Research Article

Essential Oil of Ocimum basilicum L. and (−)-Linalool Blocks the Excitability of Rat Sciatic Nerve

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The racemate linalool and its levogyrus enantiomer [(−)-LIN] are present in many essential oils and possess several pharmacological activities, such as antinociceptive and anti-inflammatory. In this work, the effects of essential oil obtained from the cultivation of the Ocimum basilicum L. (EOOb) derived from Germplasm Bank rich in (−)-LIN content in the excitability of peripheral nervous system were studied. We used rat sciatic nerve to investigate the EOOb and (−)-LIN effects on neuron excitability and the extracellular recording technique was used to register the compound action potential (CAP). EOOb and (−)-LIN blocked the CAP in a concentration-dependent way and these effects were reversible after washout. EOOb blocked positive amplitude of 1st and 2nd CAP components with IC50 of 0.38±0.2 and 0.17±0.0 mg/mL, respectively. For (−)-LIN, these values were 0.23±0.0 and 0.13±0.0 mg/mL. Both components reduced the conduction velocity of CAP and the 2nd component seems to be more affected than the 1st component. In conclusion, EOOb and (−)-LIN inhibited the excitability of peripheral nervous system in a similar way and potency, revealing that the effects of EOOb on excitability are due to the presence of (−)-LIN in the essential oil.

1. Introduction

Aromatic plants of the genus Ocimum (Lamiaceae) have been receiving widespread use in folk medicine [1]. This plant has been studied in experimental models of pain [2], inflammation [3], convulsion [4, 5], and other central nervous system disorders [6, 7]. The essential oil (EO) extracted from the leaves of different species of Ocimum is rich in several small molecules from different chemical classes (monoterpenes, cyclic sesquiterpenes, and aliphatic secondary alcohols among others) such as d-cadinol, estragole, and linalool (LIN) [8–11]. A new cultivar of the species Ocimum basilicum was derived from the Germplasm Bank North Central Regional PI Station (PI 197442, USA) and was called “Maria Bonita” [12]. In this new cultivar linalool content was increased to circa 77% (wild species 40%) and some constituents decreased to undetectable level.

The racemate LIN and its levogyrus enantiomer [(−)-LIN] have shown antinociceptive and anti-inflammatory activities [13, 14] and de Sousa et al. [15], using different models of epilepsy, concluded that LIN enantiomers and racemate have anticonvulsant activity, although with different pharmacological potencies. Previous studies about Ocimum basilicum L. (“Maria Bonita”) pharmacological effects showed that (−)-LIN is the principal constituent responsible for the antinociceptive properties of this cultivar [16, 17]. Regarding neuronal excitability, Venâncio et al. [17] showed an inhibition of neuronal excitability in hippocampal slice preparation promoted by the essential oil of Ocimum basilicum L. (EOOb) and (−)-LIN and a series of in vitro
experiments demonstrated direct actions of \((-\text{LIN})\) on ligand-gated receptors [18–20] and nitric oxide formation [21]. Additionally, Leal-Cardoso et al. [22] showed that race- 
cmate LIN concentration-dependently and reversibly blocked the compound action potential (CAP) and the excitability of 
rat sciatic nerve. However, regarding an essential oil with a 
rich content of the major constituent, the presence of other 
constituents besides the major one on the mixture might 
change (amplifying or partially inhibiting) the effect of the 
essential oil as related to effect intensity solely on basis of the percentage of the major constituent in the oil, as 
has already been demonstrated [23]. In the case of EOOb, 
neither the quantitative participation of \((-\text{LIN})\) on its effect nor 
the pharmacological potency of \((-\text{LIN})\) on nerve excitability 
is known.

Thus, in view of the fact that several EOOb pharma-
cologic effects might involve alteration of nerve excitability, 
which makes this effect very relevant, and previous study by 
Venâncio et al. [17] on hippocampus did not quantitatively 
evaluate the participation of \((-\text{LIN})\) on the effect of EOOb 
(“Maria Bonita”), this work’s objectives demonstrate the 
effects of EOOb on peripheral nerve excitability and the 
participation of \((-\text{LIN})\) as its active principle. Additionally, 
the other studies on peripheral nerves were done with the 
racemate mixture of linalool and this study also aimed to 
evaluate the effect of the pure enantiomer \((-\text{LIN})\) on peripheral 
nerve excitability.

2. Material and Methods

2.1. Plant Material and Essential Oil Extraction. Leaves were 
collected from the cultivation of the Ocimum basilicum L. 
(named “Maria Bonita”) obtained at agricultural research 
station of Federal University of Sergipe. Ocimum basilicum L. 
was derived from the accession PI 197442 of the Germplasm 
Bank (North Central Regional PI Station, USA). It is a basil 
cultivar with a rounded canopy, rose petals, and purple sepalas. It is cultivated at Brazilian northeast region [12]. Voucher 
specimens of the cultivar used in the present study were 
deposited in the Herbarium of the Federal University of 
Sergipe (Herbarium ASE) under the number 13162.

The leaves of Ocimum basilicum L. were dried in an 
oven with air renewal and circulation (model MA-037/18) 
at 40°C until complete dehydration has been achieved. The 
essential oil was obtained by hydrodistillation in a Clevenger-
type apparatus using 100 g of dried leaves. The Ocimum basilicum L. leaf essential oil obtained was dried over anhy-
drous sodium sulphate, producing yields of 4.75 mL (v/w). 
Gas chromatography-mass spectrometry (GC-MS) and gas 
chromatography-flame ionization detector (GC-FID) analy-
sis were realized to recognize the compounds of the essential 
oil of Ocimum basilicum L. (EOOb). The EOOb components 
were separated into aliphatic monoterpenes, cyclic monoterp-
enes, bicyclic monoterpenes, oxygenated monoterpenes, 
cyclic sesquiterpenes, bicyclic sesquiterpenes, oxygenated sesquiterpenes, and aliphatic secondary alcohols. The EOOb 
(Maria Bonita) consisted mainly of linalool (~69.6%), geran-
iol (~12.6%), 1,8-cineole (~75%), neryl acetate (~3.6%), and 
\(\alpha\)-trans-bergamotene (~1.2%), representing ~94.5% of total 
and the list of all compounds of EOOb is found in Venâncio 
et al. [16].

2.2. Animals. In this work we used Wistar rats weighing 250– 
350 g and the animals were provided by the animal facilities 
of State University of Ceará. Before the experiments rats were 
maintained in groups of five per cage and had free access to 
water and Purina pellets. The experimental protocols here 
employed were previously approved by the Committee on 
Ethics on Animal Use of the State University of Ceará (CEUA-
UECE, protocol # 06379067-0).

2.3. Drugs, Solutions, and Dilutions. Modified Locke’s solu-
tion was used to provide nutrition of sciatic nerve and its 
composition (in mmol/L) was NaCl 140, KCl 5.6, MgCl₂ 1.2, 
CaCl₂ 2.2, Tris-hydroxymethyl aminomethane 10, and glue 
10. The pH was adjusted to 7.40 with HCl/NaOH. The 
\((-\text{LIN})\) (>98% purity) and dimethyl sulfoxide (DMSO) were 
purchased from Sigma (USA). For this study, the range doses 
for EOOb and \((-\text{LIN})\) were 0.01 to 1.0 mg/mL. The EOOb 
and \((-\text{LIN})\) were dissolved in a mixture of DMSO and 
ethanol of 1:10 (v/v) and diluted in Locke's solution in order 
to obtain the desired doses. The DMSO-ethanol mixture was 
always added to the control solutions and did not interfere 
with neuronal excitability [24]. All other salt and drugs were 
purchased from Sigma (USA) or Reagen (Brazil, PR) and 
were of analytical grade.

2.4. Extracellular Recording of Compound Action Potential. 
Extracellular recordings of CAP were performed according 
to Leal-Cardoso et al. [22]. Rat sciatic nerve was mounted 
in a moist chamber and one of its ends was stimulated with 
a stimulus isolation unit connected to a stimulator (Model 
S48, Grass Instruments Co., Quincy, MA, USA). Stimulus 
and recording platinum electrodes were separated by 50 mm 
and the evoked CAP was continuously monitored through an 
osilloscope (Model 547, Tektronix, Inc., Portland, OR, USA). 
Computer acquisition hardware was used for data storage 
and analysis. Between stimulation and recording electrodes, 
the nerve was immersed in modified Locke’s solution used 
to maintain chamber humidity and to administer the EOOb 
and \((-\text{LIN})\). The nerves were exposed to the substances at 
least 30 minutes after stabilization of the peak-to-peak CAP 
amplitude. The period of EOOb and \((-\text{LIN})\) exposure was 
set to 60 min and the same interval was used for the washout 
recovery period. The electrophysiological parameters mea-
sured in extracellular recording were the positive amplitude 
of the 1st and 2nd components of the CAP and the conduction 
velocity. The amplitudes of the 1st and 2nd components were 
measured as the maximum positive amplitude in relation to 
the baseline (see Figure 1). The conduction velocity was 
estimated according to the equation \(v = s/t\), where \(v\) is the 
conduction velocity; \(s\) is the length of the sciatic nerve (in 
mm), measured at the end of the experiment starting from 
the second stimulating electrode; and \(t\) is the time interval (in ms) 
between the stimulus artifact and the peak amplitude of each 
CAP component (first and second components).
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Control
EOOb
Washout

(a)

EOOb (mg/mL)
0.01 0.03 0.1 0.5 1

Positive amplitude (% of control)
0
20
40
60
80
100
120

1st component
2nd component

(b)

EOOb (mg/mL)
0.01 0.1 0.3 1

Conduction velocity (% of control)
0
20
40
60
80
100
120

1st component
2nd component

∗∗∗ ∗∗∗∗∗∗
∗∗∗
∗

(c)

Figure 1: Effects of EOOb on CAP sciatic nerve. Panel (a) shows illustrative traces of CAP waves in control, EOOb, and washout conditions. Panel (b) shows the dose-response curve for 1st and 2nd CAP components and panel (c) shows the conduction velocities of CAP after 60 min EOOb exposure. Data are reported as mean ± SEM. * and ∗∗∗ indicate *<0.05 and *<0.001, respectively (ANOVA followed by Bonferroni’s post hoc test).

2.5. Statistical Analysis. Data were expressed as the mean ± SEM. The EOOb and (−)-LIN concentration-response curves on sciatic nerve were fitted by a non-linear regression sigmoidal curve. To evaluate differences on nerve conduction velocity, we used one-way analysis of variance (ANOVA) followed by appropriate comparison posttest. For all analysis we accepted *<0.05 as statistically significant.

3. Results

We investigated the effects of EOOb and (−)-LIN in the CAP of rat sciatic nerve. As seen in Figure I(a) left panel, the CAP signal shows two waves, named here as 1st and 2nd CAP components. The control values of positive amplitudes and conduction velocities of 1st CAP component were 4.1±0.3 mV and 84.0 ± 2.6 m/s. For the 2nd component the values were 3.1 ± 0.3 mV and 33.5 ± 1.9 m/s (n = 48), respectively.

Figure I shows illustrative traces of CAP in control, EOOb exposure, and washout conditions. As seen in Figure I(a), EOOb (0.5 mg/mL) was effective in blocking the 1st and 2nd CAP components of sciatic nerve and the blockade was reversible after washout. After 60 min of exposure, EOOb decreased significantly and in a concentration-dependent manner the CAP amplitudes (Figure I(b)) and calculated IC50 for 1st and 2nd CAP components were 0.38 ± 0.2 and 0.17 ± 0.0 mg/mL, respectively. EOOb also altered the CAP conduction velocity (Figure I(c)). The 2nd component was significantly reduced in doses equal to or above 0.10 mg/mL and the 1st component at doses above 0.30 mg/mL (p < 0.05, ANOVA followed by Dunn’s comparison test). Due to the great reduction in CAP amplitude promoted by EOOb 1.0 mg/mL for 1st and 2nd components and 0.3 mg/mL for 2nd component, the conduction velocity of components could not be measured.

Since the main constituent of EOOb is (−)-LIN, we decided to investigate its effects on the conductibility of CAP in sciatic nerve. Figure 2 shows the CAP in control and (−)-LIN exposure and after 60 min washout. (−)-LIN (0.5 mg/mL, Figure 2(a)), accordingly, reversibly blocked both components of the CAP sciatic nerve in a concentration-dependent manner (Figure 2(b)) with IC50 values for the 1st and 2nd components of 0.23 ± 0.1 and 0.13 ± 0.0 mg/mL,
respectively. For the CAP conduction velocities, significant inhibition promoted by (−)-LIN was seen from the concentration of 0.3 mg/mL, as shown in Figure 2(c) (p < 0.05, ANOVA followed by Dunn’s comparison test). Like EOOb, the conduction velocity of 2nd CAP component was more affected than 1st CAP component. Finally, at the concentration of 1.0 mg/mL there was such a reduction in CAP amplitude that the conduction velocities of both components could not be measured.

4. Discussion

In this study we described the effects of EO extracted from the leaves of the aromatic plant Ocimum basilicum L. and of monoterpenoid (−)-LIN, its major constituent, on the excitability of peripheral nervous system. The EOOb and (−)-LIN showed very similar results in the electrophysiological data described in this work, both being more potent on the blockade of the 2nd component of CAP than of the first. (−)-LIN showed a clear tendency to be pharmacologically more potent than EOOb at a given type of CAP component. This is coherent with the suggestion that (−)-LIN is mainly responsible for the pharmacological effects of EOOb described below, since, in a given concentration of this essential oil, (−)-LIN is diluted by the presence of the other components.

Both EOOb and (−)-LIN showed concentration-dependent effects on CAP amplitude and recovery of its effects after washout. The recovery of its effects is common to some essential oils such as Croton zehntneri Pax et Hoffm. and Lippia alba (Mill.) N. E. Brown [25, 26] but not for Croton nepetaefolius Bail. [23]. Regarding CAP amplitude, the effects of essential oils present different pharmacological potency. At the end of 180 min exposure the threshold dose (dose that produces a significant reduction of CAP peak-to-peak amplitude) of EOCn was 500 μg/mL [23]. For Lippia alba (Mill.) N. E. Brown essential oil (EOLa), the threshold dose was 30 μg/mL and complete CAP blockade was achieved in 300 μg/mL of EOLa [26]. For Croton zehntneri Pax et Hoffm. essential oil, the reduction was seen at a dose of 100 μg/mL and IC50 of 320 μg/mL [25]. The Alpinia zerumbet (Pers.) Burtt. et Smith essential oil reduced significantly the CAP amplitude at 300 μg/mL [27]. Different from these studies, the data here presented show the effect of EOOb in both
components of CAP. The IC\textsubscript{50} for 1st and 2nd CAP components (380 and 170 \(\mu g/mL\), resp.) were similar to other essential oils, excluding \textit{Lippia alba} (Mill.) N. E. Brown essential oil, although the exposure period was smaller (60 min of exposure). These facts indicate faster establishment of EOOb effects in sciatic nerve excitability and conductivity.

Regarding (−)-LIN, the effects on CAP amplitude were similar or lower than other constituents. For estragole and anethole, the IC\textsubscript{50} for CAP amplitude were \(\sim 593\) and \(220 \mu g/mL\) (4.0 and 1.5 mmol/L, resp.) [27, 28]. Leal-Cardoso et al. [22] also showed that racemic mixture of linalool reduced 1st and 2nd CAP components with an IC\textsubscript{50} of, approximately, 120 and 100 \(\mu g/mL\) (0.75 and 0.64 mmol/L, resp.) and it was similar to the doses found in this work. It is to note in those works that the necessary exposure time (to reach steady state effect) of sciatic nerve to linalool was 180 min and the necessary exposure time in this work was set to 60 min. Thus, it seems that (−)-LIN establishes its effect more rapidly than estragole, anethole, and even the linalool racemic mixture.

Regarding conduction velocity, both EOOb and (−)-LIN were effective in reducing this parameter. The 2nd component seems to be more affected by EOOb than the 1st one, since EOOb reduced significantly the 2nd CAP conduction velocity at 100 \(\mu g/mL\) and the same effect was seen in \textit{Croton zehntneri} Pax et Hoffm. [28]. The (−)-LIN acts in a similar way. The reduction in both conduction velocities was seen at 300 \(\mu g/mL\) (−)-LIN and this fact was seen for other essential oil constituents, such as citral [26] and carvacrol [29]. As seen in the illustrative traces, our CAP is composed of two waves, named here as 1st and 2nd components. The 1st component reflects the electrical activity of the fibers with the largest diameter, predominantly motor. The second component reflects the electrical activity of the fibers with intermediate diameter, predominantly sensory. The greater pharmacological potency on the 2nd CAP component thus suggests this latter type of fiber is more sensitive to EOOb and (−)-LIN than the fibers related to the first component and this effect is observed for many classical local anesthetics.

Although this work did not investigate the mechanism of action of EOOb and (−)-LIN on nerve excitability, we formulate some hypothesis. As shown for several essential oils and constituents, they could act on excitability by the blockade of ion channels responsible for action potential generation, for example, sodium channels. Estragole is a majority constituent of \textit{Croton zehntneri} Pax et Hoffm. essential oil and it was shown to inhibit Na\textsuperscript{+} current of dorsal root ganglia (DRG) in a concentration-dependent way [28]. Joca et al. [29] showed that carvacrol, present in essential oils of genera \textit{Origanum} and \textit{Thymus}, blocked the generation of action potential in intact DRG and reduced the Na\textsuperscript{+} current in dissociated DRG neurons. In a different way, 1,8-cineole, present in EOOb, blocked the generation of action potential with a depolarization of resting potential of intact superior cervical ganglion and alteration of kinetic parameters sodium channel inactivation [30, 31]. Also, Leal-Cardoso and coauthors [22] showed that racemic linalool blocked the generation of action potential (AP) and reduced the amplitude of Na\textsuperscript{+} current in DRG neurons. Additionally, the effects of linalool on the nervous system were studied by means of \textit{in vitro} [18, 20, 21] and \textit{in vivo} [14–17] preparations. The \textit{in vitro} studies of Elisabetsky’s group demonstrated a preferential action of linalool on glutamatergic related targets. The monoterpenoid inhibited glutamate uptake and release in cortical synaptosomes [18] and inhibited MK-801 binding in the rat cortical membranes [19]. These evidences were used to explain the anticonvulsant properties of linalool revealed in \textit{in vivo} seizure models [32, 33]. Moreover, linalool was shown to interact with the muscle nicotinic acetylcholine receptor [20] suggesting possible interactions with membrane proteins. Thus, it is reasonable to hypothesize that (−)-LIN could act on protein membranes responsible for AP generation, such as Na\textsuperscript{+} channels, as does its racemate or a racemic mixture [22]. However, further experiments are needed to ensure that (−)-LIN could act on Na\textsuperscript{+} channels or other voltage-gated ion channels related to excitability process.

5. Conclusions

In this study we described the effects of the essential oil extracted from the leaves of a cultivar of the aromatic plant \textit{Ocimum basilicum} L. developed to have a richer content of a pure enantiomer of the monoterpenoid, the (−)-LIN, its major constituent, on the excitability of peripheral nervous system. The EOOb and (−)-LIN inhibited the excitability of peripheral nervous system in a similar way and potency, but revealing a stronger pharmacological potency on the second CAP component which reflects predominant activity of sensory fibers. Additionally we have demonstrated that the effects of EOOb on excitability are due to the presence of (−)-LIN on the essential oil. Since \textit{Ocimum basilicum} L. is greatly used in folk medicine and linalool has several pharmacological activities which may include antieexcitability in its mechanism of action, we believe to have contributed with this study to further investigations on the effects of the \textit{Ocimum basilicum} L., of its essential oil, and of (−)-LIN.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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