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Anxiolytic and sedative properties of hydroethanolic extract of *Telfairia occidentalis* leaves in mice

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Abstract: *Telfairia occidentalis* Hook. f., Cucurbitaceae, is a leafy vegetable used in soup and folk medicine in southern Nigeria. This study was conducted to investigate the anxiolytic and sedative activities of the hydroethanolic extract of the leaves of *T. occidentalis* in mice. The hole-board, elevated plus maze, open-field, light-dark, and social interaction tests were used in this study. *T. occidentalis* (50-400 mg/kg) and diazepam (1 mg/kg) were administered *p.o*. to different groups of mice and appropriate observations were made. *T. occidentalis* increased the number of sectional crossings ($p<0.01$) and duration of head dips ($p<0.05$) at doses of 50 and 100 mg/kg respectively; increased number of entries into open arms ($p<0.01$) at the dose of 100 mg/kg; increased number of central squares crossed ($p<0.01$) at the dose of 50 mg/kg; and increased number of social interactions ($p<0.001$) at doses of 50 and 100 mg/kg. At the dose of 400 mg/kg, *T. occidentalis* reduced number of head dips and sectional crossings ($p<0.01$); reduced time spent in open arms and increased time spent in closed arms ($p<0.01$, 0.001) at doses of 200 and 400 mg/kg; reduced number of assisted rearings ($p<0.001$) at doses of 200 and 400 mg/kg; increased latency of entry into and time spent in dark box ($p<0.01$, 0.001) at doses of 200 and 400 mg/kg; and reduced number of social interactions ($p<0.001$) at the dose of 400 mg/kg. The findings in this study suggest that *T. occidentalis* possess anxiolytic property at doses of 50 and 100 mg/kg, and sedative activity at doses of 200 and 400 mg/kg.

Article

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Introduction

Central Nervous System (CNS) disorders occupy a prominent place in modern therapy. It has been reported that 10% of all drugs classified in the US pharmacopoeia pertained to CNS related disorders (Gilman et al., 1991). Facts and Comparison (1990) has estimated that 14% of the total expenditure in the USA was on CNS disorders. Health problems warranting the use of anxiolytic and sedative drugs (anxiety, nervous tension, agitation and insomnia), antidepressants and antipsychotics are a common and costly problem worldwide. Drugs acting selectively to treat anxiety, mania, depression or schizophrenia without altering consciousness are finding increasing application in modern therapy. Cox (1994) calculated the frequency of CNS type diseases treated with drugs derived from ethnopharmacological sources to be as high as 20%. This is in view of the fact that over the years, plants have generally proven to be a veritable source of drugs used in orthodox medicine. This has in recent times encouraged the search for newer and better tolerated drugs from plants, especially for disease conditions offering great challenges to orthodox medicine in respect of therapeutic options, efficacy and safety profile.

A criterion that has been used over the years for the selection of plants for pharmacological investigations is reported use in traditional medicine (TM). The effectiveness of traditional medicines in treating various CNS disorders is well documented (Gumede, 1990). A strong motivation for the renewed involvement in developing plant based drugs is the fact that plants provide a rich reservoir for the development of drugs which may offer possible alleviation for refractory CNS diseases for which effective remedies are not available in modern therapeutic system. In respect of anxiety and sedation for example, effective plant remedies may possibly have advantages over conventional drugs like the benzodiazepines by not having the tendency to evoke psychological dependence, form active metabolites, and elicit amnesic effects, in addition to being readily available.

Telfairia occidentalis Hook. f., Cucurbitaceae, is a perennial liane with herbaceous, ribbed, glabrous or pubescent stems, climbing up to 15 m or more, which becomes thickened when old (Jeffrey, 1967). It is an often

cultivated species and native of western tropical Africa in countries like Sierra Leone, Benin, Ivory Coast, Equatorial Guinea, and Nigeria (Hutchinson & Daziel, 1954). It is also found outside West Africa in Cameroon, Congo Republic, Uganda, and Angola. The plant is commonly called "fluted gourd" and "fluted pumpkin" and local names in Nigeria include "Ugu" (Igbos), "Iroko" or "Aporoko" (Yoruba), "Ubong" (Efik), "Umee" (Urhobo), and "Umeke" (Edo) (Akoroda, 1990; Badifu & Ogunsina, 1991). *T. occidentalis* is grown in West Africa as a leaf vegetable and for its edible seeds. The young shoots and leaves of the female plant are the main ingredient of a Nigerian soup ("edikang ikong"). In terms of ethnomedicinal application, preparations of the young leaves are used to treat convulsion (Gbile, 1986), anaemia and for health restoration (Ehiagbonare, 2008), and for lactating properties (Okoli & Mgbeoku, 1983). The antioxidant and free radical scavenging (Nwanna & Oboh, 2007; Adaramoye et al., 2007; Kayode et al., 2010), antiplasmodial and antimicrobial (Odoemena & Onyeneke, 1998; Oboh et al., 2006; Okokon et al., 2007), antidaibetic (Aderibigbe et al., 1999; Eseyin et al., 2007; Salman et al., 2008), and anti-inflammatory (Oluwole et al., 2003) properties of preparations of the plant have been reported.

Based on the result of ethnobotanical survey conducted among traditional medicine practitioners in Mushin area of Lagos State, Nigeria, which indicate local use of *T. occidentalis* in the treatment of CNS related disorders, this study was conducted to investigate the anxiolytic and sedative activities of the hydroethanolic leaf extract of the plant in mice.

Materials and Methods

Plant material and extraction

Fresh *Telfairia occidentalis* Hook. f., Cucurbitaceae, plants were purchased from Oje market in Ibadan, Oyo State, Nigeria, in the month of April 2011. Identification and authentication of the plant was done by Prof. J.D. Olowokudejo of the Department of Botany and Microbiology, Faculty of Science, University of Lagos, Lagos, Nigeria, and Mr. Joseph Ariwaodo of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. A voucher specimen, assigned number FHI108939, was deposited in the Herbarium of the Institute for reference.

T. occidentalis leaves were air dried until a constant weight was obtained and the dried material was grounded to fine powder. One hundred g of the plant material was macerated in 1000 mL of hydroethanol (1:1) for 48 h, after which the liquid was decanted and filtered twice to remove all debris. The residue from the process was re-macerated in same volume of hydroethanol to ensure exhaustive extraction (× 2). The filtrate from each extraction process was combined and evaporated to dryness at 40 ºC under reduced pressure. The solid extract obtained was reconstituted in distilled water before each experimental session.

Experimental animals

The animals used in this study were Swiss mice of either sex, weighing between 20-30 g, obtained from the Laboratory Animal Center of the College of Medicine, University of Lagos, Lagos, Nigeria. Mice were maintained under standard environmental conditions (23-25 ºC, 12 h/12 h light/dark cycle). The animals were fed with standard rodent diet (Livestock Feed PLC, Lagos, Nigeria) and water *ad libitum*. Acclimatization of mice was done for fourteen days before the commencement of the experiment. The procedures adopted in this study were in accordance with the provisions of the Experimentation Ethics Committee on Animal Use of the College of Medicine, University of Lagos, Lagos, Nigeria (authorization number: CM/COM/8/VOL.XXI) and the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals (2011).

The ratio of male to female mice in the different groups and pharmacological models used in this study was about 6 to 4 (3 males, 2 females when n=5; 4 males, 3 females when n=7).

Phytochemical analysis

Phytochemical screening of the hydroethanolic leaf extract of *T. occidentalis*, to determine the presence or absence of various phytochemicals, was carried out according to the methods of Sofowora (1993) and Edeoga et al. (2005).

Acute toxicity test

Mice used in this test were fasted for 12 h before the start of the procedure. *T. occidentalis* extract was administered orally (*p.o.*) up to 10 g/kg to groups of mice. In respect of the intraperitoneal (*i.p.*) route, the extract (500-5000 mg/kg) was administered to six groups of five mice each. Animals were observed for 2 h post-administration for behavioural changes and signs of toxicity. Mortality was recorded 24 h later and surviving mice were observed for a further 2 weeks for signs of delayed toxicity. The LD50 was estimated by the log dose-probit analysis method (Randhawa, 2009).

Hole-board test

The hole-board is a white painted wooden board (40 × 40 cm) with four equidistant holes (1 cm diameter × 2 cm depth). Using two thick coloured lines which intersect at the centre, the board was divided into four equal sectional

squares of 20 × 20 cm (Akindele & Adeyemi, 2010). One hour after oral treatment with distilled water (10 mL/kg), *T. occidentalis* (50, 100, 200 and 400 mg/kg) and diazepam (1 mg/kg) (n=5), each mouse was placed in turn at one corner of the board with the animal subsequently moving about and dipping its head into the holes. The number and duration of head dips and sectional crossings in 5 min. were recorded for individual mouse (File & Wardill, 1975).

Elevated plus maze test

The plus maze consists of two open arms and two closed arm (50 × 10 × 40 cm each) elevated to a height of 50 cm (Vogel & Vogel, 1997). Distilled water (10 mL/kg, *p.o*.), *T. occidentalis* (50, 100, 200 and 400 mg/kg, *p.o*.) and diazepam (1 mg/kg, *p.o*.) were administered to groups of seven mice each. One hour post-treatment, each mouse was placed in turn in the centre of the maze facing one of the closed arms. The cumulative times spent by each mouse in the open and closed arms of the maze and the respective numbers of entries were recorded for 5 min.

Open-field test

The test apparatus was made of wood 50 cm in length, 50 cm in width, and 25 cm in height. The plain floor of the box was divided into 8 cm by 8 cm, with sixteen squares on it. Sixteen squares were defined as the centre and the others adjacent to the walls as the periphery. A 60 W white bulb illuminated the apparatus. One hour after oral administration (distilled water 10 mL/kg; *T. occidentalis* 50, 100, 200 and 400 mg/kg; and diazepam 1 mg/kg, n=7), each mouse was gently placed at the centre of the open field and the number of square crossings (all four paws moved from one square to another), rearing, and assisted rearing (forepaws touching the walls of the apparatus) were determined for 5 min. (Anisman & Matheson, 2005). The floor of the open field was cleaned with ethanol after each session.

Light/dark exploration test

Natural aversion of animal for brightly lit places was evaluated in the light-dark transition model. The light-dark box is a rectangular box of 50 × 25 × 25 cm, which is divided into two compartments (light and dark). One hour after oral administration of distilled water (10 mL/kg), diazepam (1 mg/kg) and *T. occidentalis* (50, 100, 200, and 400 mg/kg) (n=7), each mouse was placed individually in the illuminated part of the light-dark box. The latency (time taken for the animal to move into the dark box for the first time), time spent in the light and dark box, were recorded during the observation period of 5 min. (Bourin & Hascoët, 2003).

Social interaction test

In this test, the amount of time a pair of mice spend socially interacting with one another reflects the level of anxiety. The duration and frequency of social interaction decreases with increased anxiety (Moy et al., 2004). Mice were placed individually in the test arena for 7 min. familiarization session on two consecutive days. On the test day, mice were orally treated with distilled water (10 mL/kg), diazepam (1 mg/kg) and *T. occidentalis* (50, 100, 200, and 400 mg/kg) (n=5 pairs). Two randomly selected mice from each group were placed in adjacent cages in the waiting area of the test room before treatment. One hour post-treatment, each pair of animals were introduced together into the centre of the test arena. Social interaction was observed remotely for 7 min. The number of social interactions (sniffing, following, and grooming the partner) was recorded for each pair of mice.

Statistical analysis

Results are expressed as mean±SEM. The data were analyzed using One-way ANOVA followed by Tukey's post-hoc test using Graphpad Prism 5 Software (GraphPad Software Inc., CA, USA). Results were considered significant at $p<0.05$.

Results

Acute toxicity test

T. occidentalis did not cause any mortality and visible signs of toxicity when administered orally up to 10 g/kg and observed for fourteen days. Behavioural manifestations observed for 2 h post-oral treatment included reduced locomotion and calmness. The LD50 of the extract administered *i.p.* was estimated to be 3162.28 mg/kg. Behavioural manifestations observed for 2 h post-intraperitoneal treatment included reduced locomotion, calmness, writhing, loss of pupillary reflex, and increased respiratory rate.

Phytochemical analysis

The results of the chemical tests performed in the preliminary phytochemical screening revealed the presence of oils, saponins, phlobatannins and tannins in the hydroethanolic leaf extract of *T. occidentalis*. Reducing sugars and anthraquinones were found to be absent.

Hole-board test

T. occidentalis at doses of 50 and 100 mg/kg

significantly increased (p<0.05, 0.01) the number of sectional crossings and duration of head dips compared to the control group. At the dose of 400 mg/kg, the extract significantly reduced (p<0.01) the number of head dips and number of sectional crossings relative to the control group. Diazepam (1 mg/kg *p.o*.) significantly reduced (p<0.05, 0.01) the number of head dips and number of sectional crossings compared to the control group. Values for *T. occidentalis* at doses of 50 and 100 mg/kg were significantly higher (p<0.05, 0.001) than those for diazepam for all the parameters recorded (Table 1).

Table 1. Effect of *Telfairia occidentalis* in hole-board test in mice.

Treatments	Dose (mg/kg)	Number of head dips	Duration of head dips (s)	No. of sectional crossings
Distilled water	(10 ml/kg)	5.20±0.37	6.20±0.58	12.20±0.80
Diazepam	1	2.40±0.40^{b}	4.20±0.80	5.20±1.59^{a}
TO	50	4.80±0.37$^{\alpha}$	8.00±0.70$^{\alpha}$	21.80±1.83$^{b,\gamma}$
TO	100	7.00±0.71$^{\gamma}$	9.80±1.16$^{a,\gamma}$	17.80±2.44$^{\gamma}$
TO	200	5.20±0.58$^{\beta}$	6.80±0.37	10.40±1.36
TO	400	2.40±0.25^{b}	3.40±0.40	2.80±0.37^{b}

Values are mean±SEM (n=5). ^{a}p<0.05, ^{b}p<0.01 vs. distilled water; $^{\alpha}p$<0.05, $^{\beta}p$<0.01, $^{\gamma}p$<0.001 vs. diazepam.

Elevated plus maze test

T. occidentalis at doses of 200 and 400 mg/kg significantly reduced (p<0.01) the cumulative time spent in the open arms and increased the cumulative time spent in the closed arms relative to the control group. The extract at the dose of 50 mg/kg significantly reduced (p<0.01) the cumulative time spent in the open arms compared to the control group. *T. occidentalis* at the dose of 100 mg/kg significantly increased (p<0.01) the number of entries into the open arms relative to the control group. Diazepam (1 mg/kg, *p.o.*) did not elicit any significant effect (p>0.05) on the parameters recorded. The value for the number of entries into the open arms produced by the extract at the dose of 100 mg/kg was significantly higher (p<0.001) than that of diazepam. At the extract dose of 400 mg/kg, the value for the cumulative time spent in the open arms was significantly lower (p<0.001) while the value for the number of entries into the closed arms was significantly higher (p<0.05) than that of diazepam (Table 2).

Open-field test

T. occidentalis at doses of 50 and 400 mg/kg significantly increased (p<0.01, 0.001) the number of central squares crossed compared to the control group. There was significant reduction (p<0.001) in the number of assisted rearings at extract doses of 200 and 400 mg/kg relative to the control group. These effects were not significantly different (p>0.05) from those elicited by diazepam. Diazepam also significantly reduced (p<0.001) the number of assisted rearings compared to control. The values elicited by *T. occidentalis* at doses of 50 and 100 mg/kg, in respect of number of squares crossed and assisted rearings, were significantly higher (p<0.05, 0.01, 0.001) than those of diazepam (Table 3).

Light/dark exploration test

T. occidentalis at doses of 200 and 400 mg/kg significantly increased (p<0.01, 0.001) the latency of entry into the dark box relative to the control group, with a more prominent effect at the lesser dose. The value obtained with the extract at the dose of 200 mg was significantly higher (p<0.001) than that elicited by diazepam. Diazepam and the extract at the dose of 400 mg/kg significantly reduced (p<0.01, 0.001) the cumulative time spent in the light box compared to the control group, with no significant difference (p>0.05) between the two effects. Also, diazepam and *T. occidentalis* at the dose of 400 mg/kg significantly increased (p<0.01, 0.001) the cumulative time spent in the dark box relative to the control group with no significant difference (p>0.05) between the values (Table 4).

Social interaction test

T. occidentalis at doses of 50 and 100 mg/kg significantly increased (p<0.001) the number of social

Table 2. Effect of *Telfairia occidentalis* in elevated plus maze test in mice.

Treatments	Dose (mg/kg)	Time spent in open arms (s)	Time spent in closed arms (s)	No. of entries into open arms	No. of entries into closed arms
Distilled water	(10 ml/kg)	106.40±3.54	167.20±7.02	4.00±0.32	5.00±0.71
Diazepam	1	97.50±9.24	191.60±16.90	2.80±0.66	2.60±0.51
TO	50	74.60±0.81^{b}	166.80 ±6.14	6.20±0.37$^{\beta}$	6.20±0.97$^{\beta}$
TO	100	97.60±6.02	167.60±7.28	7.80±0.97$^{b,\gamma}$	4.60±0.68
TO	200	75.20±5.89^{b}	232.40 ±16.69^{b}	4.40±0.40	6.00±0.45$^{\alpha}$
TO	400	50.00±3.23$^{c,\gamma}$	236.20±6.70^{b}	3.60±0.51	5.60±0.25$^{\alpha}$

Values are mean±SEM (n=7). ^{b}p<0.01, ^{c}p<0.001 vs. distilled water; $^{\alpha}p$<0.05, $^{\beta}p$<0.01, $^{\gamma}p$<0.001 vs. diazepam.

Table 3. Effect of *Telfairia occidentalis* in open-field test in mice.

Treatments	Dose (mg/kg)	No. of squares crossed	No. of central squares crossed	No. of rearings	No. of assisted rearings
Distilled water	(10 mL/kg)	67.80±9.90	2.80±0.58	1.00±0.00	23.00±1.18
Diazepam	1	51.40±6.06	6.00±0.71	0.80±0.20	5.60±0.87^{c}
TO	50	87.20±4.72$^{\alpha}$	7.60±1.08^{b}	1.20±0.20	17.20±2.40$^{\beta}$
TO	100	81.40±9.07	6.40±1.25	1.40±0.25	23.20±2.87$^{\gamma}$
TO	200	66.20±9.83	5.80±0.86	0.80±0.20	10.00±1.76^{c}
TO	400	58.20±7.88	9.80±0.58^{c}	0.80±0.37	9.60±1.54^{c}

Values are mean±SEM (n=7). $^{b}p<0.01$, $^{c}p<0.001$ vs. distilled water; $^{\alpha}p<0.05$, $^{\beta}p<0.01$, $^{\gamma}p<0.001$ vs. diazepam.

Table 4. Effect of *Telfairia occidentalis* in light/dark exploration test in mice.

Treatments	Dose (mg/kg)	Latency of entry into dark box (s)	Time spent in light box (s)	Time spent in dark box (s)
Distilled water	(10 ml/kg)	11.40±1.81	101.20±6.35	180.20±4.84
Diazepam	1	18.20±2.25	57.20±6.38^{b}	226.40±8.73^{b}
TO	50	15.80±1.72	109.80±8.16$^{\beta}$	176.80±8.18$^{\beta}$
TO	100	9.80±2.31	105.80±12.75$^{\beta}$	184.20±10.89$^{\beta}$
TO	200	43.60±4.74$^{c,\gamma}$	69.20±4.76	192.00±4.59$^{\alpha}$
TO	400	29.80±4.19^{b}	38.60±6.35^{c}	244.80±6.22^{c}

Values are mean±SEM (n=7). $^{b}p<0.01$, $^{c}p<0.001$ vs. distilled water; $^{\alpha}p<0.05$, $^{\beta}p<0.01$, $^{\gamma}p<0.001$ vs. diazepam.

Table 5. Effect of *Telfairia occidentalis* in social interaction test in mice.

Treatments	Dose (mg/kg)	No. of social interactions
Distilled Water	(10 mL/kg)	14.40±0.87
Diazepam	1	11.80±0.80
TO	50	25.40±0.93$^{c,\gamma}$
TO	100	24.40±2.16$^{c,\gamma}$
TO	200	14.20 ±1.07
TO	400	4.20±0.80$^{c,\beta}$

Values are mean±SEM (n=5 pairs). $^{c}p<0.001$ vs. distilled water; $^{\beta}p<0.01$, $^{\gamma}p<0.001$ vs. diazepam.

interactions compared to the control group, with values being significantly higher ($p<0.001$) than that elicited by diazepam. There was no significant difference ($p>0.05$) between the effects of the extract at doses of 50 and 100 mg/kg. *T. occidentalis* at the dose of 400 mg/kg significantly reduced the number of social interactions compared to control ($p<0.001$) and diazepam ($p<0.01$; Table 5).

Discussion

Expression of an anxiolytic state in animals manifest as an increase in head dipping behaviour in the hole-board test. File & Wardill (1975) reported that suppression of exploratory behaviour is an indication of CNS depressant activity. In this study, *Telfairia occidentalis* Hook. f., Cucurbitaceae, at doses of 50 and 100 mg/kg significantly increased the duration of head dips (58.06%) and number of sectional crossings (78.69%). This suggests anxiolytic activity associated with increase in locomotion. However, at the dose of 400 mg/kg, the extract elicited a sedative effect based on reduction in the number of head dips (53.85%) and number of sectional crossings (77.05%). Diazepam at the dose used in this study (1 mg/kg) demonstrated sedative rather than anxiolytic activity as it significantly reduced the number of head dips (53.85%) and sectional crossings (57.38%).

Anxiolytic compounds reduce the natural animal aversion to the open arms and promote the exploration thereof in the elevated-plus maze test. On the other hand, the forced or voluntary passages of the animal into the closed arms of the EPM are associated with hormonal and behavioral changes indicative of increased anxiety (Hogg, 1996; Santos et al., 2012). Montgomery (1955) reported that rodents consistently spend greater time in the closed arms when placed in mazes comprising of open and closed arms. Avoidance of the open arm portrays a manifestation of fear and anxiety. Based on these assertions, the elevated plus maze test is a reliable means of identifying selective anxiolytic effect of drugs. Handley & Mithani (1984) further demonstrated that rodents avoid the open arms while also reporting that open arm avoidance is reduced by diazepam (anxiolytic agent). *T. occidentalis* at the dose of 100 mg/kg increased the number of entries into the open arms (90%) without any significant effect on cumulative time spent in the open and closed arms and in the central

square, which is an indication of anxiolytic effect. The extract at doses of 200 and 400 mg/kg reduced the time spent in the open arms (29.32 and 53.01% respectively) with a corresponding increase in the time spent in the closed arms (39 and 41.27% respectively), suggesting sedative activity. Diazepam at the dose of 1 mg/kg used in this study did not elicit any effect on the parameters recorded in this model.

The open field test is utilized to evaluate the emotional state of animals. Thus, animals removed from their acclimatized cage and placed in a novel environment express anxiety and fear by showing alteration in all or some parameters, such as decreases in ambulation and exploration, immobilization or ''freezing'', reduction in normal rearing and in grooming behavior, and increased micturition and defecation due to augmented autonomic activity (Novas et al., 1988). *T. occidentalis* at the dose of 50 mg/kg produced anxiolytic effect due to the significant increase (63.16%) in the number of central squares crossed. The effect of the extract at higher doses of 200 and 400 mg/kg in significantly reducing the number of assisted rearings (56.52%, 58.26% respectively) is indicative of sedative activity. The activity of diazepam at the dose of 1 mg/kg suggests sedative effect based on reduction in the number of assisted rearings (75.65%).

The light-dark test is useful to predict the anxiolytic-like activity of drugs. Transitions have been reported to be an index of activity exploration because of habituation overtime and the time spent in each compartment to be a reflection of aversion (Belzung et al., 1987). The percent of time spent in the lit compartment is an index of the anxiety-related behaviour. Anxiety is considered to be high if the percent of time spent in the lit compartment is low. *T. occidentalis* at the dose of 200 mg/kg significantly increased the latency of entry into the dark box (282.46%) without affecting the duration of time spent in the light and dark sections. At the dose of 400 mg/kg, the extract also significantly increased the latency of entry into the dark box (161.40%) but significantly reduced the time spent in the light section (61.86%) and correspondingly increased the time spent in the dark box (35.85%). The extract thus showed a tendency for sedative activity at doses of 200 and 400 mg/kg. Consistent with the results obtained in the other models in this study, diazepam at the dose of 1 mg/kg significantly reduced the cumulative time spent in the light section (43.48%) while significantly increasing the time spent in the dark section (25.64%), indicating sedative effect.

The social interaction test was developed as the first animal test of anxiety that endeavoured to use ethologically relevant sources of anxiety, and to use a natural form of behaviour as the dependent measure and the dependent variable is the time spent by pairs of male rats in social interaction e.g. sniffing, following or grooming the partner (File & Seth, 2003). An increase in social interaction, without a concomitant increase in motor activity, is indicative of an anxiolytic effect, whereas a specific decrease in social interaction indicates an anxiogenic effect (File & Seth, 2003). At doses of 50 and 100 mg/kg, the extract elicited anxiolytic activity based on significant increase in the number of social interactions (76.39, 69.44% respectively). However, at the highest dose of 400 mg/kg, the reverse was the case (70.83% reduction) indicating sedative effect.

The results obtained in this study showed that the hydroethanolic leaf extract *T. occidentalis* largely demonstrated anxiolytic activity at lower doses of 50 and 100 mg/kg, and sedative property at higher doses of 200 and 400 mg/kg. According to Treit (1984), anxiolytics possess a biphasic profile, showing a facilitation of exploratory behaviour at low doses and an inhibition at high doses. Rodgers et al. (1997) reported that higher doses of chlordiazepoxide and diazepam (benzodiazepines) increase immobility scores. According to Santos et al. (2012), it is well known that benzodiazepines act as anxiolytics (at low doses), anticonvulsants, and also produce sedation and a myorelaxant effect at higher doses. Diazepam at the dose of 1 mg/kg used in this study principally manifested sedative activity.

The hydroethanolic leaf extract *T. occidentalis* was found in this study to contain oils, saponins, phlobatannins and tannins. The leaf of the plant has been reported to be rich in minerals, including iron, potassium, sodium, phosphorus, calcium and magnesium; vitamins like thiamine, riboflavin, nicotinamide and ascorbic acid; antioxidants and phytochemicals such as phenols (Kayode & Kayode, 2011). Iwu (1983) also reported that the leaves contain essential oils and vitamins while the root contains cucubitacine, sesquiterpene, and lactones. The plant has also been reported to be very rich in amino acids, including alanine, aspartate, glycine, glutamine, histidine, lysine, methionine, tryptophan, cystine, leucine, arginine, serine, threonine, phenylalanine, valine, tyrosine, and isoleucine (Tindall, 1968; Fasuyi, 2006). Phenolic compounds include phenylpropanoids, coumarins, benzoic acid derivatives, flavonoids, stilbenes, and tannins, and in terms of CNS function, a wide range of these compounds interact directly with neurotransmitter systems (Kennedy & Wightman, 2011). A diverse range of individual and combined flavonoids that occur in traditional medicinal extracts exert sedative/anxiolytic effects via direct binding to GABAA receptors (Dhawan et al., 2004; Ren et al., 2010), cognitive enhancement via antagonistic GABAA receptor binding and resultant cholinergic upregulation (Kim et al., 2007), and antidepressant effects via monoamine oxidase inhibition and resultant increases in levels of 5-HT, DA, and noradrenaline in select brain areas (Xu et al., 2010). Essential oils from various plants have been reported to possess anxiolytic and sedative activities (Emamghoreishi & Heidari-Hamedani, 2006; Landaverde et al., 2009;

Hajhashemi, 2010). Glutamate, aspartate, cysteine, and homocysteine are examples of excitatory amino acids which activate post-synaptic cells while GABA, glycine, alanine and taurine are examples of inhibitory amino acids which depress the activity of post-synaptic cells (D'haenen et al., 2002; Lemke & Williams, 2007). The anxiolytic and sedative activity of *T. occidentalis* observed in this study may also be due to the presence of one or a combination of amino acids reported to be present in the plant (Tindall, 1968; Fasuyi, 2006). For example, according to animal studies, taurine produces anxiolytic effect and may act as a modulator or antianxiety agent in the central nervous system by activating the glycine receptor (Chen et al., 2004; Kong et al., 2006; Zhang & Kim, 2007).

Considering the results obtained in the acute toxicity test, the hydroethanolic leaf extract of *T. occidentalis* is relatively safe when administered orally. Administered up to 10 g/kg, the extract did not produce any mortality and visible signs of delayed toxicity. It can therefore be classified as relatively non-toxic based on the assertion of Hayes (1989) that no dose-related toxicity should be considered above 5 g/kg body weight, a dose lower than the 10 g/kg administered orally in the acute toxicity study. The LD50 of the extract when administered intraperitoneally was estimated to be 3162.28 mg/kg.

Conclusion

The results obtained in this study suggest that the hydroethanolic leaf extract of *Telfairia occidentalis* possesses anxiolytic property at lower doses of 50 and 100 mg/kg, and sedative activity at higher doses of 200 and 400 mg/kg. The extract was found to be relatively non-toxic in mice; hence it is possibly safe for human consumption. Further research work to carry out bioactivity guided fractionation of the plant extract is ongoing with a view to isolate, identify and characterize the specific phytomolecules responsible for the observed biological activity and determine the precise mechanism(s) of action involved.

Authors contributions

AJA designed and supervised the study, and wrote up the manuscript for publication. MYA collected and identified the plant, and carried out the study under the supervision of AJA. Both authors read the final manuscript and approved the submission.

References

Adaramoye OA, Achem J, Akintayo OO, Fafunso MA 2007. Hypolipidemic effect of *Telfairia occidentalis* (fluted pumpkin) in rats fed a cholesterol-rich diet. *J Med Food 10*: 330-336.

Aderibigbe AO, Lawal BAS, Oluwagbemi JO 1999. The antihyperglycaemic effect of *Telfairia occidentalis* in mice. *African J Medicine Med Sci 28*: 171-175.

Akindele AJ, Adeyemi OO 2010. Anxiolytic and sedative effects of *Byrsocarpus coccineus* Schum. and Thonn. (Connaraceae) extract. *Int J Appl Res Nat Prod 3*: 28-36.

Akoroda MO 1990. Ethnobotany of *Telfairia occidentalis* (Cucurbitaceae) among Igbos of Nigeria. *Econ Bot 44*: 29-39.

Anisman H, Matheson K 2005. Stress, depression, and anhedonia: caveats concerning animal models. *Neurosci Biobehav Rev 29*: 525-546.

Badifu GIO, Ogunsina AO 1991. Chemical composition of kernels from some species of Cucurbitaceae grown in Nigeria. *Plant Food Hum Nutr 41*: 35-44.

Belzung C, Griebel G 2001. Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res 125*: 141-149.

Bourin M, Hascoët M 2003. The mouse light/dark box test. *Eur J Pharmacol 463*: 55-65.

Chen SW, Kong WX, Zhang YJ, Li YL, Mi XJ, Mu XS 2004. Possible anxiolytic effects of taurine in the mouse elevated plus-maze. *Life Sci 75*: 1503-1511.

Committee for the Update of the Guide for the Care and Use of Laboratory Animals 2011. Guide for the Care and Use of Laboratory Animals (Eighth Edition). National Academy of Sciences, National Academies Press, Washington DC, USA.

Cox AL 1994. *Ethnobotany and the Search for New Drugs*. John Wiley & Sons, New York, p. 25-41.

D'haenen H, den Boer JA, Willner P 2002. *Biological Psychiatry.* John Wiley and Sons. p. 415.

Dhawan K, Dhawan S, Sharma A 2004. *Passiflora*: a review update. *J Ethnopharmacol 94*: 1-23.

Edeoga HO, Okwu DE, Mbaebie1 BO 2005. Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol 4*: 685-688.

Ehiagbonare JE 2008. Conservation studies on *Telfairia occidentalis* Hook .f. A. indigenous plant used in enthnomedicinal treatment of anemia in Nigeria. *Afr J Agric Res 3*: 74-77.

Emamghoreishi M, Heidari-Hamedani G 2006. Sedative-hypnotic activity of extracts and essential oil of Coriander seeds. *Iran J Med Sci 31*: 22-27.

Eseyin OA, Ebong P, Ekpo A, Igboasoiyi A, Oforah E 2007. Hypoglycemic effect of the seed extract of *Telfairia occidentalis* in rats. *Pak J Biol Sci 10*: 498-501.

Facts and Comparisons Inc. 1990. *Drug facts and comparisons*, Lippincott, St. Louis, Mo. 3.

Fasuyi AO 2006. Nutritional potentials of some tropical vegetable leaf meals: Chemical characterization and functional properties. *Afr J Biotechnol 5*: 49-53.

File SE, Seth P 2003. A review of 25 years of the social interaction test. *Eur J Pharmacol 463*: 35-53.

File SE, Wardill AG 1975. Validity of head dipping as a measure of exploration in a modified hole-board. *Psychopharmacologia 44*: 53-59.

Gbile ZO 1986. Ethnobotany, Taxonomy and Conservation of Medicinal Plants. In: *The State of Medicinal Plants in Nigeria,* Sofowora A (Ed.). University of Ibadan Press, Ibadan, Nigeria.

Gilman AG, Goodman LS, Rall TW, Murad F (Eds) 1991. Goodman and Gilman's The Pharmacological Basis of Therapeutics, 7th Ed, McMillan, Philadelphia, P.A.

Gumede MV 1990. *Traditional Healers*. Skotaville, Braamfontein, South Africa.

Hajhashemi V, Rabbani M, Ghanadi A, Davari E 2010. Evaluation of antianxiety and sedative effects of essential oil of *Ducrosia anethifolia* in mice. *Clinics 65*: 1037-1042.

Handley SL, Mithani S 1984. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *Naunyn Schmiedebergs Arch Pharmacol 372*: 1-5.

Hayes AW 1989. Guidelines for acute oral toxicity testings. In: *Principles and Methods of Toxicity*, 2nd Edn. Raven Press Ltd., New York. Table 4, pp. 185.

Hogg S 1996. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacol Biochem Behav 54*: 21-30.

Hutchinson J, Dalziel JM 1954. *Flora of Tropical West Africa*. 2nd Ed., Crown Agents, London.

Iwu MW 1983. Traditional Igbo Medicine. Institute of African Studies University of Nigeria, Nsukka.

Jeffrey C 1967. *Flora of Tropical East Africa.* Cucurbitaceae: p. 1-156.

Kayode AAA, Kayode OT 2011. Some medicinal values of *Telfairia occidentalis*: A Review. *Am J Biochem Mol Biol 1*: 30-38.

Kayode AAA, Kayode OT, Odetola AA 2010. *Telfairia occidentalis* ameliorates oxidative brain damage in malnourished rats. *Int J Biol Chem 4*: 10-18.

Kennedy DO, Wightman EL 2011. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. *Adv Nutr 2*: 32-50.

Kim DH, Jeon SJ, Son KH, Jung JW, Lee S, Yoon BH, Lee J-J, Cho Y-W, Cheong JH, Ko KH, Ryu JH 2007. The ameliorating effect of oroxylin A on scopolamine-induced memory impairment in mice. *Neurobiol Learn Mem 87*: 536-546.

Kong WX, Chen SW, Li YL, Zhang YJ, Wang R, Min L, Mi X 2006. Effects of taurine on rat behaviors in three anxiety models. *Pharmacol Biochem Behav 83*: 271-276.

Landaverde NA, Juárez-Flores BI, Jiménez-Capdeville ME, Ortiz-Pérez MD 2009. Anxiolytic and sedative effects of essential oil from *Casimiroa pringlei* on Wistar rats. *J Med Plants Res 3*: 791-798.

Lemke TL, Williams DA 2007. *Foye's Principles of Medicinal Chemistry.* Lippincott Williams & Wilkins. p. 446.

Montgomery KC 1955. The relation between fear induced by novel stimulation and exploratory behavior. *J Comp Physiol Psychol 48*: 254-260.

Moy SS, Nadler JJ, Perez A, Barbaro RP, Johns JM, Magnuson TR, Piven J, Crawley JN 2004. Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. *Genes Brain Behav 3*: 287-302.

Novas ML, Wolfman C, Medina JH, de Robertis E 1988. Proconvulsant and anxiogenic effects of *n*-butyl beta carboline-3-carboxylate, an endogenous benzodiazepine binding inhibitor from brain. *Pharmacol Biochem Behav 30*: 331-336.

Nwanna EE, Oboh G 2007. Antioxidant and hepatoprotective properties of polyphenol extracts from *Telfairia occidentalis* (Fluted Pumpkin) leaves on acetaminophen induced liver damage. *Pak J Biol Sci 10*: 2682-2687.

Oboh G, Nwanna EE, Elusiyan CA 2006. Antioxidant and antimicrobial properties of *Telfairia occidentalis* (Fluted Pumpkin) leaf extracts. *J Pharmacol Toxicol 1*: 167-175.

Odoemena CS, Onyeneke EC 1998. Lipids of fluted pumpkin (*Telfairia occidentalis*) seeds. Proceedings of the 1st African Conference on Biochemistry of Lipids, (ACBL'98), Ambik Press, Benin City, Nigeria, p. 147-151.

Okokon JE, Ekpo AJ, Eseyin OA 2007. Antiplasmodial activity of ethanolic root extract of *Telfairia occidentalis. Res J Parasitol 2*: 94-98.

Okoli BE, Mgbeogu CM 1983. Fluted pumkin, Telfairia occidentalis: West African vegetable crop. *Econ Bot 37*: 145-149.

Oluwole FS, Falode AO, Ogundipe OO 2003. Anti-inflammatory effect of some common Nigerian vegetables. *Nig J Physiol Sci 18*: 35-38.

Randhawa MA 2009. Calculation of LD50 values from the method of Miller and Tainter, 1944. *J Ayub Med Coll Abbottabad 21*: 184-185.

Ren L, Wang F, Xu Z, Chan WM, Zhao C, Xue H 2010. GABAA receptor subtype selectivity underlying anxiolytic effect of 6-hydroxyflavone. *Biochem Pharmacol 79*: 1337-1344.

Rodgers RJ, Cao BJ, Dalvi A, Holmes A 1997. Animal models of anxiety: an ethological perspective. *Braz J Med Biol Res 30*: 289-304.

Salman TM, Olayaki LA, Oyeyemi WA 2008. Aqueous extract of *Telfairia occidentalis* leaves reduces blood sugar and increases haematological and reproductive indices in male rats. *Afr J Biotechnol 7*: 2299-2303.

Santos FJB, Lima SG, Cerqueira GS, Citó AML, Cavalcante AAC, Marques THC, Freitas RM 2012. Chemical composition and anxiolytic-like effects of the *Bauhinia platypetala. Rev Bras Farmacogn 22*: 507-516.

Sofowora EA 1993. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, p. 191-289.

Tindall HD 1968. Commercial Vegetables Growing. 1st Ed., Oxford University Press, Oxford, UK.

Treit D 1984. Animal models for the study of anti-anxiety agents: a review. *Neurosci Biobehav Rev 9*: 203-222.

Vogel HG, Vogel WH 1997. Elevated plus maze test. In: *Drug discovery and evaluation.* Springer-Verlag Berlin Heidelberg, New York, 1997, p 234.

Xu Y, Wang Z, You W, Zhang X, Li S, Barish P, Vernon M, Du X, Li G, Pan J, Ogle WO 2010. Antidepressant-like effect of *trans*-resveratrol: involvement of serotonin and noradrenaline system. *Eur Neuropsychopharmacol 20*: 405-413.

Zhang CG, Kim SJ 2007. Taurine induces anti-anxiety by activating strychnine-sensitive glycine receptor *in vivo. Ann Nutri Metab 51*: 379-386.

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