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Anticonvulsant and Anxiolytic Properties of the Leaves Extracts of Cymbopogon Proximus

Ibrahim MS

Abstract

Objectives: This study was conducted to evaluate the anticonvulsant and the sedative anxiolytic activity of the leaves extract of Cymbopogon proximus in rats.

Materials and Methods: The ethanolic extract of the root of C. proximus at 200, 400 and 800 mg/kg, i.p was studied for its anticonvulsant effect on four in vivo rat models (Maximal Electroshock Seizure (MES), Pentylentetrazole (PTZ), Picrotoxin (PIC) - and Strychnine (STR) - induced seizures). Simple activity meter was used for the evaluation of the anxiolytic properties. Sodium valproate (400 mg/kg) was used as a reference anticonvulsant drug for all models. The protection from tonic convulsions and the number of protected animals from seizures were noted. The number of movements between the squares in the activity meter were counted in the consecutive five minutes and the motor activity was observed.

Results: The plant showed marked sedative - anxiolytic effect and significant decrease in the motor activity (p<0.001) since the first dose (200 mg/kg) in a dose-dependent manner. The doses 400 and 800 mg/kg of the extract significantly (p<0.01 - p<0.05) reduced the duration of seizures induced by MES, MTZ and PIC while only the dose of 800mg/kg of the extract delayed the onset of tonic-clonic seizures produced by strychnine.

Conclusion: Results of the present study concluded that the ethanolic extract of Cymbopogon proximus leaves possesses strong sedative properties with moderate anticonvulsant and anxiolytic activity. It is therefore recommended for the treatment of insomnia, anxiety in case of epilepsy.

Key Words

Anxiety; Epilepsy; Extract; C. Proximus; Seizures; Traditional Medicine

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Introduction

Cymbopogon proximus is one of the important plants in African and Sudanese folk medicine known as Almahreb. It is used for the treatment of nervous and gastrointestinal disturbances, anxiety and agitation. The petroleum ether extract of Cymbopogon proximus proved to have unique antispasmodic characteristics, and could be used for the propulsion of renal and ureteric calculi. C. proximus extracts possess a valuable antihypertensive activity and it produces relaxation of the smooth muscle fibers. Many biological activities of C. proximus have been reported. Bioactivity-assisted fractionation of the C. proximus extracts led to the isolation of an active sesquiterpene, proximadiol (cryptomeridiol) which was found to have antidiabetic activity. In addition, C. proximus essential oil was found to possess a bronchodilator activity mediated via antagonising both histamine and serotonin receptors. Furthermore, it has a significant ganglionic blocking action and a mild anti-inflammatory activity. This study was intended to investigate the anticonvulsant, anxiolytic and sedative properties of this medicinal plant in rats.

2. Materials and Methods

2.1. Plant material

Cymbopogon proximus leaves were purchased from a local medical plant market in Khartoum, Sudan and authenticated by Taxonomy Department of Medicinal and Aromatic Plant Research Institute, National Center for Research, Khartoum, Sudan. Leaves were preserved in a dry and cool place for 72 hours, then were ground into a fine powder using clean dry electric blender. Ethanol extraction process was followed according to. 100g of the ground leaves were transferred to a round bottom flask and macerated in 80% ethanol. The flask was stoppered and left for 24 hours at room
temperature. The extract was then filtered using sterile cotton pieces. The filtrate thus obtained was concentrated under reduced pressure and the solvent recalled was used to extract the mark following the same procedure till exhaustion. The concentrated extract was collected and left to dry at room temperature till constant weight was obtained. The extract was then kept in a refrigerator for experimentation.

2.2. Chemicals
Pentylenetetrazole, sodium valproate, picrotoxin and strychnine were used to induce seizures and all were from Sigma Chemical, USA.

2.3. The Experimental Animals
Adult males, Wistar Albino Rats (WAR), weighing 110-125 g were housed in standard polypropylene cages in the Laboratory Animal House of the Aromatic Plants Research Institute (MAPRI), National Centre for Research (NCR), Sudan (from February 2013 to May 2014). The animals were acclimatized for seven days under standard environmental conditions (i.e., relative humidity: 40-60%, temperature: 24±2°C, and 12 h light-dark cycle), and fed with mash feed consisting of flour, meat, edible oil, sodium chloride, vitamins, minerals and tap water. Supply of food was withdrawn 12 h prior to the commencement of the experiment. However, the rats were allowed access to water always. All the experiments were carried out by using five animals in each group. The experiments were carried out between 8 am and 12 noon. Twenty five rats were used in each experiment, divided into five groups; each group received three different single doses (200, 400 and 800 mg/kg i.p) of the plant leave extract. One group was given 400 mg/kg i.p sodium valproate as a reference drug (positive control). The last group was given 10ml/kg i.p normal saline (negative control). This study was approved by the Scientific Research Committee of the College of Pharmacy, Omdurman Islamic University in accordance with good clinical practice and international guidelines for animal use in experimentations.

2.4. Experiment 1: Measurement of Anxiolytic Activity in Rats
An anxiety model, simple activity meter test, was used to explore the anti-anxiolytic effect of the tested extract[9]. The simple activity meter is a box composed of two glassy and two wooden sides stand on 625 cm² wooden plane board divided to 25 squares. Each square was 25 cm². A rat was placed on the center of the board and left to move freely for a period of five minutes. The number of movements between the squares were counted in the consecutive five minutes. Decrease in number of movements/ five minutes was taken as an indication of anti-anxiety activity. Decrease in motor activity reflected the sedative effect of the extract.

2.5. Pharmacological Tests & Assessment of Anticonvulsant Activity:

2.5.1. Experiment 2: Pentylenetetrazole (PTZ) - Induced Seizure Test:
Myoclonic jerks seizures were induced in male rats by subcutaneous injection of 70 mg/kg pentylenetetrazol (PTZ)[9,10,11,12]. The protective effect of the three tested doses of the extract was recorded. The tested extract was given 45 minutes before PTZ injection. The positive control group received 400mg/kg i.p sodium valproate 15 minutes before PTZ injection. One group received 10 ml/kg i.p normal saline and served as a negative control group.

2.5.2. Experiment 3: Picrotoxin (PIC) - Induced Seizure Test:
This model acts to disrupt the inhibition/excitation balance and creates an epileptogenic focus[13]. Clonic seizures were induced in male rats by subcutaneous injection of 10 mg/kg i.p picrotoxin. The three various doses of the extract were given 45 minutes before picrotoxin administration while the positive control received sodium valproate 15 minutes before picrotoxin injection. Another group was given 10ml/kg i.p normal saline and served as a negative control group. The protective percentage was then recorded.

2.5.3. Experiment 4: Maximal Electroshock (MES) Test:
Tonic convulsions of the hind extremities of mice were induced by passing an alternating electrical current (50 mA, of 100 Hz frequency (pulse/sec.) for 0.5 sec. duration through ear electrodes[11,12,13]. The three tested doses of the extract were given 45 minutes before the induction of the MES while the positive control received sodium valproate 15 minutes before the MES. Another group was given 10ml/kg i.p normal saline and served as a negative control group. The number of animals protected from tonic hind limb extension was determined in each dose group.

2.5.4. Experiment 5: Strychnine (STR) Test:
Convulsions followed by death were induced in male mice by the subcutaneous injection of 2.5 mg/kg strychnine (STR) nitrate. The protective effect of three different intraperitoneal treatments were given 45 minutes prior to STR was recorded. Animals that survived more than 10 minutes were classified as protected. The positive control group received 400 mg/kg i.p sodium valproate[9, 11, 15].

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2.6. Statistical Analysis

The values are expressed as mean ± SEM and the data was analysed using one way ANOVA followed by Tukey-Krammer test. The level of significance was set at P < 0.05. Median anticonvulsant dose (ED50) was calculated according to the method of Litchfield and Wilcoxon16. A computer programme was used to calculate 95% confidence limit of ED50.

3. Results

3.1. Effects of Cymbopogon Proximus Leaves Extract on the Motor Performance Using the Simple Activity Meter Test:

Cymbopogon proximus showed marked sedative - anxiolytic effect and significant decrease in the motor activity (P < 0.001) since the first dose (200 mg/kg, ip) in a dose-dependent manner (table 3.1).

<table>
<thead>
<tr>
<th>Extract dose (mg/kg)</th>
<th>200 (mg/kg)</th>
<th>400 (mg/kg)</th>
<th>800 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SEM</td>
<td>20.6 ± 2.27</td>
<td>20.6 ± 2.27</td>
<td>20.6 ± 2.27</td>
</tr>
<tr>
<td>Positive control group</td>
<td>10.0 ± 0.95**</td>
<td>8.4 ± 1.03**</td>
<td>1.0 ± 0.40***</td>
</tr>
</tbody>
</table>

Table 3.1. Effects of Cymbopogon Proximus Leaves Extract on the Motor Performance

Using the Simple Activity Meter Test:

Treatment was compared with control group. Five animals were used in each group. ** P < 0.01, *** P < 0.001

3.2. Pharmacological Tests and Assessment of Anticonvulsant Activity:

Effects of Cymbopogon Proximus Leaves Extract against Pentylenetetrazol (PTZ) - induced seizures:

3.2.1. Pentylenetetrazol (70mg/kg, s.c.) produced generalised tonic-clonic seizures in the negative control group.

40% of the animals that received 200mg/kg, i.p of the extract resisted the PTZ convulsive effect, the resistant ratio increased up to 80% in the group that received 400 mg/kg, i.p of the extract while, 800 mg/kg, i.p of the extract as well as sodium valproate (400 mg/kg, i.p) showed 100% anticonvulsant protection against PTZ. The ED50 of the extract against PTZ was found to be 248.77 mg/kg. The extract significantly (p < 0.05) decreased the recovery period in the affected animals by 20.02 ± 2.40 min. compared to the positive control group (44.50 ± 2.03 min.). (Table 3.2.1). No incidence of mortality was recorded.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sodium valproate (+ve control)</th>
<th>C. proximus (mg/kg)</th>
<th>Normal saline (-ve control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED50</td>
<td>162</td>
<td>248.77</td>
<td></td>
</tr>
<tr>
<td>95% C.L.</td>
<td>(140-185)</td>
<td>(160.42 - 385.75)</td>
<td></td>
</tr>
<tr>
<td>Time (min.) for duration of recovery (Mean ± SEM)</td>
<td>0.00 ± 0.00***</td>
<td>11.20 ± 1.10*</td>
<td>44.50 ± 2.03</td>
</tr>
</tbody>
</table>

Table 3.2.1 Effects of Cymbopogon Proximus Leaves Extract Against Picrotoxin (PIC) - Induced Seizures:

3 to 5 doses were used to calculate ED50 (in mg/kg).* p < 0.05, *** p < 0.001 significant (compared with the respective control).

3.2.2 Effects of Cymbopogon Proximus Leaves Extract Against Picrotoxin (PIC) - Induced Seizures:

Picrotoxin (10 mg/kg, s.c.) produced generalised tonic-clonic seizures in the negative control group.

The plant extract appeared slight to moderate activity against PIC-induced seizures.

The dose of 200 mg/kg, i.p showed 20% protection in the tested animals group while 400 mg/kg, ip produced 40% protection. The protection ratio increased up to 60% when the third group was injected with 800 mg/kg, ip of the extract. The ED50 of the extract against PIC was found to be 483.02 mg/kg. The extract significantly (p < 0.05) decreased the recovery period in the affected animals by 34.50 ± 2.23 min. compared to the positive control group 48.20 ± 3.12 min. (Table 3.2.2). 20% incidence of mortality was recorded in the affected animals.
Proximus Leaves Extract Against Picrotoxin (PIC) - Induced Convulsions:

3 to 5 doses were used to calculate ED50 (in mg/kg). *p < 0.05, **p < 0.01 most significant (compared with the respective control).

3.2.3. Effects of Cymbopogon Proximus Leaves Extract on Maximal Electroshock (MES)-Induced Seizures:

The anticonvulsant agent, sodium valproate, completely protected the rats against MES-induced seizures (P < 0.001). The dose of 800 mg/kg, i.p of the plant extract showed 20% protection against the MES test and significantly (p < 0.01) decreased the recovery period in the affected animals by 32.40±16.68 sec compared to the control 174.20±23.01 sec. The dose 400 mg/kg, i.p significantly (p < 0.05) shorted the duration of recovery period in the affected animals by 85.40±14.37 sec compared to the control, while 200 mg/kg of the plant extract did not appear any significant protective activity against MES test. All the affected animals were recovered and no deaths were recorded. (Table 3.2.3).

Sodium valproate completely protected the rats against STR-induced seizures (P < 0.001). Only the dose of 800mg/kg, i.p of the plant extract protected 20% of the tested animals group and showed significant (p < 0.05) increase in the latency of seizures by 16.60±2.37 min compared to the negative control 3.20 ±0.86 min while both doses of 400 and 200 mg/kg, i.p did not show any significant protective activity against STR test. Deaths were recorded in all the affected animals (Table 3.2.4).

**p < 0.05 significant, ***p < 0.01 most significant, ****p < 0.001 highly significant (compared with the respective control).

### Table 3.2.2. Effects of Cymbopogon Proximus Leaves Extract on Maximal Electroshock (MES)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sodium valproate (+ve control)</th>
<th>C. proximus</th>
<th>Normal saline (-ve control)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ED50</strong></td>
<td>192.6</td>
<td>483.02</td>
<td>-</td>
</tr>
<tr>
<td>95% C.L (mg/kg)</td>
<td>(159-207)</td>
<td>(253.46 - 920.47)</td>
<td>-</td>
</tr>
<tr>
<td>Time (min.) for duration of recovery (Mean ± SEM)</td>
<td>0.00± 0.00***</td>
<td>34.50 ± 2.23*</td>
<td>48.20 ±3.12</td>
</tr>
</tbody>
</table>

### Table 3.2.3. Effects of Cymbopogon Proximus Leaves Extract on Maximal Electroshock (MES)-Induced Seizures

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Dose rate (mg/kg)</th>
<th>Protection rate against MES (%)</th>
<th>Time (sec) for duration of Recovery/ death recovery (Mean ± SEM)</th>
<th>Recovery/ death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>(10 ml/kg)</td>
<td>174.20 ±23.01</td>
<td>0 %</td>
<td>Recovery</td>
</tr>
<tr>
<td>Sodium valproate</td>
<td>400</td>
<td>0.00 ± 0.00***</td>
<td>100%</td>
<td>Recovery</td>
</tr>
<tr>
<td>C. proximus</td>
<td>200</td>
<td>0%</td>
<td>146.00±39.45</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0%</td>
<td>85.40±14.37*</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>20%</td>
<td>32.40±16.68**</td>
<td>Recovery</td>
</tr>
</tbody>
</table>

### Table 3.2.4. Effects of Cymbopogon Proximus Leaves Extract on Strychnine (STR)-Induced Seizures

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Dose rate (mg/kg)</th>
<th>Protection rate against STR (%)</th>
<th>Time (min.) of the latency of seizures (Mean ± SEM)</th>
<th>Survive/ death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>(10 ml/kg)</td>
<td>100%</td>
<td>3.20 ±0.86</td>
<td>Death</td>
</tr>
<tr>
<td>Standard valproate</td>
<td>400</td>
<td>100%</td>
<td>6.01±1.00</td>
<td>Death</td>
</tr>
<tr>
<td>C. proximus</td>
<td>200</td>
<td>0%</td>
<td>16.60±2.37*</td>
<td>Death</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0%</td>
<td>4.20±1.01</td>
<td>Survive</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>0%</td>
<td></td>
<td>Death</td>
</tr>
</tbody>
</table>

Discussion

The results of the current study indicate that Cymbopogon proximus leaves extract has potential anxiolytic properties. This potentiation of anti-anxiety suggests the presence of anxiolytic-sedative properties in the extract of C. proximus. The sedative-anxiolytic effect of C. proximus is probably due to the essential oil which was found to have antagonising effects for both histamine and serotonin receptors. The result is in correspondence with Seth et al. who reported that Cymbopogon citratus essential oil induced hypnosis in mice.

Cymbopogon proximus also showed significant anticonvulsant properties by inhibiting convulsions induced chemically or electrically in various percentages. The extract protected rats against PTZ, PIC and STR-induced seizures in a dose-depend manner. As PTZ has been shown to interact with the gamma amino butyric acid (GABA) neurotransmitter, the antagonism of PTZ-induced seizures suggests that C. proximus interacts with...
GABAergic neurotransmission since the PTZ is a selective blocker of the chloride ionophore complex to the GABA-A receptor. Picrotoxin (PIC) - induced seizures is known to be a non-competitive GABA antagonist, exerting its effect by blocking the chloride channel in the GABA receptor complex\(^9\). It is used to induce acute simple partial seizures and generalised tonic-clonic seizures\(^8,9\). The antagonism of PIC-induced seizures suggests the interaction of the plant extract with the GABA-ergic neurotransmission.

These results agree with some studies that show that menthol, a synthetic product from the extract, acts as GABAA receptor positive allosteric modulator and increases GABAergic transmission in periaqueductal grey neurons\(^9\). Also, C. proximus essential oil was found to be a significant ganglionic blocking agent\(^10\). The mild inhibition of STR-induced seizures by C. proximus extract suggests that it possesses anticonvulsant properties\(^11\) and that glycine neurotransmission is involved in a weak way\(^12\). C. proximus antagonised MES-induced seizures probably by prolonging the activation of sodium channels\(^13\). The anticonvulsant anxiolytic activity of the plant extract is in association with some findings proved that the genus Cymbopogon has marked depressant effect on the central nervous system\(^14\).

5. Conclusion and Recommendations
Cymbopogon proximus leaves possess strong sedative properties with moderate anticonvulsant and anxiolytic activity. It is therefore for the treatment of insomnia, anxiety in case of epilepsy.

These results were obtained from experimental animals’ models, so this herbal plant should be used in appropriate formulations for further clinical trials in human.

Acknowledgement
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