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Anxiolytic effect of aridanin isolated from *Tetrapleura tetraptera* in mice

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ABSTRACT

The study was carried out to investigate the anxiolytic properties of aridanin isolated from *Tetrapleura tetraptera* in mice. Elevated plus-maze was used to investigate the effect. The possible involvement of the $GABA_A$ - benzodiazepine receptor complex was also investigated using flumazenil. Aridanin at doses of 5 and 10 mg/kg, i.p. administered 30 min prior induced anxiolytic effect expressed by increase number of entries in and time spent in the open arms and percentage of open arm entries and decrease number of entries and time spent in the closed arms. The treatment of mice with flumazenil (2.0 mg/kg, i.p.) 15 min before the administration of aridanin (10 mg/kg, i.p.) blocked the aridanin induced anxiolytic effect. It was found out that aridanin induced an anxiolytic effect in mice which may be mediated through interaction with $GABA_A$-benzodiazepine receptor complex.

Keywords: Aridanin, *Tetrapleura tetraptera*, Anxiolytic, $GABA_A$ receptor, benzodiazepine receptor.

INTRODUCTION

Tetrapleura tetraptera Taub (Mimosaceae) locally known as Aridan is a large tree growing throughout the rain forest belt of West Africa. It is generally found in the lowland forest of tropical Africa. The fruit consist of a fleshy pulp with small, brownish – black seeds. The plant has many traditional uses mainly in the management of convulsion, leprosy, inflammation and rheumatic pains, schistosomiasis, asthma and hypertension (Ojewole and Adesina, 1983). The dry fruit has a pleasant aroma (Aladesanmi, 2007). It is used as a popular seasoning spice, a medicine and a dietary supplement rich in vitamins in Southern and Eastern Nigeria (Okwu, 2003; Essien et al., 1994). The fruit is used to prepare soup for mothers from the first day of birth to prevent post partum contraction (Nwawu and Akah, 1986). The root extract

has been proven to be useful for the treatment of gastrointestinal related clinical problem (Noamesi et al., 1994). The ethanol extract and saponins from the stem bark of *Tetrapleura tetraptera* exerted an inhibitory effect on luteinizing hormone released by pituitary cells, suggesting its use as contraceptive agent (El Izzi et al., 1990). *Tetrapleura tetraptera* is a natural molluscicides as aqueous extract of it is effective against *Bulinus globosus* and *Lymnaea natalensis* (Adewunmi, 1991). The alleopathic potential of *Tetrapleura tetraptera* has led to its integration into an agro forestry system (Amoo et al., 2008). *Tetrapleura tetraptera* has been shown to improve the foaming ability of soaps (Adebayo et al., 2000). *Tetrapleura tetraptera* has no influence on cell proliferation and neither induced chromosomal aberration nor sister chromatid exchanges in Chinese hamster ovary cells (no genotoxic effect) (Adewunmi et al., 1991). *Tetrapleura tetraptera* has been shown to cause elevation in serum AST and alteration of various metabolites parameters and did not induce any marked pathological lesion in the liver (Odesanmi et al., 2009). The sedative, anticonvulsant and analgesic effect of aridanin in mice have been reported (Aderibigbe et al., 2007a; Aderibigbe et al., 2007b; Ojewole, 2005). The aqueous extract of *Tetrapleura tetraptera* fruit have been shown to possess anti-inflammatory and hypoglycaemic properties (Ojewole and Adewunmi, 2004). The ethanolic extract of *Tetrapleura tetraptera* fruit possessed antiplasmodial activity in mice (Okokon et al., 2007). One of the active constituents isolated from *Tetrapleura tetraptera* fruit is a mono – N – acetylglycoside of oleanoic acid (3β-hydroxyolean-12-en-28-oic) called aridanin (Adesina and Reish, 1985). The present study was carried out to investigate the anxiolytic effect of aridanin in mice.

MATERIALS AND METHODS

Structural elucidation and characterization of Aridanin (Figure 1) from *Tetrapleura tetraptera* was carried out by Adesina and Reish (1985). Dried fruit pulp of *Tetrapleura tetraptera* (200 g) collected in Nigeria was extracted successively with CH2CL3, MeOH and H2O. The MeOH extract (80 g) was partitioned between H_2O and BuOH. Fractionation of 20 g of the BuOH extract by column chromatography on silica gel was monitored by TLC. Seventeen fractions were obtained. Fraction 7 out of the fractions were separated by reversed-phase chromatography on RP-8 or liquid-liquid chromatography from which aridanin was obtained. Aridanin used for this experiment was collected from Prof. S. K. Adesina.

Animals

Swiss albino male mice weighing between (20-25 g) were obtained from the animal house of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. The animals were divided into five mice in each cage and were fed with a standard laboratory diet and tap water *ad libitum*. The animals were maintained at 25 $\pm$1 ^{o}C under natural 12 h daylight/night conditions. All experiment was carried out in compliance with Obafemi Awolowo University Ethics Committee on research in animals and in accordance with NIH guide for the care and use of laboratory animals.

Drugs

Diazepam and Flumazenil were obtained from Sigma Chemicals Co.St. Louis, Missouri, USA.

Drug dissolution

Aridanin, Flumazenil and Diazepam was dissolved in 5% Tween 80. Tween 80 at 5% concentration did not affect behavioural studies in rodents (Castro et al., 1995). The resulting solution, control vehicle or test materials were administered by intraperitoneal injection (i.p.).

Acute toxicity

Acute toxicity study of aridanin in mice was carried out as described by Miller and Tainter (1944) and the lethal dose was calculated by the method of Litchfield and Wilcoxon (1949). It was carried out by injecting aridanin i.p. into 5 groups of mice containing 5 animals in each group with the following dose levels 25, 37.5, 50, 75 and 100 mg/kg. The animals were observed for over 24 h and the LD_{50} was calculated.

Elevated plus-maze test (EPM)

The elevated plus-maze (EPM) test was used to evaluate the animal anxiety (Pillow and File, 1986; Lister, 1987; Nogueira and Vassilieff, 1996). The EPM for mice consisted of two open arms (30 x 5 cm) and two close arms (30 x 5 x 15 cm) that extended from a common central platform (5 x 5 cm) with an open roof, arranged in such a way that the two arms of each type were opposite to each other. The floor and the walls of each arm were wooden and painted white. The maze was elevated to a height of 38.5 cm above floor level. Testing was conducted in a quiet room that was illuminated by light. The animal's behaviour was recorded directly by an observer sitting 2 m away in the same room.

Each animal was placed in the centre of the EPM facing one of the open arms. An entry into an arm was defined as the animals placing all four paws over the line marking that area. The number of entries and the time spent in the open and closed arms were recorded during a 5 min test period. The percentages of open arm entries to total arm entries (100 x Open/Total entries) were calculated for each animal.

Initially, mice were treated with aridanin at the doses of 5, 10, 20 and 30 mg/kg, i.p. for 30 min before the evaluation in the EPM test. The control animals received 5% Tween 80 (0.2ml/20 g). Subsequently, another group of mice were treated with flumazenil (2 mg/kg, i.p.) a GABA-benzodiazepine receptor antagonist 15 min before the administration of aridanin (10 mg/kg, i.p.). The dose of flumazenil administered has been found to block the GABA receptors (Ayoka et al., 2006). The anxiety evaluation was carried out 30 min after the administration of aridanin or vehicle. Between each trial, the maze was wiped with 70% ethanol to prevent olfactory cue from animals.

Statistical analysis

Results are expressed as Mean ± standard error of the mean (S.E.M). All data were analysed by one way analysis of variance (ANOVA). Post hoc tests were then performed using Student Newman Keuls test, with the level of significant set at $P<0.05$.

RESULTS AND DISCUSSION

Acute toxicity

Acute toxicity of aridanin was calculated using graphical method of Litchfield and Wilcoxon, (1949). The intraperitoneal LD_{50} of aridanin in mice was calculated to be 60.0 mg/kg.

Elevated plus-maze

Aridanin at the doses of 5 and 10 mg/kg, i.p. increased the number of open arm entries [$F (5, 24) = 13.7$, $P < 0.001$] (Table 1), decreased the number of closed arm entries [$F (5, 24) = 7.8$ $P < 0.001$] (Table 1) and increased the percentage of the open arm entries [$F (5, 24) = 41.8$, $P < 0.001$] (Table 1). Aridanin at the doses of 20 and 30 mg/kg, i.p. decrease the percentage of open arm entries.

According to the number of entries, aridanin 5 and 10 mg/kg, i.p. increased the time spent in the open arm [$F (5, 24) = 29.5$, $P < 0.001$] (Table 2) and decreased the time spent in the closed arm [$F (5, 24) = 11.6$, $P < 0.001$] (Table 2). Aridanin at the doses of 20 and 30 mg/kg, i.p. increased the time spent in the closed arm.

According to the number of entries, the treatment of mice with aridanin (10 mg/kg, i.p.) preceded by flumazenil decreased the time spent in the open arms [$F (4, 20) = 26.4$, $P < 0.001$] (Table 3) and increased the time spent in the closed arm [$F (4, 20) = 34.6$, $P < 0.001$] (Table 3). Flumazenil blocked the aridanin (10 mg/kg, i.p.) induced anxiolytic effect, so, there was a decrease in the number of open arm entries [$F (4, 20) = 12.1$, $P < 0.001$] (Table 4) and an increase in the number of closed arm entries [$F (4, 20) = 3.6$, $P < 0.001$] (Table 4).

Table 1: Effect of aridanin on the number of entries into the open arms and closed arms and also the percentage ratio of open to total arm entries.

Treatment	Dose (mg/kg, i.p.)	NEOA	NECA	PEOA
5%TW80	0.2ml/20 g	4.2±0.6	7.8±1.1	29.0±1.1
Aridanin	5.0	6.2±0.4*	5.0±0.6	55.3±3.1*
Aridanin	10.0	6.4±0.5*	4.4±0.5	57.7±3.2*
Aridanin	20.0	3.0±0.5	6.8±0.4	34.1±2.1
Aridanin	30.0	1.8±0.4	7.1±0.4	23.1±1.1
Diazepam	1.0	8.4±1.2*	3.4±0.4	58.0±3.1*

Results are expressed as the mean ± S.E.M, (n = 5). One way ANOVA revealed that there is a significant difference between different treatment groups. NEOA: Number of entries in the open arm; NECA: Number of entries in the closed arm**;** PEOA: Percentage of entries in the open arm; 5%TW80: 5% Tween 80
*Indicate significant difference from 5% Tween 80 control. $P < 0.05$ SNK test.

Table 2: Effect of aridanin on time spent (in seconds) in open and closed arms.

Treatment	Dose (mg/kg, i.p.)	TSOA	TSCA
5%TW80	0.2ml/20g	40.0±5.6	101.2±12.2
Aridanin	5.0	135.6±17.0*	43.2±3.3
Aridanin	10.0	140.4±20.7*	50.8.4±10.0
Aridanin	20.0	38.2±4.0	125.8±15.1
Aridanin	30.0	29.8±1.7	124.0±15.0
Diazepam	1.0	170.4±6.7*	58.4±3.4

Results are expressed as the mean ± S.E.M, (n = 5). One way ANOVA revealed that there is a significant difference between different treatment groups. TSOA: Time spent in the open arm; TSCA: Time spent in the close arm; 5%TW80: 5% Tween 80
*Indicate significant difference from 5% Tween 80 control. $P < 0.05$ SNK test.

Table 3: Effect of aridanin on time spent (in seconds) in the open and closed arms in the presence of flumazenil.

Treatment	Dose (mg/kg, i.p.)	TSOA	TSCA
5%TW80	0.2ml/20g	40.0±5.6	101.2±12.2
Aridanin	10.0	140.4±20.7*	50.8±10.0
FLU	2.0	200.0±24.0*	51.2±10.2
FLU+ARI	10.0	35.8±7.9*	206.8±19.8*
FLU+DIZ	1.0	28.5±5.6*	235.6±18.8*

Results are expressed as the mean ± S.E.M, (n = 5). One way ANOVA revealed that there is a significant difference between different treatment groups. TSOA: Time spent in the open arm; TSCA: Time spent in the close arm; 5%TW80: 5% Tween 80; FLU: Flumazenil; DIZ: Diazepam; ARI = Aridanin.
*Indicate significant difference from 5% Tween 80 control. $P < 0.05$ SNK test.

Table 4: Effect of aridanin on the number of entries into the open arms and closed arms in the presence of flumazenil (2.0 mg/kg i.p.).

Treatment	Dose (mg/kg, i.p.)	NEOA	NECA
5%TW80	0.2ml/20g	4.8±0.6	7.8±1.1
Aridanin	10.0	6.0±0.5	4.4±0.5
FLU	2.0	2.7±0.4	4.5±0.5
FLU+ARI	10.0	2.4±0.5*	6.4±0.7*
FLU+DIZ	1.0	2.2±0.4*	6.7±0.9*

Results are expressed as the Mean ± S.E.M, (n = 5). One way ANOVA revealed that there is a significant difference between different treatment groups. NEOA: Number of entries in the open arm; NECA: Number of entries in the closed arm**.** FLU = Flumazenil; ARI = Aridanin; 5% TW80 = 5% Tween 80; DIZ: Diazepam.
* Indicate significant difference form Aridanin P < 0.05 SNK test

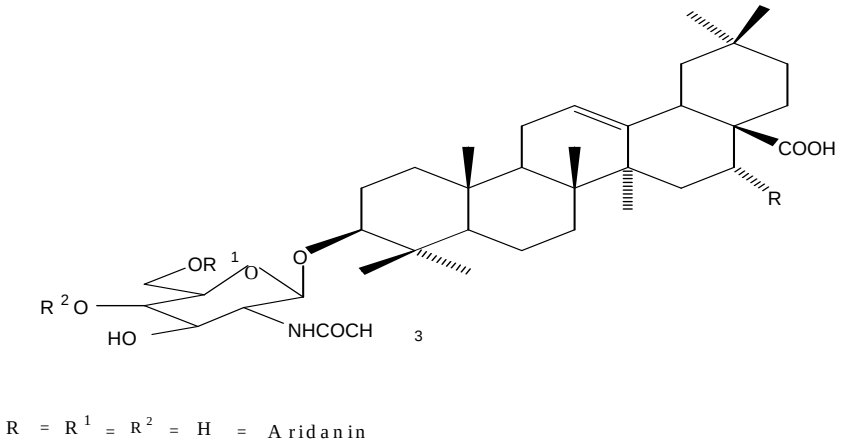

Figure 1: The chemical structure of aridanin.

The elevated plus-maze is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli (fear of a novel, brightly-lit open space and fear of balancing on a relatively narrow, raised platform) that can induce anxiety in human (Yellow and File, 1986; Lister, 1987). It has been extensively accepted as an ultimate test for anxiolytic drugs and their mechanisms of action (Rodgers et al., 1997; Cole and Rodgers, 1995).

In the present study, low doses of aridanin (5 and 10 mg/kg, i.p.) induce a dose dependent anxiolytic effect in mice. The doses increased the entries and time spent in the open arms and decreased entries and time spent in the closed arms in the EPM test. The anxiolytic effect of aridanin is similar to the one observed with diazepam, a typical benzodiazepine drug (Rall, 1990). As expected, diazepam produced significant increases in open arm time and in number of entries into the open arm. Therefore, it can be hypothesized that aridanin may be acting like a benzodiazepine like substance. Supporting this view, the treatment with flumazenil, a specific antagonist of the benzodiazepine site in the $GABA_A$ – BDZ receptor complex, was able to block completely the anxiolytic effect induced by aridanin. The anxiolytic effect of diazepam was also blocked by flumazenil. The anxiolytic effect of aridanin is similar to

that of plants such as *Cissus cornifolia, Careya anboree, Rubus brasiliensis, Stachys lavandulifolia,Scutellaria baicalensis* which has anxiolytic properties (Musa et al., 2008; Kumar et al., 2008; Hue et al., 2002; Rabbani et al., 2003; Nogueira et al., 1998). It is noteworthy that anxiolytic doses of a drug must not affect locomotion, otherwise test animals would be sedated in one arm and the result will be an error of wrong conclusion of the effect of the drug. Reddy and Kulkarni (1997) stated that; the effects of drugs seen at doses that did not markedly affect locomotor activity suggest that these changes in behaviour represent anxiolytic actions.

In conclusion, aridanin has been shown to possess anxiolytic effect which is exerted through interaction with the $GABA_A$ – BDZ receptor complex.

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