboratory. The dry plant was pulverized using an electric mill. The resultant powder sample (300g) was then extracted in a Soxhlet with methanol (MEOH) for 72 h at a temperature not exceeding the boiling point of the solvent.

The extract was filtered using Whatman filter paper (No.1) and then concentrated at 40℃ using a rotary evaporator. The extract (MECZ) was stored in a refrigerator until it was used.

2.2 Experimental Animals

Healthy Swiss albino mice of either sex weighing 20-25g were obtained from the animal house of the College of Health Sciences, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. They were housed in standard cages under normal laboratory conditions of temperature (25 ± 5℃) and a 12/12 h light/dark cycle. The animals were fed balanced animal feed and water *ad libitum*. The study was approved by the Animal Ethic Committee of LAUTECH and the 'Principle of Laboratory Animal Care' (NIH Publication No. 85-23, 1985) was followed.

2.3 Drugs and Chemicals

Some of the drugs and chemicals used for the study are pentobarbitone sodium (Sigma, USA), amphetamine (Sigma, USA), diazepam (Calmpose$^{(R)}$, Ranbaxy, India), methanol (BDH Chemicals Ltd., Poole, England),cyproheptadine (Sigma, USA), picrotoxin (Sigma, USA), pentylenetetrazole (Sigma USA).

2.4 Experimental Procedure

Anticonvulsant and CNS depressant effects of the extract were investigated using the following models: Picrotoxin and pentylenetetrazole (PTZ) –induced convulsions (Vellucci and Webster, 1984), potentiation of pentobarbitone-induced sleep (Leite et al., 1982) and amphetamine-induced stereotype behavior (Bourin et al., 1986).

2.4.1 Anticonvulsant activity

Mice were divided into seven groups of 10 animals. They were treated as follows:

Group I (Control): 2% Tween 80 (5mg/kg p.o)
Group II: MECZ (200mg/kg p.o)
Group III: MECZ (300mg/kg p.o)
Group IV: MECZ (400mg/kg p.o)
Group V: MECZ (400mg/kg p.o) + Cyproheptadine (4mg/kg i.p)
Group VI: Diazepam (1mg/kg i.p)
Group VII: Diazepam (1mg/kg i.p) + Cyproheptadine (4mg/kg i.p)

Thirty minutes after these pretreatments, the convulsant drug, picrotoxin (5mg/kg i.p) or PTZ (75mg/kg i.p) was administered. The animals were observed for 30 min for signs of tonic and clonic seizures. Time for onset of seizure and the number of death in each group were recorded.

2.4.2 Pentobarbitone-induced sleep

Mice were divided into seven groups of 6 animals each. Group I (control) was given 2% Tween 80 (5ml). Group II, III, and IV were pretreated with MECZ at graded doses of 200, 300 and 400mg/kg (p.o) respectively, while group V was given a combination of MECZ (400mg/kg p.o) and cyproheptadine (4mg/kg i.p). Group VI and VII received diazepam

(1mg/kg i.p) and combination of diazepam and cyproheptadine respectively. Thirty minutes later, animals in all the groups received pentobarbitone (30mg/kg i.p). Time interval between loss and regain of righting reflex was taken as index of hypnosis. Onset of sleep was considered to be the time when mice accepted the decubitodorsal position for three consecutive times and the duration of sleep was considered completed when mice did not accept the decubitodorsal position for three consecutive trials.

2.4.3 Amphetamine-induced stereotype behavior

Mice were divided into seven groups (n=6). Group I (control) was given 2% Tween 80 (5ml). Group II, III, and IV received MECZ at graded doses of 200, 300 and 400mg/kg (p.o) respectively. Group V was given combination of MECZ (400mg/kg p.o) and cyproheptadine (4mg/kg i.p). Group VI and VII were pretreated with chlorpromazine (2mg/kg i.p) and a combination of chlorpromazine (2mg/kg i.p) and cyproheptadine(4mg/kg i.p) respectively. Amphetamine (35mg/kg i.p) was administered to animals in all the groups 30 min after the pretreatments. Signs of stereotype behavior, mainly sniffing and gnawing were observed and rated as follows: absence of stereotype behavior = 0; occasional sniffing = 1; occasional sniffing with occasional gnawing = 2; frequent gnawing = 3; intense and continuous gnawing = 4; intense gnawing and jumping = 5. Stereotype behavior was measured 1 min after the administration of amphetamine. The scoring was done within 5 min for each animal.

2.4.4 Phytochemical screening

Investigation of the extract for the presence of phenolic compounds, flavonoids, alkaloids, terpenes, saponin, anthracyanin, tannin, anthraquinone and sterols was carried out using the methods previously described (Trease and Evans, 2002).

2.4.5 Statistical analysis

Experimental data were expressed as mean ± SEM and analyzed by one way analysis of variance (ANOVA) and Least Significant Difference (LSD) multiple range tests to determine significant differences between means. P-values <0.05 were taken to be significant.

3. RESULTS AND DISCUSSION

3.1 Anticonvulsant Activity

The extract significantly delayed onset of seizure ($p<0.05$) in a dose-dependent manner in both picrotoxin and PTZ-induced convulsions. The results are shown in table 1 and 2. For the picrotoxin-induced seizure, the onset of clonic and tonic convulsions in group 1 (control) were 21.4 ± 2.0 and 34.6 ± 1.4 sec respectively. In group IV, 400mg/kg of the extract prolonged the onset of seizures to 70.2 ± 8.1 and 96.7 ± 6.8 sec for clonic and tonic seizures respectively. All the animals in group 1 died while in group IV mortality was 80%. This is comparable to values obtained in group VI (diazepam) where 70% mortality was recorded. For PTZ-induced seizure, the onset of clonic and tonic convulsions in group1 was 26.8 ± 2.3 and 48.4 ± 3.8 respectively. In group IV, onset of seizure was significantly prolonged.

3.2 Pentobarbitone-Induced Sleep

The extract produced a dose-dependent increase in pentobarbitone sleep time and significant reduction ($p<0.05$) in the onset of sleep. As shown in table 3, the onset and duration of sleep in group 1 were 7.9 ± 1.8 and 51.4 ± 4.3 min respectively. In group IV, onset of sleep was reduced to 3.2 ± 0.9 min and duration of sleep was prolonged to 106.6 ±12.8 min. The potentiating effect of MECZ and diazepam on pentobarbitone sleep time was inhibited by cyproheptadine. In group V, onset and duration of sleep were 9.3 ± 2.1 and 55.4 ± 5.6 min respectively, while in group VII, the values for onset and duration of sleep were 8.6 ± 2.7 and 61.8 ± 5.4 min respectively. These values are comparable to those of group 1.

3.3 Amphetamine-Induced Stereotype Behavior

The extract significantly reduced stereotype behavior ($p<0.05$) in a dose-dependent manner as shown in table 4. The score in group 1 was 34.4 ± 2.7. With 400mg/kg of MECZ, the score was reduced to 11.6 ± 1.3. This is comparable to the effect of chlorpromazine (group V) which reduced the score significantly to 9.4 ± 1.7. Cyproheptadine abolished the inhibitory effect of MECZ and chlorpromazine. In group V, the score was raised to 31.9 ± 3.6 while in group VII, it was 29.2 ± 3.0. These are comparable to the score in group 1.

3.4 Phytochemical Screening

The result of the screening revealed that the extract contains flavonoids, alkaloids, terpenes, anthraquinone and sterols.

The protective effect of *Croton zambesicus* leaf extract (MECZ) against induced convulsion suggests that it has modulatory effects on neurotransmission in the CNS. Since gamma amino butyric acid (GABA) is the major inhibitory neurotransmitter in the CNS (Stanley, 1995), it is probable that MECZ promotes the activation of GABA receptors thereby potentiating GABAergic inhibition.

Table 1. Effect of MECZ on picrotoxin-induced convulsion

Group (n=10)	Onset of seizure (sec)		No of death
	Tonic	Clonic	
I	34.6 ± 1.4	21.4 ± 2.0	10
II	49.3 ± 4.0	36.2 ± 1.6	10
III	64.2 ±6.6*	45.6 ± 2.8*	9
IV	96.7 ± 6.8*	70.2 ± 8.1*	8
V	39.4 ± 3.5	27.9 ± 2.6	10
VI	118.5 ± 9.2*	89.4 ± 7.6*	7
VII	42.6 ± 5.7	24.0 ± 2.4	10

*Each value for onset of seizure represents mean ± SEM; *p<0.05 compared with the control*

Table 2. Effect of MECZ on PTZ-induced convulsion

Group (n=10)	Onset of seizure (sec)		No. of death
	Tonic	Clonic	
I	48.4 ± 3.8	26.8 ± 2.3	10
II	53.8 ± 3.2	38.1 ± 2.7	10
III	68.6 ± 5.3*	46.2 ± 3.5*	8
IV	84.9 ± 5.0*	70.5 ± 4.2*	8
V	44.0 ± 3.1	31.4 ± 2.6	10
VI	122.6 ± 6.6*	98.3 ± 8.2*	8
VII	49.1 ± 3.7	35.2 ± 3.5	10

*Each value for onset of seizure represents mean ± SEM; *p<0.05 compared with the control*

Table 3. Effect of MECZ on pentobarbitone-induced sleep

Group (n=6)	Onset of sleep (min)	Duration of sleep (min)
I	7.9 ± 1.8	51.4 ± 4.3
II	5.2 ± 1.2	73.6 ± 6.4
III	3.9 ± 1.5*	88.4 ± 9.2*
IV	3.2 ± 0.9*	106.6 ± 12.8*
V	9.3 ± 2.1	55.4 ± 5.6
VI	2.4 ± 0.5*	138.3 ± 10.3*
VII	8.6 ± 2.7	61.8 ± 5.4

*Each value represents mean ± SEM; *p<0.05 when compared with the control*

Table 4. Effect of MECZ on amphetamine-induced stereotype behavior

Group (n=6)	Score
I	34.4 ± 2.7
II	26.2 ± 1.8
III	23.4 ± 3.2
IV	11.6 ± 1.3*
V	31.9 ± 3.6
VI	9.4 ± 1.7*
VII	29.2 ± 3.0

*Values are expressed as mean ± SEM; *p<0.05 compared with control*

Picrotoxin and pentylenetetrazole produce convulsion by blocking chloride- ion channel (Desarro et al., 1999) thus preventing chloride ion conductance through the GABA-gated channels. Therefore it appears that MECZ antagonized this blockade by enhancing affinity for GABA or increasing the duration of the GABA-gated channel opening. However, cyproheptadine, a nonselective antagonist of serotonin abolished the anticonvulsant effect of MECZ. Therefore it is possible that the extract exerts its anticonvulsant effect through 5-HT receptor. If this is the case, blockade of 5-HT receptor would inhibit the anticonvulsant activities of the extract. Since enhanced GABAergic transmission is central to preventing picrotoxin and PTZ-induced seizures, it is likely that the anticonvulsant activities of the

extract involve interactions between serotonergic and GABAergic transmissions. It has been reported that the extracellular GABA level in the prefrontal cortexis regulated by 5-HT2 receptor. For instance, administration of 5-HT2 agonist, 1-(2, 5-dimethoxy-4-iodophenyl-2-amino propane (DOI) significantly increased extracellular GABA levels in a dose- dependent manner (Abi-Saab et al., 1999). Conversely, a potent 5-HT receptor antagonist, clozapine, sharply decreased extracellular GABA level in the brain (Bourdelais and Deutch, 1994). In the present study, MECZ appears to promote GABA synthesis and/or release through 5-HT receptor activation. Antagonism of this process by cyproheptadine would lead to significant decrease in GABA level resulting in the suppression of anticonvulsant effect of the extract. It is suspected that this interaction between serotonergic and GABAergic transmission is also responsible for the anticonvulsant effect of diazepam which was likewise antagonized by the administration of cyproheptadine. Benzodiazepines and barbiturates facilitate GABAergic inhibition at multiple sites in the central nervous system (Lancel, 1999). Therefore potentiation of pentobarbitone –induced sleep by diazepam and the extract indicates their interaction with $GABA_A$receptor. Reversal of these effects by cyproheptadine again suggests a possible modulatory role of serotonin. However, MECZ, like benzodiazepines and barbiturates, may also act by binding to $GABA_A$ receptors and exerts an allosteric effect on GABA-controlled opening of the chloride channels resulting in hyperpolarization. This can happen if GABA is present in sufficiently high concentration. So it is reasonable that cyproheptadine could inhibit anticonvulsant activity of MECZ and diazepam by decreasing GABA level.Amphetamine induces hyperactivity and stereotype behavior by binding to dopamine (D2) receptors (Moore and Axton, 1988). Inhibition of amphetamine-induced stereotype behavior by MECZ suggests it competitively blocked the interaction of amphetamine with D2 receptors. This effect was comparable to that produced by chlorpromazine. But again this neuroprotective benefit was lost when cyproheptadine was administered, an indication of possible involvement of serotonergic pathway. There is strong evidence indicating that the 5-HT system modulates dopaminergic activity and vice versa. This evidence is strong for the model in which 5-HT activity has an overall inhibitory effect on dopaminergic function (Meltza and Fatemi, 1996). Decreasing 5-HT function by variety of means, including lesions of the median raphe and a tryptophan-free diet, enhanced amphetamine-induced locomotor activity (Abi-Dargham et al., 1997). 5-HT2 antagonists also increase dopamine release in the prefrontal cortex by a direct action at $5\text{-}HT2_A$ receptors on dopamine nerve terminals (Pilowsky et al., 1994). Therefore, concurrent administration of cyproheptadine and the extract or chlorpromazine abolished the inhibitory effect observed when only chlorpromazine or MECZ was administered. Phytochemical screening of the plant extract revealed the presence of flavonoids. These may be responsible for the anticonvulsant and CNS depressant activities of MECZ. Flavonoids have been described as a family of benzodiazepine receptor ligands with CNS depressant activities (Medina et al., 1997; Carlini, 2003). The presence of linalool, an essential oil, in the leaves of *Croton zambesicus* (Mekkawi, 1985) may also account for its neuroprotective activities. This compound was reported to possess sedative and anticonvulsant effects (Elisabetsky et al., 1999).

4. CONCLUSION

The results of this study have shown that *Croton zambesicus* leaf extract possesses anticonvulsant and other CNS depressant activities and these activities are likely mediated by interactions between serotonergic and GABAergic transmissions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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