Bioactive Constituents and Neuropharmacological Evaluation of *Cassia sieberiana* DC (FABACEAE) Leaf Extract in Murine Models

*Abigail M. Akhigbemen, Joshua Okorie, Ifunanya C. Ehiejirikwe, Omonkhelin J. Owolabi

Department of Pharmacology & Toxicology, University of Benin, Benin- City, Nigeria

ABSTRACT

Background: Cassia sieberiana DC (Fabaceae) is claimed to be used traditionally in the treatment of several central nervous system disease. This study investigated the chemical composition of *Cassia sieberiana* leaf extract as well as its oral acute toxicity, anticonvulsant and antidepressant effects in mice models.

Methods: Cassia sieberiana leaves were collected, dried and powdered. Extraction was done by maceration in methanol to yield the whole extract. The percentage yield of the extract was calculated, chemical composition was determined using GCMS analysis and oral acute toxicity was evaluated using Lorke's method. Anticonvulsant and antidepressant studies were carried out using standard methods at doses of 100, 200 and 400 mg/kg orally.

Results: Oral acute toxicity study recorded no mortality at doses up to 5000 mg/kg. The percentage yield of the plant extract was 7.35%. GC-MS analysis showed 33 peaks corresponding to 33 different compounds. The extract at 100 and 400 mg/kg significantly (p < 0.01) increased the latency of strychnine and pentylenetetrazole induced convulsion but failed to protect the mice from mortality. The extract did not protect the mice from MES induced convulsion. Doses evaluated showed no significant increase the duration of mobility in the forced swim and tail suspension test. Similarly, the extract also did not alter the stay of mice on the rod in motor coordination test.

Conclusion: The leaf extract is safe as the LD50 was greater than 5000 mg/kg. The extract may possess anticonvulsant properties and further study is required to isolate and characterize the active component.

Key words-Acute toxicity, Depression, Epilepsy, GC-MS analysis, Motor coordination,

1. INTRODUCTION

In Africa, the use of traditional healing is an acceptable and known practice comparable with clinical medicine [1, 2] Traditional medicine is known as health practices that include plant, animal and mineral based medicines, spiritual therapies, physical methods and exercises. The World health organization (WHO) defines traditional medicine as the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation whether verbally or in writing [3]. According to the United Nations Conference on Trade and Development, 33% of total modern drugs produced by industrialized or developed countries are plant derived [4]. Epilepsy is a common non communicable disease with an incidence of 8 per 1000 persons [5] or 9.8 per 1000 [6] in Nigeria. The most common comorbid psychiatric malady in epilepsy is depression. The prevalence is estimated between 6% and 30% in population-based studies and up to 50% among patients followed up in tertiary centers [7]. Cassia sieberiana has been claimed to be used as a traditional cure for epilepsy. Various phytochemical constituents have been reported to be present in C. sieberiana. Its seeds have been found to contain moderately high crude protein (23.72%), crude fibre (10.75%), potassium (252.33 mg/L) and magnesium (52.68 mg/L) [8]. Tannin, alkaloids, phenol, oxalate, cardiac glycosides and flavonoids are also present, while saponin is absent [8]. However, the pulp (fruit) revealed the presence of saponins together with tannins, alkaloids, steroids, flavonoids, phlobatannins, cardiac glycosides, cyanogenic glycosides and reducing sugars [9]. Saponins, anthraquinones, steroils, steroidal glycosides, tannins, triterpenes have also been reported in the root [10]. The aim of this study is to evaluate the anticonvulsant and antidepressant extract of Cassia sieberiana in mice as well as identify the biological constituents present in the plant extract.

* Corresponding author: Email:abigail.omo-isibor@uniben.edu; Phone: +234 7032470846



2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Materials

The leaves of *Cassia sieberiana* were collected, identified and authenticated by Mallam Ibrahim Muazzam of National Institute of Pharmaceutical Research and Development (NIPRID) Abuja, Nigeria. The voucher specimen was deposited in the herbarium for future reference.

2.2 Methods

2.2.1Plant materials and extraction

The leaves of *Cassia sieberiana* were air dried to a constant weight and milled to a fine powder using mechanical grinder. The powdered leaves (500 g) were macerated with 2.5L of methanol for 72 hours. The material was filtered using a clean piece of cloth. The filtrate was concentrated to dryness under reduced temperature and pressure in an oven. The dried plant extract was stored at 4°C until use. The weight of the residue was used to determine the percentage yield.

2.2.2 Gas chromatography- Mass Spectrometry (GC-MS) Analysis

The extract (0.1 g) was dissolved in 10 mL of 70% methanol and was allowed to stand for 1 to 2 h in a closed test tube. The extracted sample was decanted, centrifuged and filtered using a micron filter into a 5 mL sample bottle. Analysis of the methanol extract was done using a gas chromatography instrument (model 7890A, Agilent USA) [15]. The instrument was hyphenated to a mass spectrophotometer (model 5975C) having a triple axis detector equipped with a 10 μ L syringe. Helium served as the carrier gas. All chromatographic separations were done using capillary columns with the following specification: 30 m length; 0.2 μ m internal diameter; 250 μ m thickness; and 5% phenylmethyl siloxane was used for treatment. Other conditions of the GC-MS include: ion source temperature of (ED) 250; interface temperature of 300; pressure of 16.2 psia; out time of 1.8 mm; 1 μ L injector mode with split ratio 1:50 with injection temperature of 300. The column temperature was started at 35 for 5 min and changed to 150 at the rate of 4 per min. The temperature was raised to 250 at the rate of 20 per min and was held at that temperature for 5 min. The flow rate was set at 1.5 mL/min and total elution time was 47.5 min. The system was controlled for data acquisition using the MS solution software provided by the supplier of the instrument. The compounds were identified by name, molecular formula, and molecular weight by comparing the mass spectra obtained with those of standard spectra from NIST library. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

2.2.3 Experimental animals

Male albino mice weighing (22-30 g) was obtained from the Animal House of Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The mice was kept in plastic cages and housed at room temperature. They were fed with dry rodent pellets and allowed free access to food and water. The bedding materials (wood shavings) of the cages were changed daily. All tests were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 2002. Ethical approval was obtained from the ethics committee of the Faculty of Pharmacy University of Benin (EC/FP/021/16).

2.2.4 Oral acute toxicity test

Oral median lethal dose (LD50) of the plant extract was determined using Lorke's (1983) method [11]. In the first phase, 3 groups of 3 mice each were administered 10, 100 and 1000 mg/kg of the plant extract orally. The second phase involved 3 groups of 1 mouse each administered 1600, 2900 and 5000 mg/kg of the plant extract orally. In both phases the animals were observed for signs of writhing, diarrhea, tremor and mortality within a period of 24 hours.

2.2.5 Anticonvulsant activity test

Strychnine-induced convulsion: Twenty-five male mice were divided into five groups of 5 mice each. Group 1 was treated with 0.2 mL, 5% tween 80, while groups II, III, IV and V were given 100, 200 and 400 mg/kg of the plant extract and diazepam (2 mg/kg) respectively. One hour later, 1 mg/kg strychnine was administered intraperitoneally (i.p) to all the animals. Each animal was observed for the onset of convulsion or death for a period of 30 minutes [12].

Pentylenetetrazole - induced convulsion: Twenty-five mice were divided into five groups of 5 mice each. Group 1 was treated with 0.2mL, 5% tween 80, while groups II, III, IV and V were given 100, 200 and 400 mg/kg of the plant extract and diazepam (2 mg/kg) respectively. One hour later, pentylenetetrazol (70 mg/kg) was administered intraperitoneally (i.p) to mice in all the groups. The time of onset of tonic-clonic convulsions and mortality/ protection was recorded [13].



Maximal electroshock-induced convulsion: The method described by Swinyard et al., 1952 as used. Twenty five mice were divided into five groups of 5 mice each. Group 1 was treated with 0.2 mL, 5% Tween 80 while groups II, III, IV and V were given 100, 200 and 400 mg/kg of the plant extract and phenobarbitone (30 mg/kg) respectively. One hour later, all animals were subjected to electroshock at a current of 50 mA for 0.2 seconds through a pair of ear clip electrodes. The onset of tonic hind limb extension as well as protection was noted.

2.2.6 Antidepressant test

The Forced swim test: Twenty-five mice were randomly allotted to 5 groups of 5 mice each. Group 1 received 0.2 mL of 5% Tween 80 while II, III and IV received 100,200 and 400 mg/kg of the plant extract orally, while group V received imipramine (25mg/kg) orally as well. An hour later, the mice were forced to swim in an open square tank (25cm ×25cm), filled with 15 cm of water [15]. The first 2 min was just an observation time. Total mobility was recorded for the last 4 min of a 6 - min duration test. Water in the tank was changed with each group. In this test, mice were isolated from exposure to sound.

The Tail suspension test: Twenty-five mice were randomly allotted to 5 groups of 5 mice each. Group 1 received 0.2 mL of 5% tween 80 and served as negative control, while groups II, III, IV and V received 100, 200 and 400 mg/kg of the plant extract orally, and 25 mg/kg of imipramine respectively. An hour later, the tails of mice were suspended using adhesive tape to a horizontal bar for 6 min duration test. The first 2 min was observation time while the total mobility was recorded in the last 4 min. Mice were isolated from exposure to sound.

Motor coordination test: The Rota-rod test described previously [16] but modified by Perez et al., (1998) was used to evaluate motor coordination. Male mice were screened on a treadmill device (Ugo Basile Rota-rod 7650, Italy) with slowly revolving rods of 5cm diameter at 16 rmp for 120 seconds. Mice that was able to remain on the rod for 120 seconds or longer were selected and allotted to five groups of five mice per group. Group 1 received 5 mg/kg diazepam while group II, III, IV and V received 100, 200 and 400 mg/kg of the plant extract and 0.2 mL of 5% tween 80 respectively. The animals were placed individually on the rod at 30 and 60 minutes. The time each animal spent on the rod was noted

2.3 Statistical Analysis

Data are expressed as mean \pm S.E.M (standard error of mean) and analyzed by one way analysis of variance (ANOVA). Statistical analyses were carried out using graph pad prism version 9.0 (GraphPad software, San Diego, CA, USA). p<0.05 was considered statistically significant.

3. RESULTS

3.1 Percentage yield of the extraction

The weight of the residue left behind after extraction of the leaves of *Cassia sieberiana* was 36.757g giving a percentage yield of 7.35% for methanol extract.

3.2 Acute oral toxicity of the extract

No lethal effect was observed at doses of 1600, 2900 and 5000 mg/kg of *Cassia sieberiana* extract. Physical observation showed no signs of changes in the skin, fur, eyes, writhing and behavior. There was no diarrhea or tremors in the animal. No death was recorded at the tested doses (Table 1). The oral LD_{50} of the extract was therefore estimated to be greater than 5000 mg/kg.

| | Dose(mg/kg) | Writhing | Diarrhoea | Tremor | Death | Others |
|----------|-------------|----------|-----------|--------|-------|--------|
| Phase I | 10 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| | 100 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| | 1000 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| Phase II | 1600 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 |
| | 2900 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 |
| | 5000 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 |

Table 1: Oral acute toxicity of the methanol extract in mice

Three mice were used per group in phase I while a mouse was used per group in phase II.

3.3 Biochemical constituent of cassia siberienna extract.

GC-MS analysis of the extract revealed the presence of 37 compounds as shown in (Fig 1). Table 2 shows the molecular weight and molecular formula of the bioactive constituents of *cassia siberienna* extract.



Akhigbemen et al: Bioactive Constituents and Neuropharmacological Evaluation of *Cassia sieberiana* DC (FABACEAE) Leaf Extract in Murine Models

| Table 2: GC-MS identified constituents of | Cassia sieberiana methanol leaf extract |
|---|---|
|---|---|

| SN | Name of Compound | Retention Time | Molecular Weight (g/mol) | Molecular Formula | Structure |
|----|---|-------------------|--------------------------------|--|---|
| 1 | Butyric acid,3 amino | 2.29 | 22.9 | C ₄ H ₉ NO ₂ | |
| 2 | betaD-Ribopyranoside, methyl | 2.43 | 150.13 | C ₅ H ₁₀ O ₅ | он Но он но он |
| 3 | Oxalic acid, monoamide, N-cyclopentyl-, hexyl ester | 2.67 | 241.33 | C ₁₃ H ₂₃ O ₃ | |
| 4 | Alpha-l-rhamnopyranose | 3.53 | 164.1 | C ₆ H ₁₂ O ₅ | HO OH OH |
| 5 | Hydroxylamine, O-(3- methylbutyl)- | 3.68 | 103.1 | C ₅ H ₁₃ NO | |
| 6 | 2-Hexene, 5-methyl-, (E)- | 3.84 | 98.1 | C7H14 | CH ₃ CH ₃ CH ₃ |
| 7 | Carbonyl sulfide | 4.04 | 60.0 | COS | o=c=s |
| 8 | 1-Fluorooctane | 4.62 | 132.2 | $C_8H_17_F$ | FCH ₃ |
| 9 | Acetic acid, pentyl ester | 5.37 | 130.18 | C ₇ H ₁₄ O | |



| 10 | 1 Dutanol 2 mathul | 5 5 1 | 120.1 | СНО | |
|----|---------------------------|-------|-------|----------------------|--|
| 10 | 1-Butanoi, 5-metriyi-, | 5.54 | 130.1 | $C_7 \Pi_{14} O_2$ | CH3 O |
| | acetate | | | | l l l |
| | | | | | |
| | | | | | H ₂ C |
| 11 | Unotherne | 5 72 | 80.0 | C II NO | 1.30 0 0.13 |
| 11 | Uretnane | 5.75 | 89.0 | $C_3H_7NO_2$ | НО |
| | | | | | |
| | | | | | н—мн⊔ >==о |
| | | | | | NÏ / |
| | | | | | |
| 12 | Cyclopropane, ethyl- | 6.00 | 70.1 | C_5H_{10} | |
| | | | | | ОН |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | но |
| | | | | | |
| | | | | | о́н |
| 13 | Pentane, 1-(1- | 6.74 | 60.2 | $C_9H_{20}O_2$ | |
| | ethoxyethoxy)- | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | H N O |
| | | | | | $\hat{\mathbf{I}}$ |
| | | | | | \frown |
| | | | | | |
| 14 | Hexanoic acid, ethyl | 7.22 | 144.2 | $C_8H_{16}O_2$ | |
| | ester | | | | o II |
| | | | | | |
| | | | | | |
| | | | | | |
| 15 | Cyclohexane, ethenyl- | 7.59 | 110.1 | C_8H_{14} | |
| | | | | | |
| | | | | | 0 > |
| | | | | | |
| | | | | | |
| 10 | D 112.11 | 0.00 | 1760 | C II O | INF12 |
| 16 | Propane, 1,1,3-trietnoxy- | 8.28 | 176.2 | $C_9H_{20}O_3$ | CHa |
| | | | | | |
| | | | | | —————————————————————————————————————— |
| | | | | | |
| 17 | 0.1 1 1 1 1 1 | 0.5 | 207.2 | | |
| 17 | Silylamine, 1,1,1- | 8.5 | 207.3 | $C_{12}H_{21}NS_{1}$ | ~ |
| | trimethyl-N-(.al pha | | | | |
| | methylphenethyl)- | | | | Si-HN, |
| | | | | | |
| | | | | | / |
| 18 | Heptanoic acid, ethyl | 9.8 | 158.2 | $C_9H_{18}O_2$ | |
| | ester | | | | |
| | | | | | |
| | | | | | ö |
| 19 | Fumaric acid, hexyl 2- | 10.5 | 308.4 | $C_{18}H_{28}O_4$ | |
| | methylcyclohex-1- | | | | |
| | envlmethvl ester | | | | |
| | | | | | |
| | | | | | |



Akhigbemen et al: Bioactive Constituents and Neuropharmacological Evaluation of *Cassia sieberiana* DC (FABACEAE) Leaf Extract in Murine Models

| 20 | n-Hexadecanoic acid | 10.8 | 256.4 | C ₁₆ H ₃₂ O ₂ | он |
|----|--|------|-------|--|----|
| 21 | Octadecanoic acid | 11.3 | 284.4 | C ₁₈ H ₃₆ O ₂ | |
| 22 | Decanoic acid, ethyl ester | 11.6 | 200.3 | $C_{12}H_{24}O_2$ | |
| 23 | cis-9-Hexadecenal | 14.8 | 238.4 | C ₁₆ H ₃₀ O | |
| 24 | Phthalic acid, cyclohexylmethyl 2- pentyl ester | 14.2 | 318.4 | C ₁₉ H ₂₆ O ₄ | |
| 25 | Bis(2-ethylhexyl) phthalate | 14.4 | 390.6 | C ₂₄ H ₃₈ O ₄ | |
| 26 | 3-Mercapto-2- methylpropanol | 14.7 | 106.1 | C ₄ H ₁₀ OS | H |
| 27 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 14.9 | 278.3 | C ₁₆ H ₂₂ O ₄ | |
| 28 | Hexadecanoic acid, methyl ester | 15.0 | 270.4 | C ₁₇ H ₃₄ O ₂ | |
| 29 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 15.5 | 278.3 | C ₁₆ H ₂₂ O ₄ | |
| 30 | Cyclopentane, 1,2- dimethyl-3-(1-me thylethenyl) | 16.4 | 138.2 | C ₁₀ H ₁₈ | |



| 31 | 11-Octadecenoic acid, methyl ester | 16.1 | 296.4 | C ₁₉ H ₃₆ O ₂ | |
|----|--|------|---------|---|-------|
| 32 | Heptadecanoic acid, 16- methyl- methyl ester | 16.6 | 298.5 | C ₁₉ H ₃₈ O ₂ | |
| 33 | S-1- Propenylmethanethiosulf onate | 17.0 | 152.235 | C ₄ H ₈ O ₂ S ₂ | ° s o |
| 34 | 9-Hexadecenoic acid | 17.2 | 254.4 | C ₁₆ H ₃₀ O ₂ | Н |
| 35 | Simetryn | 17.3 | 231.3 | C ₈ H ₁₅ N ₅ S | |
| 36 | Sarcosine, N-valeryl-, undecyl ester | 17.5 | 237.5 | C ₁₉ H ₃₇ NO ₃ | |
| 37 | Ethyl 2-butyramido- 3,3,3-trifluoro-2-(4- fluoroanilino)propionate | 17.5 | 350.31 | C ₁₅ H ₁₈ F ₄ N ₂ O ₃ | |



Figure 1: GC-MS spectra of *Cassia sieberiana* methanol leaf extract showing the peaks for each of the identified compounds



Akhigbemen et al: Bioactive Constituents and Neuropharmacological Evaluation of *Cassia sieberiana* DC (FABACEAE) Leaf Extract in Murine Models

3.4 Effects of extracts on models of convulsions

Strychnine induced convulsions: The methanol leaf extract of *Cassia sieberiana* offered no protection against strychnine induced convulsion at doses of 100, 200 and 400 mg/kg. When compared to the negative control group (5% tween 80), table 3 shows that the methanol extract at a dose of 400 mg/kg significantly (p<0.05) prolonged the onset of convulsion, table 3 also shows that 100 mg/kg and 400 mg/kg significantly (p<0.05) prolonged the onset of convulsion, demonstrating a non dose dependent action.

Table 3 Effects of methanol extract on Strychnine induced convulsion

| Treatment | Onset of convulsion (sec) | Duration of convulsion (sec) | Percentage (%) protection |
|---|---|---|------------------------------|
| Control 100 mg/kg | $\begin{array}{c} 117.8 \pm 24.83 \\ 341.8 \pm 78.53^{**} \end{array}$ | $14.20 \pm 3.707*$ $17.80 \pm 2.54**$ | 0 0 |
| 200 mg/kg 400 mg/kg Diazepam 2mg/kg | $\begin{array}{c} 181.8 \pm 13.90 \\ 403.3 \pm 132.6^{**} \\ 0.00 \pm 0.00 \end{array}$ | $\begin{array}{c} 16.40 \pm 5.09 * \\ 17.00 \pm 4.461 * \\ 0.00 \pm 0.00 \end{array}$ | 0 0 100 |

*p < 0.05, **p < 0.01 When compared to diazepam group n=5 per group

Pentylenetetrazol (PTZ) induced convulsions: Single dose of pentylenetetrazol (70 mg/kg, i.p) produced hindlimb tonic seizures in all the animals administered 100, 200 and 400 mg/kg of the methanol extract of *Cassia sieberiana*. With respect to the onset of convulsion table 4 showed that the extract at doses of 100 mg/kg (p< 0.01), 200 mg/kg (p< 0.05) and 400mg/kg (p< 0.01) significantly prolonged the onset of convulsion when compared with the negative control (5% Tween 80). With respect to the onset of convulsion, table 4 showed that the extract at doses of 100 mg/kg (p< 0.0001), 200 mg/kg (p< 0.0001) and 400 mg/kg (p< 0.0001) significantly prolonged the onset of convulsion table 4 showed that the extract at doses of 100 mg/kg (p< 0.0001), 200 mg/kg (p< 0.0001) and 400 mg/kg (p< 0.0001) significantly prolonged the onset of convulsion when compared with the negative control.

| Treatment | Onset of convulsion | Duration of convulsion | Percentage (%) |
|-----------------|---------------------|------------------------|----------------|
| | (sec) | (sec) | protection |
| Control | 10.80 ± 1.49 | 19.20 ± 2.29 | 0 |
| 100 mg/kg | 23.40 ± 3.12** | $10.60 \pm 2.01*$ | 0 |
| 200 mg/kg | 22.00 ± 3.91* | 15 ± 2.27 | 0 |
| 400 mg/kg | 24.40 ± 2.35** | 30.75 ± 3.25** | 0 |
| Diazepam 2mg/kg | $0.00\pm0.00*$ | 0.00 ± 0.00 | 100 |

Table 4 Effects of methanol extract on pentylenetetrazol induced convulsion

*p < 0.05, **p < 0.01. Compared to control group n=5 per group

Effects of plant extractt on maximal electroshock induced convulsions: Table 5 showed that there was no protection against hind limb extension seizure (HLES) at doses of 100, 200 and 400 mg/kg of the methanol extract of *Cassia sieberiana*

Table 5: Percentage protection of mice from maximal electroshock induced convulsion by extracts of *Cassia sieberiana*

| Treatment | Number of mice | Percentage (%) protection |
|------------------------|----------------|---------------------------|
| | 0/5 | |
| Control | 0/5 | 0 |
| 100mg/kg | 0/5 | 0 |
| 200mg/kg | 0/5 | 0 |
| 400mg/kg | 0/5 | 0 |
| Phenobarbitone 30mg/kg | 5/5 | 100 |
| | | |

Numerator = Number of mice protected; denominator = Number of mice used in the group

3.5 Effect of extract on the duration of mobility in model of depression

Forced swimming test (FST): Figure 2 shows the effect of the extract on forced swimming model of depression. At doses 100,200 and 400 mg/kg, the extract did not show any significant (p>0.05) increase in the duration of mobility in comparison with the control (5% tween 80). There was no significant difference between the extract and the control group.



Tail suspension test (TST): Figure 3 shows the effect of the extract on tail suspension model of depression. At 100,200 and 400 mg/kg, there was no significant increase in the duration of mobility in comparison with the control (5% tween 80).

Motor coordination test (MST): The extract had no effect on motor coordination as determined by the performance of mice on the Rota rod apparatus. All the mice stayed on the rod for 120 seconds without falling off during the 30 min and 1 hour observation periods (Table 6).



Fig 2 Effect of methanol extract of *Cassia sieberiana* on the duration of mobility in forced swim test. Results are represented as mean \pm SEM with n=5 in each group.







Akhigbemen et al: Bioactive Constituents and Neuropharmacological Evaluation of *Cassia sieberiana* DC (FABACEAE) Leaf Extract in Murine Models

| Treatment | Pos | | |
|-----------------------|-------------|-------------------|-------------|
| | 30 | Time on rod (sec) | 60 |
| Control (5% tween 80) | 120.00±0.00 | | 120.00±0.00 |
| 100mg/kg | 120.00±0.00 | | 120.00±0.00 |
| 200mg/kg | 120.00±0.00 | | 120.00±0.00 |
| 400mg/kg | 120.00±0.00 | | 120.00±0.00 |
| Diazepam 5mg/kg | 4.80±0.73 | | 6.80±0.97 |

Table 6 Effect of the extract on the time spent on Rota rod apparatus.

n=5 mice per group

4. DISCUSSION

Oral acute toxicity test on the plant extract of *Cassia sieberiana* showed that the plant may be relatively safe since the LD50 is greater than 5000 mg/kg in mice. According to the chemical labeling and classification of acute systemic toxicity, any substance with LD50 greater than 5000 mg/kg by oral route is assigned a class 5 status which is the least toxicity class [17]. Kennedy et al., [18] also stated that any substance with LD50 greater than 5000 mg/kg by the oral route can be said to be safe or practically harmless. Fatty acids, esters and alcohols were amongst the 37 compounds identified from Cassia sieberiana extract. Silylamine, 1,1,1-trimethyl-N-(.al pha.methylphenethyl) is a known central nervous system stimulant. The outcome of this study provides evidence that the extract of the leaf of Cassia sieberiana may possess anticonvulsant activity. The ability of the plant extract to delay the onset of convulsion and/or shorten the duration of convulsion was considered an indication of anticonvulsant activity. Strychinine a poisonous alkaloid obtained from seeds of the nux - vomica tree (S. nuxvomica) and other plants of the genus strychnos, induces convulsions by antagonizing competitively the postsynaptic inhibitory effects of glycine [19]. The fact that the extract produced no protective effect in the mice against strychnine-induced convulsions suggests it does not interact with the glycine-mediate inhibitory pathway. Pentylenetetrazol (PTZ) is a noncompetitive antagonist of GABAA receptors that acts through the t-butylbicyclo-phosphorothionate site of the receptor hence decreasing its activity [20]. Another possibility of PTZ action is via alterations in potassium and calcium channel conductance [21]. Drugs active against PTZ-induced convulsions are effective against myoclonic and absence seizures [22]. The extract of Cassia sieberiana increased the latency to PTZ induced convulsion. Phytochemicals as flavonoids present in most plant inhibit seizures generation via direct activation of inhibitory GABAergic receptors or through benzodiazepine receptor resulting in increased chloride ion influx causing neuronal hyperpolarisation [23]. Maximal electroshock (MES) induced convulsions occur through modulation of sodium (Na+) channels. The MES test is effective against generalized tonic-clonic seizures [27]. From the results obtained, the plant extract offered no protection against MES induced convulsion suggesting that the extract does not act via inhibition of voltage gated Na+ channels. However phenobarbitone which protected the mice against maximal electroshock induced convulsion acts via the potentiation of gamma aminobutyric acid (GABA). The gabaergic system is the most vital inhibitory system in the central nervous system but its roles may be altered in different conditions [28]. Forced swimming test and tail suspension test are two widely used screening tests for antidepressants. Exposure to stress plays an important role in depression [35]. The characteristic behavior evaluated in these tests, termed mobility or immobility has been considered to reflect behavioral despair similar to that seen in human depression [36]. This study, noted that the plant extract did not alter significantly the duration of mobility in both the forced swim and tail suspension test in mice. The result shows that the extract caused no alteration or effect on motor coordination of mice confirming non sedative or hypnotic like activity as was observed with diazepam, a centrally acting muscle relaxant known to be gamma aminobutyric acid (GABA) mimetics [47].

5. CONCLUSION

This study justifies the claim that *Cassia sieberiana* is used as a traditional cure for epilepsy. It also shows some of the bioactive compounds present in the plant. However, further studies will be needed to understand the exact mechanism of action of these bioactive compounds.

Acknowledgment

The authors wish to thank the Staff of Animal House, Department of Pharmacology and Toxicology, University of Benin, Benin-city.



Conflict of Interest

There is no conflict of interest associated with this work.

Contribution of the Authors

AMA conceived and designed the study. JO AND IFC collected, analyzed and collected the data. AMA, wrote the manuscript with the collaboration of JOW. All authors read and approved the manuscript for publication.

6. REFERENCES

[1].Natako L. "Honouring the African Traditional Herbalist" African Traditional Herbal Research Clinic Newsletter. Special Edition—HIV/AIDS.25 years2006: 1:10.

[2]. Ezekwesili-Ofili JO and Okaka ANC. Herbal Medicines in African Traditional Medicine, 2019

[3]. WHO/AFRO, 'African Traditional Medicine'. Report of the Regional Expert Committee Technical report series Brazzaville: 1976; (1):3-4

[4]. Raskin I, Ribnicky DM, Komarnytsky S, Ilic N and Poulev A. Plants and human health in the twenty-first century. Trends Biotechnol.2002; 20: 522-531.

[5].Owolabi LF, Owolabi SD, Taura AA, Alhaji ID, Ogunniyi A. Prevalence and burden of epilepsy in Nigeria: A systematic review and meta-analysis of community-based door-to-door surveys. Epilepsy Behav. 2019; 92:226-234.

[6]. Musa M, Salisu AB, Morenikeji AK, Stanley CI, Michael BF, Willem MO, Eric VD, Olaitan O, Anthony AM, Ibrahim A, Joseph Musaet al.Neurology 2021; 97 (7): e728-e738

[7]. Kanner AM. Depression in epilepsy: prevalence, clinical semiology, pathogenic mechanisms, and treatment. Biol Psychiatry 2003:1; 54(3):388-98.

[8]. Olapade AA, Ajayi OA., and Ajayi IA. "Physical and chemical properties of *Cassia sieberiana* seeds." International Food Research Journal, volume 2014; 21: 2

[9]. Toma, Y. Karumi and M. A.Geidam. Phytochemical screening an toxicity studies of the aqueous extract of the pods pulp of Cassia sieberiana DC.(Cassia Kotchiyana Oliv.)African Journal of Pure and Applied Chemistry2009; 3(2):026-030.

[10]. Sam GH, Mensah M.LK and Nyakoa-Ofori N . "Pharmacognostic studies and standardization of Cassia sieberiana roots," Pharmacogn. J., 2011; 3(21):12–17.

[11]. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1983; (54):275-87.

[12]. Porter RJ, Cereghino JJ, Gladding GD. Antiepileptic drug development program.Cleve Clin 1984;(51):293–305.

[13]. Vogel HG, Vogel WH. Drug discovery and evaluation, pharmacological springer.Berlin 1997:260 261.

[14]. Swinyard EA, Brown WC, Goodman LS. Comparative assay of antiepileptic drugs in mice and rats. J Pharmacol Exp Ther 1952;106:319–330

[15].Porsolt RD, Bertin A, Jalfre M. Behavioural despair in mice: a primary screening test for antidepressants. Archive Int Pharmacodynamic Therapeutics 1977; 229:327-336.

[16]. Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am pharm Ass Sci Edn. 1957;46:208-209.



Akhigbemen et al: Bioactive Constituents and Neuropharmacological Evaluation of *Cassia sieberiana* DC (FABACEAE) Leaf Extract in Murine Models

[17]. OECD (2001). Harmonized integrated classification system human health and Environmental hazards and chemical substances and mixtures. OECD testing and assessment 33:1-234.

[18]. Kennedy GL, Ferenz RLJ and Burgess BA. Estimation of acute Toxicity in rats by determination of the appromate lethal dose rather than LD50. Journal of applied toxicology 1986;(6):145-148

[19]. Bigler E.D. (1977). "comparison of effects of bicuculline, strychnine, and picrotoxin with those of pentylenetetrazol on photically evolved after discharges, "Epilepsia, 1977; 18(4):.465-470

[20]. Korpi ER, Gründer G, Lüddens H. Drug interactions at GABA (A receptors. Prog Neurobiol 2002; 67:113-159.

[21]. Madeja M, Stocker M, Mushoff V. Potassium currents in epilepsy effects of The epileptogenic agent pentylenetetrazole on a cloned potassium channel. Brain Research 1994; 656: 287-294.

[22]. Wolfgang L (2010). Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs.Seizure;20: 359-368

[23]. Singh D. Leaf phenology of Cassia sieberiana L. in Ksusta campus of Kebbi State, Nigeria. Sci Technol Public Policy 2017; 1 (1):23-28.

[24].Kasthuri S. A review: Animal models used in the screening of Antiepileptic drugs neuropsy. International research journal of pharmaceutical and applied sciences 2013; 3(3):18-23

[25]. Mody I, Pearce RA. Diversity of inhibitory neurotransmission through GABAA receptors. Trends Neuroscience 2004; 27:569-575.

[26]. De Kloet ER, Joels M, and Holsboer, F. Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 2005; 6: 463–475.

[27]. Mannan, A., Abir, A.B. & Rahman, R. Antidepressant-like effects of methanolic extract of Bacopa monniera in mice. BMC Complement Altern Med 2015; 15; 337

[28]. Charles EG, Adam MK, Franklin RB, and Alan DK. Benzodiazepine, Pharmacology and Central Nervous System–Mediated Effects. Ochsner Journal 2013; 13(2): 214223.

