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Anticonvulsant, Analgesic and Hypothermic Effects of Aridanin Isolated from *Tetrapleura tetraptera* Fruit in Mice

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Abstract: Aridanin (an N-acetylglycoside of oleanolic acid) isolated from *Tetrapleura tetraptera* fruit was investigated for anticonvulsant, analgesic and hypothermic activities in mice. Aridanin at doses of 15 and 30 mg kg⁻¹ by intraperitoneal administration was shown to protect animals in pentylenetetrazole (PTZ)-induced seizure but not in strychnine and picrotoxin induced convulsions. The same dose of aridanin equally decreased rectal temperature and acetic acid-induced writhes in mice. The hypothermic action of aridanin was reversed by pretreatment with cyproheptadine (0.1 mg kg⁻¹), atropine (2 mg kg⁻¹), naltrexone (0.25 mg kg⁻¹), but not with haloperidol (0.1 mg kg⁻¹). The effect on acetic acid-induced writhes was completely blocked by naltrexone, but not by atropine, cyproheptadine and haloperidol. The results suggest that aridanin could be acting as a Central Nervous System (CNS) depressant and that its anticonvulsant property is mediated through the membrane stabilizing property and not through GABA and glycine neurotransmitters, respectively. Analgesic and hypothermic actions were mediated through opioids and cholinergic, 5-HT receptors, respectively.

Key words *Tetrapleura tetraptera* fruit, aridanin, anticonvulsant, analgesic, hypothermic activities

INTRODUCTION

The plant *Tetrapleura tetraptera* Taub Mimosaceae (Leguminosae) popularly known as Aridan in Yoruba is a semi-wild tree with several ethnomedicinal indications. Various parts of the plant have been claimed to be therapeutically useful in the treatment of convulsion, leprosy, inflammation, fever and rheumatoid pains (Dalziel, 1955; Thomas, 1989).

Many chemical compounds such as coumarin, saponin, oleanolic acid and essential oil (Ojewole, 1984; Adesina and Reisch, 1985; Maillard *et al.*, 1989) have been isolated and identified from the fruit. The local use of the plant in South-Western Nigeria for convulsion by the Yorubas made us to examine the anticonvulsant, analgesic and hypothermic properties of aridanin with a view of confirming its folkloric uses.

MATERIALS AND METHODS

Plant materials: Freshly-fallen dark reddish brown fruits of *T. tetraptera* were collected in September, 2000 at the Obafemi Awolowo University campus, Ile-Ife. This tree has been previously identified at the Forestry Research Institute of Nigeria, Ibadan to be *Tetrapleura tetraptera* Taub (Ojewole, 1984).

Preparation, isolation and identification of aridanin:

Isolation and identification of Aridanin from the fruits were carried out as described by Adesina and Reisch (1985) and Millard *et al.* (1989). Freshly prepared solution of aridanin, (2% v/v) in Tween 80 (Polysorbate 80) was used for this study. All preparations were administered through intraperitoneal (i.p.) route.

Animals: Swiss albino mice of either sex weighing between 20-26 g were used. The animals were maintained at 25±1°C under natural day light/night condition for at least 5 days before experimentation during which food and water were given *ad libitum*.

Drugs: The following drugs were used for the experiments: Polysorbate 80 (Tween 80) (Sigma), Picrotoxin (Sigma), Strychnine (BDH), Pentylenetetrazol (Sigma), Diazepam (Roche), Glacial acetic-acid (May and Baker), Morphine hydrochloride (Sigma), Methanol (Sigma), Atropine (Sigma), Cyproheptadine (Sigma), Naltrexone (Research Biochemical Inc. MA. USA) and Haloperidol (BDH).

Test for motor coordination

Rotarod test The ability of aridanin to interfere with motor coordination was tested on a Rotarod Threadinill model 700A (Ugo Basile, Italy) using the method of Dunham and

Miya (1957). Mice used for this experiment were trained twice daily for 3 days by placing them on the rotating rod for a total of 30 min. Mice which remained for more than 2 min on a rotating rod with a speed of 16 rev min⁻¹ were selected and divided into three groups. Groups 1 and 2 were given 15 and 30 mg kg⁻¹ of aridanin. while the control group received 2% Tween 80. Each animal from each group was placed on the rotarod mill at intervals of 30, 60, 90, 120 and 180 min after drug administration and observations recorded.

Traction test: The ability of a mouse hanging with its fore paws on a small twisted wire rigidly supported and placing at least one hind foot on the wire within 5 sec was determined (Rudzik *et al.*, 1973). Mice were divided into three groups. Treatment was done as in the rotarod test above. After 30, 60, 90, 120 and 180 min of drug administration, each animal was suspended by means of their fore paws, the number of animals in each groups that could not touch the wire with their hind-paws within 5 sec after placement were recorded.

Anti-convulsant activity

Effect on pentylenetetrazol induced convulsion: Pentylenetetrazol (PTZ) at 85 mg kg⁻¹ i.p. was used to induce clonic-tonic convulsion in mice according to Swinyard *et al.* (1989). Three groups of mice were used (n = 6). Groups 1 and 2 were pre-treated with two doses of aridanin (15 and 30 mg kg⁻¹ i.p.) for 30, 60 and 120 min, while the 3rd group was given 2% Tween 80 (20 mL kg⁻¹) for 30 min. After these treatments, they were followed by PTZ administration. The mice were then observed for latency tonic convulsion and monitored for mortality with 24 h.

Effect on strychnine- induced convulsion: The method described by Elisha *et al.* (1988) was used in this case. Three groups of mice were used (n = 6). The first two groups were given aridanin (15 and 30 mg kg⁻¹ i.p.) and to the third group, 2% Tween 80 (20 mL kg⁻¹) given to the control. This was followed by the administration of strychnine at 2 mg kg⁻¹ i.p. The animals were then observed for latency tonic convulsion and were also monitored for 24 h.

Effect on picrotoxin induced convulsion: For the picrotoxin-induced convulsion, Stone and Javid (1979) method was employed. Aridanin and 2% Tween 80 were administered into 3 groups of mice in the same trend as described in strychnine-induced convulsion above.

However, instead of strychnine, picrotoxin 7.5 mg kg⁻¹ i.p. was administered to induced convulsion. After this, the animals were observed for 30 min to determine the onset, duration of convulsion and death.

Effect on body temperature: The recording of the body temperature was carried out using a thermoprobe inserted 1.5 cm into the rectum of each mouse. After the administration of aridanin (15 and 30 mg kg⁻¹ i.p.) into groups of mice, the temperature of the animals was recorded at 0, 30, 60, 90, 120, 180, 240 and 300 min of drug administration. Pre-drug recording serves as the reference point for the determination of temperature changes. The effect of various antagonists such as cyprohetadine (0.1 mg kg⁻¹), atropine (2 mg kg⁻¹), naltrexone (0.25 mg kg⁻¹) and haloperidol (0.1 mg kg⁻¹) on aridanin -induced body hypothermia was also studied on another 4 different groups of mice. The antagonists were administered 1 h before aridanin (30 mg kg⁻¹ i.p.).

Analgesic activity

Hot-plate: Nociception was evaluated by using the hot plate method previously described by Campos *et al.* (2002). The hot plate (Gallenkamp Technico, Compenstat Cat. No. SWT-500-010L) was maintained at 55±1°C. Tween 80 solution at 20 mg kg⁻¹ and aridanin, at 15 and 30 mg kg⁻¹ i.p. were injected into the control and 2 test groups, respectively. Pain response latency on the hot plate (described as jump latency) was measured in seconds at 30 min interval for 1.5 h and compared to the control.

Acetic acid- induced writhing in mice: The writhing test was performed as described by Koster *et al.* (1959). The mice were fasted overnight but had water *ad libitum*. They were randomly divided into 3 groups (n = 5). The control group received 20 mL kg⁻¹ of 2% Tween 80 while the other 2 groups received 15 and 30 mg kg⁻¹ i.p. aridanin, respectively. Thirty min after each drug pre-treatment, each mouse was further dosed with 0.1 mL of 3% acetic acid solution i.p. Animals were then observed singly in a transparent glass box (45×25×25 cm) and the number of writhes counted 20 min after administration of acetic acid.

Statistical analysis: Data were analyzed using Kruskal-Wallis ANOVA followed by Mann-Whitney U-test. Other parameters measured were analyzed using Student-t-test (*p<0.05, 0.005).

RESULTS

Effect on motor function: Aridanin at the dose of 15 and 30 mg kg⁻¹ at 30 min after administration did not produce ataxia or motor in-coordination in the rotarod test and in the traction test.

Effect of aridanin on PTZ-induced seizure: Pentylenetetrazol (PTZ) 85 mg kg⁻¹ i.p. produced 100% convulsion and death in mice. A dose of 15 mg kg⁻¹ aridanin did not prevent convulsion induced by PTZ, while a dose of 30 mg kg⁻¹ aridanin caused a significant delay in the onset of convulsion. A 50% protective effect was observed at 30 min after treatment (Table 1).

Effect of aridanin on strychnine and picrotoxin induced convulsion: In all the two doses of aridanin administered, no protective effect was observed on convulsed animals. All the experimental animals died (Table 2).

Body temperature recording: In the control animals, no significant variations of rectal temperature were found. However, pre-treatment with aridanin at 15 and 30 mg kg⁻¹ significantly produced a fall in body temperature (Table 3) which increased with increase in dose administered which indicate that the fall in body temperature was dose-dependent. However the temperature was reversed back to normal after 5 h for the 2 doses tested (Table 3). The effect of the antagonists showed that atropine, cyproheptadine and naltrexone reversed the effect of aridanin on hypothermia significantly while haloperidol had no effect (Table 4).

Hot plate test: Aridanin did not show any analgesic activity using the hot plate test. With the 2 doses of aridanin, there was no significant difference in aridanin response time as compared with the control group.

Writhes induced by acetic acid: Aridanin significantly reduced acetic acid induced writhing in mice. Thirty milligram per kilogram of aridanin completely suppressed the writhes (Table 2). With 15 mg kg⁻¹, few writhing response was observed, hence the reduction was dose dependent. Similarly, morphine 0.5 mg kg⁻¹, completely suppressed the writhing response. However, in the presence of naltrexone, both aridanin and morphine exhibited writhing responses similar to that in the control.

Table 1: Anticonvulsant effect of aridanin in pentylenetetrazol (85 mg kg⁻¹ i.p.) induced seizure in rats

Treatment	Dose (mg kg ⁻¹)	Onset (sec)	Protection of Control (%)
2% Tween 80	20.0 mL kg ⁻¹	43.3±1.05	-
Aridanin	15.0	57.8±1.50	-
Aridanin	30.0	108.6±0.26*	50
Diazepam	3.0	NC	100

Each valve represent mean±SEM (n = 6) * p<0.05, NC = No convulsion

Table 2: Effect of Aridanin on the acetic acid induced writhing response in mice

Treatment	Dose (mg kg ⁻¹)	Writhing Response (No./20 min)	Inhibition (%)
2% Tween 80	20.0 mL kg ⁻¹	36.2±3.15	-
Aridanin	15.0	12.0±0.56*	66.9
Aridanin	30.0	-	100.0
Morphine	0.5	-	100.0
Naltrexone + Aridanin	0.25+30	25.8±1.30	28.8
Naltrexone + Morphine	0.25+30	29.2±0.86	19.3

(Values are mean±SEM, Significantly different from control at *p<0.05, n = 5)

Table 3: Effect of aridanin on body temperature of mice

Treatments (mg kg ⁻¹)	Pre-drug	Time (min)						
		30	60	90	120	180	240	300
2% Tween 80(20 mg kg ⁻¹)	38.0±0.20	37.8±0.10	37.6±0.05	37.1±0.20	37.6±0.30	37.1±0.10	37.1±0.40	37.2±0.32
Aridanin15	37.8±0.15	36.5±0.1*	36.5±0.1*	36.9±0.30	37.1±0.25	37.2±0.15	37.2±0.15	37.2±0.15
Aridanin30	38.2±0.14	35.1±0.08*	35.2±0.09*	35.9±0.24*	36.2±0.32*	36.7±0.10*	36.8±0.16	37.4±0.12

(Values are mean±SEM, Significantly different from control at *p<0.005, n = 5)

Table 4: the hypothermic effect of aridanin in the presence of the antagonists

Treatment	Dose (mg kg ⁻¹)	Pre-drug	Time (min)			
			30	60	90	120
2% Tween 80	20 mg kg ⁻¹	38.0±0.2	37.8±0.1	37.6±0.30	37.1±0.02	37.6±0.3
Aridanin	30	38.2±0.1	35.1±0.08	35.2±0.09	36.2±0.32	36.7±0.1
Atropine + Aridanin	2.0	37.8±0.15	37.4±0.12	37.5±0.11	37.7±0.13	37.8±0.15
Cyproheptadine + Aridanin	0.1	37.9±0.14	37.4±0.12	37.2±0.32	37.5±0.17	37.9±0.14
Naltrexone + Aridanin	0.25	37.9±0.14	37.2±0.15	37.5±0.22	37.7±0.3	37.9±0.14
Haloperidol + Aridanin	0.1	37.9±0.14	35.3±0.09	35.2±0.08	35.3±0.09	36.0±0.30

(Values are mean±SEM, Significantly different from control at *p<0.005, n = 5)

DISCUSSION

The present study was carried out in order to investigate the anti-convulsant, analgesic and hypothermic activities of aridanin. The rotarod test is often used as a method to determine the central nervous system potential of any anticonvulsant agent. The results of this test showed that aridanin causes central nervous system depression but there is no loss of muscular coordination. Similarly, the traction test result showed that there is no ataxia in mice and there is no loss of muscular coordination. Aridanin appears to have a selective anti-convulsant effect as it produced significant protection against PTZ-induced seizure but not against strychnine and picrotoxin induced seizures. PTZ-induced clonic-tonic convulsion in rodents is the valuable model for studying the effect of putative anti-convulsant drugs in the propagation of seizure activity (Goodman *et al.*, 1953). According to Kendall *et al.* (1981) the anti-convulsant potential of a drug is not only determined by its ability to prevent convulsion and mortality, but also by its ability to delay the onset of convulsion, shorten the frequency and duration of tonic-clonic seizure. Aridanin appears to exhibit this property on PTZ induced seizure only. Earlier, the *in vitro* activity of scopolin isolated from *Tetrapleura tetraptera* on isolated tissues suggested that this compound has central effect that are mediated mainly by its local anaesthetic property (membrane stabilizing activity) (Ojewole, 1984) which PTZ has been implicated to evoke in the induction of convulsion. The convulsant action of picrotoxin and strychnine are frequently ascribed to their capacity to antagonize GABA and glycine mediated inhibition, respectively (Meldrum, 1975). Since aridanin failed to protect the mice against convulsion induced by picrotoxin and strychnine, it can be deduced that aridanin is not acting through GABA and glycine pathway in the CNS. The action of aridanin was compared favourably with that of diazepam. This agent is a very effective standard anticonvulsant agent against PTZ.

The hypothermia observed in this study after intraperitoneal administration of aridanin suggests both central and peripheral mechanisms of action. The antagonistic study provides an evidence to show that the hypothermic effect of aridanin was found to be mediated through the cholinergic, the serotonergic and the opioid receptors but not with the dopaminergic receptor system. Aridanin produced an inhibition in acetic acid induced writhing. This model has been used to screen drugs for their analgesic activity (Koster *et al.*, 1959). The

effect shows that aridanin has potential analgesic activity. The location of the antinociceptive action of aridanin appears to be the mu (μ) receptor in the brain. It has been reported that the writhing assay is sensitive to (μ) opioid and non-steroidal anti-inflammatory drugs which act primarily by a central and peripheral mechanism, respectively (Millian, 1994). This opioidergic and/or monoaminergic mechanism may be involved in the anti-writhing effect of aridanin.

CONCLUSIONS

The present studies allow us to conclude that aridanin has anticonvulsant property with PTZ and not with strychnine and picrotoxin. Aridanin did not cause ataxia and motor incoordination in mice. The compound has both analgesic and hypothermic activities. The results validate the popular use of *Tetrapleura tetraptera* fruits for the treatment of convulsion.

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REFERENCES

- Adesina, S.K. and J. Reisch, 1985. A Triterpenoid glycoside from *Tetrapleura tetraptera* fruit. *Phytochemistry*, 24: 3003-3006.
- Campos, A.R., F.A.A. Albuquerque, V.S.N. Rao, M.A.M. Maciel and A.C. Pinto, 2002. Investigations on the antinociceptive activity of crude extracts from *Croton cajucara* leaves in mice. *Fitoterapia*, 73: 116-120.
- Dalziel, J.M., 1955. *The Useful Plants of West Tropical Africa*. Oxford London, pp: 223.
- Dunham, N.W. and T.S. Miya, 1957. A note on simple apparatus for detecting neurological deficit in rats and mice. *Pharm. Assoc.*, 46: 208-209.
- Elisha, E., S.J. Al-Maliki and D.K. Ibrahim, 1988. Effect of *Jasoninum officinale* flowers on the CNS of the mouse. *Intl. J. Crude Drug Res.*, 26: 221-227.
- Goodman, L.S., M.S. Grenal and W.C. Brown, 1953. Comparison of maximal seizure evoked by pentylenetetrazol and electroshock in mice and their modification by anticonvulsants. *J. Pharmacol. Exp. Therapeu.*, 108: 168-176.

- Kendall, D.A., D.A. Fox and S.J. Enna, 1981. Anticonvulsant profile of Gamma-vinyl GABA. *Neuropharmacology*, 20: 4-10.
- Koster, R., M. Anderson and E.J. De-Beer, 1959. Acetic acid for analgesic screening. *Fed. Proc.*, 18: 412-418.
- Maillard, M., C.O. Adewunmi and K. Hostettman, 1989. New triterpenoid N-acetylglycoside with molluscicidal activity from *Tetrapleura tetraptera* Taub *Helvetica*. *Chim. Acta*, 72: 668-674.
- Meldrum, B.S., 1975. Epilepsy and Gamma-amino butyric acid mediated inhibition. *Intl. Rev. Neurobiol.*, 17: 1-36.
- Millian, M.J., 1994. Serotonin and pain: Evidence that activation of 5-HT_{1A} receptor does not elicit antinociceptive against noxious thermal, mechanical and chemical stimuli in mice. *Pain*, 58: 45-61.
- Ojewole, J.A.O., 1984. Effects of scopoletin on autonomic transmissions. *Intl. J. Crude Drug Res.*, 22: 81-93.
- Rudzik, A.O., J.B. Hesten and A.H. Tang, 1973. Triazolobenzo Diazepines, a New Class of Central Nervous System-Depressant Compounds. In: Garattini, S., E. Mussini and L.O. Randall (Eds.). *The Benzodiazepines*. Raven Press, New York, pp: 285-297.
- Stone, W.E. and M.J. Javid, 1979. Quantitative evaluation of the actions of anticonvulsants against different chemical convulsions. *Arch. Intl. Pharmacodyn.*, 240: 66-78.
- Swinyard, E.A., J.H. Woodhead and M.R. Franklin, 1989. Experimental Selection, Quantification and Evaluation of Anticonvulsants. In: Levy, R., R. Mattson, B.S. Meldrum, J.K. Perry and F.E. Dreifuss (Eds.). *Antiepileptic drugs: 3rd Edn*. Raven Press, New York, pp: 85-102.
- Thomas, O.O., 1989. Perspectives on ethno-phytotherapy of Yoruba medicinal herbs and preparations. *Fitoterapia*, LX: 49-60.