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Anxiogenic-like Effects of a Root Extract of *Sphenocentrum jollyanum* Pierre in Murine Behavioural Models

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Abstract: This study has characterized the effect of an ethanolic extract of roots of *Sphenocentrum jollyanum* (SJE) which are chewed or taken in alcoholic bitters in Ghana for its stimulant effect on the CNS and as an aphrodisiac agent. Four widely used animal models of anxiety: the open field test, elevated plus maze, hole-board and light/dark box were employed. Results were compared qualitatively to those obtained for diazepam and caffeine which served as anxiolytic and anxiogenic drugs, respectively. Acute administration of SJE (100-1000 mg kg^{-1}, p.o.) exhibited anxiety-like effects dose-dependently, which were qualitatively similar to those induced by caffeine (10-100 mg kg^{-1}). Both drugs decreased the number of entries and time spent on the open arms of the elevated-plus maze and increased the number of visits to the corners of the open field. In addition, SJE decreased the number and duration of head dips compared to vehicle-treated mice. Also, the extract exhibited anxiogenic properties in hole-board and light/dark box by significantly decreasing the number of head-dips and the time spent in the dark portion of the light/dark box, respectively. In contrast, diazepam (0.1-1 mg kg^{-1}) exhibited a typical profile of an anxiolytic drug. At all doses tested, SJE produced no motor deficits in animals using the rotarod test but decreased spontaneous locomotor activity in the activity cage apparatus. In conclusion, the results indicate that the root extract of *S. jollyanum* has anxiogenic like effects in mice and thus supports the use of the plant in traditional medicine.

Key words: Anxiety, elevated plus-maze, open field, hole-board, light/dark box, caffeine, diazepam

INTRODUCTION

Sphenocentrum jollyanum Pierre (family Menispermaceae) popularly known as *Aduro kokoo* (red medicine) or *Okramankote* (dog's penis) in the Akan language of Ghana is a small erect sparsely branched shrub which grows up to 1.5 m in height. Different parts of the plant have been used extensively for the treatment of various ailments in the West African sub-region. Extracts from the root

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have been used for the relief of constipation, as stomachic, as a cough medicine, for sickle cell disease, rheumatism, aphrodisiac and other inflammatory conditions (Burkill, 1985; Iwu, 1993; Moody *et al*., 2006). Decoctions prepared from the fruits, together with the fruits of *Piper guineense* and lime juice, are used for the relief of cough. The plant is reputed to possess exceptional wound healing properties, (Raji *et al*., 2006). The fruits are used as an antifatigue snack (Raji *et al*., 2006). It is also perceived to have unusual haemostatic and stomachic properties as well as an emetic for poisonings by traditional medical practitioners in the Ivory Coast (Abbiw, 1990; Irvine, 1961).

Some scientific study has been done on this plant in relation to its antiviral and anti-inflammatory activities (Moody *et al*., 2006), anti-oxidant and anti-angiogenic property (Nia *et al*., 2004), aphrodisiac property (Owiredu *et al*., 2007) and also, Raji *et al*. (2006), have shown that the methanolic extract of the root of *S. jollyanum* increased the testosterone levels in a dose-dependent manner and also reduced the count, motility and viability of spermatozoa in albino rats.

Since, the roots of *S. jollyanum* are chewed as a stimulant, the primary objective of the present study was to assess the neuropharmacological actions of an ethanolic extract of the roots of *S. jollyanum* in animal models of anxiety. Comparable data for caffeine, an anxiogenic and diazepam an anxiolytic, obtained under the same experimental conditions were also obtained.

MATERIALS AND METHODS

Plant Material

The sun-dried roots of the *S. jollyanum* Pierre (family Menispermaceae) were bought from the Central Market, Kumasi, Ashanti Region, Ghana and identified by Dr. T.C. Fleischer, Department of Pharmacognosy, KNUST, Kumasi, Ghana and a voucher sample was deposited at the Department. This study was conducted between January and April, 2007 at the Department of Pharmacology, KNUST, Kumasi, Ghana.

Preparation of the Root Extract

The roots were pulverized with a hammer-mill to obtain a coarse powder and 5 kg of the powder was extracted with 70% (v/v) ethanol in a Soxhlet apparatus for 24 h. Using a vacuum rotary evaporator, the hydro-alcoholic filtrate was concentrated under reduced pressure to obtain a yellowish-brown syrupy mass which was then air-dried at room temperature (28°C) for 36 h. The yield was 478 g (9.56%). This was kept in a dessicator at room temperature and is subsequently referred to as extract or *Sphenocentrum jollyanum* extract (SJE).

Phytochemical Screening

The methods of Trease and Evans (1989) were used for the detection of phytochemicals in the ethanolic extract of the plant.

Animals

ICR mice (25-35 g; 2-3 months old) were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Accra and housed at the animal facility of the Department of Pharmacology, KNUST, Kumasi, Ghana. The animals were housed in groups of 6 in stainless steel cages (34×47×18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions. All animals used in these studies were treated in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services Publication No. 85-23, revised 1985) and the study was approved by the Faculty Ethics Committee.

Drugs and Chemicals

Diazepam and caffeine hydrochloride were obtained from Sigma Chemicals (St. Louis, MO, USA).

Spontaneous Locomotor Activity

Spontaneous locomotor activity was quantitatively estimated in an automated activity cage (Model 7401, Ugo Basile, Milan, Italy; dimensions 19×23×35 cm^3). The movement of a mouse inside the box was detected by 29 stainless steel bars placed 1 cm apart on the floor of the cage. Activity for each mouse was counted automatically for 5 min.

Open-Field Test

The test was based on that described earlier by Erdogan *et al*. (2004) and Kasture *et al*. (2002). Testing was conducted in clear Plexiglas boxes (40×40×30 cm) whose floor was divided into 16 equal squares by black lines. For behavioral analysis, the arena of the open field was designated as (i) corner (one of the four corner squares), (ii) periphery (the squares along the walls), or (iii) center (the four inner squares). The animals were divided into ten groups of six animals each and received either the extracts (100, 300 or 1000 mg kg^{-1}, p.o.), the vehicle or the standard reference drugs diazepam (0.1, 0.3 or 1 mg kg^{-1}, i.p.) or caffeine (10, 30 or 100 mg kg^{-1}, p.o.). Thirty minutes after i.p. and 1 h after oral administration of the test compound, mice were placed individually in the centre of the open field and allowed to explore freely for 5 min. Each session was recorded by a video camera suspended approximately 100 cm above the arena. After each session, the number of fecal pellets was noted in the open-field arena. All animals were regularly handled before individual tests in order to minimize handling-related stress. Videotapes of the arena and the following variables of motor activity were recorded: locomotor activity, fine movement and rearing. Furthermore, distance traveled, total time, rest time, number of entries and head pokes in individual zones were also recorded. The exploration activity in the open field was determined as distance covered of horizontal movements and vertical (rearings) explorations. Thereafter, behaviour in the open field was analyzed for 5 min. Mean±Standard Error of the Mean (SEM) were calculated for each and compared to vehicle-treated animals.

To compute total distances travelled by the mice, the software Behavior Collect (http://cas.bellarmine.edu/tietjen/DownLoads/computer_programs_for_data_colle.htm) was used to obtain raw XY data from the videos. These data were then exported into Microsoft® Office Excel 2007 and further analyzed. Distance between two X-Y coordinate pairs was calculated from the formula:

$$\sqrt{[(X_1 - X_2)^2 + (Y_1 - Y_2)^2]}$$

Behavioural parameters for all the tests were scored from videotapes with the aid of a computer program, Behavior Tracker Version 1.5 (http://www.behaviortracker.com/).

In this experiment and subsequent ones, the apparatus was cleaned between each session with 70% w/v ethanol to preclude possible cueing effects of odors left by previous animals (Phillips, 1982).

Elevated Plus-Maze Test

The method used was as described for rats by Pellow *et al*. (1985) with some modifications. The elevated plus maze was made from opaque Plexiglas. It consisted of two opposite open arms (15×5 cm) without side walls and two enclosed arms (15×5×30 cm), extending from a central square platform (5×5 cm^2). A rim of Plexiglas (0.5 cm in height) surrounded the perimeter of the open arms to provide additional grip and thus prevent the mice from falling off (Rodgers and Johnson, 1995). The maze was elevated to the height of 80 cm from the floor and placed in a lit room (~750 lux). The animals were divided into ten groups of six animals each and received treatments similar to that

described for the open field test. Animals were placed individually in the central platform of the EPM for 5 min and their behavior recorded on a videotape with a digital camera placed 100 cm above the maze. Behavioural parameters were scored from the videotapes as follows: (1) number of closed and open arm entries-(absolute value and percentage of the total number), (2) time spent in exploring the open and closed arms of the maze- absolute time and percentage of the total time of testing, (3) number of head-dips- protruding the head over the ledge of an open arm and down toward the floor, (4) number of stretch-attend postures-the rat stretches forward and retracts to original position, (5) number of nose pokes, (6) number of grooming and (7) number of rearing. An arm entry was counted only when all four limbs of the rat were within a given arm.

Hole-Board Test

This test was based essentially on that described by File and Wardill (1975). The hole-board apparatus consisted of a square Plexiglas chamber (30×30×30 cm^3) with four holes (2.5 cm diameter) each situated 5 cm from each of the corners, elevated 5 cm from the ground so that the mice could peep through the holes. The animals were grouped and treated as described above for the open field and EPM experiments. Each mouse was placed individually in the center of the field and videotaped for 5 min. The number of times the mouse dipped its head in a hole and the duration were determined from the videotapes as described earlier.

Light/Dark Test

The light-dark exploration test is typically used to more directly assess anxiety-related responses. This apparatus was based on the initial model described by Crawley (1981) and as modified by Belzung and Pape (1994) and Belzung *et al.* (1987). It consists of wooden boxes (45 cm long × 30 cm wide × 30 cm deep), which are divided into two equal compartments by a wooden board with a 7×7 cm^2 opening located centrally at the floor level, connecting the compartments. One compartment was painted black and covered with a wooden lid. The other box (not covered) was painted white and lit by a 60 W light bulb set 30 cm above the box. Mice were grouped and treated with drugs as described for the other behavioral tests described above. At the beginning of the experiment, mice were placed individually in the center of the illuminated box, facing the opening away from the dark compartment. Behaviors of the animals were recorded for 5 min with a digital camera placed 1 m above the box. Videotapes were scored manually with the aid of a computer program, Behavior Tracker Version 1.5 for the following parameters: (1) initial latency to enter dark compartment, (2) frequency of compartment entries, (3) total time spent by mice in each compartment and (4) total number of transitions between compartments.

Rotarod Test

The effect on motor co-ordination was assessed using rotarod apparatus (Model 7600, Ugo Basile, Cormerio, Italy) (Ribeiro *et al.*, 2002) rotating at a speed of 12 rpm. This apparatus consists of a base platform and a rotating rod of 3 cm diameter with a non-skid surface. The rod, 50 cm in length, is divided into five equal sections by six disks. Five mice were tested simultaneously. The mice were placed individually on the cylinder. Before the start of the experiment, animals were trained to stay on the rotarod for 300 sec. Mice that failed to learn the test or did not reach the criterion (300 sec endurance) were excluded from the study. On the test day, latencies to fall from the rod were measured after administration of the test compounds or vehicle.

Statistical Analysis

In all experiments, a sample size of six (n = 6) was utilized. All data are presented as Mean±SEM. To compare differences between groups, one-way ANOVA was performed with

Newman-Keuls's test as post hoc. Also, the behavioural data from the light/dark box maze were analyzed using two-way Analysis of Variance (ANOVA) with groups as a between-subject factor and compartment (light vs. dark) as a within-subject factor followed Bonferroni's as post hoc. GraphPad Prism for Windows Version 4.03 (GraphPad Software, San Diego, CA, USA) was used for all statistical analysis and ED_{50} determinations. $p<0.05$ was considered statistically significant.

RESULTS

Phytochemical Analysis

The phytochemical analysis of SJE showed the presence of terpenoids, flavonoids and alkaloids, with alkaloid as the most dominant chemical constituent.

Spontaneous Locomotor Activity

Activity of the mice treated with caffeine and SJE were significantly decreased as compared to control animals in a dose dependent manner [$F_{3,31} = 27.96$, $p<0.0001$ and $F_{3,31} = 14.26$, $p<0.0001$, respectively]. Though spontaneous locomotor activity of the mice treated with diazepam was lower than the control group, there was gradual increase in activity with increasing dose [$F_{3,31} = 13.05$, $p<0.0001$] (Fig. 1a-c).

Open Field Test

Acute administration of SJE (100-1000 mg kg^{-1}; p.o.) to mice significantly decreased the percentage visits to the center [$F_{3,31} = 7.132$, $p = 0.0008$] (Fig. 2a) as well as the percentage time spent in the center [$F_{3,31} = 7.357$, $p = 0.0007$] (Fig. 2c) in a dose-dependent manner. Caffeine-treatment (10-100 mg kg^{-1}; p.o.), similarly decreased the percentage visits to the center [$F_{3,31} = 5.334$, $p = 0.0044$] (Fig. 2b) and the percentage time spent in the central portion of the field [$F_{3,31} = 3.670$, $p = 0.0226$] (Fig. 2d). Further analysis of the data using 2-way ANOVA (treatment×zone) revealed significant effect of type of zone visited i.e., corner, peripheral or center. Post hoc analysis (Bonferroni's test) revealed significantly less visits to the center when compared with visits to the corner for the SJE-treated groups [$F_{2,96} = 1058.24$, $p<0.0001$] (Fig. 2a) and the caffeine-treated groups [$F_{2,96} = 1058.24$, $p<0.0001$] (Fig. 2b).

In contrast to SJE and caffeine, diazepam increased significantly the number and percentage of visits to the center of the open field [$F_{3,30} = 3.38$, $p<0.039$ and $F_{3,30} = 22.73$, $p<0.0001$, respectively] (Table 1). Furthermore, treatment of mice with diazepam decreased the percentage of visits to the

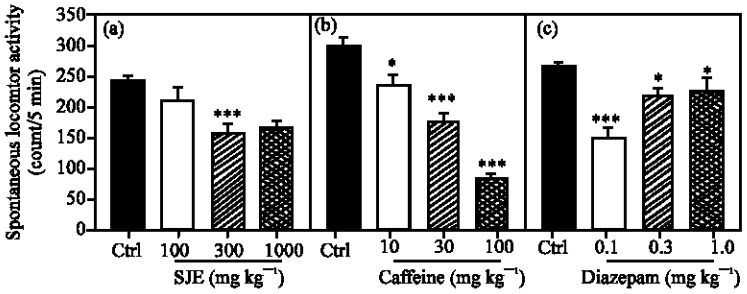

Fig. 1: Effects of acute (a) SJE (100, 300 and 1000 mg kg^{-1}), (b) caffeine (10, 30 and 100.0 mg kg^{-1}) and (c) diazepam (0.1, 0.3 and 1.0 mg kg^{-1}), all 30 min pretest on spontaneous locomotor activity in the activity cage. Data are presented as group Mean±SEM. Significantly different from control (Ctrl): *$p<0.05$, ***$p<0.001$ by Newman-Keuls test

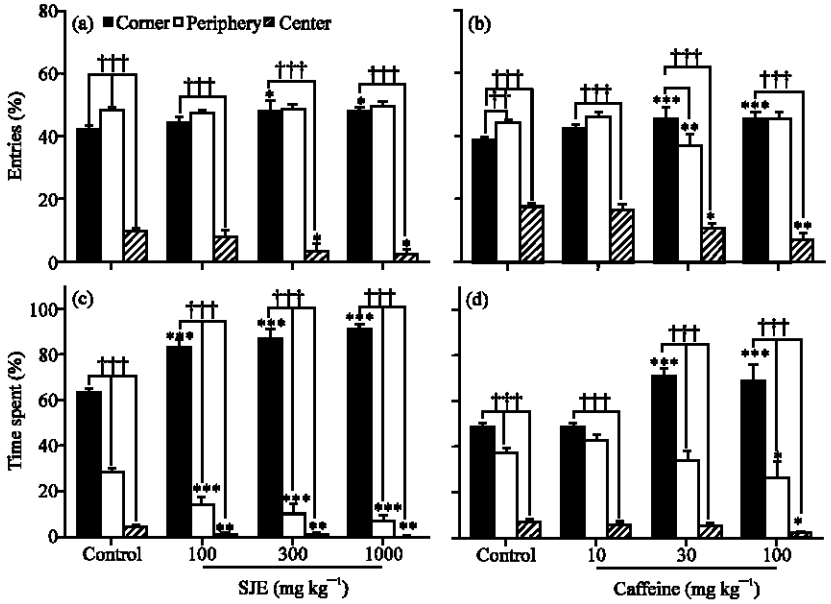

Fig. 2: Effects of acute SJE (100, 300 and 1000 mg kg^{-1}) and caffeine (10, 30 and 100 mg kg^{-1}) 30 min pretest on the % entries for SJE (a), caffeine (b) and % time spent for SJE (c), caffeine (d) in the open field test. Data are presented as group Mean±SEM. Significantly different from control: *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test and significant difference when the zonal entries where compared to each other: †p<0.05, ††p<0.01, †††p<0.001 (two-way repeated measures ANOVA followed by Bonferroni's post hoc)

Table 1: Effects of acute diazepam administration for 30 min pretest on the parameters of open field

		Diazepam (mg kg^{-1})			
Parameters	Vehicle	0.1	0.3	1.0	p-value
Entry into zones (%)					
Corner	47.37±0.96	40.26±0.28***	36.73±0.91***	37.25±0.16***	<0.0001
Periphery	43.51±0.64	48.62±1.57**	44.42±0.60	38.23±0.98**	<0.0001
Center	9.12±1.58	11.12±1.83	18.86±1.50***	24.52±0.90***	<0.0001
Time spent in zones (%)					
Corner	63.11±2.84	52.89±3.28*	48.22±3.47**	44.61±2.89**	0.0029
Periphery	31.39±4.45	38.50±5.35	42.33±5.91	43.83±5.14	0.3623
Center	4.20±1.15	9.87±2.00	10.93±2.37	12.93±2.19*	0.038

Data are presented as group Mean±SEM; Significantly different from control; *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test

corners [$F_{3,30}$ = 6.556, p<0.0029] and though there was a dose-dependent decrease in the time spent in the corners, the decrease did not reach statistical significance [$F_{3,30}$ = 2.699, p<0.0732]. SJE (100-1000 mg kg^{-1}, p.o.) and caffeine (10-100 mg kg^{-1}, i.p.) dose-dependently decreased the locomotor activity [$F_{3,31}$ = 27.29, p<0.0001 and $F_{3,31}$ = 41.42, p<0.0001, respectively], which was evident in the decreases in the distance travelled in the arena (Fig. 3). Diazepam, on the other hand, increased locomotor activity in the open field test [$F_{3,31}$ = 1059, p<0.0001]. Overall exposure to SJE and caffeine made the mice highly thigmotactic, spending most of the time along the walls (Fig. 3).

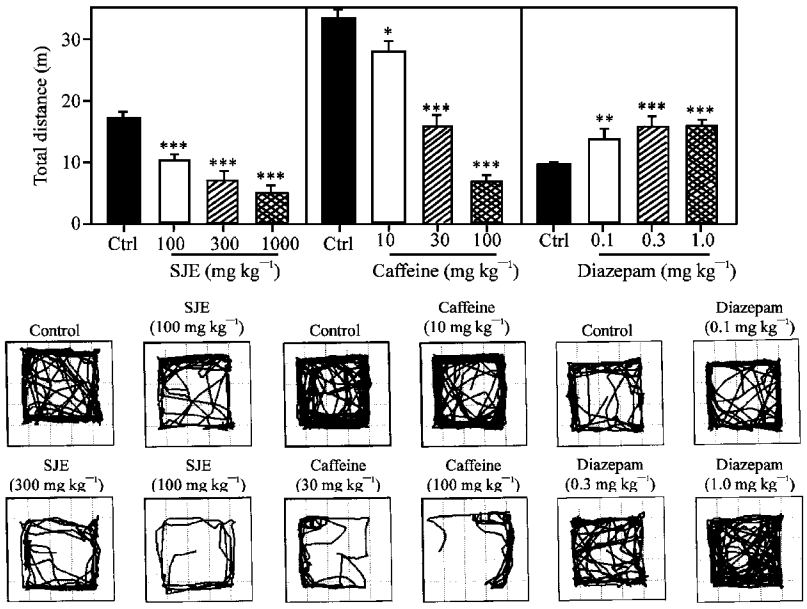

Fig. 3: Effects of acute SJE (100, 300 and 1000 mg kg^{-1}), caffeine (10, 30 and 100 mg kg^{-})^{1}and diazepam (0.1, 0.3 and 1.0 mg kg^{-1}), all 30 min pretest on total distance travelled in the open field test. Data are presented as group Mean±SEM. Significantly different from control (Ctrl): *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test. Line plots (lower panels) 3D plots were generated from the time and XY data obtained using SigmaPlot Version 10 (Systat Software Inc., Point Richmond, CA, USA)

Elevated Plus Maze

Results obtained in the elevated plus maze test have been presented as spatio-temporal and ethological measures of anxiety. Figure 4 shows the effect of SJE on the spatio-temporal measures of anxiety. Administration of SJE significantly increased anxiety in mice. A one-way ANOVA showed that there was a significant decrease [$F_{3,38} = 5.67$; $p = 0.0026$] in the absolute number of open-arm entries compared to vehicle-treated mice (Fig. 4a). The decrease was dose-dependent and achieved statistical significance ($p<0.5$ and 0.01; Neuman-Keuls test) at doses of 300 and 1000 mg kg^{-1}. However, when entries into the arms are expressed as percentage open-arm entries, a significant decrease is achieved only at a dose of 1000 mg kg^{-1} (Fig. 4b). Two-way ANOVA (treatment group×arm type (open or close) revealed a significant arm-type effect [$F_{1,76} = 35.54$; $p<0.0001$]. Post hoc analysis (Bonferroni's test) revealed that all the treatment groups, except the 300 mg kg^{-1} group, made significantly less entries into the open-arm when compared with entries into the closed arm (Fig. 4a). Also, the administration of SJE significantly decreased [$F_{3,38} = 5.83$; $p = 0.0022$] both the absolute time spent in the open-arms (Fig. 4c) and the percentage of time spent in the open-arm (Fig. 4d). Furthermore, two-way ANOVA revealed a significant arm type effect [$F_{1,76} = 439.4$; $p<0.0001$] with the post hoc analysis revealing that all the treatment groups spent significantly less time in the open-arm when compared to the time spent in the closed arm (Fig. 4c). For the ethological measures (Fig. 5), SJE caused significant effect on the frequencies of stretch attends [$F_{3,38} = 8.41$; $p = 0.0002$] (Fig. 5a), nose pokes [$F_{3,38} = 27.98$; $p<0.0001$] (Fig. 5b), head dips [$F_{3,38} = 2.87$; $p = 0.0488$] (Fig. 5c) and rearings [$F_{3,38} = 9.54$; $p = 0.0001$] (Fig. 5d).

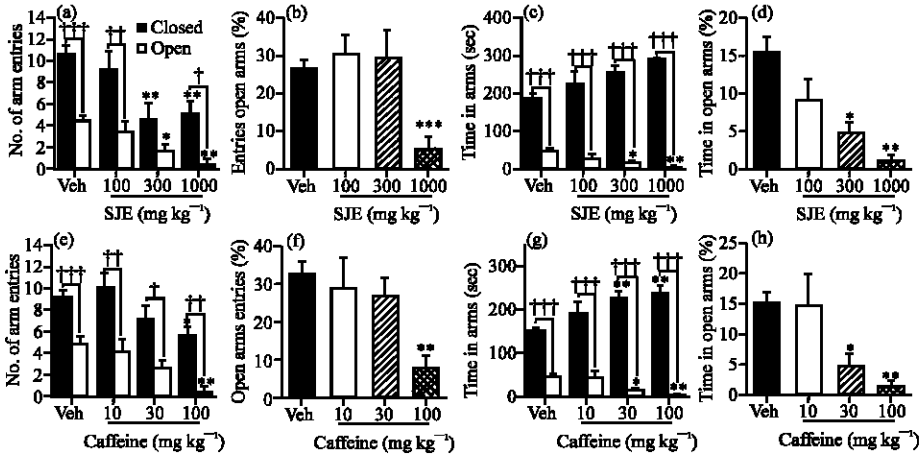

Fig. 4: Effects of acute SJE (100, 300 and 1000 mg kg^{-1}) 30 min pretest on number of arm entries (a), % entries open arm (b), time in arms (c) and % time open arm (d) and acute caffeine (10, 30 and 100 mg kg^{-1}) 30 min pretest on number of arm entries (e), % entries open arm (f), time in arms (g) and % time open arm (h) in the EPM. Data are presented as group Mean±SEM.Significantly different from control: *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test and significant difference when open arm and closed arm where compared: †p<0.05, ††p<0.01, †††p<0.001 (two-way repeated measures ANOVA followed by Bonferroni's post hoc)

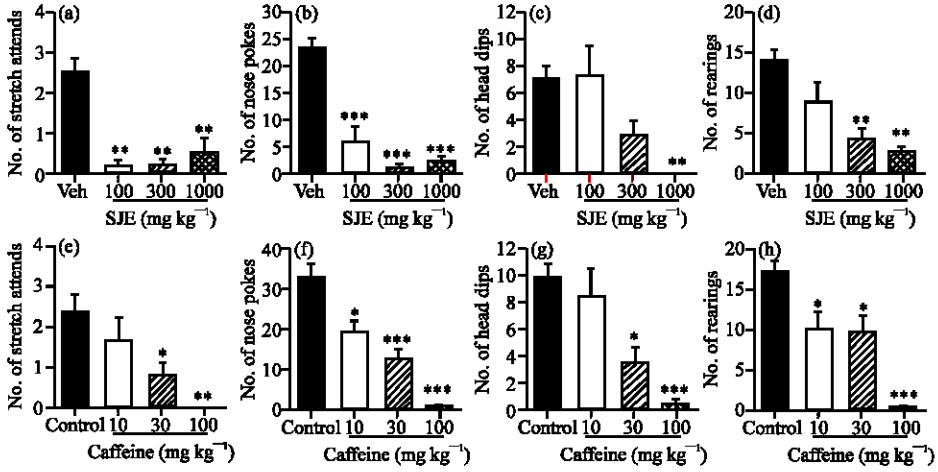

Fig. 5: Effects of acute SJE (100, 300 and 1000 mg kg^{-1}) 30 min pretest on number of stretch attend (a), number of nose poke (b), number of head dips (c) and number of rearing (d) and acute caffeine (10, 30 and 100 mg kg^{-1}) 30 min pretest on number of stretch attend (e), number of nose poke (f), number of head dips (g) and number of rearing (h) in the EPM. Data are presented as group Mean±SEM. Significantly different from control: **p<0.01, ***p<0.001 by Newman-Keuls test

In comparison, treatment of mice with the anxiogenic drug, caffeine produced effects that were qualitatively similar to SJE in both the spatio-temporal and ethological measure of anxiety (Fig. 4, 5).

Table 2: Effects of acute diazepam administration for 30 min pretest on the parameters of elevated plus maze test

		Diazepam (mg kg^{-1})			
Parameters	Vehicle	0.1	0.3	1.0	p-value
Arm entries					
Closed arm	19.17±1.97	27.83±2.76*	33.33±2.43**	33.50±3.53**	0.0042
Open arm	2.83±0.60	5.67±1.31	7.83±1.05	13.67±2.36***	0.0004
Open arm entry	23.90±3.15	32.46±4.39	47.90±3.72**	56.65±6.70***	<0.0001
Time spent in arms					
Closed arm	173.30±15.58	159.70±23.47	81.83±16.35**	70.67±10.43**	0.0004
Open arm	36.83±9.57	72.60±15.97	97.00±17.15*	145.70±10.31***	0.0001
Time in open arm (%)	9.50±1.84	20.39±5.78	32.33±5.72**	48.56±3.45***	<0.0001
Other parameters					
Head dip	8.00±0.83	9.83±1.42	15.00±1.32***	15.83±1.47***	<0.0001
Rearing	16.56±2.05	10.83±2.94	8.67±1.20	7.17±1.38*	0.0220
Grooming	1.67±0.33	2.33±0.42	0.17±0.17**	0.67±0.21*	0.0002
Stretch attend	4.50±0.62	1.33±0.72**	1.83±0.70*	0.00±0.00***	0.0003

Data are presented as group Mean±SEM; Significantly different from control; *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test

Caffeine caused a dose-dependent decrease in the frequency of entries [$F_{3,32} = 4.46$; $p = 0.01$] and the percentage entries [$F_{3,32} = 9.54$; $p = 0.012$] into the open-arms (Fig. 4e, f). Similarly, caffeine also significantly decreased the time spent [$F_{3,32} = 5.85$; $p = 0.0026$] as well as the percentage of total time spent [$F_{3,32} = 5.89$; $p = 0.0026$] in the open arms (Fig. 4g , h) Also, all ethological measures of anxiety were decreased by caffeine treatment (Fig. 5e-h). In contrast to SJE and caffeine, diazepam decreased anxiety in mice. Diazepam increased dose-dependently the frequency of entries as well as the percentage of total entries into the open arms (Table 2). Furthermore, diazepam also increased both the time spent and percentage of total time spent in the open arms. Surprisingly, almost all the ethological measures were decreased by diazepam with the exception of the effect on head dips which were increased.

In terms of horizontal exploration, SJE and caffeine caused significant ($F_{3,32} = 41.42$, $p<0.0001$ and $F_{3,32} = 30.23$, $p<0.0001$, respectively) reductions in locomotor activities in a dose-dependent manner, as reflected in the total distance traveled on the maze as a whole. Total distances travelled in meters by the mice in the SJE group were as: 9.44±0.64, 7.69±1.52, 3.87±1.39 and 3.57±0.94, respectively for control, 100, 300 and 1000 mg kg^{-1}. Distances for the caffeine groups were as 11.67+0.62, 12.27±1.24, 7.31±1.54 and 3.56±0.40, respectively for control, 10, 30 and 100 mg kg^{-1} treatment groups. Diazepam, in contrast, significantly ($F_{3,32} = 10.03$, $p<0.0001$) increased locomotor activity in a dose-dependent manner: 10.27±0.34, 12.12±1.07, 12.78 and 14.97, respectively for control, 0.1, 0.3 and 1 mg kg^{-1} treatment groups.

Hole-Board Test

Figure 6 shows results obtained in the hole-board test. Acute administration of SJE (100-1000 mg kg^{-1}, p.o.) significantly reduced the number and duration of head dips (Fig. 6a; $F_{3,20} = 14.48$, $p<0.0001$ and Fig. 6e; $F_{3,20} = 16.14$, $p<0.0001$) and as well as locomotor activity (total distances travelled) in mice ($F_{3,20} = 7.60$, $p = 0.014$; data not shown). Though, the administration of caffeine did not have any significant effect on the number of head dips (Fig. 6b; $F_{3,20} = 0.70$, $p<0.07$) and locomotor activity, as measured by total distance travelled ($F_{3,19} = 1.82$, $p = 0.17$; data not shown), the duration of head dipping was significantly decreased in a dose-dependent manner (Fig. 6f; $F_{3,18} = 9.88$, $p = 0.0005$).

In contrast, diazepam caused a slight but not significant increase in the number of head dips (Fig. 6c; $F_{3,20} = 1.91$, $p = 0.16$) and a significant increase in the duration of head dips (Fig. 6d; $F_{3,20} = 0.70$, $p<0.07$) as well as the locomotor activity ($F_{3,20} = 7.47$, $p = 0.0015$; data not shown).

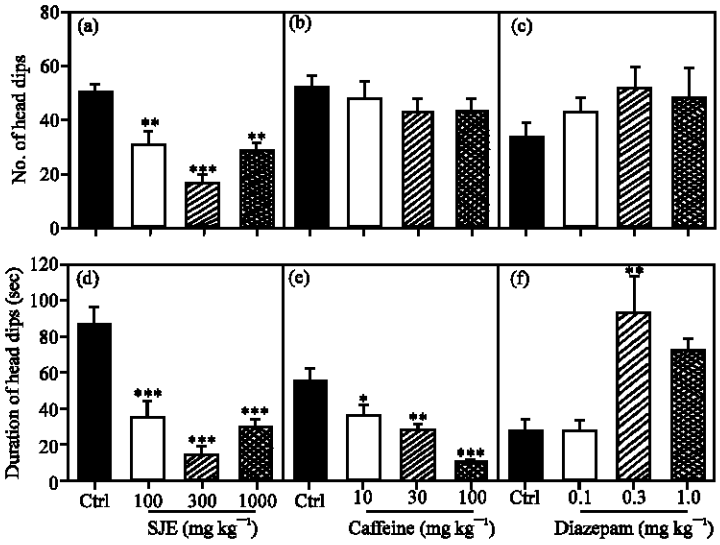

Fig. 6: Effects of acute SJE (100, 300 and 1000 mg kg^{-1}), caffeine (10, 30 and 100 mg kg^{-1}) and diazepam (0.1, 0.3 and 1.0 mg kg^{-1}), all 30 min pretest on the (a-c) number of head dips and (d-f) duration of head dipping in the hole board test. Data are presented as group Mean±SEM. Significantly different from control (Ctrl): *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test

Light/Dark Box Test

Results for the light/dark test are shown in Fig. 7. SJE (100-1000 mg kg^{-1} p.o.) decreased significantly [$F_{3,20} = 6.424$; $p = 0.0032$] the latency to enter the dark box in a dose-dependent manner (Fig. 7a). A similar decrease was observed in caffeine-treated mice [$F_{3,20} = 6.523$; $p = 0.0030$; Fig. 7b] and in diazepam-treated mice [$F_{3,20} = 15.52$; $p<0.0001$; Fig. 7c]; the effects of diazepam was bell-shaped. However, with regards to other measures, SJE (100-1000 mg kg^{-1}; p.o.) induced anxiety-like effects by significantly decreasing dose-dependently the time spent in the lit box [$F_{3,20} = 4.461$; $p = 0.0149$] and increasing the time spent in the dark box [$F_{3,20} = 4.461$; $p = 0.0149$]; (Fig. 7d). Two-way ANOVA (treatment group×box type (lit or dark) revealed a significant box type effect [$F_{1,30} = 298.07$; $p<0.0001$]. Post hoc analysis (Bonferroni's test) revealed that all the treatment groups, spent significantly less time in the lit box compared with time spent in the dark box. There were no significant changes in the number of entries into the light [$F_{3,20} = 1.499$; $p = 0.2454$] and dark box [$F_{3,20} = 2.092$; $p = 0.1334$; Fig. 7g] as well as the frequency of transition between compartments.

Similarly, acute administration of caffeine (10-100 mg kg^{-1}; i.p.) induced anxiety-like effects (Fig. 7e). Caffeine significantly decreased [$F_{3,20} = 4.839$; $p = 0.0108$] the time spent by mice in the lit box and increased significantly [$F_{3,20} = 4.839$; $p = 0.0108$] the time spent in the dark box. Furthermore, two-way ANOVA revealed a significant arm type effect [$F_{1,30} = 250.99$; $p<0.0001$] with the post hoc analysis revealing that all the treatment groups spent significantly less time in the lit box compared to time spent in the dark box. However, unlike SJE, treatment with caffeine significantly increased the frequencies of entry into both the lit [$F_{3,20} = 6.310$; $p = 0.0035$] and dark [$F_{3,20} = 4.373$; $p = 0.0160$] boxes compared to control (Fig. 7h). Also caffeine increased significantly [$F_{3,20} = 4.307$; $p = 0.0169$] the frequency of transition between the compartments.

In contrast to SJE and caffeine, diazepam (0.1-1.0 mg kg^{-1}, i.p.) induced anxiolytic-related behaviors in the light/dark test (Fig. 7f). Diazepam significantly increased the duration of time spent

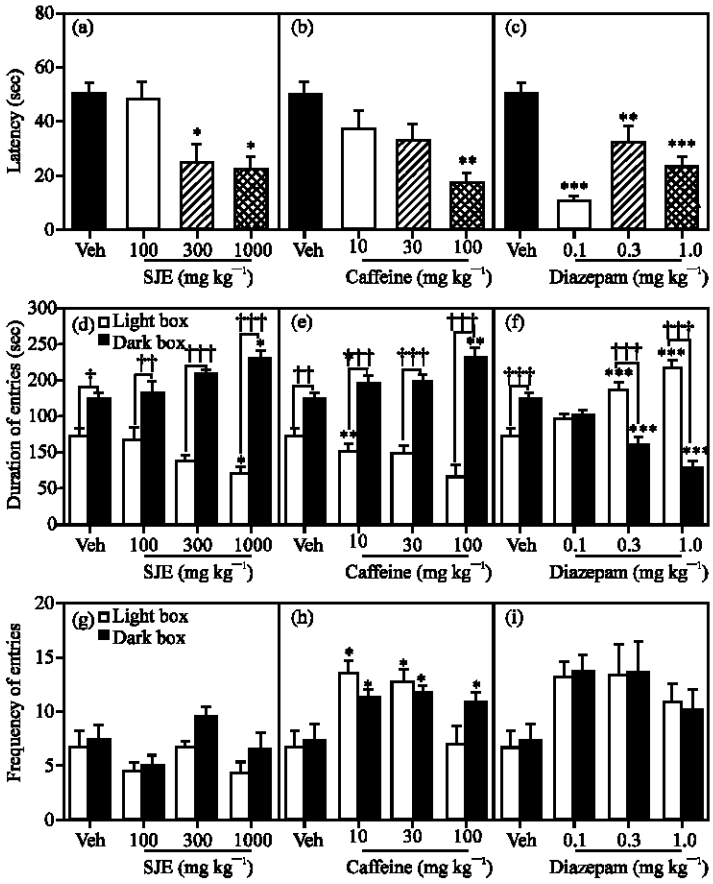

Fig. 7: Effects of acute SJE (100, 300 and 1000 mg kg^{-1}), caffeine (10, 30 and 100 mg kg^{-1}) and diazepam (0.1, 0.3 and 1.0 mg kg^{-1}), 30 min pretest in the light/dark test. (a-c) Latency(s), (d-f) Duration of entires and (g-i) Frequncy pf entires. Data are presented as group Mean±SEM. Significantly different from control (Ctrl): *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test and significant difference when open compartment and closed compartment where compared: †p<0.05, ††p<0.01, †††p<0.001 (two-way repeated measures ANOVA followed by Bonferroni's post hoc)

in the lit box [$F_{3,20} = 26.73$; $p<0.0001$] and decreased the time spent in the dark box [$F_{3,20} = 26.73$; $p<0.0001$]. Two-way ANOVA revealed a significant arm type effect [$F_{1,30} = 65.13$; $p<0.0001$] with the post hoc analysis revealing that the two high dose-groups (0.3 and 1 mg kg^{-1}) spent significantly more time in the lit box compared to time spent in the dark box. There was no effect at the lowest dose of 0.1 mg kg^{-1}. Though, there were increases in the frequencies of entry into the light and dark boxes (Fig. 7i) and the transition between compartments, the increases did not reach statistical significance.

Effects on Motor Coordination in Rota-Rod

At all doses tested SJE (100-1000 mg kg^{-1}) did not alter the performance of trained mice in comparison to vehicle treated animals. Similar results were obtained for caffeine (10-100 mg kg^{-1}) and diazepam (0.1-1 mg kg^{-1}).

DISCUSSION

Results of the present study show that an ethanolic extract of the roots of *S. jollyanum* induce anxiety-like behaviors in murine models of anxiety. Roots of *S. jollyanum* are chewed in Ghana and elsewhere in the West African sub-region for its aphrodisiac and central stimulatory activity. Behavioral models used in the study are based on unconditioned responses to stimuli which are thought to be indicative of human generalized anxiety symptoms (Crawley, 1999; Ohl, 2005; Rodgers *et al*., 1997).

In this study, SJE decreased both the frequency and duration of open-arm exploration in the EPM indicative of an anxiety-like state. The EPM test is based on two conflicting innate tendencies; the rodent's drive to explore a novel environment and it's aversion to heights and brightly-lit open spaces (Borsini *et al*., 2002; Gordon and Hen, 2004; Lister, 1987; Trullas and Skolmick, 1993). This test has been demonstrated to be bi-directionally sensitive to both anxiolytic drugs; in particular benzodiazepines (Handley and Mithani, 1984; Lister, 1987; Pellow *et al*., 1985), as well as compounds which induce anxiety in man (Lister, 1987; Pellow *et al*., 1985; Pellow and File, 1986). In agreement with other researchers such as Bhattacharya *et al*. (1997) and El Yacoubi *et al*. (2000), caffeine induced behaviors typical of an anxiogenic drug. Caffeine decreased in a dose-dependent manner, the number of entries in the open arm and decreased locomotor activity. In contrast, diazepam caused anxiolysis in the mice as earlier reported by Borsini *et al*. (2002) by causing an increase in the number of entries and percentage time spent in the open arm.

In addition to using the spatio-temporal indicators of anxiety in the EPM, ethological measures of risk assessment, such as stretched-attend postures, nose-pokes, rearing and head-dipping, which have been validated and shown by factor analysis to be a more predictive determinant of anxiety (Rodgers *et al*., 1997; Rodgers and Johnson, 1995) was also used. In agreement with its anxiogenic properties in mice, SJE like caffeine decreased dose-dependently all ethological measures of anxiety. However, with diazepam, in terms of the ethological measures, only head dipping showed anxiolysis. Cruz *et al*. (1994) and Griebel *et al*. (1997) also reported on some of such inconsistencies with these ethological measures which are very dependent on species and dose. Other behavioral parameters such as number of fecal pellets and grooming were also measured but these were at best inconsistent and were therefore not included in the analysis.

The open-field model examines anxiety-related behavior characterized by the normal aversion of the animal to an open, brightly lit area (Asano, 1986; Choleris *et al*., 2001; Mechan *et al*., 2002). When placed into a brightly lit open field, rats and mice tend to remain in the periphery of the apparatus or against the walls (thigmotaxis). Benzodiazepines and anxiolytics increase exploration time in the center of the open field while anxiogenic drugs and stressful stimuli decrease the number of center crossings. Open field activity, therefore, represents a valid measure of anxiety-like behavior in drug-treated and genetically manipulated animals (Choleris *et al*., 2001; Prut and Belzung, 2003). SJE displayed anxiety-like behaviors in the open-field test. It is to be noted that in this experiment, SJE increased the percentage of corner entries, an index of anxiety and reduced the number of entries into the central portion of the arena. At the highest dose used, SJE induced positive thigmotaxis completely avoiding the central portion of the arena. Similarly, caffeine-treated mice also exhibited anxiety-like behaviors in the open field test. Diazepam, in contrast, induced behaviors in the open-field test in agreement with studies done by Wolfman *et al*. (1994) and Yasumatsu *et al*. (1994).

The hole-board test, introduced by Boissier *et al*. (1964) measured the response of an animal to an unfamiliar environment. This test has been used to assess emotionality, exploratory activity and anxiety (File and Wardill, 1986; Takeda *et al*., 1998). Results from earlier studies have shown that changes in head-dipping behavior reflect the anxiogenic and/or anxiolytic state of animals-head-dipping is decreased in the anxiogenic state and increased in an anxiolytic state (File and Wardill, 1986; Takeda *et al*., 1998). SJE decreased exploratory behavior (head dipping) in the hole-board test, a result

characteristic with the profile of anxiogenic drugs. Moreover, SJE also dose-dependently decreased locomotor activity as indicated by the total distance travelled. Caffeine has been reported to decrease locomotor activity (Bhattacharya *et al*., 1997; El-Yacoubi *et al*., 2000) with the possibility that it would decrease the animal's tendency of taking risk.

Furthermore, the effect of SJE on anxiety-related behavior was examined using the light/dark box. The apparatus is an arena divided into dark and brightly lit compartments and is an ethologically-based approach-avoidance conflict test which is sensitive to drugs that affect anxiety (Chaouloff *et al*., 1997; Costall *et al*., 1989; Crawley *et al*., 1997; Ohl, 2005). When given a choice between dark, enclosed areas and brightly lit open areas, rodents naturally tend to explore the dark, enclosed environment. Avoidance of brightly lit spaces is believed to reflect their aversive or anxiety-provoking properties. Anxiogenic agents decrease, while benzodiazepines and other anxiolytic drugs increase the duration of time spent in the light half of the chamber (Crawley, 1999; Kliethermes, 2005). In the light/dark test, SJE and caffeine decrease in a dose-dependent manner the total time spent in the light compartment. Decrease in the first time entry from the light half of the chamber to the black chamber (latency) reflects the aversive properties of the brightly lit area, an indication for anxiogenic effect (Crawley, 1999; Kliethermes, 2005). Surprisingly, all the drugs influenced this parameter in the same way. However, this effect has been said to be non-specific (Crawley, 1999; Kliethermes, 2005). Moreover, SJE-treated mice displayed a significant increase in the number of light/dark transitions which is also indicative of an anxiolytic effect. In contrast to this finding, caffeine has been reported to decrease the frequency of transitions in the light/dark box (El Yacoubi *et al*., 2000). As stated earlier by Belzung and Le Pape (1994) showed by component analysis that hyperactivity probably is a component of the expression of anxiolysis. In fact, in the present study, the anxiolytic drug diazepam displayed such an association by increasing the time spent in the light compartment as well as the number of transitions between the compartments. A possible explanation for the dissociation in the case of SJE and caffeine may be the evidence suggesting that similar rodent behavioral tests may measure different forms of anxiety-like behavior (Belzung and Le Pape, 1994; Van Gaalen and Streckler, 2000). Furthermore, it has also been shown that in EPM or the zero-maze, anxiolytics and psychostimulants increase open arm exploration and stimulate locomotor activity thus confounding the interpretation of results obtained using these models (Weiss *et al*., 1998). It must be noted however that caffeine may generally increase locomotor activity (Bhattacharya *et al*., 1997; El-Yacoubi *et al*., 2000).

An earlier study has demonstrated that SJE increased plasma levels of testosterone (Owiredu *et al*., 2007) confirming results obtained by Raji *et al*. (2006). It has been postulated that testosterone may increase dopamine (DA) release in the CNS by upregulating nitric oxide synthase, which produces nitric oxide, which in turn increases DA release (Du and Hull, 1999; Hull and Dominguez, 2007; Hull *et al*., 1999). Therefore, this may explain in part the CNS stimulant effect of the extract. However, it must be pointed out that several mechanisms and neurotransmitters such as dopaminergic, serotonergic, GABAergic and glutamatergic neurotransmissions may be involved in CNS stimulation and as such further experiments, may be necessary to confirm the exact mechanisms.

CONCLUSION

From the results, it is clear that the ethanolic extract of *S. jollyanum* roots has anxiogenic properties similar to that of caffeine in murine models of anxiety.

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