Toxicity Assessment and Anxiolytic Properties of Methanol Stem Bark Extract from Khaya senegalensis in Wistar Rats

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ABSTRACT

Background and Objective: Mental illnesses such as anxiety are characterized by impairment in cognition, emotional regulation, or behavior and are predisposed by a combination of genetic, biological, environmental and psychological factors. Khaya senegalensis is reported to have a wide variety of medicinal uses such as in diarrhea, bacterial infections, helminthiasis, trypanosomiasis, diabetes and cancer. This study evaluated the anxiolytic effect of methanol stem bark extract of Khaya senegalensis in Wistar rats. Materials and Methods: Acute toxicity studies were conducted using Lorke’s Method. After which thirty rats were randomly divided into five groups of six each, group 1 and 5 received distilled water and diazepam, respectively as control groups and 2nd to 4th group received graded doses of methanol stem bark extract of Khaya senegalensis for anxiety studies using Hole-board Test and Elevated Plus-Maze. Results: The extract significantly (p<0.05) increased the number of entries into open arms and the time spent there in the Elevated Plus-Maze Test and increased the number of head dips in the Hole-board Test. Conclusion: This study revealed that the methanol stem bark extract of khaya senegalensis is practically nontoxic and also demonstrated significant and dose-dependent anxiolytic activities in Wistar rats.

KEYWORDS
Khaya senegalensis, methanol effect, toxicity, anxiolytic effect, rats, stem bark

INTRODUCTION

The history of using plants as a source of medicine is as old as humankind1. There has been increasing interest in the utilization of herbal medicines over the last few decades. This is because medicinal plants are useful in treating so many diseases, including mental illnesses such as anxiety and depression.

Mental illness refers to a wide range of conditions characterized by abnormalities of mood, thinking and behavior2. Worldwide, mental illness accounts for 14% of the global disease burden with 75% of the
affected in underdeveloped countries\textsuperscript{3}. Mental disorders such as anxiety, depression and substance abuse cause disability in more people than complications due to AIDS, heart disease, accidents and wars combined\textsuperscript{4}.

Anxiety disorder is characterized by excessive fear or avoidance of perceived threats that are persistent and impairing\textsuperscript{5}. Anxiety is known to be caused by environmental, genetic and biological factors. Anxiety disorder is the most prevalent mental health disorder, with a global prevalence of 7.3\% having early adulthood onset\textsuperscript{6}.

Global Burden of Disease 2019 estimated that seven of the top 25 causes of years lived with disability worldwide were mental disorders, with anxiety disorders ranked ninth. It has been shown that about 85\% of patients with depression also experience anxiety symptoms and about 90\% of patients with anxiety disorders also have symptoms of depression\textsuperscript{7}. Females are two times more likely to have depression and anxiety than males due to various biological and social factors\textsuperscript{8,9}. With the increasing rate of psychosocial and socioeconomic stressors such as poverty, insecurity, conflicts, wars, displacement, disasters and rape, especially in Nigeria and other parts of the world, the prevalence of anxiety and depression is higher\textsuperscript{10}.

Current pharmacological agents for treating anxiety are expensive and not readily accessible to rural African communities. They also have limited efficacy and are associated with many adverse effect and those who benefit only do so after the delayed onset of action of several days\textsuperscript{11-14}.

The ranking of mental illness, especially anxiety, as the top contributor to the Global Burden of Disease and with anticipation of the rise in incidence and prevalence due to increasing psychosocial and socioeconomic stressors, especially in the developing countries like Nigeria, there is an urgent need to search for a new drug from plant source with anxiolytic effects. It is hoped that the new drug will address the drawbacks of the existing drugs. It should be fast acting, devoid of deleterious side effects, cheap and accessible even in rural African communities. It is speculated that \textit{Khaya senegalensis} is used for treating mental illness and therefore has the potential neuro-pharmacological effect that may be beneficial in the treatment of anxiety. There is a need to validate this assumption scientifically\textsuperscript{15-17}.

This study aims to evaluate the anxiolytic effects and the toxicity profile of methanol stem bark extract of \textit{Khaya senegalensis} in Wistar rats.

**MATERIALS AND METHODS**

**Study area:** The study was conducted at the laboratory of the Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences. College of Health Sciences, Usmanu Danfodiyo University Sokoto, Nigeria from October, 2021 to January, 2022.

**Experimental animals:** Ninety-six male Wistar rats weighing 120-180 g used in this study were obtained from the animal house of Usmanu Danfodiyo University Sokoto, Nigeria. The rats were maintained in a well-ventilated cage with an alternating 12 hrs light/dark cycle and were acclimatized to the environment for 1 week. The conditions of animal use were according to the National Institute of Health Guide for the Care and Use of Laboratory animals.

**Plant collection:** The stem bark of \textit{Khaya senegalensis} was obtained from Sokoto, Sokoto State, Nigeria, in the month of October, 2020. A taxonomist in the Department of Pharmacognosy department identified and authenticated the sample at Usmanu Danfodiyo University Sokoto, Nigeria.
Extract preparation: The stem bark was dried under shade then chopped into small pieces and pulverized into a fine powder using a wooden pestle and mortar. Five hundred grams of the dried powder was subjected to cold maceration using 95% methanol as solvent, allowed for 72 hrs and filtered using Whatman filter paper. The filtrate was evaporated in an oven at 40°C. The dried extract was stored in an air-tight container. The percentage yield was calculated by dividing the total weight of the dried extract by the total weight of the dried powdered plant, then multiplying the value by 100.

Acute toxicity studies: Lorke’s Method was adopted for this study. The test was conducted in two phases. In the initial phase, nine rats were divided into three groups with three rats each. First, second and third groups received 10, 100 and 1000 mg kg$^{-1}$ of *Khaya senegalensis* stem bark extract, respectively and were observed for 24 hrs for possible mortality. In the second phase, three rats, one from each group used and received 1600, 2900 and 5000 mg kg$^{-1}$ of the plant extract respectively. The LD50 was determined by calculating the geometric mean of the highest dose the rat survived and the lowest dose that killed the rat.

Anxiolytic test

Elevated Plus-Maze Model: Lister’s Method was adopted for this study. The apparatus comprised two open arms and two closed arms extending from a common central platform. The maze was entirely elevated to a height of 50 cm from the ground. Thirty rats were grouped into five groups with six each being used for the experiment. Group I was labelled positive control, given 1 mg kg$^{-1}$ of diazepam orally and allowed 30 min for the drug to act. The rat was then put into the maze and observed for 3 min. The subsequent groups of rats were subjected to similar tests after administering 100, 200 and 400 mg kg$^{-1}$ of *Khaya senegalensis* stem bark extract and distilled water via oral route, respectively. The parameters observed include (1) Number of entries into the open arm, (2) Number of entries into the closed arm and 3 times spent in the arms, respectively.

Hole-board Test: The method described by Takeda et al. was adopted. The hole-board consists of a wooden box with sixteen holes evenly distributed on the floor in a grid pattern. The apparatus was elevated to about 50 cm above the ground. Thirty rats were grouped into five groups with six each being used for the experiment. Group I was labelled positive control and 0.5 mg kg$^{-1}$ of diazepam was given to each of the rats in the group orally and allowed 30 min for the drug to act. Then each rat was put on the board and observed for 3 min. The subsequent groups of rats were subjected to similar tests after administering 100, 200 and 400 mg kg$^{-1}$ of *Khaya senegalensis* stem bark extract and distilled water via oral route, respectively. The number of head dips was observed and recorded.

Sub-chronic toxicity studies: The Organization for Economic Co-operation and Development (OECD 407) method was adopted for the study’s 28-day sub-chronic toxicity test. Twenty four rats were allocated into four groups of six at random. Orally administered to the first group was 10 mL kg$^{-1}$ of distilled water. The second, third and fourth received daily oral administration of *Khaya senegalensis* stem bark extract in graduated doses (100, 200 and 400 mg kg$^{-1}$) for 28 days. The general signs of toxicity and death were monitored daily. Every week, the rats were weighed. After a night of fasting, the animals were sacrificed under general anesthetic on the 29th. To conduct hematological and biochemical studies, blood samples were obtained.

Ethical considerations: Ethical clearance was obtained from the Department’s Committee and ethical standards were strictly observed in line with international standards and protocols in handling the animal subjects.
Statistical analysis: The data were analyzed using SPSS Version 24 and summarized as mean±standard error of mean (SEM). One-way Analysis of Variance (ANOVA) and Mann-Whitney U Test were used to compute the differences. After obtaining statistical differences, the Dunnett’s Test was used for multiple comparisons depending on the nature of the data. The p<0.05 was accepted as significant.

RESULTS

Percentage yield of methanol stem bark extract of *Khaya senegalensis*: The percentage yield of methanol stem bark extract of *K. senegalensis* was 9.2%.

Acute toxicity studies: No mortality is observed in both phases of the acute toxicity study (Table 1). The median Lethal Dose (LD₅₀) of methanol stem bark extract of *K. senegalensis* was calculated to be more than 5000 mg kg⁻¹ orally.

Effect of methanol stems bark extract of *K. senegalensis* on liver function test following 28 days’ sub-chronic oral treatment in Wistar rats: There was no significant change in all the liver function parameters following 28 days of oral administration of graded doses (100, 200 and 400 mg kg⁻¹) of methanol stem bark extract of *K. senegalensis* in Wistar rats (Table 2).

Effects of methanol stem bark extract of *K. senegalensis* on renal function test following 28 days sub-chronic oral treatment in Wistar rats: There was no significant change in all the renal function parameters following 28 days of oral administration of graded doses (100, 200 and 400 mg kg⁻¹) of methanol stem bark extract of *K. senegalensis* in Wistar rats (Table 3).

Effects of methanol stem bark extract of *K. senegalensis* on hematological indices following 28 days sub-chronic oral treatment in Wistar rats: There was no significant change in all the hematological parameters following 28 days’ oral administration of graded doses (100, 200 and 400 mg kg⁻¹) of methanol stem bark extract of *K. senegalensis* in Wistar rats (Table 4).

Effect of methanol stem bark extract of *K. senegalensis* on anxiety behavior using Hole-Board Test in Wistar rats: The oral administration of methanol stem bark extract of *K. senegalensis* to Wistar rats at a dose of 400 mg kg⁻¹ showed significant (p<0.05) increase in head dips on hole-board test compared to control, but not as much as the standard anxiolytic (diazepam) at a dose of 1 mg kg⁻¹ as shown in Fig. 1.

Table 1: Oral acute toxicity studies of methanol stem bark extract of *K. senegalensis*

<table>
<thead>
<tr>
<th>Dose (mg kg⁻¹) PO</th>
<th>Number of animal mortality</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td>1000</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td>Phase II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1600</td>
<td>0/1</td>
<td>0</td>
</tr>
<tr>
<td>2900</td>
<td>0/1</td>
<td>0</td>
</tr>
<tr>
<td>5000</td>
<td>0/1</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: Effect of methanol stem bark extract of *K. senegalensis* on liver function indices following 28 days’ sub-chronic oral treatment in Wistar rat

<table>
<thead>
<tr>
<th>Treatment/dose (mg kg⁻¹)</th>
<th>TB (mg%)</th>
<th>DB (mg%)</th>
<th>ALK (m L⁻¹)</th>
<th>AST (m L⁻¹)</th>
<th>ALT (m L⁻¹)</th>
<th>TP (g L⁻¹)</th>
<th>Albumin (g L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0.71±0.47</td>
<td>0.21±0.30</td>
<td>65.50±4.75</td>
<td>6.16±0.75</td>
<td>8.00±1.00</td>
<td>60.50±1.72</td>
<td>37.66±0.80</td>
</tr>
<tr>
<td>KSE 100 mg kg⁻¹</td>
<td>0.75±0.34</td>
<td>0.20±0.25</td>
<td>17.00±5.56</td>
<td>6.33±0.66</td>
<td>8.66±0.66</td>
<td>60.66±0.95</td>
<td>37.66±0.87</td>
</tr>
<tr>
<td>KSE 200 mg kg⁻¹</td>
<td>0.76±0.42</td>
<td>0.25±0.22</td>
<td>71.33±4.08</td>
<td>6.00±0.77</td>
<td>7.66±0.55</td>
<td>58.66±1.38</td>
<td>37.83±2.01</td>
</tr>
<tr>
<td>KSE 400 mg kg⁻¹</td>
<td>0.75±0.42</td>
<td>0.23±0.21</td>
<td>57.50±3.15</td>
<td>5.66±0.82</td>
<td>8.50±0.71</td>
<td>61.66±0.76</td>
<td>39.33±0.98</td>
</tr>
</tbody>
</table>

Data expressed as mean±SEM, SEM: Standard Error of Mean, n = 6, TB: Total Bilirubin, DB: Direct Bilirubin, ALK: Alkaline Phosphatase, AST: Aspartate Transaminase, ALT: Alanine Transaminase, TP: Total Protein and KSE: *Khaya senegalensis* extract Dunnett’s post-hoc Test

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Table 3: Renal function parameters following 28 days’ oral treatment of methanol stem bark extract of *Khaya senegalensis*

<table>
<thead>
<tr>
<th>Treatment/dose</th>
<th>Urea (mg kg⁻¹)</th>
<th>Creatinine (mg%)</th>
<th>Na⁺ (mmol L⁻¹)</th>
<th>K⁺ (mmol L⁻¹)</th>
<th>Cl⁻ (mmol L⁻¹)</th>
<th>HCO₃⁻ (mmol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>5.28±0.20</td>
<td>1.06±0.13</td>
<td>139.16±0.60</td>
<td>3.90±0.19</td>
<td>101.10±0.47</td>
<td>24.83±0.30</td>
</tr>
<tr>
<td>KSE 100 mg kg⁻¹</td>
<td>4.91±0.21</td>
<td>1.23±0.94</td>
<td>140.33±2.53</td>
<td>4.03±0.90</td>
<td>100.3±0.61</td>
<td>25.10±0.42</td>
</tr>
<tr>
<td>KSE 200 mg kg⁻¹</td>
<td>6.53±0.74</td>
<td>1.01±0.06</td>
<td>138.00±2.90</td>
<td>4.06±0.10</td>
<td>99.83±2.78</td>
<td>24.66±0.41</td>
</tr>
<tr>
<td>KSE 400 mg kg⁻¹</td>
<td>6.53±0.74</td>
<td>1.40±0.45</td>
<td>138.50±0.88</td>
<td>4.15±0.19</td>
<td>24.00±1.20</td>
<td>100.8±4.17</td>
</tr>
</tbody>
</table>

SEM: Standard Error of Mean, n = 6, Na⁺: Sodium ion, K⁺: Potassium ion, Cl⁻: Chloride ion, HCO₃⁻: Bicarbonate and KSE: *Khaya senegalensis* extract Dunnett’s post-hoc Test

Fig. 1: Effect of methanol stem bark extract of *Khaya senegalensis* on anxiety behavior using Hole-Board Test in Wistar rats

KSE: *Khaya senegalensis* extract, data expressed as Mean±Standard Error of Mean, Dunnett’s post-hoc Test and *p<0.05

Fig. 2: Effect of methanol stem bark extract of *Khaya senegalensis* on anxiety behavior using Elevated Plus-Maze (open arm) in Wistar rats

KSE: *Khaya senegalensis* extract, data expressed as mean±SEM, *p<0.05 and Dunnett’s post-hoc Test
Fig. 3: Effect of methanolic stem bark extract of *Khaya senegalensis* on anxiety using Elevated Plus-Maze (close arm) in Wistar rats

KSE: *Khaya senegalensis* extract, data presented as mean±SEM, *p*<0.05 and Dunnett’s post-hoc Test

Table 4: Effect of methanol stem bark extract of *K. senegalensis* on hematological indices following 28 days sub-chronic oral treatment in Wistar rats

<table>
<thead>
<tr>
<th>Treatment/dose (mg kg⁻¹)</th>
<th>WBC (x10³ µL)</th>
<th>RBC (x10³ µL)</th>
<th>HGB (g dL⁻¹)</th>
<th>HCT (%)</th>
<th>PLT ((x10³ µL)</th>
<th>MCV (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>12.32±1.60</td>
<td>8.27±0.42</td>
<td>15.41±0.71</td>
<td>43.28±1.48</td>
<td>722.66±2.10</td>
<td>60.33±0.41</td>
</tr>
<tr>
<td>KSE 100 mg kg⁻¹</td>
<td>8.42±0.16</td>
<td>8.95±0.41</td>
<td>17.11±0.23</td>
<td>47.81±1.20</td>
<td>655.50±4.14</td>
<td>55.66±1.8</td>
</tr>
<tr>
<td>KSE 200 mg kg⁻¹</td>
<td>10.84±0.65</td>
<td>8.75±0.20</td>
<td>14.78±2.28</td>
<td>44.35±0.71</td>
<td>635.00±5.49</td>
<td>52.3±1.05</td>
</tr>
<tr>
<td>KSE 400 mg kg⁻¹</td>
<td>9.67±0.11</td>
<td>8.49±0.23</td>
<td>15.00±3.61</td>
<td>44.35±1.90</td>
<td>800.00±5.21</td>
<td>52.50±1.11</td>
</tr>
</tbody>
</table>


Dunnett’s post-hoc Test

Effect of methanol stem bark extract of *K. senegalensis* on anxiety behavior using Elevated Plus Maze in rats: Oral administration of methanol stem bark extract of *K. senegalensis* at a dose of 400 mg kg⁻¹, when compared to the control, showed a significant increase (*p*<0.05) in the number of entries into the open arms and the time spent there (Fig. 2), as well as a reduction in the number of entries into the closed arm (Fig. 3), though not as much as with the conventional anxiolytic (diazepam) at a dose of 1 mg kg⁻¹.

DISCUSSION

The oral acute toxicity studies of the methanol stem bark extract of *K. senegalensis* were estimated to be greater than 5000 mg kg⁻¹ b.wt. In grading the toxicity of a chemical substance, Lorke¹⁸, indicated that toxicity at 1 mg kg⁻¹ is considered highly toxic, 10 mg kg⁻¹ is considered toxic, 100 mg kg⁻¹ moderately toxic, 1000 mg kg⁻¹ slightly toxic and 5000 mg kg⁻¹ not toxic. Therefore, the extract is practically non-toxic. The LD₅₀ of the aqueous leaf extract of the same plant was reported to be greater than 3000 mg kg⁻¹.²²

This study shows no significant difference in liver enzymes after administration of various doses (100, 200 and 400 mg kg⁻¹) of methanol stem bark extract of *K. senegalensis* to Wistar rats for 28 days compared to the control group. This was contrary to the findings of Kolawole²³, who used aqueous stem bark extract
of the same plant at 100 and 200 mg kg\textsuperscript{-1} body weight for 18 days and discovered elevated serum levels of liver enzymes namely Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST) and Alkaline Phosphatase (ALP) in rats. Also Abubakar et al.\textsuperscript{24} with much lower doses (10-40 mg kg\textsuperscript{-1}) administered for 28 days in albino rats. Yakubu et al.\textsuperscript{25} also reported an increased concentration of liver enzymes in rats following daily administration of ethanolic extract of \textit{K. senegalensis} at 2 mg kg\textsuperscript{-1} for 18 days. These authors, therefore, concluded the cytolytic effect of the extract on the liver. However, some authors reported reduced serum AST, ALT and ALP effects after using the extract on rodents and therefore highlighted the possible hepato-protective effect of the extract\textsuperscript{26-28}. The discrepancy in the above findings could be due to impurities such as heavy metals in the extract, which may be toxic.

The current study showed no significant statistical difference in renal parameters following graded doses (100, 200 and 400 mg kg\textsuperscript{-1}) of methanol stem bark extract of \textit{K. senegalensis} compared to the control after 28 days. El Badwi et al.\textsuperscript{29} revealed the nephro-protective effect of \textit{K. senegalensis} aqueous extract in Wistar rats with gentamycin-induced renal dysfunction after receiving 250-500 mg kg\textsuperscript{-1} b.wt., of the extract, having significant improvement in their renal function. However, Kolawole\textsuperscript{23}, reported a dose-dependent increase in the blood level of urea, creatinine, total protein and globulin, in rats following 100 and 200 mg kg\textsuperscript{-1} administered for 18 days. On the contrary, Ali et al.\textsuperscript{26} reported reduced concentration of the exact parameters after using higher doses (250 and 500 mg kg\textsuperscript{-1}) for a shorter duration of five days. The disparity between the two observations suggested that a longer course of treatment can cause histological damage to the kidneys, liver and other organs. Some authors have also reported increased serum sodium and potassium after administering the extract to the rats\textsuperscript{23,30}.

This study also has not shown any significant change in hematological parameters. However, Kolawole\textsuperscript{23} revealed a decrease in red blood cells, packed cell volume and hemoglobin concentration. This may indicate toxicity to the erythrocytes.

This study showed that methanol stems bark extract of \textit{K. senegalensis} exhibited anxiolytic activity as demonstrated by Hole-Board Test (HBT) and Elevated-Plus Maze (EPM). Rodents have a natural aversion to new, bright, open and elevated places. The exploratory activity reflects the combined effects of these tendencies in novel situations. The number of head dips is assumed to be inversely proportional to the anxiety state\textsuperscript{31}. Therefore, the HBT increase in head dips is suggested as an index of anxiolytic activity. Similarly, the EPM is based on a similar principle: Rodents have a natural tendency to explore the novel environment and their innate avoidance of unprotected (open), bright and elevated places. Confinement to the open arm caused physiological stress which manifests as increased defecation, whereas exposure to classical anxiolytics such as diazepam or extract with anxiolytic properties increased exploration of these arms\textsuperscript{32}. Other studies in different parts of the world used similar animal models, especially EPM and HBT, to evaluate the anxiolytic effects of different plants and found similar results\textsuperscript{33-35}.

The results obtained from the present study using the HBT and EPM models for anxiety, revealed that oral administration of methanol stems bark extract of \textit{Khaya senegalensis} at a high dose of 400 mg kg\textsuperscript{-1} produces a significant increase in the number of head dips in HBT and the number of open arm entries and the time spent there in EPM, this suggested that the extract possess anxiolytic effects.

**CONCLUSION**

Methanol stem bark extract of \textit{Khaya senegalensis} has significant anxiolytic activity at a 400 mg kg\textsuperscript{-1} dose. It is safe for short-term use but care should be taken at higher doses for the long term. Activity-guided fraction of the extract should be evaluated in the subsequent studies to identify the best-performing fraction and further isolate, characterize and elucidate the structures of the phytochemical(s) responsible for the observed effects. Further mechanistic studies need to be done to disclose the exact mechanism of action of the isolated active phytochemical(s) responsible for the observed activities. Also, a chronic toxicity study is needed to determine the extract’s safety for long-term use.
SIGNIFICANCE STATEMENT
The work set out to investigate the anxiolytic effects and safety profile of the stem bark extract of *Khaya senegalensis* in Wistar rats. It was found to have substantial anxiolytic activity and is safe for short-term use. These findings are important in the design and development of new drugs for the management of anxiety disorders. The active ingredient responsible for these findings should be isolated and characterized and the mechanism of action should be determined.

REFERENCES