Central activities of *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum

James Oluwagbamigbe FAJEMIROYE1*, José Luís Rodrigues MARTINS1, Adriane Ferreira de BRITO1, Pablinny Moreira GALDINO1, José Realino de PAULA2, Joelma Abadia Marciano de PAULA3, Elson Alves COSTA1

1Institute of Biological Sciences, Department of Physiological Sciences, Federal University of Goiás, Campus Samambaia, Goiânia-GO, Brazil
2Faculty of Pharmacy, Federal University of Goiás, Setor Universitário, Goiânia-GO, Brazil
3Sciences and Technology Unit, Goias State University, BR153, Nº.3105, Fazenda Barreiro do Meio, C.P. 459, 75132-903 Anápolis, GO, Brazil

*Corresponding Author, Tel: 55 62 3521 1491, Fax: 55 62 352 11204

Article History: Received 23rd November 2011, Revised 10th January 2012, Accepted 10th January 2012.

Abstract: Folkloric medicinal application of *Pimenta pseudocaryophyllus* popularly known as “craveiro” as calming agent is a commonplace in Campos do Jordão, SP, Brazil. Pharmacological screening of ethanolic leaf extract of *Pimenta pseudocaryophyllus* (EEPp) and its active fraction (s) for possible anxiolytic like effect became necessary in order to confirm its therapeutic claims by the populace. Preliminary investigation with oral administration of EEPp (0.5, 1.0 and 2.0 g/kg) was devoid of any sign of neurotoxicity. In barbiturate induced – hypnosis, EEPp 1.0 g/kg potentiated the pentobarbital-induced sleep (32% reduction in sleep latency and 20% increase in sleep duration) while only dichloromethane and aqueous fractions increases sleep duration. Based on the parameters evaluated in both the open field and light dark box models, only dichloromethane fraction among others showed consistent anxiolytic like effect.

Keywords: Behavioural alterations; *Pimenta pseudocaryophyllus*; Anxiolytic-like.

Introduction

*Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum (Family: Myrtaceae) is popularly known as craveiro. In Brazil, about 1000 species and 23 genera of this family are found (Landrum and Kawasaki 1997). The genus *Pimenta* has 15 species of which only *P. pseudocaryophyllus* occurs in the Brazilian flora. Two different Chemotypes of *P. pseudocaryophyllus* have also been reported by Paula et al. (2010).

This species is a tree whose height varies from 4-10 m with almost upright trunk (20 to 30 cm in diameter). Its leaves are elliptical or elliptic-ob lanceolate. Its inflorescence is a dichasium or a panicle of usually 7 to 15 flowers (Landrum 1986; Lorenzi and Matos 2002).

Based on the folkloric perceptions of *P. pseudocaryophyllus*, its leaf extracts is used in Brazilian traditional medicine in different preparations. In São Gonçalo do Abaeté, MG, Brazil, the leaves are used to make tea for influenza (Paula et al. 2008) while in the county of Campos do Jordão, SP, Brazil, the leaves are infused to make a soothing tea (Nakaoka-Sakita et al. 1994). Considering alarming increase in the cases of neural disorders like anxiety, depression among others and lack of drugs with tolerable side effect, this research work was aimed at screening the ethanolic leaf extract of *P. pseudocaryophyllus* (EEPp) and its active fraction (s) for possible anxiolytic like effect.

*Corresponding author. (E-mail) olulolo.A.T@yahoo.com
©2012 Open Access Science Research Publisher http://www.openaccessscience.com
ijmap@openaccessscience.com
Materials and methods

Plant material

Leaves of *P. pseudocaryophyllus* (Gomes) L.R. Landrum were collected in São Gonçalo do Abaeté, MG, Brazil, 18º20’58.4” S, 45º55’23.4” W, 864 m altitude in February 2008. The plant material was identified by Carolyn Elinore B.P. PhD, University of Brasilia; the voucher specimen (Nº 27159) was deposited in the Herbarium of Universidade Federal de Goiás - UFG.

Crude ethanol extract preparation and liquid-liquid partition

The pulverized material (leaves) of *P. pseudocaryophyllus* (464 g) were macerated in ethanol (95%, v/v, 1:5 w/v) at room temperature, followed by filtration and concentration on the rotary evaporator at a temperature below 40°C. This process was repeated with the residue thrice to ensure exhaustive extraction. The extract was collected, concentrated to constant weight and termed crude extract (EEPp). 50.0 g of EEPp was dissolved in 200 mL of methanol/water (7:3). The solutions obtained were subjected to liquid/liquid solvent partition (hexane, dichloromethane and ethyl acetate) as described by Ferri 1996. The solvent of each fraction were later evaporated in rotary evaporator and each fraction (HF, DF and EAF) was dried to constant weight. For the fraction of methanol/water, the methanol was removed in rotary evaporator and subsequently freeze-dried to obtain aqueous lyophilized fraction (AF). The fractions were stored at -10°C until their experimental use.

Animals

Male albino *Swiss* mice weighing between 30 to 40 g, provided by the Central Animal House of UFG were used. The animals were maintained under controlled conditions of temperature, light (12 h dark/light), with water and food *ad libitum*. Experiments were carried out between 9:00 a.m. – 12:00 noon. The animals were drawn at random and interspersed into test and control groups during treatment. All proceedings and experimental models were executed in accordance with the experimental protocols designed according to ethical principles in animal research adopted by the Brazilian Society of Science of Laboratory Animal (SBCAL) and were approved by Research Ethics Committee of UFG (Nº 104/08).

Drugs

Tween 80® (2%, Sigma – EUA). Sodium Pentobarbital (Abbott – Brazil) dissolved in 0.9% sodium chloride isotonic solution. EEPp and all fractions were prepared by dissolving in distilled water and 2% Tween 80®. All solutions were prepared freshly on test days and administered in a volume of 0.1 mL/10 g body weight.

General Pharmacological Evaluation

Subcutaneous, intraperitoneal or oral route were explored in extract administration. Control group were treated with vehicle (10 mL/kg) in volume proportional to the dose of EEPp (0.5, 1.0, or 2.0 g/kg) administered. Observation were made at 5, 10, 20, 30 and 60 min; 2, 4, 8, 24 and 48 h; as well as after 4 and 7 days of treatment (Malone, 1977).

Barbiturate-induced hypnosis

After 60 min of oral treatments with vehicle (10 mL/kg), EEPp (1.0 g/kg), AF (0.64 g/kg), EAF (0.42 g/kg), DF (0.25 g/kg) or HF (0.16 g/kg), all groups (n = 12) received sodium pentobarbital (40 mg/kg, i.p.). The time elapsed between the administration of sodium pentobarbital till the loss of the righting reflex and the time taken for recovery of the righting reflex were recorded as sleep latency and sleep duration respectively (Galdino et al. 2010).

Open-field Test

Experimental groups of 12 mice were treated (p.o.) with vehicle (10 mL/kg), AF (0.64 g/kg), EAF (0.42 g/kg), DF (0.25 g/kg) or HF (0.16 g/kg). Sixty min after the treatment, the animals were placed at the centre of the open-field arena, with the bottom divided into eight areas; the observed parameters are as follows:
total crossing, central area crossing, time spent in central area and immobility time.

**Light dark box test**

Experimental groups of 12 mice were treated (p.o.) with vehicle (10 mL/kg), AF (0.64 g/kg), DF (0.25 g/kg) or HF (0.16 g/kg). Sixty minutes after the treatment, the animals were placed at the centre of the light area facing the opening of dark area. The latency to the first transitions, number of transitions between the two compartments and time spent in the light area were recorded over a 5 minutes period (Crawley and Goodwin 1980).

**Statistical analysis**

Data were showed as mean ± SEM and analyzed statistically with Student’s t-test.

**Results and Discussion**

In respect of the extraction and fractionation process, EEPp yielded 24.0 % relative to plant material while HF, DF, EAF and AF yielded 8.2, 13.2, 21.3 and 31.3% respectively in respect to the EEPp. The doses of the fractions were established based on their respective yield in relation to the highest dose of EEPp that was used in the general pharmacological test.

Biological activity of *P. pseudocaryophyllus* had been reported in the research earlier conducted in our laboratory (Fajemiroye et al. 2010 and 2011). The results obtained demonstrated neuropharmacological activity of the essential oil and the fractions obtained from the EEPp.

According to Malone (1983), general pharmacological test is an approach that permits the detection of central effect (depression or stimulation) of a pure compound, combinations of drugs, plant extracts or other pharmaceutical product preparations.

In the general test of pharmacological activity, the EEPp elicited a rapid-onset, dose-related decrease of exploratory activity, abdominal contortion and alienation within 10 min of i.p. (0.5, 1.0 and 2.0 g/kg) and 30 min of p.o. (0.5, 1.0 and 2.0 g/kg) administration. Subcutaneous administration of EEPp 2.0 g/kg elicited decrease in spontaneous motor activity and piloerection within 30 min and ataxia at 60 min. Intraperitoneal and Subcutaneous administration of EEPp 2.0 g/kg resulted in the death of experimental subject after 12 and 24 h of treatment respectively.

On the other hand, no death was recorded in the group treated orally at this dose besides the absence of palpebral ptosis, aggression, tremors, convulsions or behavioural alterations that could suggest neurotoxicity (results not shown). Meanwhile, the observed alterations can be associated with CNS depression.

Except for dichloromethane fraction (DF), treatments with crude leaf extract (EEPp), aqueous fraction (AF), ethyl acetate fraction (EAF) or hexane fraction (HF) decreased the sleep latency, meanwhile, only EEPp, AF and DF increased the sleeping time (Table 1). This central effect is a pointer to popularly acclaimed soothing effect of the leaves extract of *P. pseudocaryophyllus* (Nakaoka-Sakita et al. 1994).

**Table 1:** Effect of ethanolic leaf extract of *P. pseudocaryophyllus* leaves and its fractions on latency and duration of barbiturate induced hypnosis in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (p.o.)</th>
<th>Latency (s)</th>
<th>Sleeping time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10 mL/kg</td>
<td>139.3 ± 6.80</td>
<td>41.4 ± 3.60</td>
</tr>
<tr>
<td>EEPp</td>
<td>1 g/kg</td>
<td>111.0 ± 7.80*</td>
<td>54.7 ± 3.49*</td>
</tr>
<tr>
<td>AF</td>
<td>0.64 g/kg</td>
<td>118.4 ± 2.86**</td>
<td>55.7 ± 6.28*</td>
</tr>
<tr>
<td>EAF</td>
<td>0.42 g/kg</td>
<td>115.6 ± 2.19 **</td>
<td>42.2 ± 3.20</td>
</tr>
<tr>
<td>DF</td>
<td>0.25 g/kg</td>
<td>142.7 ± 6.31</td>
<td>67.0 ± 3.10***</td>
</tr>
<tr>
<td>HF</td>
<td>0.16 g/kg</td>
<td>114.0 ± 3.30**</td>
<td>49.1 ± 3.14</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM (n = 12). *p < 0.05, **p < 0.01, ***p < 0.001 vs. control group.

In the open-field test, the sedative effect of the aqueous fraction, which was manifested by reduction in total crossing and increase in freezing time (Table 2) and reduction in number of transition in the LDB (Table 3), is possibly due to the compromise of motor activity. The dichloromethane fraction showed an anxiolytic-like effect without any significant decrease in exploratory activity of the animal. An increase in activity or time spent at the centre of the open-field.
field without any sign of motor incoordination as observed with dichloromethane fraction (Table 2) is indicative of anxiolysis (Prut and Belzung 2003).

Table 2: Effect of ethanolic leaf extract of *P. pseudocaryophyllus* fractions on the parameters evaluated in open-field test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (p.o.)</th>
<th>Total crossing</th>
<th>Central area crossing (%)</th>
<th>Time spent in the centre (s)</th>
<th>Immobility time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10 mL/kg</td>
<td>121.9 ± 7.8</td>
<td>69.5 ± 2.3</td>
<td>127.0 ± 13.0</td>
<td>16.6 ± 4.4</td>
</tr>
<tr>
<td>AF</td>
<td>0.64 g/kg</td>
<td>96.6 ± 9.3*</td>
<td>76.2 ± 1.8*</td>
<td>168.8 ± 4.0**</td>
<td>33.3 ± 5.1*</td>
</tr>
<tr>
<td>EAF</td>
<td>0.42 g/kg</td>
<td>89.2 ± 10.0*</td>
<td>67.9 ± 3.4</td>
<td>124.2 ± 18.0</td>
<td>12.5 ± 7.1</td>
</tr>
<tr>
<td>DF</td>
<td>0.25 g/kg</td>
<td>160.3 ± 8.0**</td>
<td>75.1 ± 1.6**</td>
<td>175.3 ± 8.3**</td>
<td>2.1 ± 1.1**</td>
</tr>
<tr>
<td>HF</td>
<td>0.16 g/kg</td>
<td>103.9 ± 7.6</td>
<td>63.5 ± 5.4</td>
<td>163 ± 13.3</td>
<td>10.2 ± 3.8</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM (n = 12).*p < 0.05, **p < 0.01 vs. control group.

Table 3: Effect of Aqueous, dichloromethane and Hexane fractions obtained from ethanolic leaf extract of *P. pseudocaryophyllus* on the mice in light dark box

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (p.o.)</th>
<th>Latency</th>
<th>Transitions</th>
<th>Time spent (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dark area</td>
</tr>
<tr>
<td>Vehicle</td>
<td>10 mL/kg</td>
<td>2.70 ± 0.4</td>
<td>10.7 ± 0.9</td>
<td>232.6 ± 7.9</td>
</tr>
<tr>
<td>AF</td>
<td>0.64 g/kg</td>
<td>6.82 ± 1.0***</td>
<td>4.3 ± 0.4***</td>
<td>231.0 ± 7.8</td>
</tr>
<tr>
<td>DF</td>
<td>0.25 g/kg</td>
<td>8.80 ± 0.6***</td>
<td>18.0 ± 1.0***</td>
<td>191.7 ± 5.3**</td>
</tr>
<tr>
<td>HF</td>
<td>0.16 g/kg</td>
<td>2.62 ± 0.4</td>
<td>10.3 ± 0.9</td>
<td>221.3 ± 7.5</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM (n = 12).**p < 0.01, ***p < 0.001 vs. control group.

Interestingly, in the LDB anxiolytic and sedative-like effect of AF and DF respectively were confirmed at the dose tested (Table 3) unlike HF that failed to show consistent central effect based on the data obtained in this model.

In conclusion, the overall activities of ethanolic leaf extract of *P. pseudocaryophyllus* are suggestive of central nervous system depression while the effect of dichloromethane and aqueous fractions treatments were characterized by anxiolytic and anxiolytic/sedative-like effects respectively. Future research is required to elucidate the mechanisms of action and the active principles responsible for these biological activities.

Acknowledgements: Special appreciations to CAPES, CNPq, FAPEG and UFG for all the financial supports.

References


