BEHAVIOURAL STUDIES ON THE METHANOLIC STEM BARK EXTRACT OF FICUS INGENS (MIQUEL) MIQUEL (MORACEAE) IN MICE

OFFIAH RO1, 4, ANUKA JA2, MAGAJI MG3, SALAWU OA3, TIJANI AY3
1Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria
2Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria
3Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria
4Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development Idu Industrial Area, P.M.B 21, Garki –Abuja, Nigeria

ABSTRACT
Ficus ingens (Miquel) Miquel belongs to the Moraceae family. It is claimed to be used by traditional practitioners in North-western Nigerian for the management of mental illnesses. In this study, the behavioural effects of methanolic extract of Ficus ingens (Miquel) Miquel stem bark were investigated in mice at doses of 75, 150 and 300 mg/kg, using diazepam-induced sleeping time, hole board test, beam walking assay, elevated plus maze, elevated zero maze and staircase test in mice. The results revealed that the extract significantly (P<0.0005) prolonged the duration of diazepam-induced sleep without any effect on the latency to sleep at all the doses tested. The extract slightly decreased the number of head dips in the exploratory behaviour of mice in the hole board test. It did not significantly alter the time taken to complete task and the number of foot slips in the beam walking assay. The extract significantly (P< 0.001) reduced the total number of rearing as well as the number of upward stairs climbed in the staircase test. The extract had no significant effect on the number of open arm entries nor the time spent in the open arms as compared to the control group in both the plus maze and the zero maze. The Oral and intraperitoneal LD50 were both found to be greater than (>) 5,000 mg/kg. The preliminary phytochemical screening revealed the presence of saponins, triterpenoids, alkaloids, flavonoids, phlobatansins and anthraquinones. These results suggest that the extract contains biologically active principles that are sedative in nature and may be responsible for the ethno medicinal use of the plant in mental illnesses.

INTRODUCTION
The World Health organization (WHO) has recommended, especially in developing countries, the initiation of programmes designed to use medicinal plants more effectively in traditional health care systems [1]. Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plants, animals and mineral based medicine, spiritual therapies, manual techniques and exercises, applied singularly, or in combination to treat, diagnose and prevent illnesses or maintain well-being [2]. A number of countries are exploring the possibility of developing their own traditional health care system (with plants playing a central role) as an alternative to conventional health care systems which are usually expensive and unaffordable to the vast majority of their citizenry. A number of medicinal plants have been used by traditional healers in the management of many ailments for many years [3]. According to WHO’s estimation, about 450 million people worldwide suffer from a mental or behavioural disorder [2]. Majority of such patients in the developing countries still rely on traditional healing practices and medicinal plants for treatment of these conditions. Alternative treatments are increasingly being used to alleviate affective
disorders [4] and the plant kingdom can be a rich source of bioactive compounds which can serve as lead compounds in the production of potent agents that can be used in the management of these disorders. *Ficus ingens* which belongs to the Moraceae family [5], is one of the many medicinal plants with long historical use in traditional medical practices. The bark is considered a tonic. Extracts of the bark are administered to cows with low milk production [6]. Zulus in South Africa also give the same to people suffering from anaemia. In Borno, Nigeria, preparations of the bark, roots and leaves are used for piles and diarrhea and as laxative and diuretic. Nevertheless, scientific research is needed to provide evidences of its safety and efficacy [7]. Although there are no documented claims of the use of the plant for the management of neuropsychiatric disorders in traditional medicine, however, the claim of the use of the root of the plant by some traditional practitioners in north-western Nigeria for mental illnesses (Muhammad Maiwada, Personal Communication) and the preponderance of information on the ethnomedicinal uses of other species of the genus, *Ficus* informed the investigation of the methanolic stem bark extract of *Ficus ingens* for CNS depressant activity.

MATERIALS AND METHODS  

**Plant material**  

**Collection and Identification**  
The whole plant (stem bark, leaves and flower) of *Ficus ingens* was collected from Sabo Wuse in Niger state of Nigeria and identified by staff of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Idu- Abuja, Nigeria where a voucher sample with specimen number (NIPRD/H/6590) was prepared and deposited for future references.

**Extraction**  
The stem bark was air-dried at room temperature and crushed. About 100 g of the dry plant material was macerated in 70% methanol for 48 h. The resulting mixture was filtered using muslin cloth followed by Whatman filter paper (No. 1). The aliquots obtained were dried on water bath at 40°C and stored at 4°C until required for use.

**Phytochemical screening of the extract**  
The extract obtained was screened phytochemically for the presence or absence of secondary metabolites using standard conventional protocols [8][9].

**Experimental animals**  

Mice were used in this study. The mice were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The mice were housed in standard polypropylene cages and maintained on laboratory animal feed and water ad libitum. All experimental procedures were in accordance with the Ahmadu Bello University Research policy; and guideline for the care and use of experimental animals as obtainable in “Principles of laboratory animal care” (NIH Publication no. 85-23, revised 1996). The experiments were conducted between hours of 900 h to 1600 h.

**Drugs/chemicals used in this experiment**  

Diazepam (Roche, France; Batch no F0134F51) and Normal saline (Batch no 012062)  

**Groupings and Treatments**  
The animals were grouped into 4 or 5 (depending on the studies), each consisting of six (6) mice. The first group received normal saline (10 ml/kg). The second, third and the fourth group received the extract (75, 150 and 300 mg/kg) while the fifth group received diazepam (0.5 mg/kg). All treatments were by intraperitoneal (i.p) route.

**Acute toxicity study**  
The acute toxicity of the methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel was investigated in mice via oral and intraperitoneal route of drug administration. The method used was as described by [10]. The study was carried out in two phases. In the initial phase, three groups of three mice each were used. The first group received extract at a dose of 10mg/kg, while the second and third groups received extract at doses of 100 and 1,000 mg/kg respectively. The animals were observed for signs of toxicity and death within 24 hours after which they were monitored for fourteen (14) days. In the second phase, three groups of one mouse each were used. Extract at respective doses of (which depended on the result of the first phase) 1,600 mg/kg, 2,900 mg/kg and 5,000 mg/kg were administered. The animals were observed for signs of toxicity and death within 24 hours and monitored for 14 days. The LD₅₀ value was determined as the geometric mean of the highest non-lethal dose (that did not cause death) and the lowest lethal dose for which the animal died (0/1 and 1/1).

**Diazepam-Induced Sleep in Mice**  
The method described by [11] was adopted in this study. Thirty (30) minutes post-treatment with
normal saline or various doses of the extracts: the mice were administered diazepam at a dose of 25 mg/kg body weight. The mice were placed individually in cages. The onset and the duration of sleep were determined for each animal. Loss of lightening reflex was considered as the criterion for sleep while the interval between the loss of lightening reflex and the recovery of straightening was regarded as the duration of sleep [12].

**Test for Exploratory Behaviour in Mice**
The apparatus used was a white painted wooden board (60 cm x 30 cm) with 16 evenly spaced holes (1 cm diameter x 2 cm depth) [13]. Thirty (30) minutes post-treatment with normal saline, extract (75, 150, and 300 mg/kg) or diazepam (0.5 mg/kg), each mouse was placed at a corner of the board and the number of head dips on the hole was counted using a tally counter during a 5 minute period [14]. A head dip was considered when the mouse dipped its head into the hole to the level of the eyes.

**Elevated Plus Maze Test in Mice (EPM)**
The EPM test was conducted according to the method previously described by [15] and [16]. The maze comprised of two open arms (35 cm x 5 cm) and two closed arms (30 cm x 5 cm x 15 cm) which were connected by a common central area (5 cm x 5 cm) and elevated to a height of 60 m above the floor. The floor and the walls of each were wooden and painted black [17]. The test was conducted in a room illuminated by a 60Watt red bulb. Thirty (30) minutes-post treatment with normal saline, extract or diazepam, each Mouse was placed at the centre platform facing one of the open arms and its behaviour videotaped over a 5 minutes period. A Mouse is said to have entered an arm when it has placed all four paws over the line separating the area and the centre. The maze was wiped with 70% ethyl alcohol and dried between trials to remove olfactory cues. The numbers of open and closed arm entries as well as the time spent in both open and closed arms were recorded.

**Elevated Zero-Maze Test (EZM)**
The maze comprised of black Perspex annular platform (105 cm in diameter, 10 cm width) elevated to 65 cm above the ground level and divided equally into four quadrants. The two opposite quadrants were enclosed by a black Perspex wall (27 cm high) on both the inner and outer edges of the platform, while the remaining two opposite quadrants were surrounded by Perspex "lip" (1 cm high) which served as a tactile guide to animals on these open areas. The apparatus was illuminated by dim white light arranged in such a manner to provide similar lux levels in open and enclosed quadrants for 5 min test period. Thirty (30) minutes-post treatment with normal saline, extract or diazepam, each mouse was placed on one of the enclosed quadrants and its behaviour videotaped over a 5 minutes period. The maze was cleaned with 5% ethanol/water solution and dried thoroughly between test sessions. Animals were scored as being in the open area when all four paws were in the open quadrants and in the enclosed area only when all four paws had passed the open-closed divide. The numbers of open and closed quadrants entries as well as the time spent in both open and closed quadrants were recorded [18].

**Mouse Beam Walking Assay**
Mice were trained to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the benches by metal support to a goal box. Three trials were performed for each mouse, and were designed such that the mouse tested would be aware that there was a goal box that could be reached. The goal box was a Perspex glass cage (with wood chippings beddings) with a small hole at the bottom [13]. The mice that successfully walked along the ruler were randomly grouped into five groups each containing six mice. The beam was made of wood, 8 cm in diameter, 60 cm long and elevated 30 cm above the bench by metal support. Thirty (30) minutes post-treatment, each mouse was placed on the beam at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from, with a maximum time of 60 s allowed on beam. The number of foot slips (one or both hind limb slipping from the beam) was recorded with the aid of a tally counter. The time taken to complete the task was also recorded [19].

**Staircase Test in Mice**
The device used in this study consists of a wooden staircase similar to the one previously described [20]. The staircase was enclosed in transparent Perspex vertical walls (45 cm x 12 cm x 25 cm) and had 5 identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. Thirty (30) minutes before placing each mouse individually on the floor of the Perspex box (with its back to the staircase), animals were treated with normal saline, extract or diazepam. The behaviour of each mouse videotaped and number of upward steps climbed and rearings were recorded over a 5 min period. A step was considered climbed if a mouse placed all its four paws on it. Rearing was counted when a mouse rose on its hind limb both against the wall and on a step. The staircase
was wiped with 70% ethyl alcohol and allowed to dry between tests to remove any olfactory cue.

**Statistical Analysis**

Results were expressed as mean ± standard error of mean (SEM), quantal effect or percentages, where applicable. Statistical analysis was conducted using SPSS package 17.0. Analysis of variance followed by post hoc dunnett’s t-tests for multiple comparisons. Differences were considered significant at P less than 0.05.

**RESULTS**

**Acute Toxicity Study**

The median lethal dose (LD50) value of methanolic extract of *Ficus ingens* stem bark in mice was found to be greater than (> ) 5,000 mg/kg body weight, orally and intraperitoneally.

**Preliminary Phytochemical Constituents of Ficus ingens** (Miquel) Miquel Methanolic Stem Bark Extract

The result of preliminary phytochemical screening of the extract revealed the presence of alkaloids, triterpenoids, flavonoids, saponins, phlobatannins, anthraquinones and reducing sugars, while steroids and tannins were absent (Table 1).

**Table 1** Phytochemical constituents of *Ficus ingens* (Miquel) Miquel methanolic stem bark extract

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>++</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinolones</td>
<td>++</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>++</td>
</tr>
</tbody>
</table>

++ = Present  + = little traces  - = Absent

**Effects of Methanolic Stem Bark Extract of Ficus ingens** (Miquel) Miquel on Diazepam Induced Sleep in Mice

The extract at the doses tested showed a no significant decrease in the number of head dips in the hole-board test compared with the control group. The standard drug, diazepam 0.5 mg/kg caused a significant (P<0.001) increase in the exploratory behaviour (Table 3).

**Table 2** Effects of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel on diazepam induced sleep in mice.

<table>
<thead>
<tr>
<th>Treatment/Dosage</th>
<th>Mean onset of sleep (mins) ± SEM</th>
<th>Mean Duration of sleep (mins) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/Saline 10 ml/kg</td>
<td>3.17 ± 0.40</td>
<td>169.67 ± 34.50</td>
</tr>
<tr>
<td>Extract 75 mg/kg</td>
<td>3.17 ± 0.60</td>
<td>335.17 ± 64.65*</td>
</tr>
<tr>
<td>Extract 150 mg/kg</td>
<td>4.16 ± 0.91</td>
<td>336.83 ± 63.13*</td>
</tr>
<tr>
<td>Extract 300 mg/kg</td>
<td>3.67 ± 0.21</td>
<td>364.50 ± 39.59*</td>
</tr>
</tbody>
</table>

*Values are Mean ± SEM; n = 6
*P< 0.0005 student’s t-test
SEM = Standard Error of Mean

**Effects of Methanolic Stem Bark Extract Of Ficus ingens** (Miquel) Miquel On Exploratory Behavioural Patterns In Mice

The extract at the doses tested showed a no significant decrease in the number of head dips in the hole-board test compared with the control group. The standard drug, diazepam 0.5 mg/kg caused a significant (P<0.001) increase in the exploratory behaviour (Table 3).

**Table 3** Effect of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel on exploratory behaviour (head dip test) in mice.

<table>
<thead>
<tr>
<th>Treatment/Dosage</th>
<th>Mean number of head dips in 5 min ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/Saline 10 ml/kg</td>
<td>19.83 ± 1.99</td>
</tr>
<tr>
<td>Extract 75 mg/kg</td>
<td>16.50 ± 1.59</td>
</tr>
<tr>
<td>Extract 150 mg/kg</td>
<td>16.83 ± 4.09</td>
</tr>
<tr>
<td>Extract 300 mg/kg</td>
<td>16.80 ± 4.07</td>
</tr>
<tr>
<td>Diazepam 0.5 mg/kg</td>
<td>58.50 ± 4.93*</td>
</tr>
</tbody>
</table>

*Values are Mean ± SEM; n = 6
*P<0.001 Dunnet-post t-test for multiple comparison
SEM = Standard Error of Mean

**Effects of Methanolic Stem Bark Extract Of Ficus ingens** (Miquel) Miquel On Beam Walking Assay (Motor Coordination) In Mice

Diazepam at 0.5 mg/kg significantly (P<0.001) impaired motor coordination while the extract showed no significant difference in the number of foot slips compared to the control group (Table 4).
Table 4 Effect of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel on beam walking assay (motor coordination) in mice

<table>
<thead>
<tr>
<th>Treatment/Dosage</th>
<th>Mean Duration of Beam walk (s) ± SEM</th>
<th>Mean Number of Foot Slips ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/Saline 10 ml/kg</td>
<td>49.17 ± 6.57</td>
<td>0.43 ± 0.48</td>
</tr>
<tr>
<td>Extract 75 mg/kg</td>
<td>43.50 ± 8.87</td>
<td>0.39 ± 0.22</td>
</tr>
<tr>
<td>Extract 150 mg/kg</td>
<td>34.17 ± 9.59</td>
<td>0.36 ± 0.48</td>
</tr>
<tr>
<td>Extract 300 mg/kg</td>
<td>44.50 ± 9.55</td>
<td>0.38 ± 0.33</td>
</tr>
<tr>
<td>Diazepam 0.5 mg/kg</td>
<td>50.17 ± 7.38</td>
<td>6.17 ± 1.42</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± SEM; *P< 0.001 Dunnet post hoc t-test for multiple comparison; n = 6

Effect of Methanolic Stem Bark Extract of *Ficus ingens* (Miquel) Miquel in Staircase Test in Mice

The extract significantly (*P<0.001*) decreased the number of rearing and the number of upward steps climbed when compared to the control group. Diazepam when compared to the control group, exhibited a no significant reduction in the number of rearing but significantly (*P<0.01*) increased the upward steps climbed (Table 5).

Table 5 Effect of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel on staircase test in mice

| Treatment/Dosage | Mean No. of Upward steps climbed ± SEM | Mean No. of Rearings ± SEM |
|------------------|======================================|---------------------------|
| N/Saline 10 ml/kg| 39.17 ± 4.12                         | 37.33 ± 2.47              |
| Extract 75 mg/kg | 27.67 ± 3.54**                       | 24.17 ± 4.86**            |
| Extract 150 mg/kg| 19.33 ± 2.86**                       | 19.50 ± 3.93**            |
| Extract 300 mg/kg| 17.16 ± 2.56**                       | 14.33 ± 2.62**            |
| Diazepam 0.5 mg/kg| 46.83 ± 6.19*                      | 33.83 ± 3.26              |

Values are presented as Mean ± SEM; *P<0.01; **P<0.001 Dunnet post hoc t-test for multiple comparison; n = 6;

Effect of Methanolic Stem Bark Extract of *Ficus ingens* (Miquel) Miquel on Elevated Plus Maze and Elevated Zero Maze in Mice

In the elevated plus and zero mazes, the extract showed no significant difference in both the number of entries in the open arm/quadrant and the time spent in the open arm/quadrant as compared to the control group. However, with the zero maze, the extract at 150 mg/kg showed a statistically significant (*P<0.001*) increase in the amount of time spent in the open arm/quadrant. In the elevated plus and zero mazes, diazepam showed a statistically significant (*P<0.001*) increase in both the amount of time spent in the open arm/quadrant and the number of entries in the open arm/quadrant (Table 6 and 7).

Table 6 Effect of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel on elevated plus maze test in mice

<table>
<thead>
<tr>
<th>Treatment/Dosage</th>
<th>Mean No. of Open Arm Entries ± SEM</th>
<th>Mean Time spent in Open Arm (sec) ± SEM</th>
<th>Mean No. of Closed Arm Entries ± SEM</th>
<th>Mean Time spent in Closed Arm (sec) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/Saline 10 ml/kg</td>
<td>5.83 ± 1.80</td>
<td>82.33 ± 27.03</td>
<td>6.83 ± 0.87</td>
<td>148.50 ± 29.99</td>
</tr>
<tr>
<td>Extract 75 mg/kg</td>
<td>1.67 ± 0.76</td>
<td>24.83 ± 11.91</td>
<td>3.83 ± 0.91</td>
<td>206.000 ± 22.12</td>
</tr>
<tr>
<td>Extract 150 mg/kg</td>
<td>1.67 ± 1.09</td>
<td>25.00 ± 19.15</td>
<td>4.67 ± 1.28</td>
<td>243.50 ± 23.97</td>
</tr>
<tr>
<td>Extract 300 mg/kg</td>
<td>3.50 ± 0.76</td>
<td>38.83 ± 13.01</td>
<td>6.67 ± 1.33</td>
<td>228.83 ± 12.45</td>
</tr>
<tr>
<td>Diazepam 0.5 mg/kg</td>
<td>13.69 ± 0.75*</td>
<td>239.83± 10.86*</td>
<td>1.83 ± 1.91</td>
<td>25.50 ± 10.31</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± SEM; *P<0.001 Dunnet post hoc t-test for multiple comparison; n = 6

Table 7 Effect of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel on elevated zero maze tests in mice

<table>
<thead>
<tr>
<th>Treatment/Dosage</th>
<th>Mean no of open arm entries ±SEM</th>
<th>Mean no of time spent in open arm ±SEM (sec)</th>
<th>Mean no of closed arm entries ±SEM</th>
<th>Mean no of time spent in close arm ±SEM (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/Saline 10ml/kg</td>
<td>1 ± 0.28</td>
<td>5 ± 0.85</td>
<td>4.4 ± 0.46</td>
<td>295 ± 0.85</td>
</tr>
<tr>
<td>Extract 75mg/kg</td>
<td>1.6 ± 0.64</td>
<td>25.6 ± 1.15</td>
<td>3.2 ± 0.33</td>
<td>274 ± 1.15</td>
</tr>
<tr>
<td>Extract 150mg/kg</td>
<td>1.02 ± 0.46</td>
<td>156 ± 0.63*</td>
<td>1.17 ± 0.52</td>
<td>144 ± 0.63</td>
</tr>
<tr>
<td>Extract 300mg/kg</td>
<td>0.8 ± 0.19</td>
<td>5.2 ± 0.91</td>
<td>4.6 ± 0.54</td>
<td>294.8 ± 0.91</td>
</tr>
<tr>
<td>Diazepam 0.5mg/kg</td>
<td>6.6 ± 0.53*</td>
<td>286.4 ± 1.89*</td>
<td>1.4 ± 0.13</td>
<td>13.6 ± 1.89</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± SEM; *P<0.00; Dunnet post hoc t-test for multiple comparison; n = 6
DISCUSSION
The present study showed that the methanolic stem bark extract of *Ficus ingens* possesses CNS depressant activity. The preliminary phytochemical screening of the extract revealed the presence of saponins, triterpenoids, alkaloids, flavonoids, phlobatannins and anthraquinones. Several plants have been reported to have CNS depressant and anxiolytic activities due to the presence of triterpenoids, saponins and flavonoids [21]. Triterpenoids and saponins are reported to have agonistic/facilitatory activities at GABA<sub>A</sub> receptor complex [22] [23] and led to the hypothesis that they act in similar way to benzodiazepine-like molecules. This is supported by their behavioural effects in animal models of CNS depression and anxiety [21]. The extract prolonged the duration of sleep induced by diazepam. The prolongation of diazepam-induced sleeping time may be attributed to an action on the central mechanism involved in the regulation of sleep [24], thus suggesting *Ficus ingens* as a neurosedative drug [25].

The determination of exploratory behaviour in animals is measured by the hole board experiment [26] and is an accepted parameter for evaluating anxiety conditions in animals [27]. The extract produced a non-significant decrease in exploratory behaviour as shown by the decrease in the number of head dips in the hole-board test. A decrease in number of head dips reveals sedative behavior [28] and is thus a measure of CNS depressant activity [29] [30].

The number of foot slips made by mice in the motor coordination test has been found to be a sensitive measure at determining benzodiazepine induced motor coordination deficits and is a good predicator of doses producing clinical sedation [19]. The extract had no observable effect on motor coordination when compared with the negative control suggesting that the inhibition effect observed in the other tests might be elicited centrally and not due to a peripheral neuromuscular blockade [31-33].

The staircase test is a simple, quick and differential test based on the inherent tendency of rodents to explore a novel environment which gives a general idea on the level of emotivity of the rodent [34][35]. In the staircase test, the numbers of steps climbed and rearing are measured as behavioural parameters of exploratory/locomotor activity and anxiety, respectively [20][36-38]. Some studies have shown that anxiolytic drugs decrease the number of rearing without any effect on step climbing behavior [20] [36-38]. The extract exhibited dose dependent decrease in the number of rearing and this is suggestive of an anxiolytic effect.

The important parameters in the elevated plus maze and elevated zero maze tests are the frequency and amount of time spent on the open arms/quadrants. Both diazepam and the 100 mg extract/kg body weight in the zero maze significantly increased the open arm/quadrant entries and open arm/quadrant duration. It also increased the total number of entries. This is agreement with other studies [39] [40]. The extract did not increase the number of open arm/quadrant entries nor increase the duration in the open arms/quadrants. It is known that some clinically used anxiolytic drugs show no effects or even anxiogenic effects on the elevated plus maze. For instance, some serotonergic agents such as fluoxetine were found to show no effects and anxiogenic effects after chronic and single administration respectively [41] [42]. Behavioural and pharmacological validation of the elevated maze has shown that the increase in avoidance latency is a function of the aversive character of the open arm, and suggests that the pharmacological profile of the avoidance task is similar to that of GAD [43][44] hence, consistent with literature data, serotonergic agents are less reliable as compared to the benzodiazepines. The extract may possess bioactive compounds that act via a different mechanism from diazepam.

The results obtained from the elevated plus maze and the elevated zero maze revealed that the zero maze is an improvement over elevated plus maze and has several advantages over it. The zero maze has no centre square which can produce ambiguous measures as was observed in this study; and, it has a circular design that allows for uninterrupted exploration. The extract does contain bioactive compound that possess CNS depressant properties and possible anxiolytic properties.
Further research should involve isolation of the bioactive constituents responsible for the observed pharmacological activities and elucidation of the mechanism of action.

REFERENCES


