The central nervous effects of *Mitragyna africanus* (Willd) stembark extract in rats

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Received 16 September 2000; received in revised form 5 May 2001; accepted 12 May 2001

Abstract

The acute toxicity and the central effects of *Mitragyna africanus* (*M. africanus*) stembark methanol extract were studied in rats. The extract did not produce any death in the treated rats even at the highest dose (6400 mg kg⁻¹) used. It produced depressant effects on the central nervous system. The stembark extract potentiated amylobarbitone sleeping time in rats dose-dependently, induced sleep in rats and also produced significant local anaesthetic effect on rabbits, the effects being comparable to that of xylocaine. The extract protected rats treated with a convulsive dose of strychnine (2 mg kg⁻¹) and increased the period of onset of convulsions and decreased the number of spasms. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *M. africanus*; Acute toxicity; Sleeping time; Anticonvulsant; Local anaesthetic

1. Introduction

*Mitragyna africanus* (*M. africanus*, Willd) belonging to the Rubiaceae family is a large tree widely distributed in Nigeria, where it is known among the natives as ‘uburu’ in Igbo; ‘abura’ in Yoruba; ‘guljeya or gyayya’ in Hausa and ‘Kawui’ in Kanuri. The bark and the leaves are used in West Africa for the treatment of bacterial infections especially gonorrhoea (Jinju, 1990), dysentery, mental disorder and epilepsy (Von Maydell, 1990). The extract from this plant is usually used as a decoction either alone or with other plant materials. The decoction of the stem bark mixed with *Garcina kola* seed extract is used in southeastern Nigeria for treatment of African sleeping sickness (trypanosomiasis). The plant is listed by Walker, (1953) as one of the ingredients in the treatment of sterility, where it is combined with other plant materials such as *Coula edulis*, *Isolana letestui*, *Bertiera fistulosa* and *Alchornea cardifolia* in a decoction by the Bupunus of Gabon. In Northeastern Nigeria, it is combined with *Ficus thomningii* (stem bark) and *Ziziphus spina-christi* (stem bark) for treatment of mental illness. (Abdulrahman, 1992).

In Nigeria, scientific information on the effects of stem bark extract of *M. africanus* on the nervous system in man is lacking. The herb may be of pharmacological importance in psychiatry, hence the need for its screening.

The objective of this study therefore, is to investigate the effects of the stembark extract of *M. africanus* on the nervous system using experimental rats and rabbits since the decoction from the plant is known to be used by traditional healers for the treatment of mental illness and epilepsy (Von Maydell, 1990).

2. Materials and methods

2.1. Plant identification and collection

The plant *M. africanus* was identified by a traditional medical practitioner-Fugu Mustapha as ‘Kawui’ in Kanuri and confirmed by Mr S.A. Sanusi, a plant taxonomist in the Biological Sciences Department, University of Maiduguri. The plant is located along
lake Alau in Alau village of Konduga local government area of Borno State, some 12 km from the state capital Maiduguri. The stem bark was collected in the month of May 1998, it was air dried in the laboratory at the Department of Veterinary Physiology and Pharmacology, University of Maiduguri. A voucher herbarium specimen (Aji I) for reference was deposited in the Department of Biological Sciences, University of Maiduguri, Maiduguri.

### 2.2. Preparation of the extract

The dried stem bark was pounded into powder with mortar and pestle. 50 g of the powdered stem bark were extracted by boiling with 350 ml of 50% methanol for 15 min and allowed to cool. It was then filtered and the filtrate was concentrated in a water bath (Philip Harris Ltd, England) at a temperature of 80°C for 7 h. The concentrated extract was stored at 4°C until used.

### 2.3. Test animals

Male New Zealand rabbits (2.4–2.5 kg) and Wistar rats (150–160 g) of both sexes purchased from the animal house of the Faculty of Veterinary Medicine, Usman Danfodio University, Sokoto, were used for the studies. They were housed in cages and were given food (Nutrifeeds, Nig. Kano, Nigeria) and water ad libitum and were allowed to adjust to the laboratory environment for one week before the commencement of the experiments.

### 2.4. Acute toxicity testing

Thirty-five Wistar rats were used for this study. They were randomly separated into seven groups of 5 rats and were allowed free access to food and water. The animals in groups 1–7 were injected intraperitoneally (i.p.) with varying doses (100, 200, 400, 800, 1600, 3200 and 6400 mg kg\(^{-1}\)) of *M. africanus* stem bark extract in distilled water. The symptoms of toxicity in each rat were observed, scored in ascending order of severity and recorded. The number of rats that died within 24 h was recorded.

### 2.5. Effect on amylobarbitone sleeping time

Thirty rats of both sexes were randomly divided into five groups containing six rats each and treated as follows:

- **Group A**: is the control. The rats in this group were treated with amylobarbitone (35 mg kg\(^{-1}\)) intraperitoneally only. Animals in groups B, C, D and E were treated with varying doses (25, 50, 100 and 200 mg kg\(^{-1}\)) of *M. africanus* i.p. 30 min prior to treatment with amylobarbitone (35 mg kg\(^{-1}\)) by the same route.

All the rats were given food and water ad libitum during the experiment. The time of amylobarbitone administration, time of onset of sleep and the time of awakening were recorded. The data obtained were subjected to analysis of variance (ANOVA).

#### 2.6. Local anaesthetic effect

The method described by Shetty and Anika, (1982) was adopted. Three male rabbits obtained from the Department of Veterinary Physiology and Pharmacology were used for this study. Four identical, symmetrical and circular regions were shaved on the dorsum of male rabbits with two shaved circles on the thoracic region and the other two on the lumbar region, 24 h before the experiment. Two concentrations (1.0 and 0.3 mg ml\(^{-1}\)) of xylocaine and the extract (100 and 25 mg ml\(^{-1}\)) were prepared and 0.2 ml each of 0.3 and 1.0 mg ml\(^{-1}\) of xylocaine was injected intradermally in right thoracic and left lumbar shaved regions, respectively, to form weals which were also encircled with a marker.

Likewise, 0.2 ml each of 25 and 100 mg ml\(^{-1}\) of the *M. africanus* stem bark extract was injected intradermally in the shaved right lumbar and left thoracic regions, respectively, to form weals which were also encircled with a marker.

The encircled regions were each pricked with a needle six times at 5 min interval for 30 min, starting at time zero (0) i.e. before the injection of the drug or extract. The number of responses to pain by the rabbits when pricked with a needle was recorded. The response at the site of the injection indicated the degree of anaesthesia, which is expressed as the number of positive responses, i.e. failure to twitch.

### 2.7. Effect on strychnine induced convulsions

Two groups (A and B) of 5 rats each were housed in 2 cages and were given food and water ad libitum. A convulsive dose (2 mg kg\(^{-1}\) i.p.) of strychnine was given to both groups A and B, group B was however, pre-treated with a therapeutic dose (400 mg kg\(^{-1}\) i.p.) of the stem bark extract 30 min before treatment with the convulsant. The onset of convulsion, number of convulsions per minute and duration of convulsions were recorded (Takagi et al., 1960; Meada et al., 1981). The data obtained were analysed by unpaired Student’s *t* test.

## 3. Results

### 3.1. Plant extraction

The methanol extract of *M. africanus* stem bark was dark brown in colour. The yield of the extract was 6.7% (w/w)
3.2. Acute toxicity

The clinical symptoms observed in rats following the administration of methanol stem bark extract of *M. africanus* include depression, recumbency, hind limb paralysis, sleeping and difficulty in respiration, which increased with increasing doses of the extract. The effect of the extract lasted for 8–12 h, after which the rats returned to normal activity. No mortality was recorded in any of the treatment groups hence the LD$_{50}$ could not be calculated. Scores were allocated for each of the observed symptoms according to the severity of the effect; and these scores were summed up (Fig. 1). The scores ranged from 6 ± 0.5 in rats given 100 mg kg$^{-1}$ to 20 ± 3 mg kg$^{-1}$ in those given 6400 mg kg$^{-1}$ of the extract.

3.3. Amylobarbitone sleeping time

The stem bark extract significantly ($P < 0.05$) increased the sleeping time of amylobarbitone dose dependently (Fig. 2), the time lapse between dosing with amylobarbitone and onset of sleep significantly ($P < 0.05$) decreased with increasing dose of the extract (Fig. 3).

3.4. The local anaesthetic effect

The administration of 0.2 ml of methanol stem bark extract of *M. africanus* intradermally to rabbits at the concentrations of 25 and 100 mg ml$^{-1}$ resulted in a local anaesthetic effect of 86.1 and 100%, respectively. Xylocaine administered intradermally (0.2 ml) at con-
centrations of 0.3 mg ml\(^{-1}\) was observed to produce 44.4 and 100% local anaesthetic activity, respectively (Table 1).

3.5. Effect on strychnine-induced convulsions

The stembark extract of *M. africanus* provided 40% protection to rats against strychnine-induced convulsion (Table 2) when compared to rats which were not pretreated with the stembark extract. The mean number of spasm per minute was reduced by 52%, and the mean onset of convulsion was increased by 77%. All the rats in group A and 3 rats in group B died.

4. Discussion

The methanol extract of the stembark of *M. africanus* showed profound depressant effect on the central nervous system. The extract potentiated amylobarbitone
Fig. 3. The effect of *M. africanus* stem bark extract on mean on-set of sleep. Group A, control; B, 25 mg kg$^{-1}$; C, 50 mg kg$^{-1}$; D, 100 mg kg$^{-1}$; E, 200 mg kg$^{-1}$. *n* = 6.

Table 1
The local anaesthetic effect of *M. africanus* stem bark extract in rabbits

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (mg ml$^{-1}$)</th>
<th>Number of positive <em>a</em> responses overtime (min)</th>
<th>Total out of 36</th>
<th>Anaesthetic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylocaine</td>
<td>0.3</td>
<td>0  6  6  4  0  0  0  16</td>
<td></td>
<td>44.4</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0  6  6  6  6  6  6  36</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td><em>M. africanus</em></td>
<td>25</td>
<td>0  6  6  6  5  4  4  31</td>
<td></td>
<td>86.1</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0  6  6  6  6  6  6  36</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

6. Maximum anaesthesia; 0, no anaesthesia; *n* = 3.

* Positive responses indicate failure to twitch.
Table 2
The effect of *M. africanus* stem bark extract on strychnine induced convulsions

<table>
<thead>
<tr>
<th>Group</th>
<th>Extract pretreatment (mg kg(^{-1}) i.p.)</th>
<th>Convulsant (treatment) (2 mg kg(^{-1}), i.p.)</th>
<th>Mean (^a) number of spasm per min ± SD (min)</th>
<th>Mean (^a) onset of convulsion ± SD (min)</th>
<th>Mean (^a) onset of death ± SD (min)</th>
<th>Quantal death</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Nil</td>
<td>Strychnine</td>
<td>6.8 ± 1.72</td>
<td>6.6 ± 1.35</td>
<td>3.75 ± 1.47</td>
<td>5/5</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>400 mg kg(^{-1})</td>
<td>Strychnine</td>
<td>3.2 ± 2.3 (^b)</td>
<td>29.2 ± 5.84 (^b)</td>
<td>4.5 ± 1.5</td>
<td>3/5</td>
<td>40</td>
</tr>
</tbody>
</table>

\(n = 5\).

\(^a\) Values are means ± SD.

\(^b\) \(P < 0.05\) versus strychnine alone; Student’s *t* test.
sleeping time in a dose dependent manner indicating pharmacological effect. Amylobarbitone an intermediate barbiturate is known to induce anaesthesia for a longer period compared to the short acting pentobarbitone (Booth, 1984). Telle, (1999) observed that rabbits treated with 12 mg/100 g body weight of amylobarbitone slept for 366 ± 133.2 min with onset of 7.25 ± 3.9 min. In the present study treatment of rats with amylobarbitone (35 mg kg⁻¹) resulted in a shorter duration of sleep. However the duration of sleep increased when combined with the stem bark extract (dose dependently). The extract appears to have a sedative action, as some rats dosed with the extract went to sleep. This may be the reason why the traditional healers make use of the decoction from the plant in treating psychiatric illness.

The M. africanaus stem bark was observed to produce local anaesthesia in rabbits, which agrees with earlier reports on other Mitragyna species (Oliver-Bever, 1986; Annoa, 1986).

The local anaesthetic effect commenced five minutes after the extract was injected intradermally (just as observed for xylocaine), and the effect lasted for over 30 min even with the lower concentration (Table 1), the extract has comparable effect with xylocaine.

The extract conferred 40% protection against a convulsive dose of strychnine, and also increased the period of onset of convulsions and decreased the number of spasms. The 40% protection conferred on rats treated with strychnine may be due to the low dose of the extract used. Moreover, anticonvulsant activity of some plant extracts as reported by some researchers (Gupta et al., 1990; Akah and Nwambie, 1993); was based on the ability of these extracts to delay the onset of seizures (Yidya et al., 1990; Diwan et al., 1991; Martin and Rao, 1991), as was observed in the present study. In addition, it has been observed that anticonvulsant drugs at doses that did not block convulsion only increased the latency of clonic seizures (Loscher et al., 1991).

The toxic effects of the extract observed in the treated rats may be an extension of its pharmacological activity. In lower doses signs of depression, drowsiness and anaesthesia (sleep) occurred, while in higher doses recumbency and difficulty in respiration were noticed. The absence of death in rats treated with the highest dose of 6400 mg kg⁻¹ i.p. may be an indication of high safety margin of the extract.

The present work did not include the identification of the active principles and its mechanism of action. This will be the subject of future work.

In conclusion, the stem bark extract of M. africanaus induced significant depressant effect on the central nervous system. It potentiated amylobarbitone sleeping time, induced local anaesthesia and produced anticonvulsant activity.

Acknowledgements

The authors wish to thank Drs P.A. Nwafor and K.D. Effrain for their technical assistance. The work was supported in part by University of Maiduguri Senate Research grant R/ACA. 32/C9.

References

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