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Research Report

Anxiolytic-*like* effects of phytol: Possible involvement of GABAergic transmission

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ABSTRACT

Phytol, a branched chain unsaturated alcohol, is particularly interesting because it is an isolated compound from essential oils of different medicinal plants. The aim of this study was to evaluate the anxiolytic-*like* effects of phytol in animal models to clarify their possible action mechanism. After acute intraperitoneal treatment with phytol at doses of 25, 50 and 75 mg/kg behavioral models of open-field, elevated-plus-maze, rota-rod, light–dark, marble-burying and pentobarbital sleeping time tests were utilized. In open field test, phytol (25, 50 and 75 mg/kg) [$p<0.01$] increased the number of crossings and rearings. However, the number of groomings [$p<0.01$] was reduced. Likewise, the number of entries and the time spent in light space were increased [$p<0.01$] while the number of marble-burying was decreased [$p<0.001$], in elevated-plus-maze, light–dark and marble-burying tests, respectively. In motor activity test, phytol (75 mg/kg) impaired the rota-rod performance of mice [$p<0.01$]. In pentobarbital sleeping time test, phytol 75 mg/kg decreased for latency of sleeping and phytol (25, 50 and 75 mg/kg) increased the sleep time when compared to negative control [$p<0.05$]. All these effects were reversed by pre-treatment with flumazenil (2.5 mg/kg, i.p.), similarly to those observed with diazepam (2 mg/kg, i.p.; positive control) suggesting that the phytol presents mechanism of action by interaction with the GABAergic system. These findings suggest that acute administration of phytol exerts an anxiolytic-*like* effect on mice. Furthermore, suppose that phytol interacts with $GABA_A$ receptor, probably at the receptor subtypes that mediate benzodiazepines effects, to produce sedative and anxiolytic activities.

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1. Introduction

Anxiety is generally a normal reaction to stress and there will always be situations that create stress and discomfort (Higgins and George, 2010; Mackenzie et al., 2011). There are several different types of anxiety disorders. Benzodiazepines (BDZ) are the first-line pharmacological anxiolytics drugs, and more psychoactive medications are being developed in the last 45 year. However, despite the clinical efficacy, most drugs in this class have many problems, including sedation, muscle relaxation, anterograde amnesia and risk of accidents (Mitte et al., 2005; Cunningham et al., 2010). In addition, chronic drug use can lead to psychomotor effects, paradoxical reactions, tolerance, teratologic risk and dependence (Dell'osso and Lader, 2013).

Although conventional anxiolytics have been used for treatment of anxiety disorders, recent clinical evidences have shown that selective serotonin reuptake inhibitors (SSRIs) are also effective on various anxiety disorders. They act by preventing the reuptake of 5-hydroxytryptamine (5-HT), thereby increasing 5-HT levels within the synaptic cleft and modulating neurochemical signaling (Nishikawa et al., 2007; Tsapakis et al., 2012). However, paradoxically they may increase symptoms of anxiety when treatment is initiated and despite extensive research over the past 30 years focused on SSRIs treatment, the precise mechanisms by which SSRIs exert these opposing acute and chronic effects on anxiety remain unknown (Burghardt and Bauer, 2013). Some clinical studies have reported adverse outcomes, such as premature birth, neonatal cardiovascular abnormalities, reduced bone mineral density and an increased risk of bone fracture (Haney et al., 2010; Olivier et al., 2011).

Given the side effects of BDZ and reuptake inhibitors, numerous research groups are looking for new forms of pharmacological treatment for anxiety that can replace the conventional ones. In addition, the search for new compounds which are more effective and safer, with less possibility of adverse reactions is extremely necessary as a large number of users become dependent on chemical and physical conditions. Thus, numerous scientific researchers have been exploring for example several species of medicinal plants both in terms of chemical and pharmacological compounds in search for new anxiolytics (Brito et al., 2012 Oyemitan et al., 2013).

Several studies have shown that many plants containing essential oil possess medicinal properties. Recent research shows that the vast majority of biological activities are derived from terpenes, which are the main chemical components in these oils essential (Souto-Maior et al., 2011; Melo et al., 2013; Russo et al., 2013). Previous studies have shown that terpenes can affect the Central Nervous System (Guimarães et al., 2010; Sousa, 2011; Machado et al., 2013).

The diterpene phytol (3,7,11,15-tetrametilhexadec-2-en-1-ol) (Fig. 1) is a member of branched-chain unsaturated alcohols whose common characteristic structural elements are one hydroxyl group per molecule and a twenty-one double bond carbon atoms ($C_{20}H_{40}O$), molecular weight 296.54 mol/L. It is liquid at room temperature, with a density of 0.8533 g/cm^3, colorless with a boiling point of 202 °C, flash point >200 °C and refractive index 1.460–1.466. It is optically active inasmuch as it contains three asymmetric carbon atoms. The aqueous solubility of phytol is about 0.00327 mg/L (Belsito et al., 2010; McGinty et al., 2010; Karunagoda, 2010). This compound is particularly interesting because it is a component of the chlorophyll molecule, present in green leaves of various medicinal plants, hence it is present in nature in abundance (Rontani and Volkman, 2003). However, phytol has not yet been submitted to neuropharmacological evaluation.

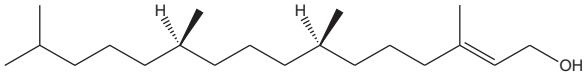

Fig. 1 – Chemical structure of phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol).

Thus, to pursuit the development and introduction of new drugs with greater efficacy and safety is essential to enhance treatment of anxiety. Importantly, the search for alternatives with less toxicity has resulted in decreased introduction of synthetic substances, which underscores the importance of pharmacological studies of natural products such as phytol. In this perspective, in view of its abundance and few data in neuropharmacological literature, the aim of study was evaluate the anxiolytic-*like* effects of phytol in animal models to clarify their possible action mechanism.

2. Results

2.1. *Open-field test*

Phytol (PHY), at doses 25, 50 and 75 mg/kg, (i.p.) showed sedative effects as assessed by open-field test in mice. Significant effects were detected with doses tested of PHY, which produced similar percentages of increased number of crossings (71%, 70% and 69.5%) and rearings (70%, 70% and 71%) when compared with to positive control [$p<0.01$]. The number of groomings (41%, 43% and 48% respectively) [$p<0.01$] were reduced with PHY 25, 50 and 75 mg/kg, whose results were similar to those observed with diazepam (2 mg/kg, i.p.), used as a positive controls [$p<0.01$]. Flumazenil was used for evaluating the possible mechanism of action of the sedative effect of phytol. The group receiving flumazenil (2.5 mg/kg, i.p.) and PHY (75 mg/kg, i.p.) exhibited similar behaviors to those from the positive control [$p<0.01$], indicating that phytol produced a benzodiazepine-type sedative effect (Table 1).

2.2. *Motor coordination test (rota-rod test)*

The rota-rod test was used for evaluating motor coordination and presence of any muscle relaxation effect. Results showed that there was no change after PHY administration (25 and 50 mg/kg, i.p.), when compared with to positive control [$p<0.01$]. PHY (75 mg/kg, i.p.) and diazepam significantly impaired the rota-rod performance of mice. PHY 75 mg/kg and diazepam 2 mg/kg reduced the take-time to fall-down (63% and 66%, respectively) [$p<0.01$]. Flumazenil was used for evaluating the possible mechanism of action of muscle