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Anticonvulsant and behavioral effects observed in mice following treatment with an ester derivative of ferulic acid: Isopentyl ferulate

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ABSTRACT

The objective of this study was to evaluate the potential anticonvulsant effect of isopentyl ferulate, a new ester derived from ferulic acid in mice (*Mus musculus*) subjected to two models of induced seizures. According to the results obtained, the IF at doses of 25, 50 and 75 mg/kg (i.p.) showed protective effect against induced seizures by pilocarpine (400 mg/kg, i.p.) and pentylenetetrazole (70 mg/kg, i.p.). In the two animal models of seizures, the pretreatment of the IF (25, 50 and 75 mg/kg) with flumazenil blocked the anticonvulsant effect, suggesting that the mechanism of action of this ester derived of ferulic acid may be related to activity in the benzodiazepine-binding site of the $GABA_A$ receptor (γ-aminobutyric acid, type A). In addition to the anticonvulsant effect, behavioral changes as neurotoxicity indication were assessed by using the rota rod and open field tests. The results obtained showed that the IF (25, 50 and 75 mg/kg) does not induce significant changes in locomotor activity and motor coordination when compared with the control group, unlike the results presented by diazepam. Thus, these results demonstrate a new pharmacological knowledge of IF with potential application against epileptic seizures. However, further studies are needed to elucidate other neurobiological mechanisms underlying epilepsy.

1. Introduction

Epilepsy is a severe neurological disorder characterized mainly by recurrent seizures due to an abnormal neuronal hyperexcitability in the brain and according to the World Health Organization, affects approximately 50 million people worldwide [1,2]. Even with the significant advances made by several research groups for the treatment of epilepsy, approximately 30% of patients treated with antiepileptic drugs showed recurrence of uncontrolled seizures [3]. Furthermore, the main problems associated with antiepileptic drugs (e.g. - carbamazepine, ethosuximide, phenobarbital, pheny toin, primidone, and valproate) involve to exhibit some side effects such as cognitive dysfunction, ataxia, sedation, hypersensitivity or worsening of seizures [4,5].

Way forward, there is a need for the development of new research with the objective of evaluating new molecules with a better therapeutic effectiveness than antiepileptic drugs already existing. The natural products derived from plants represent a great opportunity for the discovery of new substances with potential therapeutic interest for the treatment of epilepsy [6,7]. Among the groups of substances from natural origin with potential antiepileptic effect, the essential oils play an important role in scientific research due to their high level of bioactive components, by which can be detached the derivatives of phenylpropanoid and terpenoid [8].

The phenylpropanoids are a group of compounds derived from the carbon skeleton of phenylalanine, which have various neuropharmacological activities [9–11]. Among these compounds, stands out the ferulic acid that presents several pharmacological activities such as neuroprotective [12], antidiabetic [13], anticancer [14] anti-inflammatory [15], antidepressant [16,17] and mainly antioxidant [18]. In addition, the development of their derivatives that

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comprise similar chemical structure are promising in relation to a range of new pharmacological properties on the central nervous system, which may be related to neuroprotective action [19–21]. Despite its promising pharmacological activities, few researches have directed a pharmacological study on phenylpropanoids for the treatment of epilepsy.

Thus, the present study has the objective of providing information on potential anticonvulsant effect and the possible mechanism of action of Isopentyl ferulate (IF), a ester derivative of ferulic acid in mice subjected to two seizure models (Pilocarpine-induced seizures and Pentylenetetrazole-induced seizures). This compound was also tested for the muscular relaxation effect and locomotor activity using the rota rod test and open field test, respectively.

2. Materials and methods

2.1. Reagents and drugs

Polyoxyethylene sorbate (Tween 80), pilocarpine, pentylenetetrazole and flumazenil were obtained from Eg Sigma Chem Ex. Co. St. Louis, Missouri, USA. Diazepam was purchased from Union Chemical (Brazil). All the other chemicals were of the analytical grade.

2.2. Preparation of the substance

Isopentyl ferulate (IF, Fig. 1) has a molecular formula of $C_{15}H_{20}O_4$, refractive index of 1.544 ± 0.02, surface tension of 40.3 ± 3.0 dyn/cm and density of 1.104 ± 0.06 g/cm^3. The process of developing the product consists of a reaction of esterification of ferulic acid [22,23]. The process of esterification of ferulic acid consists in a stirred mixture of ferulic acid (5 mmol) in isoamyl alcohol (200 ml). Ethanol was added to concentrated sulfuric acid (0.067 mL, 1.25 mmol) and the reaction mixture was subjected to reflux for 3 h in a 500 ml flask. After cooling to 25 °C, ethyl acetate was added and the solution was washed with water and brine. The ethyl acetate fraction was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by chromatography on a silica gel column using 20% ethyl acetate in hexane to produce isopentyl ferulate (yield 55%) [24]. Subsequently, the IF was emulsified with 0.05% Tween 80 dissolved in 0.9% saline and administered via intraperitoneal (i.p.) in doses of 25 mg/kg (IF 25), 50 mg/kg (IF 50) and 75 mg/kg (IF 75).

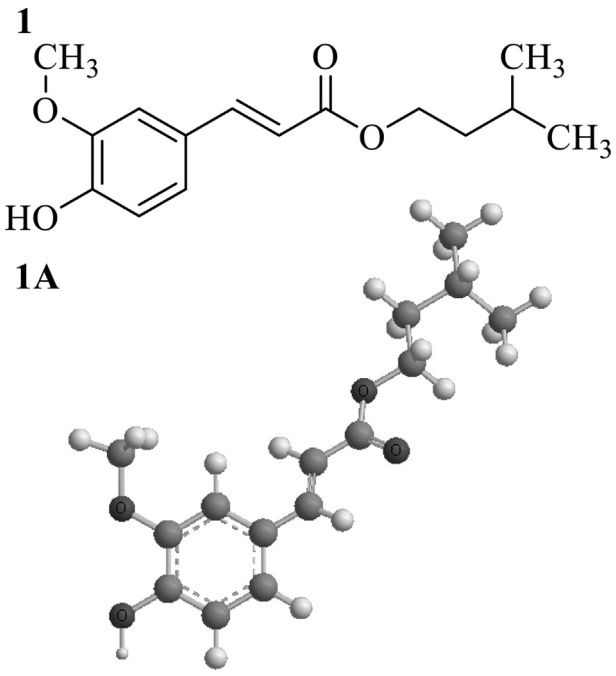

Fig. 1. Chemical structure of isopentyl ferulate (3-methylbutyl (E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate (**1** and **1A**).

2.3. Animals and evaluation of behavioral seizure

Male Swiss (*Mus musculus*) adult mice (25–30 g; 2 months-old) were used in this study (Central Animal Laboratory of the Federal University of Piaui). Animals were housed in cages (35 cm L × 52 cm W × 17 cm H) with free access to food (Purina® pellets) and water and were kept under standard artificial light–dark cycle (lights on at 07:00 a.m.) with controlled temperature (25 ± 2 °C). The animals were acclimated for three days before the experiments and were housed in groups during the experiments. Animals were tested during the light period and observed in a closed room with controlled temperature (25 ± 2 °C).

Protocol and procedures were approved by the Ethics Committee in Animal Experimentation of UFPI (CEEA/UFPI - authorization number: #030/13). All protocols were designed aiming to reduce the number of animals used to a minimum, as well as to minimize their suffering. The experiments were performed according to the Guide for Care and Use of Laboratory of US Department of Health and Human Services, Washington, DC (1985).

To investigate the effects of IF, an acute treatment to the test animals received by intraperitoneally. Vehicle group was treated with 0.05% Tween 80 dissolved in 0.9% saline (0.1 mL/kg, negative control). IF 25, IF 50 and IF 75 groups were treated with emulsified IF in the vehicle mentioned at doses of 25, 50 and 75 mg/kg, respectively. The doses used were based on a preliminary pharmacological screening protocol [25] with different doses of the IF administered to mice. At all doses used, no signs of acute toxicity or behaviors suggestive of neurotoxicity were observed (data not shown). Diazepam (DZP) group was treated with diazepam (2 mg/kg, positive control) emulsified in vehicle. The selection of the dose of diazepam was based on previous studies [26,27].

In turn, to clarify the mechanism of action of IF, other groups were treated with flumazenil (FLU), DZP and IF at a dose of 75 mg/kg and associations. The FLU group was treated with flumazenil (5 mg/kg) emulsified vehicle. The DZP + FLU group was pretreated with flumazenil (5 mg/kg) and, after 15 min treated with DZP (2 mg/kg). The IF 75 + FLU group (n = 8) was pretreated with FLU (5 mg/kg) and, after 15 min, treated with IF 75.

2.4. Pilocarpine-induced seizure

This model was developed by Turski et al. [28]. Briefly, after 30 min from the doses of 25, 50 and 75 mg/kg (i.p.), all the groups received pilocarpine (400 mg/kg, i.p.). Direct observation was made for 4 h to monitor latency to the first seizure (tonic-clonic seizures, with or without raising) and the number of animals that seized and/or death [29–31].

2.5. Pentylenetetrazole-induced seizure

Pentylenetetrazole (PTZ) was used to induce clonic convulsions [32]. Briefly, after 30 min from the doses of 25, 50 and 75 mg/kg (i.p.), all groups received PTZ (70 mg/kg, i.p.) and animals were observed for 4 h to monitor the same parameters of the previous test [7].