

## Anticonvulsant and depressant activities of the seed extracts of *Adnanthera parvonina*

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### ABSTRACT

In the present study, we have examined the central nervous system activities of the methanol extract of the seed of *Adnanthera parvonina* (MESAP). The central nervous system activities were evaluated in the picrotoxin (PCT)-, pentylenetetrazole (PTZ)-, strychnine (STC)-induced convulsions and phenobarbitone (PBT)-induced sleep in mice. Diazepam and chlorpromazine were used as reference anticonvulsant and sedative drugs for comparison respectively. Like the reference anticonvulsant and sedative agents used, MESAP (50-200 mg/kg) protected mice against PCT- and PTZ-induced seizures and prolonged phenobarbitone-induced sleeping time dose-dependently. It would appear that seed extract of *A. parvonina* produces its anticonvulsant activity by enhancing GABAergic neurotransmission or facilitating GABAergic action and/or prevention of cell membrane destabilization in the brain. In general, the average onset of convulsion was delayed, while the average duration of convulsion was markedly reduced. The seed's extract also depressed the central nervous system (CNS) activity by prolonging the phenobarbitone (PBT)-induced sleeping time in mice. It is, therefore, thought that the anticonvulsant property of the herb may be linked at least in part, to its ability to depress the CNS activity. However, the results of this experimental animal study revealed the anticonvulsant and depressant activities of *A. parvonina*.

**Keywords:** *Adnanthera parvonina* Crude extract, Central nervous system

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### INTRODUCTION

*Febrile convulsion among infants and young children is a common phenomenon, especially in the rural areas in Nigeria, which often result to death.*

*Phytomedicines are used by the traditional healers, in the management, control and/or treatment of infantile and childhood convulsions.*

*Adenanthera parvonina* Linn. (Family: Leguminsae-Mimosoideae), is a deciduous tree, 18-24 m tall, bole erect and 60 cm in diameter (Bouquet and Debray, 1974; Burkill, 1966). It is used in perfumery and also as an ornamental tree (Howes, 1974). Traditionally, the ground seed's extract is widely used in Nigeria for the treatment, management and/or control of various human ailments such as t of boils, inflammation, blood disorders, arthritis, rheumatism, cholera, paralysis, epilepsy, convulsion, spasm and indigestion (Burkill, 1985; Balogun and Fetuga, 1985) Phytochemically, the seed and its pod contain glycosides, saponins and steroids (Howes, 1974; Gennaro, et al., 1972; Yadav, et al., 1976; Moreira, et al., 1998). A new five-membered lactone ring compound, parvonin was isolated from the methanol soluble part of *A. parvonina* (Muhammad, et al., 2005). Oil extracted from the seed has been reported to have membrane-stabilizing activity by reducing lytic effect on erythrocytes, exhibited by many intravenous drugs (Anna, et al., 2006). The methanol seed extract has also been reported to demonstrate anti-inflammatory and analgesic activities (Olajide, et al., 2003). In this study, we report the anticonvulsant and depressant activities of the seed extract of *A. parvonina*.

## MATERIALS AND METHODS

**Plant material:** The seeds of *A. parvonina* were collected from the trees located at the staff school of the University of Ibadan, Ibadan, Nigeria in the month of November, 2007, and were authenticated by Mr. E.A Ogunduyile; a taxonomist in the Botany Department of the University of Ibadan, Nigeria. Where voucher specimen was deposited. The seeds were sun-dried and reduced to powdery form using an electric blender.

**Preparation of Adenanthera parvonina seed extract:** 400 g of powdered sample of the seeds was extracted with 500 ml of 100 % methanol using a soxhlet extractor. The resulting crude methanol extract was then concentrated under reduced pressure at 40 °C in a rotary evaporator (Rota vapor) to obtain a solid sample giving 7.2 % yield. This was stored in the refrigerator at 4 °C and was used throughout the experiment.

**Animals:** Male mice used for this study weighing between 20-23g, strain Swiss Albino were housed in a well ventilated pre- clinical animal house, College of Medicine, University of Ibadan. The animals were acclimatized in the laboratory for two weeks before experimentation, and were fed with standard diet (Ladokun Feeds Nigeria Ltd) and water *ad libitum*. The animals were divided into seed's extract- and reference anticonvulsant drug-treated 'test', and 2 % tween 80-treated 'control' groups of 6 animals per group.

The experimental protocols and procedures used in this study were approved by the Ethical committee, University of Ibadan, Ibadan, Nigeria and conform to the guideline of the care and use of animals in research and teaching (NIH publications no 85-93, revised 1985).

**Evaluation of anticonvulsant activity:** The anticonvulsant testing method of Velluci and Webster 1984, modified by Amabeoku, et al. 1998, was used to evaluate the anticonvulsant activity of the seed's extract ion mice. Standard convulsant agents, pentylenetetrazole (PTZ, 3mg/kg i.p), picrotoxin (PCT, 10mg/kg i.p) and strychnine (STC, 3mg/kg i.p) were used to induce convulsions in mice. Diazepam (DZP, 5mg/kg

i.p) was used as a reference drug for comparison. Following intraperitoneal (i.p) injection of standard convulsant agents, the animals were observed for 30 min for signs of neurological deficits, especially hind-limb tonic seizures or convulsions. Hind-limb tonic extensions in mice were regarded as manifestation of seizures or convulsions. The ability of the seed's extract to prevent the seizures or delay/prolong the latency of or onset of the hind-limb extensions was considered as an indication of anticonvulsant activity (Amabeoku, et al., 1998; and Navarro-Ruiz, et al., 1995). In the absence and in the presence of each seed's extract dose and reference drug used, the onset and duration of convulsions in mice were noted and recorded, and the percentage protection by each of the seed's extract doses and the reference anticonvulsant drug were determined. Because the seed's extract and the reference drugs used in these study were dissolved in 2% tween 80, each day at the beginning of the experiments, 2% tween80(10ml/kg)-treated mice were used as 'control' animals.

**Phenobarbitone-induced sleep:** The method used is as described by Leite, et al., 1982. Thirty mice, divided into five groups of six animals per group Group1 mice received 2%tween80 (10 ml/kg) each, serving as 'control' group. Group 2, 3 and 4 received seed's extract, at graded doses of 50, 100 and 200 mg/ kg (i.p) respectively, while group 5 received chlorpromazine(CPZ, 10mg/kg i.p), a standard sedative drug. Thirty minutes later, each animal in all groups received phenobarbitone at the dose of 47 mg/ kg (i.p).The duration of loss and gain of righting reflex was taken as a measure of sleeping time.

**Data analysis:** Data are presented as Mean±S.E.M. Data from 2% tween80 (10 ml/kg)-treated 'control' mice were used as base values. The differences between the data obtained with the seed's extract- and reference anticonvulsant drug-treated 'test' mice and the data obtained with 2%tween80-treated 'control' animals were subjected one-way analysis of variance(ANOVA). Values of  $P<0.05$  were taken to imply statistical significance.

## RESULTS

MSEAP {50-200 mg/ kg i.p}, protected mice significantly ( $P<0.05$ ) and dose-dependently against picrotoxin(PCT, 10 mg/kg)- and pentylenetetrazole (PTZ, 3 mg/kg)-induced seizures (Tables-1A; B), while there was no protection against strychnine-induced convulsion (Table-1C).Furthermore, the seed's extract {MSEAP, 50-200 mg/ kg i.p} significantly ( $P<0.05$ ) delayed the onset of PCT- and PTZ-induced seizures. The reference anticonvulsant drug (DZP, 5 mg/kg i.p) profoundly delayed the onset of, and significantly antagonized ( $P<0.001$ ), PCT- and PTZ-induced seizures (Tables-1A; B). The extract {MSEAP, 50-200 mg/ kg i.p}, also significantly ( $P<0.05$ ) and dose-dependently prolonged phenobarbitone (PBT, 47 mg/kg i.p))-induced sleeping time (Table-2).

## DISCUSSION

There are a number of synthetic anticonvulsant drugs available in the market for use in the management, control and/or treatment of individuals with epilepsy. However, most of these synthetic drugs are not only inaccessible and unaffordable, but they possess many toxic effects. It is therefore necessary to look inward for the development of cheap, effective and safe anticonvulsant agents from plants and other natural resources.

*Adenanthera parvonina* seed's extract is widely used in Nigeria for the treatment of various human ailments, relatively little scientific information exists in biomedical

literature on the therapeutic efficacy of the plant product. The result of the present laboratory animal study provide evidence in favor of the anticonvulsant activity of the herb and show that the seed's extract possesses anticonvulsant activity in the experimental animal model used. The effectiveness of the seed's extract in the experimental convulsion paradigm used probably suggests that the herb could be used in both the *petit* and *grand mal* types of epilepsy. The seed's extract appears to be relatively more effective in PCT- and PTZ-induced convulsions. This observation probably suggests that the seed's extract could be useful in the management, control and/or treatment of *grand mal* convulsions. Picrotoxin (PCT), strychnine (STC) and pentylenetetrazole (PTZ) are convulsants drugs used to induce convulsions, while ability of an agent to inhibit convulsion in comparison with the untreated mice is taken as a measure of an *in-vivo* protection level of the agent. Picrotoxin (PCT) and strychnine (STC) produce their convulsions by blocking gamma-aminobutyric acid (GABA) and glycine receptors respectively, while pentyltetrazole (PTZ) destabilizes nervous cell membrane to produce convulsion (Curti, et al., 1971; Ryall, 1975; Gnyther, 1986) Pentylenetetrazole (PTZ) has also been reported to produce seizures by inhibiting GABA neurotransmission (De Sarro, et al., 1999; Okada, et al., 1989) GABA is the predominant inhibitory neurotransmitter in the mammalian CNS, and is widely implicated in epilepsy, mediating inhibition of neuronal responsiveness (excitability) and activity by increasing the chloride ion conductance through opening of the chloride-ion channel (Meldrum, 1975; Olsen, 1981; Gale, 1992; Leonard, 2000). It follows therefore that picrotoxin (PCT) and pentylenetetrazole (PTZ), antagonists of GABA receptors close up the channel preventing chloride-ion conductance to induce seizures. It would appear, therefore, that complete protection of the mice by the reference anticonvulsant (DZP) drug used against PCT- and PTZ-induced seizures is in consonance with the above hypothesis, since the standard anticonvulsant drug used have been shown to exert its action by enhancing GABAergic neurotransmission and activity (Gale,1992; Olsen, 1981; Rang and Dale, 2000) The findings of the present study, therefore, tend to suggest that *Adenantha parvonina* seed's extract might have inhibited and/or attenuated PTZ-induced seizures by enhancing, or in some ways interfering with GABAergic neurotransmission. Protection of mice against PTZ-induced seizures by the seed's extract might also be due to the stabilization activity of the nerve cell membrane in the brain, since PTZ has been reported to produce its convulsion by destabilizing nerve cell membrane (Curtis, et al., 1971; Ryall, 1975; Gnyther, 1986). This action of nerve cell membrane stabilization by the seed's extract may also results in inhibition of the glutamatergic excitotoxic membrane breakdown, an effect which may be beneficial to brain hypoxia and/or neuronal hyperactivity (Weichel, et al., 1999). It appears from this experimental animal study that, the anti-convulsant property of the seed's extract is probably mediated through GABAergic pathway and/or membrane stabilizing action. The observed anticonvulsant activity of the seed's extract may also be due, at least in part, to its ability to depress the central nervous system (CNS) by one or more of the known anticonvulsant action (MacDonald and Kelly,1994), which may include Na<sup>+</sup>-K<sup>+</sup>ATPase expression (Kang, et al., 2004), pyridoxine5' -phosphate (PMP) (An, et al., 2004) and inhibition of expression of inducible nitric oxide (iNO) (Wellard and Morgan, 2004). Regarding the results on phenobarbital-induced sleep in mice, there is possibility of pharmacokinetic interaction between phenobarbitone and the extract which might has effect on effective metabolism of phenobarbitone (Gilman and Goodman, 1995). This might probably lead to the prolongation of sleeping time. Barbiturates, one of which is phenobarbitone produce many of their actions by interacting with GABA receptors through clear interaction

with distinct allosteric site (MacDonald, et al., 1989; Twyman and MacDonald, 1992), 34. It would therefore, appear that the seed's extract may interact with barbiturate allosteric site on GABA receptors, potentiating the action of phenobarbitone, the effect of which might cause prolonged sleeping time observed in this study .

## CONCLUSION

In conclusion, the findings of the present laboratory study lend pharmacological credence to the suggested uses of *A. parvonina* seed extract as natural remedy for management and/or control of childhood convulsions and epilepsy.

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**Table-1A: Effects of methanol seed extract of *A. parvonina* on picrotoxin-induced convulsion in mice.**

Treatment	Dose (mg/kg or ml/kg i.p)	Mean onset of seizure (min)	%Protection
Control (2 %Tween 80)	10	1.3±0.21	0
<i>A. Parvonina</i>	50	7.6±1.0*	60
<i>A. parvonina</i>	100	14.0±2.0*	80
<i>A. parvonina</i>	200	20.5±2.0 *	90
Diazepam	5	0±00**	100

**Table-1B: Effects of methanol seed extract of *A. parvonina* on pentylenetetrazole-induced convulsion in mice.**

Treatment	Dose (mg/kg or ml/kg i.p)	Mean onset of seizure (min)	%Protection
Control (2 %Tween 80)	10	1.6±0.024	0
<i>A. Parvonina</i>	50	7.0±0.23*	40*
<i>A. parvonina</i>	100	15.2±0.16**	80**
<i>A. parvonina</i>	200	0±00 *	100***
Diazepam	5	0±00**	100***

**Table-1C: Effects of methanol seed extract of *A. parvonina* on strychnine-induced convulsion in mice.**

Treatment	Dose (mg/kg or ml/kg i.p)	Mean onset of seizure (min)	%Protection
Control (2 %Tween 80)	10	1.1±0.024	0
<i>A. Parvonina</i>	50	1.6±0.23	0
<i>A. parvonina</i>	100	1.2±0.16	0
<i>A. parvonina</i>	200	1.81±0.10	0
Diazepam	5	0±00*	100

- Each value is the mean ± SEM of six mice.
- \*P<0.05 vs. control; \*\*P< 0.01 vs. control; \*\*\*P< 0.001.

**Table- 2: Effects of methanol seed extract of *A. parvonina* on Phenobarbitone (PBT)-induced sleep in mice.**

Treatment	Dose (mg/kg or ml/kg i.p)	Mean sleeping time (min)
Control (2 %Tween 80)	10	33.3±4.3
<i>A. Parvonina</i>	50	76.6±4.3*
<i>A. parvonina</i>	100	102.4±5.1**
<i>A. parvonina</i>	200	117.2±3.7**
Chlorpromazine	10	152.6±8.9***

- Each value is the mean ± SEM of six mice.
- \*P<0.05 vs. control; \*\*P< 0.01 vs. control; \*\*\*P< 0.001.

