The Pharma Innovation Journal 2020; 9(7): 294-297

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(7): 294-297

www.thepharmajournal.com
Received: 10-05-2020
Accepted: 12-06-2020

Bakou Niangoran François
Unit of Animal Physiology, University Jean Lorougnon GUEDE of Daloa, Côte d'Ivoire

BA Abdoulaye
Unit of Animal Physiology, Jean Lorougnon GUEDE University, Daloa, Côte d'Ivoire,

Guiro Hamidou
Laboratory of Neuroscience, UFR Biosciences, Felix HOUPHOUET-BOIGNY University, Abidjan, Côte d'Ivoire

Atayi E
Neurology Service, Functional Exploration Unit of the Nervous System, C.H.U. from Cocody-Abidjan, Côte d'Ivoire

Corresponding Author:
Bakou Niangoran François
Unit of Animal Physiology, University Jean Lorougnon GUEDE of Daloa, Côte d'Ivoire

Evaluation of anxiolytic effect of hydro alcohol extract of stem bark from *Cassia sieberiana* in mice

Bakou Niangoran François, BA Abdoulaye, Guiro Hamidou and Atayi E

Abstract
Background: To study the anxiolytic activity of the hydro ethanolic extract of stem bark from *Cassia sieberiana* (*CS*). Methods: Male Swiss albino mice were used. Hydro ethanolic extract of stem bark from *Cassia sieberiana* (*CS*) was administered in the doses of 100, 200 and 400 mg/kg i.p. Hole board (HB), open field (OF), elevated plus maze (EPM) tests were used for determination of anxiolytic activity. **Results:** The hydro ethanolic extract of stem bark from *Cassia sieberiana* (*CS*) significantly increased the number and duration of head poking in HB test. The extract also significantly increased the time spent and the number of entries in open arm in EPM. In OFT, the extract showed significant increase in number of rearing, assisted rearing and the squares crossed. Conclusions: In the present study, *CS* exhibited anxiolytic activity which might be attributed to its phytoconstituents like alkaloid, steroid and triterpenes. Since *Cassia sieberiana* (*CS*) is ubiquitous and abundantly grown, it could be a fairly economical therapeutic agent for management of anxiety disorders.

Keywords: *Cassia sieberiana,* anxiety, mice

Introduction
Anxiety disorders are among the most common psychiatric disorders that affect all age groups of the general population [1]. Approximately 450 million people suffer from a mental or behavioral disorder [2]. It is a feeling of apprehension, uncertainty, and fear characterized by physical symptoms such as palpitations, sweating, and feelings of stress [3]. These disorders are widely treated with benzodiazepine anxiolytic agents. However, the clinical use of benzodiazepines is limited by their side effects such as respiratory depression, motor coordination deficits, memory, cognitive dysfunctions, and dependence liability [4,5]. Therefore, finding novel therapeutic agents with fewer complications in the treatment of anxiety disorder, is of major interest to researchers [6]. Medicinal plant with traditional background of use in neurological diseases could be good candidates to find new anxiolytic agents. *Cassia sieberiana*, *Cassia kotschyana* Oliv.; Fam. Caesalpinaceae, is a savannah tree that grows to about 15 m tall and is commonly fairly cultivated because of its attractive blossom and curious fruits (commonly referred to as the African laburnum. *C. sieberiana* has a very wide range of phytotherapeutic application in Ghana including the use of its roots in the management of hernia, leprosy, indigestion and gastric ulcer [7]. At the Centre for Scientific Research into Plant Medicine (CSRPM) in Ghana, an aqueous suspension of the powdered roots bark is used to manage abdominal colic and pains associated with the joints. Earlier studies we conducted indicated that the aqueous roots bark extract of *C. sieberiana* possesses anti-ulcerogenic properties against gastric ulcers induced by various methods [8]. In traditional medicine, the plant is used as antimicrobial, antiviral, antibacterial, anti-inflammatory, antitrypanosomal and antioxidant agent, as a strong purgative, diuretic, abortifacient, anti-schistosomiasis, anti-dysentery and antihaemorrhoid [9,10]. No scientific report regarding the *in vivo* anxiolytic activity of *Cassia sieberiana* (*CS*) has been published. That's why, the present study was undertaken to assess the possible anxiolytic effects following single administration of hydro ethanolic extract of stem bark from *Cassia sieberiana* (*CS*) in mice. For this purpose, we used the elevated plus-maze, open field and Hole board tests**.**

Materials and Methods
Plant material
Cassia sieberiana stems bark were harvested in October, 2019 at the Jean Lorougnon GUEDE university from Daloa, (Cote d'Ivoire). The plant was identified and verified by botanist Professor from Jean Lorougnon GUEDE university of Daloa (Cote d'Ivoire).

Extract preparation
The stems bark of *Cassia sieberiana* were dried for four weeks. The drying process of the stems barks of *Cassia sieberiana* was done in the absence of light to avoid the principle of the clear phase of photosynthesis which is for the plant (*Cassia sieberiana*) to capture the light energy Photons and to transmit it by way of the electrons charged with this energy, to a chain of electron acceptors (molecules with variable oxidoreduction potentials). Then the dried stem bark of *Cassia sieberiana a* made powder using an electric grinder IKAMAG RCT®. 100 grams of powder of *Cassia sieberiana* were macerated for 24 hours in 1 liter of ethanol (ethanol and distilled water mixture: 70/30). The macerated obtained was then filtered twice on white cotton and once on Whatman filter paper N°4. The filtrate obtained in 70% ethanol was evaporated to dryness at reduced pressure at temperature of 40 °C using a rotary evaporator type Buchi 161 Water Bath.

Animals
25 healthy adult male Swiss albino mice weighing (20–30 g) were obtained from the animal house of Jean Lorougnon GUEDE University, Daloa. These animals were housed under standard environmental conditions. The rats were fed with FACI® (Fabrication d'Aliments de Côte d'Ivoire) pellets, groundnuts and dried fish. They had free access to drinking water ad libitum.

Drugs and chemicals
The standard drugs Diazepam and saline water were collected from Square Pharmaceuticals Ltd., Cote d'Ivoire. Distilled water which was used for dilution purpose was prepared was obtained from Jean Lorougnon GUEDE university of Daloa (Cote d'Ivoire).

Behavioral parameters used to test anxiolytic activity
Open field test
Locomotor activity and exploratory behavior were assessed in an open field by the method described by Souza [11]. The apparatus consisted of a wooden box (60 × 60 × 30 cm^3) with the floor divided into 16 squares (15 × 15 cm^2). The apparatus was illuminated with a 40-W lamp suspended 100 cm above. Twenty-five mice were randomly divided into five groups of five mice each (n=5/group). One hour before test session, mice were treated with graded doses of *CS* (100, 200, and 400mg/kg, i.p.) while the control received 0,1ml normal saline/kg i.p. 30 min later each mouse was placed individually in the center of the apparatus and observed for 5 min to record the locomotor (number of squares crossed with four paws) and exploratory activities (indicated by frequency of rearing and assisted rearing) [11].

Elevated plus-maze test
The elevated plus-maze (EPM) test consisted of two open arms (30 × 5 × 0.25 cm) and two closed arms (30×5×15 cm) emanating from a common central platform (5×5 cm). Two pairs of identical arms were opposite to each other. The entire apparatus was elevated to a height of 40 cm above floor level. At the beginning of the session, a mouse was placed at the centre of the maze, its head facing an open arm and allowed to explore the maze for 5 minutes, and the following parameters were scored: the time spent and number of entries in each type of arms. The plus maze was carefully cleaned with a wet towel after each animal test. The control group received vehicle (saline water 0.1mL/mice). Diazepam (1 mg/kg b.w., IP) was used as the positive control or standard group and *CS* extract at doses of 100,200 and 400 mg/kg body weight, in the three remaining groups. After each trial, the EPM apparatus was wiped clean with alcohol (70%) Solution.

Hole board test
The hole board apparatus consisted of a wooden chamber (40 × 40 × 25 cm^3) with 16 holes (each of 3 cm diameter) evenly distributed on the floor. The apparatus was elevated to a height of 25 cm from the ground so that the mouse could peep through the holes. The mice were treated with *CS* (100, 200 and 400 mg/kg body weight i.p), diazepam (1 mg/kg body weight i.p) or saline water (0,1ml/kg body weight i.p) 30 min prior to test and kept in the apparatus. The numbers and the duration of head poking were recorded during the 5 min observation period

Statistical analysis
Results are expressed as mean ± S.E.M. The statistical analysis of data was done using the one-way analysis of variance (ANOVA) followed by Dunnett's test. A probability level less than 0.05 was considered statistically significant.

Results
Elevated plus maze test
The saline-treated mice spent 35.8 ± 1.25 s in the open arm and 247 ± 1.6 s in the closed arm, with 7.3 ± 4.5 entries into the open arm and 11.7 ± 2.6 entries into the closed arm. *CS* (100 and 200 mg/kg) and diazepam (1 mg/kg) induced significant (P<0.01) increase in the occupancy in the open arm. *CS* in the dose of 200 and 400 mg/kg did not cause a significant decrease in the time spent in the closed arm, whereas *CS* at a dose of 100 mg/kg and diazepam brought about a significant (P<0.01) decrease in the time spent in the closed arm. The animals treated with diazepam and *CS* (100 mg/kg) showed a decreased preference for the closed arm and significantly (P<0.01) increased entries into the open arm. *CS* at 200 and 400 mg/kg did not produce any significant increase in open arm entries

Open field test
The salne water-treated mice traversed 82.2 ± 15 squares and showed 11.8 ± 1.25 assisted rearing and 4.5 ± 1.2 self-rearing during the test interval of 5 min. *CS* at 100 and 200mg/kg and diazepam brought about a significant (P<0.01) and dose-dependant increase in the number of squares traversed. The assisted rearing and self-rearing was significantly (P<0.05 and P<0.01, respectively) increased by *CS* (100 and 200 mg/kg) and diazepam; *CS* at 400 mg/kg did not a produce significant effect

Hole board test
Each mouse was placed individually in the hole-board apparatus and the number of head pokes and the duration of head poking were noted. With the dose of 400mg/kg, i.p., of *CS* there was no significant increase in number of head pokes when compared with vehicle. *CS* at 100 and 200 mg/kg, i.p., increased the number of head pokes significantly (P<0.01) and dose dependently. The duration of head poking was also significantly (P<0.01) increased by *CS* at all doses. The reference standard (diazepam, 1 mg/kg, i.p.)-treated group showed significant increase in exploratory activity (P<0.01)

Table 1: Effect of (CS) on animals' stay in the open and enclosed arms of the elevated plus-maze in mice

Treatment	Time spent in the open arm (s)	Time spent in the enclosed arm (s)	Entries into open arm	Entries into enclosed arm
NaCl 0,1ml	35.8±1.25	247±1.6	7.3±4.55	11.7±2.6
DZP1mg/Kg	101±6.54**	159±1.55**	20.5±1.85**	7.7±15
*CS*100 mg/Kg	113±1.25**	136.2±2.45**	18.5±1.27**	8.5±2.15
*CS*200 mg/Kg	87.8±12**	219.8±1.85	8.3±4.15	9.5±2.47
*CS*400 mg/Kg	28.5±1.65	217.7±2.1	10.2±4.25	8.7±2.25

Table 2: Effect of (CS) on rearing and locomotion in open field test model

Treatment	Rearing	Assisted rearing	Number of square traversed
NaCl 0,1ml	4.5±1.2	10.8±1.5	82.2±15
DZP1mg/Kg	13±6.45**	21.7±6.45**	155±3.45**
*CS*100 mg/Kg	27.5±2.65**	18.5±2.7*	116.2±2.2**
*CS*200 mg/Kg	21.8±5.5**	17.8±2.35*	137.8±2.27**
*CS*400 mg/Kg	12±2.2	11.3±1.25	149.5±2.37**

Table 3: Effect of (CS) on hole board

Treatment	Duration on head poking (s)	Number of head poking
NaCl 0,1ml	25.8±1.2	26.8±1.2
DZP1mg/Kg	71±6.4**	74±6.5**
*CS*100 mg/Kg	52±1.2**	48±1.35**
CS200 mg/Kg	76.8±1.27**	76.8±1.25**
CS400 mg/Kg	91.5±2.65**	27.1±1.67

Discussion

The elevated plus maze (EPM) test represents one of the most widely used animal models for screening anxiolytics [12]. This test is able to reproduce anxiolytic or anxiogenic effects in rodents such that anxiolytics produce increase the time spent in the open arm of the elevated plus maze, while anxiogenic substances produce the opposite effect [12,14]. The indices of anxiety (number of open-arm entries, and time spent in the open arm) are sensitive to agents and are thought to act via the GABAA receptor complex, justifying the use of diazepam (DZP) as a positive control in this study. Diazepam, a benzodiazepine binds to GABAA receptors to increase the frequency of chloride channel openings resulting in hyperpolarization. It increased the frequency of open-arm entries and the time spent in the open arms [13], confirming its anxiolytic effects. In our study, we observed that *CS* (100, 200 and 400mg/kg) induced significant increases in the both the number of entries and time spent in the open arms. The number of entries and the time spent in the closed arms were reduced in the extract-treated group as compared to the control group. The open-field apparatus provides information on anxiety-related behaviour characterized by natural aversion of rodents to an open brightly lit area [15]. Animals are thus afraid of the centre and spend more time in the protective corners and in freezing state. Anxiolytics increase total locomotive activity resulting in a reduction of time spent in corners, an increased time spent in the center and a decreased time spent in freezing state. The extract of *CS* at 100, 200 and 400 mg/kg body weight increased total locomotive activity and increased rearing of treated mice in our study. Hole-board test indicated that the head-dipping behavior was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state may be reflected by an increase in head-dipping behavior [16]. In our study, *CS* (100, 200 and 400 mg/kg) significantly increased the numbers and duration of head poking compared to the control group. These results confirm the anxiolytic effects of *Cassia sieberiana*. They are to be compared with the work of Nsour [17], who in a similar study showed the anxiolytic effect of *Rauvolfia Serpentina* [18].; from Aidee, which highlighted the anxiolytic effects of the ethanolic extracts of *Argemone mexicana* [19]; from Carla, which demonstrated anxiolytic properties of aqueous extracts of *Salvia miltiorrhiza* in rats; Charles [20] and Carnevale [21] who showed anxiolytic properties of extracts of *Maerua angolensis* in mice and *Griffonia simplicifolia* in rat.

Conclusion

In conclusion, the results obtained in our study suggest that hydro ethanolic extract of stem bark from *Cassia sieberiana* possesses anxiolytic activity, which is possibly mediated through the GABA A -BZD mechanism. Thus, *Cassia sieberiana* has potential clinical application in the management of anxiety disorders. Further investigation of the mechanism(s) of action of the plant extract, as well as the active substance(s) responsible for its biological actions, is necessary.

Reférences

1. Kessler RC, Angermeyer M, Anthony JC, Lifetime prevalence and age-of-onset distributions of mental disorders in the World Health Organization's World Mental Health Survey initiative. World Psychiatry. 2007, 6:168-76.
2. World Health Report. Mental health: New understanding new hope. WHO, Geneva, 2001.
3. Silvaraman D, Muralidharan P, Habibur R. Evaluation of the anxiolytic effect of methanolic leaf extract of *Ficus hispida* Linn. in corticosterone induced anxiety in young adult rats. Pharmacologia. 2012, 3:467-471.
4. Venault P, Chapouthier G, de Carvalho LP, Benzodiazepine impairs and beta-carboline enhances performance in learning and memory tasks. Nature. 1986, 321:864-6.
5. Greenblatt DJ, Shader RI. Dependence, tolerance, and addiction to benzodiazepines: clinical and pharmacokinetic considerations. Drug Metab. Rev. 1978; 8:13-28.
6. Kent JM, Mathew SJ, Gorman JM. Molecular targets in the treatment of anxiety. Biol. Psychiatry. 2002; 52:1008-30.
7. Abbiw DK. Portions and Medicine. London: Intermediate

Technology Publications; 1990, 118-205. (Useful plants of Ghana).
8. Nartey ET, Addo PG, Ofosuhene M, Okine LK, Nyarko AK. The effect of Cassia sieberiana roots bark extract on various experimental gastric ulcer models in rats. J Phytomed Ther. 2009; 14:53-59.
9. Kerharo J, Adam JG. La Pharmacopée Sénégalaise Traditionnelle: Plantes médicinales et toxiques. Paris: Editions Vigot frères; 1974. In Burkill, H.M. The useful plants of West Tropical Africa. Families J-L. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 2nd Ed. 1995; 3:857.
10. Elujoba AA, Abere AT, Adelusi SA. Laxative activities of Cassia pods sourced from Nigeria. Nigerian Journal Natural Products and Medicine. 1999; 3:51-53.
11. Souza G, Christina A, Cesar AB, WN. Diphenyl diselenide improves scopolamine-induced memory impairment in mice. Behav Pharmac, 2010; 21:556-562.
12. Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacol (Berl), 1987; 92:180-185.
13. Choleris E, Thomas AW, Kavaliers. A detailed ethological analysis of the mouse open field test: Effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci Behav Rev, 2001; 25:235-260.
14. Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus maze: a novel test of anxiety in rats. Pharmacol Biochem Behav, 1986, 24:525-529.
15. Crawley JN. Behavioral phenotyping of transgenic and knockout mice: Experimental design and evaluation of general health, sensory functions, motor abilities and specific behavioural tests. Brain Res, 1999; 835:18-26.
16. Chandana CB, Archana T, Shameem AB. Anxiolytic activity of methanol leaf extract of *Achyranthes aspera* Linn in mice using experimental models of anxiety. Indian J Pharmacol. 2012; 44(1):63-67.
17. Nsour WM, Lau CB, Wong IC. Review on phytotherapy in epilepsy Seizure. 2001; 9:96-107.
18. Aideé IA, Omar DM, Miguel ÁD. Anxiolytic-like effect of ethanolic extract of *Argemone mexicana* and its alkaloids in Wistar rats. 2016; 6:476-488.
19. Carla L, Giancarlo C, Gian LG. anxiolytic effect of an extract of *Salvia miltiorrhiza* roots in rats 2018; 81:390-397.
20. Charles KB, Robert PB, Donatus WA. Anxiolytic and Antidepressant Effects of *Maerua angolensis* DC. Stem Bark Extract in Mice. 2018; 5:1-17.
21. Carnevale G, Di Viesti V, Zavatti M. Anxiolytic-like effet of *Griffonia simplicifolia* Baill. Seed extract in rats. Phytomedicine. 2011; 18(10):848-851.