



Rosewood oil induces sedation and inhibits compound action potential in rodents

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ABSTRACT

Aim of the study: *Aniba rosaeodora* is an aromatic plant which has been used in Brazil folk medicine due to its sedative effect. Therefore, the purpose of the present study was to evaluate the sedative effect of linalool-rich rosewood oil in mice. In addition we sought to investigate the linalool-rich oil effects on the isolated nerve using the single sucrose-gap technique.

Materials and methods: Sedative effect was determined by measuring the potentiation of the pentobarbital-induced sleeping time. The compound action potential amplitude was evaluated as a way to detect changes in excitability of the isolated nerve.

Results: The results showed that administration of rosewood oil at the doses of 200 and 300 mg/kg significantly decreased latency and increased the duration of sleeping time. On the other hand, the dose of 100 mg/kg potentiated significantly the pentobarbital action decreasing pentobarbital latency time and increasing pentobarbital sleeping time. In addition, the effect of linalool-rich rosewood oil on the isolated nerve of the rat was also investigated through the single sucrose-gap technique. The amplitude of the action potential decreased almost 100% when it was incubated for 30 min at 100 µg/ml.

Conclusions: From this study, it is suggested a sedative effect of linalool-rich rosewood oil that could, at least in part, be explained by the reduction in action potential amplitude that provokes a decrease in neuronal excitability.

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1. Introduction

The rosewood oil was obtained from one of the species of the Lauraceae family, the *Aniba rosaeodora* Ducke, a large tree reaching up to 30 m in height, occurring in the Amazon region. All parts of the tree are fragrant although only the trunkwood is harvested and distilled. The oil is a colourless to pale yellow liquid with a woody-floral fragrance. The main constituent of rosewood oil is the monoterpene alcohol, (–)-linalool, which can be transformed into a number of derivatives of value to the flavour and fragrance industries (Letizia et al., 2003; Simic et al., 2004). Linalool occurs in nature in both dextrorotatory (+)-

linalool (also called coriandrol) and levorotatory (–)-linalool (called licareol) forms. In rosewood oil, the prevalent form is the levorotatory isomer with the absolute configuration (3R)-(–)-linalool (Letizia et al., 2003). The pharmacological effects attributed to linalool include anti-inflammatory, sedative and hypothermic effects (Elisabetsky et al., 1995; Peana et al., 2002) and opioid antinociception (Peana et al., 2003, 2004). Additionally, neurochemical studies have shown that linalool exerts an inhibitory effect on glutamate receptors in the rat cortex (Elisabetsky et al., 1995). Linalool also inhibited nitric oxide (NO) formation in vitro (Peana et al., 2006) and the blockade of N-methyl-D-aspartate glutamate receptors (Batista et al., 2008). Similarly, some monoterpene alcohols present in many essential oils possess neuronal-depressant activity in animal models, such as α-terpineol (De Sousa et al., 2007a), isopulegol (Silva et al., 2007), and neoisopulegol (De Sousa et al., 2007b).

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Because rosewood essential oil and linalool show effects on neuronal activity we hypothesize that the sedative/anesthetic effect of linalool-rich rosewood oil could be associated with nerve conduction blockade.

2. Materials and methods

2.1. Plant processing

The trunkwood essential oil of *Aniba rosaeodora* Ducke was sent by a private company to the Faculty of Chemical Engineering, Federal University of Pará, City of Belém, state of Pará, for the analysis of its chemical composition. The trunkwood collected in the area of Jatapu River, municipality of Novo Airão, state of Amazonas, Brazil, was also required to complete the analysis. It was submitted to hydro-distillation using Clevenger-type apparatus (100 g, 3 h). The oil was dried over anhydrous sodium sulfate and its percentage content was calculated on the basis of the plant dry weight.

2.2. Oil analysis

The analysis of volatile constituents was performed on a Finnigan Mat Incos XL GC-MS instrument, with the following conditions: a WCOT DB-5ms (30 m × 0.25 mm; 0.25 μm film thickness) fuse silica capillary column; temperature programmed, 60–240 °C (3 °C/min); injector temperature, 220 °C; carrier gas, helium; injection type, splitless (2 μL, of a 1:1000 hexane sol.); electron energy, 70 eV, ion source temperature and connection parts, 180 °C. The quantitative data were obtained by peak area normalization using a HP 5890 GC/FID operated under the same conditions, except for the gas that was hydrogen. Individual components were identified by comparison of both mass spectra and their retention time with those existing in the data system libraries and cited in the literature (Adams, 2007). The specific rotation was determined by a PerkinElmer polarimeter using 1 mL chloroform oil (20 μL) solution at 25 °C.

2.3. Animals

Male Swiss albino mice, three months old weighing 25–35 g and Wistar rats (3 months of age) weighing 350–450 g, were obtained from our animal facility. Animals were kept on a 12 h light/dark cycle (light period: 06:00–18:00 h) at 23 ± 2 °C with free access to food (Purina, Brazil) and water. Experimental protocols and procedures were approved by the Animal Ethics Committee of the Pharmaceutical Technology Laboratory.

2.4. Behavioral studies

Mice were divided into five groups ($n=10$). The first group received intraperitoneal injections of 0.9% saline solution (0.1 ml/10 g body weight) plus pentobarbital (40 mg/kg), while the second group received rosewood oil (100 mg/kg) plus pentobarbital (40 mg/kg). The three remaining groups received an injection of rosewood oil of 100, 200 and 300 mg/kg, respectively. The time to loss of righting reflex (LORR) was recorded. We considered the time from i.p. injection of the drug to LORR as the latency, and the time between the LORR and the time mice regained the ability to right themselves was considered the duration of sleeping time (Mattei et al., 1998).

2.5. The single sucrose-gap technique

Procedures for these experiments were similar to those already described in previous papers (De Sousa et al., 2006; Gonçalves et al., 2008). Briefly, the sciatic nerves were carefully removed

and de-sheathed ($n=10$ rats). The nerve bundle was positioned across the five electrically isolated compartments of the experimental chamber. Compartments 1 and 2 were used to apply supramaximal stimulation of fast-conducting myelinated fibers ($A\alpha$). The supramaximal stimulation consisted of 100 μs isolated square voltage pulses, delivered by a manually triggered stimulator (CF Palmer, Model 8048, UK). All compartments were filled with physiological solution: NaCl 150 mM; KCl 4.0 mM; CaCl₂ 2.0 mM; MgCl₂ 1.0 mM; glucose 10 mM; [N-(2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid] (HEPES) 10 mM, adjusted to pH 7.4 with NaOH. The fourth compartment was filled with isotonic (280 mM) sucrose solution that was continuously renewed, to electrically isolate the neighboring recording compartments. Rosewood oil at concentrations of 2, 5, 10, 50 and 100 μg/ml was introduced into the test (central) compartment. We used different preparations for each tested concentration and total incubation time with rosewood oil was 30 min where the data were taken for analysis. In these experiments rosewood oil was dissolved in the physiological solution containing 0.1% Tween 80. The dispersant agent at that concentration did not promote any alteration on CAP ($n=5$, data not shown). Data were converted to digital form using a microcomputer-based 12-bit A/D converter at a rate of 10.5 kHz and were later analyzed using a suite of programs (Lynx, São Paulo, Brazil). Parameters used to quantify the effects of rosewood oil were the compound action potential (CAP) amplitude, which is the potential difference between the baseline and the maximal voltage of the CAP.

2.6. Drugs

Sodium pentobarbital (PTB) and Tween 80 (polyoxyethylene sorbitan monooleate) were purchased from Sigma–Aldrich (USA). All drugs used in vivo were injected intraperitoneally (i.p.). Unless otherwise stated, all reagents used were of the best quality available.

2.7. Statistical analysis

All results are reported as the arithmetic mean ± standard error. Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's test. The sucrose-gap data were statistically analyzed using the two-tailed Student's *t*-test. Differences were considered to be statistically significant when $p < 0.05$.

3. Results

The oil yield obtained in the field distillation process was 1.0% while in the laboratory it was 2.1%. The oil was analyzed by GC and GC-MS resulting in high content of linalool (87.7%) followed by minute amounts of α-terpineol (3.1%), geraniol (1.2%), *trans*-linalool oxide and *cis*-linalool oxide (1.5%) and some oxygenated sesquiterpenes (4.7%). The value of specific rotation was $[\alpha]_D = -16.2^\circ$.

The sedative effects of rosewood oil in mice were first investigated using the LORR test and estimating the latency (Fig. 1A). The time taken for mice to lose their righting reflex after i.p. injection of rosewood oil was dose-dependent. Likewise, another class of sedative agent (pentobarbital, a barbiturate at 40 mg/kg) produced similar latency durations as rosewood oil at 100 mg/kg. Surprisingly, when injected together, pentobarbital plus rosewood oil, there was a clear potentiation. These results suggest that rosewood oil may have an additional pharmacological target. Rosewood oil dose-dependently increased the mice sleeping time (Fig. 1B). As we reported in the last set of results the combination of pentobarbital and rosewood oil produced a significantly longer sleeping time when compared with either rosewood oil or pentobarbital alone. Altogether, we may conclude that rosewood oil has additional effects on those elicited by pentobarbital.

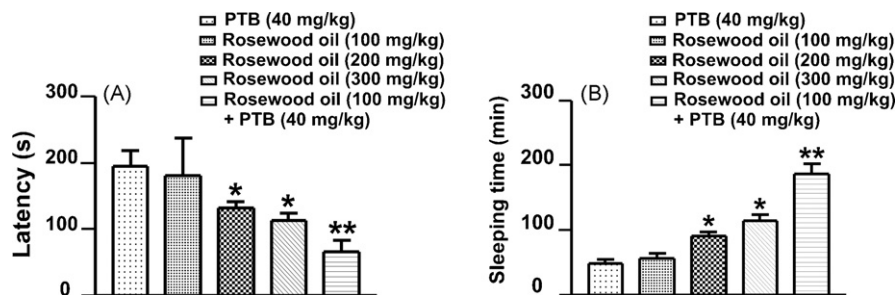


Fig. 1. Effect of rosewood oil on sleeping time test (A) latency and (B) sleeping time duration. Each bar represents the results expressed as mean \pm standard deviation from 10 animals per group. All the treatments were performed by intraperitoneal (i.p.) route. Significant differences from control group are indicated by * $p < 0.05$; ** $p < 0.01$.

The way in which sedative agents provoke their effects is still a matter of debate. A number of ion channels that control neuronal excitability have now been shown to be potential targets for a variety of natural products especially terpenoids.

It is well known that distinct ion channels participate in the generation of neuronal action potentials (Waxman, 1995). As linalool is presumably the major component of the rosewood oil (see Section 3) and because there are in vitro studies indicating that linalool acts as a glutamate NMDA receptor antagonist (Linck et al., 2009) an observation compatible with the sedative effects described by this study and by others (Linck et al., 2009). However, this fact does not rule out the possibility of linalool-rich rosewood oil to act on other targets. Therefore, to investigate whether linalool-rich rosewood oil affects neuronal excitability we used the isolated sciatic nerve preparation.

The isolated sciatic nerve is a neural preparation that is especially useful for studying the effects of drugs on electrical activity arising from nervous tissue in vitro (Docherty et al., 2005; Almeida et al., 2008).

Fig. 2A shows compound action potentials from isolated rat nerve preparation using the single sucrose-gap technique (De Sousa et al., 2006; Gonçalves et al., 2008). After control recordings were obtained (Fig. 2A, black trace), the rat sciatic nerves were exposed to different concentrations of linalool-rich rosewood oil for 30 min and then compound action potentials were recorded (Fig. 2A, code colour represent different concentrations). The blocking effect of linalool-rich rosewood oil on rat sciatic nerve was studied at concentrations of 2, 5, 10, 50 and 100 $\mu\text{g/ml}$ ($n = 4$ nerve bundles per concentration). Fig. 2B shows that by increasing linalool-rich rosewood oil concentration the CAP amplitude decreases. The blocking effect varied from 75% (at 2 $\mu\text{g/ml}$) to 95% (at 100 $\mu\text{g/ml}$) and it was irreversible. Additionally, we evaluated if linalool-rich rosewood oil (at the concentrations that did not elicit full blockade) could have an effect on the repolarization phase of the compound action poten-

tial. We, then, calculated the repolarization time constant and we did not find any significant change (data not shown, $n = 4$).

4. Discussion

Natural products, especially those isolated from plants, have long been a traditional source of unique drug molecules (Molinski et al., 2009). With this idea in mind the discovery of selective pharmacological activities from natural sources is of significant importance. Our present results show that linalool-rich rosewood oil provoked sedative effect in vivo and depressed sciatic nerve excitability. The latter effect is a novel finding that may be important to the understanding of essential oils' biological activity.

Even though linalool is the major constituent of rosewood oil it is difficult to discard the possibility that some of the effects observed in this study could be elicited by the other chemical components. Further experiments will be necessary to clarify this point.

The current results extend the findings of Linck et al. (2009) by demonstrating the significant blockade of the compound action potential and the potentiation of pentobarbital effects. Recent studies have provided handful of evidence concerning the role of GABA receptors on sedation (Atack, 2003; Savić et al., 2008). Essential oils show a variety of pharmacological effects and there are reports indicating a possible inhibitory effect on GABA transaminase (Koo et al., 2003), an enzyme responsible for the GABA degradation, which per se leads to augmentation of GABA levels provoking sedative effects. This possibility could explain our results assuming that GABA potentiates pentobarbital effects which have been already shown (Skolnick et al., 1980; Chu et al., 2007).

Pentobarbital activates GABA receptors to produce sedation. Rosewood oil potentiated the pentobarbital effect on LORR and sleeping time suggesting that rosewood oil could affect the interaction between GABA receptor and pentobarbital or alternatively

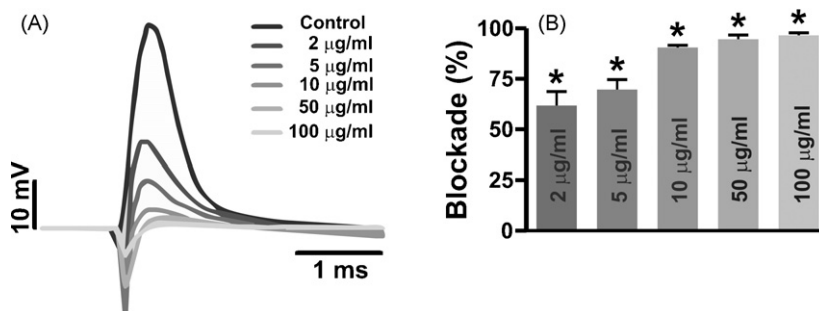


Fig. 2. Effects of rosewood oil on the CAP. (A) Representative CAP recordings showing the trace without rosewood oil incubation (control, black trace) and after 30 min of different linalool-rich rosewood oil concentration, from 2 to 100 $\mu\text{g/ml}$, added to the test compartment of the recording chamber. (B) Shows the percentage blockade of CAP amplitude using different concentrations from 2 to 100 $\mu\text{g/ml}$ and taken at 30 min incubation. Each value represents the mean \pm S.D. of four nerve bundles. * $p < 0.05$ when compared to controls.

through other mechanism. At this point we do not know how this is occurring in detail but the results add important information. It is worth noting that anesthetics can produce sedative effects via a specific sleep pathway in the hypothalamus (Nelson et al., 2002). As we demonstrated rosewood oil also increases the sleeping time. It could be reasonable to speculate that the two processes investigated in this study may share a common mechanism most likely involving GABA receptors modulation.

Although cellular mechanisms linked to these actions have not been totally uncovered we reasoned that would be interesting to explore the idea that sedative drugs alter neuronal excitability. We showed that linalool-rich rosewood oil dose-dependently and significantly inhibited the nerve compound action potential (Fig. 2A). The question that should be raised is: how do our electrophysiological findings in vitro correlate with sedative actions of linalool-rich rosewood oil? It is rather difficult to draw any substantial conclusion at this stage, but we are quite comfortable with the inhibitory effect observed in this study. As far as we know there is no report indicating the presence of functional neurotransmitter receptors (for example to GABA and/or Glutamate) in the sciatic nerve trunk (i.e. at the Ranvier Nodes) therefore linalool-rich rosewood oil would lead the inhibitory action on compound action potentials probably through a blockade of voltage-dependent Na⁺ channels and/or increase in resting K⁺ conductance. The latter mechanism can be ruled out due to the fact that we did not see any significant change in baseline level throughout the experiments.

From this study, it is suggested a sedative effect of linalool-rich rosewood oil that could be important to delineate its therapeutic potential.

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