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Sedative effects of *Cassia singueana* root bark

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Abstract

Objective: To investigate the effects of *Cassia singueana* on the central nervous system in mice. Materials and methods: The effects of methanol extract of *Cassia singueana* on motor coordination, spontaneous motor activity, pentobarbital sleeping time and exploratory behaviour were evaluated in mice. Results: The extract failed to exhibit activity on motor coordination test, but significantly reduced spontaneous motor activity and exploratory behaviour, and prolonged barbital hypnosis. Conclusion: The extract contains potent neuropharmacological principle with sedative effect.

Keywords: *Cassia singueana*, Sedation.

1. Introduction

Cassia singueana (syn: *Cassia goratensis* Fresen) (Fabaceae) is a medicinal plant reputed to be of beneficial effect in the traditional system of medicine. The plant is used for various ailments that include: sterility in women [1], abortive [2], urinary schistosomiases [3], hernia, abdominal pain, fever [4], anticonvulsant, constipation, heartburn and snake bite [5]. Previous studies on the plant showed weak antimicrobial activity [6-9] and phytochemical constituents that include alkaloids, antraquinones, quinoid, steroid and triterpenes [2, 5, 10, 11]. Studies on the effect of the plant on the central nervous system to our knowledge had not been reported. On this basis, we investigated the activity of the methanol extract of the root bark of the plant *Cassia singueana* on motor coordination test, spontaneous motor activity, pentobartital sleeping time and exploratory behaviour in mice.

2. Materials and methods

2.1 Plant material

Mal. Achaba Lugudu collected roots of the plant *Cassia singueana* at Midlu, Adamawa State, Nigeria, in October 2001. The identity of the plant was authenticated at the Taxonomy, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (No. 4598) was deposited at

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the Herbarium of the Institute for future reference. The plant materials were dried, grounded into powder using pestle and mortar, and 185g of the powdered material were extracted with 500 ml of distilled methanol using 250-ml soxhlet apparatus. It was then evaporated under reduced pressure. This gave a dark brown extract with a yield of 27.2g (14.7 % w/w).

2.2. Drugs

Sodium pentobarbital (Sigma Chemical Co., USA), sodium chloride (BDH, England) and methanol (M & B, England) were used. Normal saline (10 ml/kg p.o.) was used as control in all experiments.

2.3 Animals

Swiss albino mice weighing 18 - 26g obtained from the Animal Facility Centre, NIPRD, were used. The animals were housed in plastic cages under standard condition of 12:12h light and dark circle, and were fed with standard diet (Ladokun Feeds Ltd., Ibadan) and tap water *ad libitum.* All experiments involving the use of mice were performed according to the "principle of laboratory animal care" [NIH Publication No. 85-23, revised (1985)]. Mice were fasted overnight prior to study. Experiment were carried out between 9:00am and 12:00 noon local time (+1 GMT)

2.4 Pharmacological evaluations

2.4.1 Motor coordination test

Ugo Basile (Italy) biological research apparatus (No. 7600) was used for the test. The instrument (a horizontal rotation device) was set at a rate of 16 revolutions per min. Mice were placed on the rod and those that were able to remain on the rod longer than 3 min were selected and group into 3 (n = 4). Group 1 was treated with saline, while groups 2 and 3 received the extract (100 and 200 mg/kg p.o.). They were again placed on the rod 30 min after treatment. Mouse unable to remain on the rod was considered as a positive test and the time it falls (endurance time) was recorded [12].

2.4.2 Spontaneous motor activity

The study was performed using Letica (Spain) activity cage (LE 886). The instrument consists of 4 ventilated motor cages connected to a multicounter (LE 3806). Mice were grouped into 3 (n = 4) and treated with saline or the extract (100 and 200 mg/kg p.o.). Activity was automatically recorded 30 min after treatment and at every 2 min for 6 min. The experiments were repeated at an interval of 30 min, for a total of 120 min. Results of the treated groups were compared with those of saline group at each time interval [13].

2.4.3 Pentobarbital hypnosis

A total of 18 mice grouped into 3 (n = 6) were used for this study. They were treated as follows; Group 1 received normal saline, while groups 2 and 3 received the extract (100 and 200 mg/kg p.o.). The animals were given sodium pentobarbital (25 mg/kg i.p.) 30 min later and index of hypnotic effect recorded. The effects were recorded as follows: Time elapsed between the administration of pentobarbital until loss of righting reflex was recorded of as the onset of sleep, while the time from the loss to its recovery was considered as the sleeping time [14].

Table 1
Effects of methanol extract of *C. singueana* root on motor coordination in mice

Experimental time (min)	Endurance time (s)		
	Saline 10 ml/kg, p.o.	100 mg/kg, p.o.	200 mg/kg, p.o.
30	180	178 ± 1.23	179 ± 1.33
60	180	175 ± 2.00	169 ± 2.08
90	180	176 ± 2.78	170 ± 1.52
120	180	175 ± 1.95	173 ± 0.54

Table 2
Effect of methanol extract of *C.singueana* on spontaneous motor activity in mice

		Experimental time (min)				
Treatment	**Dose**	**0**	**30**	**60**	**90**	**120**
Saline	10ml/kg, p.o.	114.7 ± 1707	85.13 ± 11.63	80.14 ± 13.3	71.83 ± 12.69	64.14 ± 29.12
C.singueana	100mgkg, p.o.	155.33 ± 21.63	64.33 ± 18.63*	60.83 ± 17.11*	57.57 ± 18.89*	57.17 ± 15.14*
	200mg/kg, p.o.	157.92 ± 19.26	57.89 ± 16.34*	48.42 ± 13.05*	32.34 ± 7.5*	25.83 ± 6.84*

Value in parenthesis indicate mean activity count + SEM; * Indicates significant different F[(2,11) = 4.24; p< 0.05]

Table 3
Effects of methanol extract of *C.singueana* root on pentobarbital induced sleeping time

Treatment	**Dose**	**Onset of sleep (min)**	**Duration of sleep (min)**
Saline	10ml/kg, p.o.	4.96 ± 0.21	38.65 ± 1.68
C. singueana	100mg/kg, p.o.	5.24 ± 0.19	45.81 ± 2.17*
	200mg/kg, p.o.	5.15 ± 0.32	54.17 ± 1.14*

Values are means ± SEM; *Significantly different F [(2,15) = 3.94; p< 0.05]

2.4.4 Test for exploratory behaviour

The study was carried out using Letica (Spain) board measuring 40 x 40cm with 16 evenly distributed holes attached to a multicounter (LE 3333). Mice were grouped into 3 (n = 6) and treated with saline or extract (100 and 200 mg/kg p.o.). Each mouse was placed on the board 30 min after treatment and the instrument automatically counted the number of times they dipped they heads into the holes during the 5 min trial [15]. The experiments were again repeated 60 min after treatment.

2.5 Data analysis

Results were expressed as mean ± SEM. Analysis of Variance (ANOVA) was used to compare and analyze results followed by Dunnet's test for multiple comparisons. Effects were considered significant at $p < 0.05$ in all cases.

3. Results and discussion

The extract failed to affect the motor coordination test as determine by treadmill performance (Table 1). This failure shows that the activity of the extract may not be attributed to peripheral neuromuscular joint blockade [17] but rather elicited centrally [16].

However, it significantly reduces spontaneous motor activity (Table 2). The activity is a measure of the level of excitability of the CNS [18]. The evidence that extract suppressed this activity therefore suggests sedative effect. This suggestion is collaborated by the extract's potentiation of barbital-induced sleep (Table 3). Earlier studies have related prolongation of barbital hypnosis to pentobarbital metabolic inhibition or action on the CNS involved in the regulation of sleep [19, 20].

The result of the exploratory behaviour test (Table 4) further supports the neurosedative activity and its possible application in anxiety condition [13].

Table 4
Effects of methanol extract of *C.singueana* on exploratory behaviour pattern in mice

		Mean head-dips in 5 min[a]		
Treatment	**Dose**	**Predose**	**30 min**	**60 min**
Saline	10ml/kg, p.o.	75.5 ± 3.14	58.21 ± 4.18	34.23 ± 2.23
C.singueana	100mg/kg, p.o.	81.23 ± 5.14	46.95 ± 2.71	18.21 ± 1.74
	200mg/kg, p.o.	77.54 ± 3.1	43.32 ± 4.10	9.42 ± 3.14

a Values are mean ± SEM
* Significantly different F [(2,15) = 4.02; $p < 0.05$]

It is evident therefore, from the above studies that the methanol extract of *Cassia singueana* contain agent with sedative/anxiolytic properties, which merits further investigation.

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