

Anxiolytic effect of aqueous root extract of *Citrus aurantium* in Wistar albino rats

Abstract

Anxiety is a very common mental disorder among neurological disorders. *Citrus aurantium* decoction has been used to treat anxiety-like behaviour, by traditional medicine practitioners in Nasarawa State, Nigeria. The aim of this study was to evaluate the effect of aqueous root extract of *Citrus aurantium* (AECA) on anxiety, spontaneous alternation behaviour, and locomotor activity. The open field apparatus was used to evaluate effects of AECA and on locomotion. The effect of AECA (50-200 mg/kg, p.o.), diazepam (2.5 mg/kg, i.p.), and 10 ml normal saline/kg on anxiety-like behaviour, spontaneous alternation behaviour, locomotor and exploratory activity were evaluated in rats on hole-board, elevated plus maze (EPM), elevated zero maze (EZM), Y-maze, and open field apparatus, respectively. The results showed that AECA significantly ($p < 0.05-0.001$) and dose-dependently increased time spent on hole-board, open arm of elevated plus maze, EPM and open arm of elevated zero maze, EZM. The administration of AECA produced dose-dependent and significant ($p < 0.05-0.001$) increase in spontaneous alternation behaviour in rats. Locomotor and exploratory behaviour was significantly ($p < 0.05-0.001$) increased in the open field apparatus.

The present results provided evidence for anxiolytic effects of the AECA, hence may be developed as a safe alternative for the continuous use in the therapeutic management of neurological disorders characterized by anxiety and amnesia.

Key words: Anxiety, *Citrus aurantium*, spontaneous alternation behaviour, anxiolytic.

Abbreviations: AECA: Aqueous root extract of *Citrus aurantium*; mg/kg, po: milligram per kilogram of body weight per os (orally); mg/kg, i.p: milligram per kilogram of body weight intraperitoneal; EPM: elevated plus maze; EZM: elevated zero maze; OF: Open field; WHO: World Health Organisation; BZDs: benzodiazepines; GABA_A: gamma-Aminobutyric acid; NVRI: National Veterinary Research Institute.

1. Introduction

Anxiety is a mental disorder characterised by apprehension and persistent fear that is not associated to any real danger or risk (Sivaraman *et al.*, 2012). Hence, result in the feeling of uncertainty that may affect the normal routine of the life style of an individual. About 450 million people are reported to suffer from a mental or behavioural disorder including anxiety (WHO, 2001). The increasing number of individuals suffering from anxiety and other neurological disorders has led to the rise in the use of antidepressant and anxiety drugs. The commonest, safest and widely used antidepressant drug are benzodiazepines (BZDs) used as anxiolytic agents which are known to act on receptors known as BZD-GABA_A receptors (Yadav *et al.*, 2008). However, some side effects such as violent behaviour, nervousness, loss of appetite and sexual dysfunction have been reported to be associated with anxiolytic drugs (Sivaraman *et al.*, 2012).

The utilisation of plants in the treatment of certain human diseases is evidence of man's ingenuity. All these make the knowledge of therapeutic, biochemical and molecular activities of medicinal plants used in traditional medicine, become necessary and led researchers to scientifically study the possible beneficial effects of medicinal plants. The search for a novel and alternative treatment from medicinal plants is still progressing. Some effective medicinal treatment for anxiety related disorders have been reported elsewhere (Aliyue *et al.*, 2014; Tijani *et al.*, 2014).

Citrus aurantium which belongs to the family *Rutaceae* is a widely distributed and most commonly found in the tropics, including Nigeria. The leaves and essential oil obtained from *Citrus aurantium* peel is reported to have anxiolytic and sedative effects in mice (Costa *et al.*, 2013). *Citrus aurantium* decoction has been used to treat anxiety related disorders in the

traditional medicinal practice in Nasarawa State, Nigeria. This study is aimed at evaluating the effects of aqueous root extract of *Citrus aurantium* (AECA) on anxiety, spontaneous alternation behaviour, and locomotor activity.

2. Materials and Methods

Chemicals

Diazepam (Roche, Lagos, Nigeria), normal saline (0.9% sodium chloride), 70% methanol and distilled water were used in the current study.

2.1 Experimental Animals

Male Wistar albino rats weighing 100 to 120 g, obtained from National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria were used in this study. The animals were grouped and housed in cages with four animals per cage and maintained under standard laboratory conditions of humidity ($60 \pm 2\%$), temperature ($25 \pm 1^\circ\text{C}$) and a controlled 12h dark and light cycle. The animals were fed with standard vital diet supplied by Grand Cereals Ltd., Jos, Plateau State, Nigeria and fresh water *ad libitum*. All the animals were acclimatized to laboratory condition for one week before commencement of experiment. Food was withheld for 24 h prior to each experiment. All the guidelines of Institutional Animal Ethics Committee were followed while handling the animals.

2.2 Plant Material

The root of *Citrus aurantium* was collected from Keffi (Nasarawa State, Nigeria) by a traditional medical practitioner. The leaves, fruits and roots were identified and authenticated by Mr Musa Mara Likita of Plant Science and Biotechnology Unit, Biological Sciences Department, Nasarawa State University, Keffi and voucher specimen (NSH 312) was deposited at the herbarium. The roots were cut into small pieces, cleaned, air-dried at room temperature ($35\text{--}37^\circ\text{C}$) to minimize loss of volatile constituents, and pounded into fine powder using mortar and pestle. The powder was stored in an airtight container and kept at 25°C for subsequent use.

2.3 Extraction method

Five thousand grams (5000g) of the powdered sample was then soaked in 5000ml of water for 48h through infusion method at room temperature. The extract was then filtered twice through cotton wool, then through Whatman filter paper (No 1). The filtrate was allowed to evaporate to dryness using a water bath at 45°C and dried under reduced pressure to obtain aqueous extract of *Citrus aurantium* (AECA).

2.4 Phytochemical screening

Phytochemical studies of AECA were done using the standard method previously described by Tiwari *et al.* (2011) with some modifications for detecting the presence of secondary metabolites: alkaloids, phenol, flavonoids, carbohydrates, triterpenes, saponin, and tannins.

2.5 Behavioural tests

All the behavioural procedures were carried out between 8:00am and 12:00pm in a temperature controlled room ($25 \pm 1^\circ\text{C}$). The rats were grouped such that each group consisted of equal number of rats; five rats per group.

2.6.1 Hole-Board

The hole-board test is an anxiety paradigm based on novelty and uncertainty on the animal behaviour. Head-dipping is generally considered to provide a measure of exploration that was distinct from motor activity. Thirty six rats were divided into six groups, seven per group. The animals were placed on board (40×40) with 16 holes (symmetrically distributed in four rows) 1 h after oral administration of 10 ml normal saline/kg, standard reference drug

diazepam (2.5 mg/kg, i.p.) and different doses of AECA (50, 100 and 200 mg/kg, p.o) were administered 30 minutes before the test. The frequency of head dips into the holes during 10 minutes was registered immediately after the administration of AECA at different doses used and the total locomotion (number of floor units entered-the floor of the squares crossed with all paws) was also recorded. They are represented as mean total number of head dips (Lister, 1987).

2.6.2 Elevated plus maze

This study was carried out according to the method described by Akanmu *et al.*, (2011) with slight modification. Thirty six rats were divided into six groups. The animals were administered the crude sample of AECA (50, 100, and 200 mg/kg, p.o.). The control group received 10 ml normal saline/kg while standard reference drug diazepam (2.5 mg/kg, i.p.) was administered 30 minutes before the test. At the start of the session the rat was placed at the centre of the maze with the head facing an open arm and allowed to explore the maze for 5 minutes. During the 5 minute observation period, the following measurements were recorded: the number of entries and the time spent in open and closed arms, and the exploratory behaviour (total number of arm entries). An entry with all feet with the exception of the tail put into one arm is defined as an arm entry in this experiment; four groups of mice were tested as described in the experimental procedures above. All the animals were also subjected to same experimental model for anxiety study. The maze apparatus was carefully wiped between tests with a cotton wool moistened with 70% methanol after each animal to prevent odour bias.

2.6.3. Elevated zero maze

This study was carried out according to the method described by Tijani *et al.*, (2014). Rats were randomly divided into five groups of four rats each. One hour before this test, rats were treated with graded doses of AECA (50, 100, and 200 mg/kg, p.o.). The control group received 10ml of normal saline/kg while standard reference drug diazepam (2.5 mg/kg, i.p.) was administered 30 minutes before the test. 1 h after drug administration, each rat was placed at the centre of the open arm (facing toward the closed chamber). The times spent in both open and closed arms of the maze were manually recorded. The maze apparatus was thoroughly wiped between tests with a cotton wool moistened with 70% methanol after each animal to prevent odour bias.

2.6.4 Open-field test (OF)

The Open Field (OF) test was carried out using the method described by Herrera-Ruiz *et al.*, (2008). The OF apparatus consists of a transparent glass box (45×45 cm). The floor was divided by lines drawn into 9 equally sized squares. Twenty five rats were randomized into five groups of rats each. An hour before test session, rats were treated orally with graded doses of AECA (50, 100, and 200 mg/kg, p.o.). Another group received diazepam (2.5mg/kg, i.p.) while the control received 10 ml normal saline/kg orally. One hour after extract and 30 minutes after diazepam administration, each rat was placed individually in the centre of the apparatus. The locomotor (number of squares crossed with four paws) and exploratory activities (indicated by frequency of rearing) was recorded manually for 5 minutes period (Souza *et al.*, 2010; Walsh and Cummins, 1976). The apparatus were cleaned between tests with cotton wool moistened with 70% methanol after each animal to prevent odour bias.

2.6.5 Y-maze task

The Y-maze test was carried out using the method described by Tijani *et al.* (2014). Y-maze is used to measure immediate general locomotor activity, spatial working memory and stereotypic behaviour (Heo *et al.*, 2009). The Y-maze is made up of three-arm horizontal maze of equal size (45cm long and 7 cm wide with walls 14 cm high) in which the floor of

each arms consist of wood 5cm wide. Rats were initially placed within one arm (A), and the arm entry sequence (e.g., ABC BCA, CAB, where letters indicate each arm codes) and the number of arm entries were mutually recorded for each rat over a 6 minute period. The maze arms were cleaned with cotton wool moistened with 70% methanol after each animal to prevent odour bias. The spontaneous alternation determined from successive entries into the three arms on overlapping triplet sets in which three different arms are entered. An actual alternation percentage was defined as entries into all three arms consecutively except for its tail (i.e., each arm is labelled A, B or C: ABC, A, B, CAB, BCA, B, A, ABC, B, C). In the above example, the rat entered 18 arms, twelve of which were alternations. An entry was defined as placing all four paws within the boundaries of the arm. Hence, an hour before this test, rats were treated with graded doses of *Citrus aurantium* aqueous root extract (50, 100, and 200 mg/kg, p.o.). The control group received 10 ml normal saline/kg and diazepam (2.5mg/kg, i.p.). The percentage spontaneous alternation was calculated. The number of maximum spontaneous alternation is therefore the total number of arms entered minus two and the percentage (Heoet *al.*, 2009).

Statistical analysis

All values were expressed as mean $\pm$ SEM from six animals. Statistical analysis was carried out using one way analysis of variance (ANOVA) using Smith statistical package (SSP). Student's t-test was used to calculate the significant difference if any at 95% probability level.

3. Results and Discussion

Phytochemical screening

Results of phytochemical screening of the aqueous root extract of *Citrus aurantium* (AECA) are shown in Table 1. As illustrated, the major phytochemical constituents of AECA are phenol, saponins and tannins.

Table 1: Results of phytochemical screening of the aqueous root extract of *Citrus aurantium* (AECA)

Secondary metabolites	Inference
Alkaloids	+
Phenol	++
Flavonoids	+
Carbohydrates	-
Triterpenes	+
Saponin	++
Tannins	++

+ Slightly present, ++ present, - Absent.

Effect of AECA on Hole-board model

The result showed that the AECA extract ((50, 100 and 200 mg/kg) significantly ($p < 0.05$ -0.001) increased the frequency of head dips on hole-board model as dose decreases. Diazepam showed no significant difference when compared to the control, hence more potent in increasing the frequency of head dips on the hole-board than the AECA (figure 1). The

decrease observed in the AECA extract is said to be dose-dependent on this model. The frequency of the head dips decreases as the concentration increases.

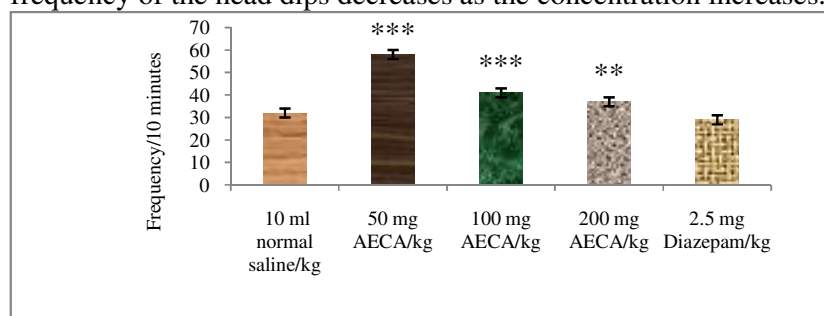


Figure 1: Effect of AECA on frequency of head dips in hole-board model. **, *** Significantly different from the control at $p<0.01$ or $p<0.001$ respectively

Effects of AECA on elevated plus maze (EPM)

The AECA extract (50 and 100 mg/kg) significantly ($p<0.05-0.001$) decreased the time spent in the close arm of the elevated plus maze (EPM). However, 200 mg AECA/kg significantly increased the time spent in the closed arm of the EPM. Diazepam was more potent in reducing the time spent in closed arm of the EPM than the AECA except at conc.200mg (Figure 2). AECA significantly ($p<0.001$) increased the time spent in the open arm of the elevated plus maze except for 200 mg AECA/kg which decreased time spent in the open arm of elevated plus maze significantly, while diazepam increased time spent on open arm of the maze (Figure 3).

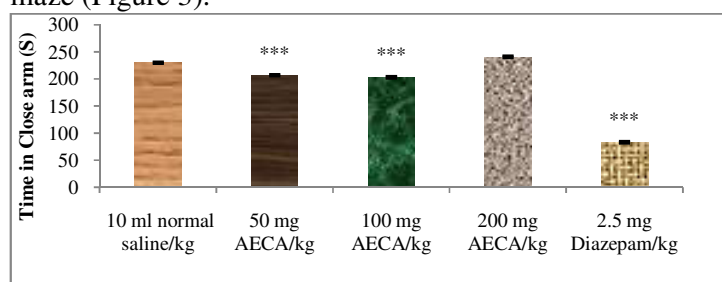


Figure 2: Effect of AECA on time spent in close arm of the EPM. *** Significantly different from the control at $p<0.001$.

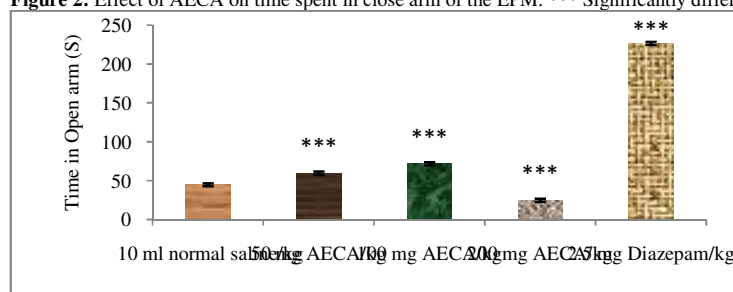


Figure 3: Effect of AECA on time spent in opened arm of the EPM. *** Significantly different from the control at $p<0.001$

Effect of AECA on elevated zero-maze

AECA significantly ($p<0.001$) decreased the time spent in the closed arm of the elevated zero maze except for 200 mg AECA/kg where significant increase in time spent in the open arm was observed (Figure 4). AECA significantly ($p<0.001$) increased the time spent in the open arm of the elevated zero maze except for 200 mg AECA/kg which decrease time spent in the open arm of elevated zero maze significantly, while diazepam increased time spent on open arm of the maze (Figure 5).

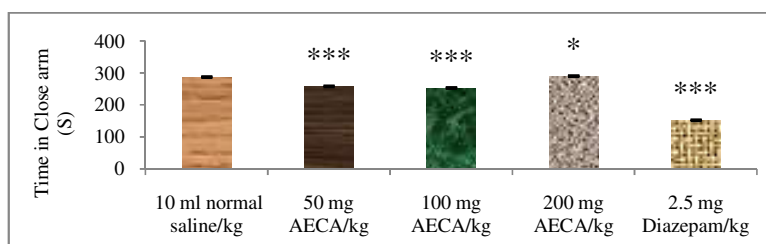


Figure 4: Effect of AECA on time spent in closed arm of zero-maze. *, *** Significantly different from the control at $p<0.05$, or $p<0.001$ respectively.

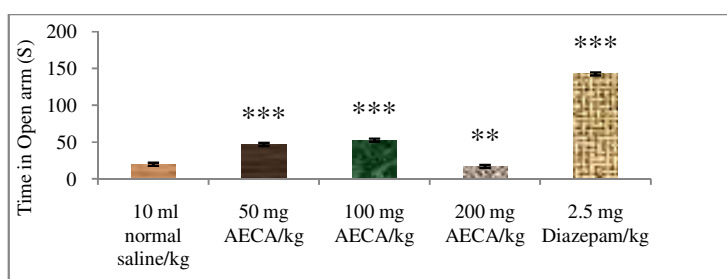


Figure 5: Effect of AECA on time spent in open arm of zero-maze. **, *** Significantly different from the control at $p<0.01$ or $p<0.001$ respectively.

Effect of AECA on total loco motor activity

The AECA extract at 50 and 100 mg/kg significantly ($p<0.05$ -0.001) increased the total locomotive activity of rats on open field apparatus (Figure 6). However, at 200 mg AECA/kg there was observed significant decrease in the locomotive activity of rats while diazepam increased time spent on open arm of the maze (Figure 6).

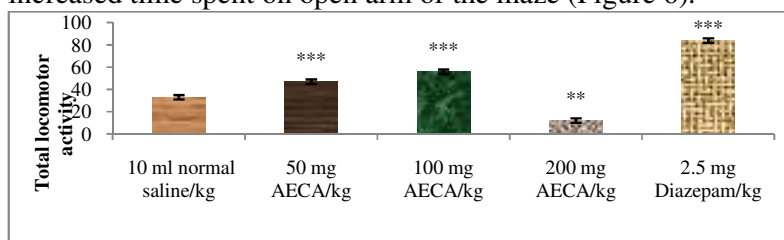


Figure 6: Effect of AECA on total locomotor activity on open field apparatus. **, *** Significantly different from the control at $p<0.01$ or $p<0.001$ respectively.

Effect of AECA on rearing

AECA significantly ($p<0.05$ -0.001) increased the frequency of rearing at dose concentration of 50 and 100 mg/kg except for 200 mg AECA/kg which decrease the frequency of rearing while diazepam increased the frequency of rearing (Figure 7).

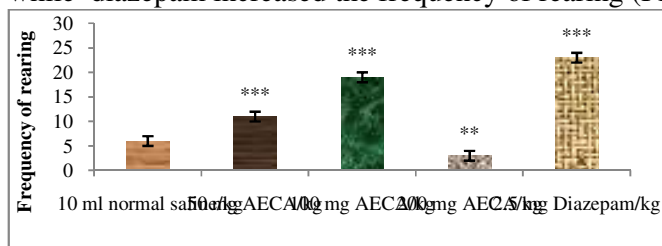


Figure 7: Effect of AECA on rearing. **, *** Significantly different from the control at $p<0.01$ or $p<0.001$ respectively.

Effect of *Citrus aurantium* on spontaneous alternation behaviour of rat in Y-maze

The AECA (50 and 100 mg/kg) and ($p<0.01$ -0.001) increased the spontaneous alternation behaviour in rats compared with the control except for 200 mg AECA/kg which showed no

effect. However, diazepam showed a significant increase in the spontaneous alternation behaviour (Figure 8).

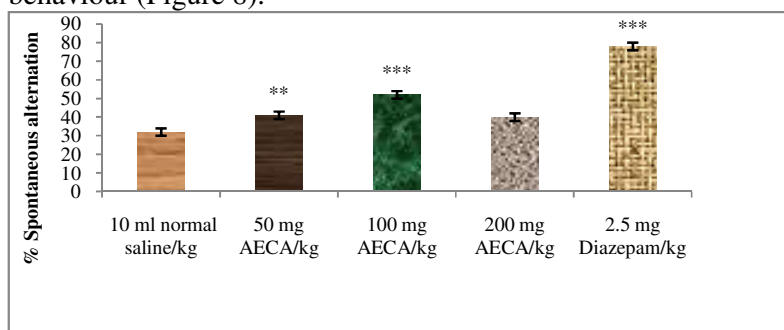


Figure 8: Effect of AECA on spontaneous alternation behaviour. **, *** Significantly different from the control at $p<0.01$ or $p<0.001$ respectively.

The phytochemical screening of the crude extract revealed the presence of alkaloids, phenol, flavonoids, triterpenes, saponin, and tannins. The therapeutic potential of medicinal plant extracts used as traditional remedies have been attributed to a combination of active secondary metabolites which might be involved in inhibiting some mediators that could cause anxiety (Akindele and Adeyemi, 2010). The alkaloids, erysodine and erysothrine are reported to possess anxiolytic-like effects in mice (Serrano *et al.*, 2011). Montanine, anisoquinoline alkaloids are reported to have anxiolytic, antidepressant and anticonvulsant-like effect in mice (Da silver *et al.*, 2006). The behavioural effect of carvacrol, a monoterpenic phenol was reported (Melo *et al.*, 2010). However, flavonoids have the ability to bind to the benzodiazepine site of GABA_A receptor (Griebe *et al.*, 1999). Salgueiro *et al.* (1997) reported anxiolytic effects of two flavonoids including chrysin and apigenin on central benzodiazepine receptors. Moreover, triterpene rich fractions were shown by Chen *et al.* (2006) to possess anxiolytic-like effects. Saponins have been shown to possess anxiolytic properties (Wei *et al.*, 2007). Tannins may also be responsible for the observed neurological effect of this plant due to its possible effect against many CNS disorders (Bhattacharya and Satyan, 1997).

The results of the behavioural studies revealed that the aqueous root extract of *Citrus aurantium* produced significant anxiolytic effect in rats on hole-board, elevated plus maze, elevated zero-maze, and spontaneous alternation behaviour on the open field as well as locomotors and exploratory behaviour of rats on Y-maze. The anxiolytic activities of some agents have been assessed by using the hole-board test (Akanmu *et al.*, 2011). However, increased number of head dips into the holes on the board means reduced anxiety state. In this study, hole-board paradigm showed that exploratory behaviour was decreased with the administration of AECA with increase in the dose. Therefore further investigations on the effects of AECA on anxiety state using the elevated-plus maze may be necessary. The elevated plus-maze (EPM) test represents one of the most widely used animal models for screening anxiolytics (Lister, 1987; Pellow *et al.*, 1985). This test is able to reproduce anxiolytic or anxiogenic effects in animals such that anxiolytics produce increase in the number of entries into the open arms of the maze and the time spent there, while anxiogenic activity of drugs produces the opposite effect (Lister, 1987; Pellow *et al.*, 1985). In this study, *Citrus aurantium* aqueous root extract at 50 and 100 mg/kg significantly increased the time spent in the open arm of the elevated plus-maze. However, the extract produced a significant increase in time spent in the closed arm of the maze at 200 mg/kg. This study is consistent with standard anxiolytics behaviour similar to benzodiazepines, a metabolite of diazepam

with anxiolytic effect at low doses and anxiogenic or sedative effect at higher doses (Aliyuet *et al.*, 2014). Diazepam, a benzodiazepine (receptor agonist) binds to GABA_A receptors to increase the frequency of chloride channel openings resulting in hyperpolarization (Tijani *et al.*, 2012). It increased the frequency of open-arm entries and the time spent in the open arms confirming its anxiolytic effects (Matsubara and Matsushita, 1982). However, at higher dose, the decrease activity was concluded to have a sedative effect. The *Citrus aurantium* aqueous root extract had similar effects on these parameters at 200 mg/kg body weight whereas at lower dose (50-100 mg/kg body weight) confirm its anxiolytic effect.

The anxiolytic effect of the AECA was further confirmed by the results obtained from the use of the elevated zero-maze. The zero-maze has two major advantages over the elevated plus maze: lack ambiguity associated with the interpretation of the time spent in the central area of the elevated plus maze and allowance of uninterrupted exploration (Singh *et al.*, 2007). The extract at 50 and 100 mg/kg produced anxiolytic-like effect which is clearly defined by the increased time spent in the open quadrant of the zero-maze apparatus. However, at 200 mg/kg body weight, the extract exerted anxiogenic-like effect in the various test methods used in the study. This might be due to the extreme spectrum of anxiolytic sedative effects characterized by sedation like behaviour (Tijani *et al.*, 2014). This is consistent with the effect of sedative-anxiolytics.

The open-field apparatus provides information on anxiety-related behaviour characterized by natural aversion of animals to an open brightly lit area (Tijani *et al.*, 2012; Mechanet *et al.*, 2002;). Animals express their anxiety and fear of the centre and spend more time in the protective corners and in freezing state (Tijani *et al.*, 2014). Anxiolytics increase total locomotor activity resulting in a reduction of time spent in corners, an increased time spent in the centre and a decreased time spent in freezing state hence decreasing the exploratory behaviour. The extract at 50 and 100 mg/kg body weight increased total locomotive activity and increased frequency of rearing in treated rats. This study further confirmed the anxiolytic potential of AECA. Natural products of plant origin may elicit anxiolytic effects via interaction with some endogenous mediators such as GABAergic and serotonergic pathways in the body (Tijani *et al.*, 2012). The present study showed that AECA possessed potent anxiolytic effects as evidenced in the results obtained from the various used models.

Spontaneous alternation behaviour determined using Y-maze test is regarded as a measure of spatial short-term memory in animals including rats (Pena *et al.*, 2014). A rat must remember the least recently visited arm in order to alternate the arm choice (Lee *et al.*, 2010). AECA extracts at 50 and 100 mg/kg body weight administered to healthy rats produce significant increase in spontaneous alternation behaviour. Thus, the secondary metabolites such as tannins and saponins present in AECA may be responsible for the observed anxiolytic-like effects in treated rats. Similarly, tannins are reported to possess a significant effect against Alzheimer's disease (Ono *et al.*, 2004) and epilepsy (Yokoi *et al.*, 1989).

In conclusion, the present results provided evidence for anxiolytic effects of the AECA in rats which might be possible through BZD-GABA_A mechanism. This activity may be attributed to the presence of the secondary metabolites presence thus providing rational scientific evidence for its continuous use in the therapeutic management of neurological disorders characterized by anxiety, and amnesia. Further studies on identifying the specific metabolite responsible for the observed pharmacological potential and possible mechanism(s) of actions of these bioactive compounds should be investigated.

References

- [1] Akanmu MA, Olowookere TA, Atunwa SA, Ibrahim BO, Lamidi OF, Adams PA, Ajimuda BO, Adeyemo LE. 2011. Neuropharmacological effects of Nigerian honey in

- 343 mice. African Journal of Traditional, Complementary and Alternative Medicine, 8(3):
344 230-249.
- 345 [2] Akindele AJ, Adeyemi OO. 2010. Anxiolytic and sedative effects of
346 *Byrsocarpuscoccineus*Schum. And Thonn. (Connaraceae) extract. International Journal
347 of Applied Research in Natural Products, 3(1): 28-36.
- 348 [3]Aliyu M, Anuka JA, Yaro AH, Magaji MG. 2014. Evaluation of the Anxiolytic Effect of
349 Methanolic Leaves Extract of *Paulliniapinnata* Lin in Mice. British Journal of
350 Pharmaceutical Research, 4(13): 1638-1646.
- 351 [4] Chen SW, Wang WJ, Li WJ, Wang R, Li YL, Yan Ni Huang YN, Liang X. 2006.
352 Anxiolyti-like effect of asiaticoside in mice. Pharmacology Biochemistry and
353 Behaviour, 85: 339-344.
- 354 [5]Costa CA, Cury TC, Cassettari, BO, Takahira RK, Florio JC, Costa M. 2013. *Citrus*
355 *aurantium* L. essential oil exhibits anxiolytic-like activity mediated by 5-HT (1A)-
356 receptors and reduces cholesterol after repeated oral treatment. BMC Complementary
357 and Alternative Medicine, 13: 42.
- 358
- 359 [6] Dasilver AF, d'Andrade JP, Bevilacqua LR, de Souza MM, Izquierdo I, Henriques AT,
360 Zuanazzi JA. 2006. Anxiolytic-, antidepressant- and anticonvulsant-like effects of the
361 alkaloid montanine isolated from *Hippeastrum vittatum*. Pharmacology Biochemistry
362 and Behaviour, 85(1): 148-154.
- 363 [7] De Angelis L, Bertolissi M, Nardini G, Traversa U, Vertua R. 1982. Interaction of
364 caffeine with benzodiazepines: behavioural effects in mice. Archive International of
365 Pharmacodynamics and Therapy, 255: 89-102.
- 366 [8] Griebel G, Perrault G, Tan S, Schoemaker H, Sanger DJ. 1999. Pharmacological studies
367 on synthetic flavonoids: comparison with diadepam. Neuropharmacology, 38: 965-977.
- 368 [10]Herrera-Ruiz M, Roman-Ramos R, Zamilpa A, Tortoriello J, Jimenez-Ferrer JE. 2008.
369 Flavonoids from *Tiliaamericana* with anxiolytic activity in plus-maze test. Journal of
370 Ethnopharmacology, 118: 312-317.
- 371 [11]Hritcu L, Clicinschi M, Nabeshima T. 2007. Brain serotonin depletion impairs short-term
372 memory, but not long-term memory in rats. Physiology & Behaviour, 91: 652-657.
- 373 [12]Kim DH, Hung TM, Bae KH, Jung JW, Lee S, Yoon BH, Cheong JH, Ko KH, Ryu JH.
374 2006. Gomisins A improves scopolamine induced memory impairment in mice.
375 European Journal of Pharmacology, 542: 129-135.
- 376 [13]Lee M, Yun B, Zhang D, Liu L, Wang Z, WangC, Gu L, Wang C, Mo E, Ly S, Sung C.
377 2010. Effect of aqueous antler extract on scopolamine-induced memory impairment
378 in mice and antioxidant activities. Food Science and Biotechnology, 19: 655-661.
- 379 [14]Lister RG. 1987. The use of a plus-maze to measure anxiety in the mouse.
380 Psychopharmacology (Berl), 92: 180-185.
- 381 [15]Matsubara K, Matsushita A. 1982. Changes in ambulatory activities and muscle
382 relaxation in rats after repeated doses of diazepam. Psychopharmacology, 77: 279-283.
- 383 [16] Ono K, Hasegawa K, Naiki H, Yamada M. 2004. Anti-amyloidogenic activity of tannic
384 acid and its activity to destabilize Alzheimer's beta-amyloid fibrils *in vitro*.
385 BiochimicaetBiophysicaActa, 1690(3): 193-202.
- 386 [17] Pellow S., Chopin P., File S.E., Briley M. (1985). Validation of open: closed arm entries
387 in an EPM as a measure of anxiety in the rat. Journal of Neuroscience Methods, 14 (3),
388 149-167.
- 389 [18] Pena ID, Yoon SY, Kim HJ, Park S, Hong EY, Ryu JH, Park IH, Cheong JH. 2014.
390 Effects of ginsenoside Rg3, an Rg3-enriched fraction, on scopolamine-induced memory
391 impairment and learning deficit in mice. Journal of Ginseng Research, 38(1): 1-7.

- 392 [19]SalawuO.A., Tijani, A. Y., Akingbasote, J. A., Oga, F. E., (2010): Acute and sub-
 393 acuteToxicological study of the ethanolic stem bark extract of *Faidherbiaalbidain* rats.
 394 *African Journal of Biotechnology*, 9: 1218-1224.
- 395 [20] Salgueiro JB, Ardenghi P, Dias M, Ferreira MBC, Izquierdo I, Medina, JH. 1997.
 396 Anxiolytic Natural and Synthetic Flavonoid Ligands of the Central Benzodiazepine
 397 Receptor Have No Effect on Memory Task in Rats. *Pharmacology Biochemistry and*
 398 *Behaviour*, 58(4): 887-891.
- 399 [21] Serrano MAR, Batista AND, Bolzani VD, Santos LD, Nogueira PJD, Nunes-De-Souza
 400 RL, Latif A, Arfan, M. 2011. Anxiolytic-like effects of erythrinian alkaloids from
 401 *Erythrinasuberosa*. *Quimica Nova*, 34(5): 808-811.
- 402 [22]Silvaraman D, Muralidharan P, Habibur R. 2012. Evaluation of the anxiolytic effect of
 403 methanolic leaf extract of *Ficushispida*Linn. In corticosterone induced anxiety in young
 404 adult rats. *Pharmacologia*, 3: 467-471.
- 405 [23] Singh K, Bishnoi M, Kulkarni SK. 2007. Elevated Zero-maze: A paradigm to evaluate
 406 anti-anxiety effects of drugs. *Methods and Findings in Experimental and Clinical*
 407 *Pharmacology*, 29(5): 343.
- 408 [24]Souza G, Christina A, Cesar AB, Marlon RL, Gilson Z, Christina WN. 2010.
 409 Diphenyldiselenide improves scopolamine-induced memory impairment in mice.
 410 *Behavioural Pharmacology*, 21: 556-562.
- 411 [25]Tijani AY, Okhale SE, Salawu TA. 2014. Neuropharmacological effects of standardized
 412 aqueous stem bark extract of *Parkiabiglobossain*Wistar rats. *Avicenna Journal of*
 413 *Phytomedicine*, 4(1): 59-71.
- 414 [26]Tijani AY, Salawu OA, Anuka AJ, Isah MH. 2012. Sedative and Anxiolytic effects of
 415 *Crinum zeylanicum*. *Medicinal Chemistry and Drug Discovery*, 3: 20-29.
- 416 Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. 2011. Phytochemical screening and
 417 Extraction: A Review. *InternationalePharmaceuticaScientia*, 1; 98-106.
- 418 .
- 419 [27] Wei XY, Yang JY, Wang JH, Wu CF. 2007. Anxiolytic effect of saponins from
 420 *Panaxquinquefolium* in mice. *Journal of Ethnopharmacology*, 111(3): 613-618.
- 421 World Health Report. 2001. Mental health: New understanding new hope. WHO, Geneva.
- 422 Wu CF, Liu YL, Song M, Liu W, Wang JH, Li X, Yang JY. 2003. Protective effects of
 423 pseudoginsenoside-F11 on methamphetamine-induced neurotoxicity in mice.
 424 *Pharmacology Biochemistry and Behavior*, 76(1): 103-109.
- 425 [28]Yadav AV, Kawale LA, Nade VS. 2008. Effect of *Morusalba* L. (mulberry) leaves on
 426 anxiety in mice. *Indian Journal of Pharmacology*, 40(1): 32-36.
- 427 [29]Yokoi I, Kabuto H, Akiyama K, Mori A, Ozaki M. 1989. Tannins inhibit the occurrence
 428 of epileptic focus induced by FeCl₃ injection in rats. *The Japanese Journal of Psychiatry*
 429 *and Neurology*, 43(3): 552-553.