



CNS activity of ZS-1A: a phytoceutical from *Zizyphus spina-christi* root bark

Bulus ADZU^{1,2,3,*}, Abdu Kaita HARUNA¹, Mohammed ILYAS¹ and
Karniyus Shingu GAMANIEL²

¹Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU), Zaria, Nigeria.

²Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

³Department of Pharmacy, School of Health Sciences, Kampala International University (KIU), Western Campus, Ishaka, Uganda.

* Corresponding Author, E-mail: bulusadzu@yahoo.com, Tel.: +256 752659615; +234 7088858008

ABSTRACT

Zizyphus spina-christi (ZS) Willd is reputed to have medicinal values. Studies on the phytochemistry, and some pharmacological activity of the plant's rootbark were initiated in our laboratories. The present study is a report on sedative effect of a fraction obtained from the hexane extract (numbered ZS-1A) of the plant material. The fraction (25, 50 and 100 mg/kg, p.o.) were tested against pentobarbital sleeping time, motor coordination (rota-rod performance) test and exploratory behaviour in mice. Results show that ZS-1A prolonged pentobarbital induced hypnosis and decrease the head-dip responses in the exploratory behaviour tests dose dependently. It however failed to give a positive test on the motor coordination test. The results demonstrate potent central depressant effect by ZS-1A.

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INTRODUCTION

Zizyphus spina-christi (ZS) Willd belongs to the family Rhamnaceae (Cavalier-Smith, 1986; Brands (Compl.), 1989 - 2007). The plant grows wild in Asia and tropical Africa, and is reputed to have nutritional and medicinal values throughout these regions (Dweck, 2005). Preliminary studies in our laboratory show that crude extracts of the plant exhibited analgesic, antidiarrhoeal and CNS (central nervous system) activities (Adzu et al., 2001; 2002; 2003). Recently, we initiated bioassay-guided fractionation and pharmacological testing of components of the plant's rootbark in line with technical need to standardize herbal medicines (Farnsworth, 1980; Patwardhan, 2005). Part 1 and 2 of the study were on analgesic/anti-inflammatory and antidiarrhoeal efficacy of the plant that

was traced to fractions (numbered ZS-4D and ZS-4E respectively) of the methanol extract (Adzu and Haruna, 2007; Adzu et al., 2007a). Part 3 was on ZS-2A a potent antiplasmodial agent from chloroform extract (Adzu et al., 2007b). This continuation is a report on CNS effect of a fraction obtained from the hexane extract. The effect was evaluated using three standard experimental models: pentobarbital induced sleeping time; rota-rod (motor coordination) test and exploratory behaviour test in mice. The safety level (acute toxicity test) of the fraction was also tested.

MATERIALS AND METHODS

Phytochemical evaluation

Zizyphus spina-christi Willd -- Sp. Pl., ed.4 [Willdenow] 1 (2): 1105. 1798 [Jul 1798] (IK) (*The International Plant Names Index*

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www.ipni.org) was collected between April and May 2004 from Midlu, Adamawa State, Nigeria. It was authenticated at Taxonomy Unit, Department of Medicinal Plant Research and Traditional Medicine, NIPRD, Abuja, and voucher specimen (NIPRD # 4108) was kept at the Herbarium Unit of the Department. The roots were collected and dried under shade, powdered and a total of 1.29 kg was sequentially extracted along polarity gradient using hexane, chloroform, ethylacetate and methanol. The extracts gave a total yield of 21.86% w/w, and phytochemical screening (Harborne et al., 1998) gave positive tests for alkaloids, balsams, carbohydrates, saponins, tannins, steroids and terpenes. The various extracts were subjected to fractionalization using different chromatographic techniques, and various pharmacological testing as presented graphically in figure 1.

Animals

Swiss albino mice weighing 20–28 g obtained from the Animal Facility Centre, NIPRD, Abuja, Nigeria were used. They were housed in standard polypropylene cages with saw dust as beddings, and have access to food and water *ad libitum*. The animals were used in accordance with the ethical norms of *NIH Guide for the Care and Use of Laboratory Animals [NIH Publication No. 85 – 23, revised (1985)] The National Academic Press, Washington DC, (1985)*.

Acute toxicity test

The safety level of ZS-1A was tested by determining the LD₅₀ using Lorke's (1983) method. The LD₅₀ was estimated as the square root of the lowest lethal dose multiplied by the highest non-lethal dose, obtained from the second stage of dosing (Vongtau et al., 2004).

Pentobarbital sleeping time

The test (Fujimori, 1965) was carried out in mice as previously described by Amos et al. (2001) and modified for our laboratory setting (Adzu et al., 2008). Mice were grouped into five (n = 5) and treated with aqueous ZS-1A (25, 50 and 100 mg/kg, p.o.), vehicle (10ml/kg, p.o.) and diazepam (2 mg/kg, p.o.). The animal received sodium pentobarbital (25 mg/kg, i.p.) 30 min after the treatment. Hypnotic effect index was recorded as follows: time between the injections of

pentobarbital until loss of righting reflex was taken as the onset of sleep, while the time from this loss of reflex and its recovery was taken as the duration of sleep (Soulimani et al., 2001).

Motor coordination (Thread Mill) test

This test was performed using a horizontal rotatory rod thread mill device (Ugo Basile, No. 7500, Italy). The instrument was first set at a rate of 16 rpm, and mice that were able to remain on the rod longer than 3 min were selected and grouped into four (n = 5). Groups 1-3 were treated with the ZS-1A (25, 50 and 100 mg/kg, p.o.) while Group 4 received normal saline. Each mouse was again singly placed on the rod 30 min after the treatment and every 30 min interval for duration of 120 min (Adzu and Gamaniel, 2003). The test was adjudged positive if mouse was unable to remain on the rod for 180 seconds duration of the test and the endurance time recorded (Amos et al., 2005)

Exploratory behaviour test

This test was carried out using LETICA (Spain) (LE3333), a hole-board apparatus 40 X 40 cm with an automatic multicounter. The experiment was a head dip test similar to those described by Perez et al. (1998) and Amos et al. (2001). Mice were grouped into five (n = 5). Groups 1–3 were treated with aqueous ZS-1A (25, 50 and 100 mg/kg, p.o.) and the control group treated with the vehicle. Each mouse was placed on the board and the number of times the mouse dipped head into the hole during a 5 minutes trial was automatically counted by the instrument.

Data analysis

Results were expressed as mean ± SEM. Analysis was performed using ANOVA followed by Dunnett's test using GraphPad Prism Version 4.00 for Windows, GraphPad software, San Diego California USA, www.graphpad.com

RESULTS

Acute toxicity tests

The LD₅₀ of the fraction was established to be 871.78 mg/kg, i.p. in mice. This indicates that the experimental doses used (25, 50 and 100 mg/kg, p.o.) were within safe limit.

Pentobarbital induced sleep

There was a significant prolongation of the sleeping time among the treated group when compared with the control. The standard drug used (diazepam) however showed a superior activity (Table 1).

Motor coordination test

The fraction failed to give a positive test on the thread mill test (Table 2).

Exploratory behaviour test

The fraction reduced the head-dip in the treated groups between 25.98–66.90% compared with control (Table 3).

DISCUSSIONS

Results showed that ZS-1A prolonged the sleeping time induced by injection of pentobarbital to mice in the treated group compared to those in the control group. Pentobarbital-induced sleep model (in laboratory animals) is used to test centrally acting effect of agents (Carpando et al., 1994).

Prolongation of pentobarbital sleep is due to sedative/hypnotic effect of such agents, due to inhibition of pentobarbital metabolism or central mechanisms involved in regulation of sleep (Fujimori, 1965; Kaul and Kulkarni, 1978; N’Gouemo et al., 1994), an indication of central depressing effect on the CNS.

Table 1: Effect of ZS-1A on pentobarbital induced sleep in mice.

Treatment	Dose (mg/kg, p.o.)	Onset of sleep (min)	Duration of sleep (min)
Saline	10 ml/kg	6.8 ± 0.9	43.4 ± 3.2
ZS-1A	25	5.6 ± 0.4	47.4 ± 2.1*
	50	4.2 ± 0.5	51.4 ± 3.7*
	100	3.96 ± 0.4	58.2 ± 2.5*
Diazepam	2	3.41±0.4	64.6±5.4*

* Significant difference; F [(4,24 = 5.12); p < 0.05]

Table 2: Effect of ZS-1A on motor coordination (rota-rod performance) test in mice.

Experimental Time (min)	Cut-off time (s)	Endurance time(s)		
		25	50	100 mg/kg, p.o.
30	180	180	180	180
60	180	180	180	180
90	180	180	180	176
120	180	180	174	160

Table 3: Effect of ZS-1A on exploratory behaviour test in mice.

Treatment	Dose (mg/kg, p.o.)	Mean head dip (in 5 min)	% Inhibition
Saline	10ml/kg	56.2 ± 5.3	-
ZS-1A	25	41.6 ± 3.5	25.98
	50	28.4 ± 2.2	49.47
	100	18.6 ± 2.7	66.9

* Significant difference; F [(3,19 = 4.02); p < 0.05]

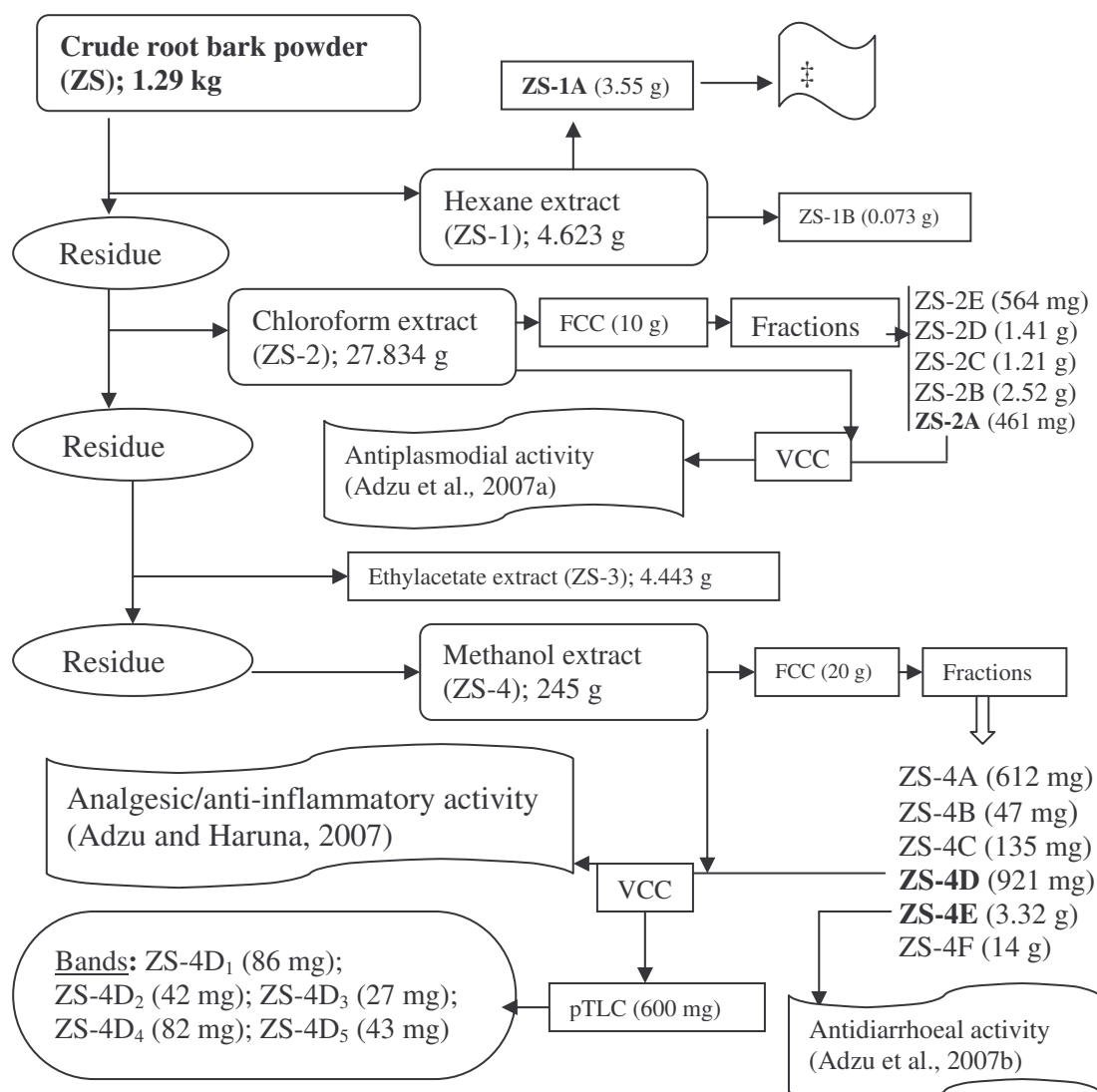


Figure 1: Flow chart: phytochemical evaluation of *Zizyphus spina-christi* root bark.

ZS-*Zizyphus spina-christi*; FCC-Flush Column Chromatography (Still et al., 1978); VCC-Vacuum Column Chromatography (O'Donell et al., 2006); pTLC-Preparative Thin Layer Chromatography (Ncube et al., 1998; Ahmad et al., 2005);[‡]Fraction used for the current study.

ZS-1A failed to give a positive test on the rota-rod test. This indicates that the observed depressant effect is devoid of peripheral neuromuscular joint blockade (Capasso et al., 1996). The fraction however induced a decrease in the exploratory behaviour as shown by the decrease in head dip responses compared with the control. In this test, decrease in head dip reveals sedation (File and Wardill, 1975) while an increase is indicative of anxiolytic effect (File and Pellow, 1985). The observed decrease in the head dip by the treated groups further supports depressing effect of ZS-1A. Conclusively, the fraction has potent agent which has CNS activity that is sedative in nature.

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