The Decoction of Leaves of Phyllanthus discoideus Possesses Anticonvulsant and Sedative Properties in Mice

Department of Biological Science, Faculty of Science, University of Ngaoundéré, P.O. Box 454, Ngaoundéré, Cameroon
Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon
Department of Chemistry, Faculty of Science, University of Ngaoundéré, P.O. Box 454, Ngaoundéré, Cameroon

Abstract: The aim of this study is to scientifically look for sedative and anticonvulsant properties of the decoction of Phyllanthus discoideus Baill (P. discoideus) in mice. The in vivo models of epilepsy were used to evaluate the anticonvulsant properties of the plant. These models were maximal electroshock-, N-methyl-D-aspartate-, pentylenetetrazol-, isonicotinic hydrazide- acid and strychnine- induced convulsions or turning behavior in mice. The potentiation of sleep induced by diazepam in mice was used for the determination of the sedative properties. Four doses of the plant in the decoction were used: 17.1, 42.7, 85.5 and 171 mg kg⁻¹. The decoction of the leaves of P. discoideus strongly increased the total sleep time (p<0.001) induced by diazepam and precipitated its onset (p<0.001). The decoction also protected mice against maximal electroshock- (p<0.001), pentylenetetrazol- (p<0.001), strychnine- (p<0.001) and N-methyl-D-aspartate- induced seizures or turning behavior (p<0.001). Finally, the decoction increased the latency to the onset of seizure in isonicotinic hydrazide acid test (p<0.001). In conclusion the decoction of P. discoideus possesses anticonvulsant and sedative properties in mice. The presence of these properties could explain its use in traditional medicine in Cameroon in the treatment of insomnia and epilepsy.

Key words: Medicinal plant, epilepsy, insomnia, treatment

INTRODUCTION

In Africa, phytotherapy still plays an important role in the management of diseases, mainly among populations with very low income. Phyllanthus discoideus Baill also named Margaritaria discoidea Baill or Cicca discoidea Baill is a small tree of 5-6 m high. It is very common in equatorial secondary forests close to rivers (Arbonnier, 2000). The tree is called Nikkveh in the Bamoun language in Cameroon, Baakonkon and Keri in Malinke and Poular languages in West Africa (Carrière, 2000). Personal communications with some Cameroonian Healers showed that the decoction of leaves of P. discoideus is used to treat various nervous system diseases: anxiety, convulsion, epilepsy and madness. In Guinea Conakry, the bark of this plant is used in the treatment of diarrhea and belly worms (Currière, 2000). Some pharmacological studies showed that P. discoideus possesses antibacterial activity (Mensah et al., 1990). It is also an intestine muscle relaxant interacting through adrenergic receptors. P. discoideus is used in traditional medicine in Africa and particularly in Cameroon to treat insomnia and epilepsy at a negligible price. The objective of this study is to scientifically look for sedative and anticonvulsant properties of this plant.

MATERIALS AND METHODS

Animals: Adult male mice of Mus musculus Swiss weighing 20-24 g were used. The animals were housed in standard cages, at 25°C, on a 12/12 h light-dark cycle. They were provided with food and water ad libitum. For the anticonvulsant tests, mice were divided in 6 groups of 6 mice. One negative control group received distilled water, one positive control group received appropriate substance and four tested groups received the decoction. For the diazepam- induced sleep test, mice were divided in 5 groups of 6 mice. One negative control group received distilled water and four tested groups received the decoction. In general, drugs were administered intraperitoneally in a volume of 10 mL kg⁻¹ of mice, except for diazepam (per os) in isonicotinic hydrazide acid test.
and N-methyl-D-aspartate (subcutaneous injection). The study was conducted in accordance with the nationally and internationally accepted principles for laboratory animal use and care.

**Chemicals:** Clonazepam, D-2-amino-7-phosphonoheptanoate (D-AP7), diazepam, isonicotinic acid hydrazide, N-methyl-D-aspartate (NMDA), pentylenetetrazol and strychnine are from Sigma Chemical, USA. Diazepam is from Roche.

**Plant material:** The plant specimens of *P. discoides* used were collected in Cameroon in the vicinity of Foumban in June 2006. A voucher specimen of the plant (417247/HNC) was authenticated at the National Herbarium of Cameroon.

The decoction was obtained according to the method close to the one used in traditional medicine. The dried leaves of *P. discoides* were ground. The powder (10 g) was put for maceration in 50 mL of distilled water for 1 h. The mixture was boiled for 20 min. After cooling, the supernatant (decoction) was collected and filtered with Whatman paper No. 1. The stock solution obtained (38 mL) corresponds to a concentration of 0.263 g mL⁻¹, representing a 6.5% yield. The following doses were used: 17.1, 42.7, 85.5 and 171 mg kg⁻¹. The decoction was administered intraperitoneally (i.p.) 1 h before the test.

**Chemical characterization of the decoction:** The chemical characterization of the decoction was done using the methods already described for the determination of alkaloids, anthraquinones, flavonoids, glycosides, phenols, saponins, bufadienolides and tannins (Harborne, 1973).

**Anticonvulsant tests**

**Maximal electroshock test:** Tonic convulsions of mice were induced by passing alternating electrical current (50 Hz, 30 mA, 0.2 sec) through eyes electrodes. Animals that did not convulse were declared protected (Ngo Bum et al., 2001, 2004a). The number of animals protected was determined in each group of mice. The positive control group received 5 mg kg⁻¹ of diazepam, i.p. The four tested groups received four doses of the decoction: 17.1, 42.7, 85.5 and 171 mg kg⁻¹.

**N-methyl-D-aspartate (NMDA) test:** Mice were injected subcutaneously with NMDA, 75 mg kg⁻¹, 1 h after administration of the decoction. They were observed for 30 min. Animals that did not exhibit turning behavior within the 30 min of observation were declared protected. Turning behavior was characterized by two consecutive 360° cycles fulfilled by the same animal. The positive control group received 33 nmol kg⁻¹ of D-AP7 (Croucher et al., 1982, Ngo Bum et al., 2008). The doses of the decoction tested were 17.1, 42.7, 85.5 and 171 mg kg⁻¹.

**Strychnine test:** Convulsions followed by death were induced in mice by the i.p. injection of 2.5 mg kg⁻¹ strychnine nitrate. The different treatments were given i.p. 1 h before strychnine injection. The animals which survived more than 10 min after strychnine injection were qualified protected. The positive control group received 3 mg kg⁻¹ of clonazepam (Ngo Bum et al., 2001). The doses of the decoction tested were 17.1, 42.7, 85.5 and 171 mg kg⁻¹.

**Pentylenetetrazol test:** Clonic seizures were induced in mice by the i.p. injection of 70 mg kg⁻¹ pentylenetetrazol. The different treatments were given 1 h before the injection of pentylenetetrazol. The animals that do not convulse within the 10 min from the injection of pentyleneetrazol were qualified protected (Ngo Bum et al., 2001). The positive control group received 0.1 mg kg⁻¹ of clonazepam. The doses of the decoction tested were 17.1, 42.7, 85.5 and 171 mg kg⁻¹.

**Isonicotinic hydrazide acid test:** Animals were injected i.p. with isonicotinic hydrazide acid 250 mg kg⁻¹ (Ngo Bum et al., 2001) 1 h after the administration of the different treatments. The time to the onset of seizures was recorded. The positive control group received diazepam 10 mg kg⁻¹ (per os). The doses of the decoction tested were 17.1, 42.7, 85.5 and 171 mg kg⁻¹. Data of the control group (treated with distilled water) were compared to data of the groups treated with the decoction.

**Diazepam-induced sleep in mice:** The method described by Rakotonirina et al. (2001) was used. Distilled water and the different doses of the decoction were given to mice 1 h before the injection of diazepam at a dose of 50 mg kg⁻¹. The sleeping time of mice was taking. The time between the loss of the straightening reflex and the regain of this reflex measured the sleeping time. The loss or the regain of the straightening reflex was measured by stimulating the external ear. When the mouse anterior paw does not move after stimulation with horsehair, the animal is sleeping. When the mouse is awakened, it moves and shakes its paw. The doses of the decoction tested were 17.1, 42.7, 85.5 and 171 mg kg⁻¹.

**Statistical analysis:** In anticonvulsant tests, the percentage of protection of mice in the control groups were compare to the percentage of protection of mice in groups treated with the decoction and in positive control
group. In isonicotinic hydrazide acid-induced convulsions and in diazepam-induced sleep tests, the mean value of the control group was comparable to the mean value of other groups. The statistical analysis were done using Anova followed by Dunnett (REGWQ). p<0.05 was considered significant.

**RESULTS AND DISCUSSION**

**Chemical characterization:** The chemical characterization of the decoction of the plant showed the presence of flavonoids, tannins, polyphenols and triterpenes. The decoction of *P. discoides* does not contain alkaloids, saponins, anthraquinones and steroids.

**Effect of *P. discoides* on NMDA-induced turning behavior:** The decoction of *P. discoides* (from 17.1 to 171 mg kg⁻¹) dose dependently and significantly antagonized NMDA-induced turning behavior in mice. Animals were strongly protected by the decoction (83.33% of protection at a dose of 171 mg kg⁻¹, (p<0.001)). D-AP7, the NMDA antagonist protected 100% of mice at a dose of 33 μmol kg⁻¹ (p<0.001) (Table 1).

**Effect of *P. discoides* on maximal electroshock-induced seizures:** The anticonvulstant compound diazepam completely protected mice against maximal electroshock-induced seizures (p<0.001). The decoction of *P. discoides* provided moderate protection. The dose of 171 mg kg⁻¹ protected 50% of mice (p<0.05) (Table 1).

**Effect of *P. discoides* on pentylentetrazol- and strychnine-induced seizures:** Clonazepam completely protected mice against both pentylentetrazol- and strychnine-induced seizures (p<0.001). The decoction of *P. discoides* protected 66.7% of mice against pentylentetrazol- and strychnine-induced seizures (p<0.01) (Table 1).

**Effect of *P. discoides* on diazepam-induced sleep:** Animals injected with diazepam (50 mg kg⁻¹, i.p.) showed the loss of the straightening reflex within 2 to 5 min after its administration. The decoction of *P. discoides* (from 17.1 to 171 mg kg⁻¹) strongly potentiated in a dose-dependent manner the sleeping time induced by diazepam (the decoction multiply by a factor of 3 to 5 times the sleeping time of the control group) (p<0.001) (Table 2). In the same time the decoction precipitated the loss of the straightening reflex from 4.7 min in the control group to 1.2 min in the group treated with the decoction at the dose of 171 mg kg⁻¹ (Table 3).

The decoction of *P. discoides* significantly protected mice against pentylentetrazol- and strychnine-induced seizures in mice. The inhibition by the decoction of strychnine-induced seizures suggests the presence of anticonvulsant properties (Fisher, 1989; Park et al., 2007; Trailovic and Varagic, 2007) and the involvement of glycine receptors (Findlay et al., 2002; Salih and Mustafa, 2008). The antagonism of pentylentetrazol-induced seizures suggests the interaction of the decoction of *P. discoides* with the GABA-ergic neurotransmission (Löschter and Schmidt, 1988; De Deyn et al., 1992). The decoction of *P. discoides* also antagonized NMDA-induced turning behavior. Since NMDA and non-NMDA receptors antagonists have been shown to possess anticonvulsant and antiepileptic properties in several animal models of epilepsy (Davies et al., 1986; Meldrum, 1992; Ngo Bun et al., 1996), it can be suggested that the decoction of *P. discoides* possesses anticonvulsant

---

**Table 1:** The effect of the decoction of *P. discoides* on maximal electroshock-, NMDA-, pentylentetrazol-, strychnine- and isonicotinic hydrazide acid-induced convulsions or turning behavior.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Maximal electroshock</th>
<th>NMDA</th>
<th>Pentylentetrazol</th>
<th>Strychnine</th>
<th>Isonicotinic hydrazide acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decoction (mg kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.71</td>
<td>0</td>
<td>33.3</td>
<td>33.3</td>
<td>33.3</td>
<td>48.4±6.7*</td>
</tr>
<tr>
<td>42.7</td>
<td>16.7</td>
<td>66.7**</td>
<td>50*</td>
<td>50*</td>
<td>55.4±17.5</td>
</tr>
<tr>
<td>85.5</td>
<td>16.7</td>
<td>66.7**</td>
<td>50*</td>
<td>50*</td>
<td>61.2±19.2</td>
</tr>
<tr>
<td>171</td>
<td>50*</td>
<td>83.3**</td>
<td>66.7**</td>
<td>66.7**</td>
<td>72±14.4**</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>39±8.1*</td>
</tr>
<tr>
<td>Diazepam</td>
<td>100***</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>83±15.3***</td>
</tr>
<tr>
<td>D-AP7</td>
<td>100***</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>--</td>
<td>100***</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Data represent the percentage of protected mice and the time to the onset of seizures in min (Mean ± SEM) in isonicotinic hydrazide acid test, n=6. *p<0.05, **p<0.01, ***p<0.001 compared to the control groups, Anova followed by Dunnett (REGWQ). Clonazepam, 0.1 mg kg⁻¹ for pentylentetrazol test and 3 mg kg⁻¹ for strychnine test. Diazepam, 5 mg kg⁻¹ for maximal electroshock test and 10 mg kg⁻¹ for isonicotinic hydrazide acid test. D-AP7, 33 μmol kg⁻¹ for NMDA test.
Table 2: Total sleep time (min) induced by diazepam in the presence of *P. discoides*

<table>
<thead>
<tr>
<th>Decoy (mg kg⁻¹)</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.1</td>
<td>59±5</td>
</tr>
<tr>
<td>42.7</td>
<td>70±11</td>
</tr>
<tr>
<td>85.5</td>
<td>90±14</td>
</tr>
<tr>
<td>171</td>
<td>110±21</td>
</tr>
<tr>
<td>Control</td>
<td>20±8</td>
</tr>
</tbody>
</table>

Data are presented as Mean ± SEM, n = 6. **p<0.001 compared to the control group, Anova followed by Dunnett (REGWQ)

Table 3: The onset time to sleep (min) induced by diazepam in the presence of *P. discoides*

<table>
<thead>
<tr>
<th>Decoy (mg kg⁻¹)</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.1</td>
<td>4.80±0.7</td>
</tr>
<tr>
<td>42.7</td>
<td>3.10±0.7</td>
</tr>
<tr>
<td>85.5</td>
<td>1.81±0.8</td>
</tr>
<tr>
<td>171</td>
<td>1.23±0.4</td>
</tr>
<tr>
<td>Control</td>
<td>4.70±0.5</td>
</tr>
</tbody>
</table>

Data are presented as Mean ± SEM, n = 6. *p<0.05, **p<0.01, ***p<0.001 compared to the control group, Anova followed by Dunnett (REGWQ)

properties. The effect of the decoction of *P. discoides* was moderate against maximal electroshock-induced seizures. The effects of the decoction in maximal electroshock and pentylentetrazol tests suggest anticonvulsant efficacy against generalized tonic-clonic seizures, generalized clonic seizures and partial seizures in man (De Deyn et al., 1992; Kupferberg and Schmutz, 1997; Löscher and Schmidt, 1988). The decoction of *P. discoides* strongly increased the total sleep time induced by diazepam and precipitated the onset of sleep. These effects suggest the presence of sedative properties in the decoction of *P. discoides* (Rakotonirina et al., 2001; Ngo Bun et al., 2004b, 2005, 2008). The sedative properties of *P. discoides* could be related to the presence of some components in the decoction activating the benzodiazepine and/or GABA sites in the GABA receptor complex (Rang et al., 1999). Flavonoids and tannins found in the decoction could be the active components of *P. discoides* since they are known to possess anticonvulsant and sedative properties (Bruneton, 1999).

In conclusion, the decoction of *P. discoides* possesses sedative and anticonvulsant properties in mice. These properties could explain the use of this plant in traditional medicine in Africa, particularly in Cameroon in the treatment of insomnia and epilepsy.

REFERENCES


