Involvement of anti-inflammatory, antioxidant, and BDNF up-regulating properties in the antipsychotic-like effect of the essential oil of *Alpinia zerumbet* in mice: a comparative study with olanzapine

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**Abstract**

The current drug therapy for schizophrenia effectively treats acute psychosis and its recurrence; however, this mental disorder’s cognitive and negative symptoms are still poorly controlled. Antipsychotics present important side effects, such as weight gain and extrapyramidal effects. The essential oil of *Alpinia zerumbet* (EOAZ) leaves presents potential antipsychotic properties that need further preclinical investigation. Here, we determined EAOZ effects in preventing and reversing schizophrenia-like symptoms (positive, negative, and cognitive) induced by ketamine (KET) repeated administration in mice and putative neurobiological mechanisms related to this effect. We conducted the behavioral evaluations of prepulse inhibition of the startle reflex (PPI), social interaction, and working memory (Y-maze task), and verified antioxidant (GSH, nitrite levels), anti-inflammatory [interleukin (IL)-6], and neurotrophic [brain-derived neurotrophic factor (BDNF)] effects of this oil in hippocampal tissue. The atypical antipsychotic olanzapine (OLZ) was used as standard drug therapy. EOAZ, similarly to OLZ, prevented and reversed most KET-induced schizophrenia-like behavioral alterations, i.e., sensorimotor gating deficits and social impairment. EOAZ had a modest effect on the prevention of KET-associated working memory deficit. Compared to OLZ, EOAZ showed a more favorable side effects profile, inducing less cataleptic and weight gain changes. EOAZ efficiently protected the hippocampus against KET-induced oxidative imbalance, IL-6 increments, and BDNF impairment. In conclusion, our data add more mechanistic evidence for the anti-schizophrenia effects of EOAZ, based on its antioxidant, anti-inflammatory, and BDNF up-regulating actions. The absence of significant side effects observed in current antipsychotic drug therapy seems to be an essential benefit of the oil.

**Keywords** Schizophrenia · Ketamine model · *Alpinia zerumbet* · Anti-inflammatory effects · Antipsychotic effects · Extrapyramidal side effects

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Introduction

Alpinia zerumbet (Alpinia zerumbet (Pers) Burtt. et Smith), popularly known in Brazil as “colônia”, is a tropical ornamental plant used in some regions to control depression, psychomotor agitation, and anxiety (Satou et al. 2010).

Despite the prevalent use of Alpinia zerumbet in controlling neuropsychiatric symptoms, its oil’s central effects started to be investigated only in the last decade. In this context, the oil presents an anxiolytic-like effect in mice (Satou et al. 2010, 2011). Additionally, our research group demonstrated a putative antipsychotic-like effect of the EOAZ with dopaminergic mechanisms’ involvement (de Araújo et al. 2009). To this end, we first used screening models to evaluate novel antipsychotic drugs based on acute administration of dopaminergic drugs such as amphetamine and ketamine. In this initial protocol, we observed that the EOAZ counteracted the stereotypies and hyperlocomotion induced by these drugs (de Araújo et al. 2009, 2011). In vitro analysis revealed that this oil presents a marked brain antioxidant action, which, hence, can partially justify its neuroprotectant effects (De Araújo et al. 2011).

To date, schizophrenia drug treatment is not ideal (Gaebel et al. 2019). Antipsychotic drugs present a good control of positive symptoms but are of limited efficacy for treating cognitive and negative symptoms, presenting important side effects (Campbell et al. 1999; Carpenter and Davis 2012; Ben-Azu et al. 2018c). One common mechanism of these drugs is antagonizing dopaminergic receptors (Li et al. 2016). Despite this, the pathophysiology of schizophrenia, besides not fully understood, includes, but is not limited to alterations in glutamatergic neurotransmission (Javitt et al. 2012; Howes et al. 2015; Ben-Azu et al. 2018b), and in neurotrophic mechanisms (Favalli et al. 2012; Ben-Azu et al. 2018a), as well as oxidative damage (Ben-Azu et al. 2018b) and neuroinflammation (Muller and Schwarz 2006; Ben-Azu et al. 2019). Therefore, new anti-schizophrenia therapies targeting other pathophysiological alterations observed in schizophrenia and with proven efficacy against negative/cognitive symptoms with a safe and more tolerable side effects profile are challenging topics in the schizophrenia research field (Ben-Azu et al. 2019).

Ketamine (KET) repeated administration is a widely used animal model of schizophrenia. This model based on repeated administration can mimic this mental disorder’s positive, negative, and cognitive symptoms (Monte et al. 2013; Frohlich and Van Horn 2014; Ben-Azu et al. 2018a, b). Dysfunctions of glutamatergic transmission and increased dopaminergic tone in the mesolimbic pathway accompany these behavioral alterations observed in this model (Irifune et al. 1991; Tan et al. 2012; Ben-Azu et al. 2018b), as well as increased oxidative stress and neuroimmune changes (Monte et al. 2013; Araújo et al. 2016). Ketamine-induced behavioral and neurochemical abnormalities are attenuated by atypical antipsychotics, confirming this model’s predictive validity (Vasconcelos et al. 2015; Araújo et al. 2016).

Hippocampal dysregulation is observed since the premorbid stage of schizophrenia. Indeed, disruption of glutamate neurotransmission in the CA1 region of the hippocampus is implicated in the induction of attenuated psychotic symptoms and the onset of the transition to syndromal psychosis (Lieberman et al. 2018). Furthermore, neuronal activity in the hippocampus and prefrontal cortex (PFC) is frequently correlated or synchronized in time, indicating interactions between the two structures. This interaction is important for functions such as working memory (Bühner et al. 2015), and regulation of motivational and emotional behaviors (Bannerman et al. 2004). Finally, the hippocampus seems to regulate striatal, particularly mesolimbic, dopamine release (Grace et al. 2007). Importantly, phasic activation of dopaminergic neurons is critical for reward-based learning and the assigning of incentive salience to sensory stimuli, functions that are disturbed in schizophrenia (Kapur et al. 2005). Taken together, hippocampus and its interactions with the PFC and striatum are relevant to the regulation of behavioral alterations observed in patients with schizophrenia.

In the present study, we hypothesized that the repeated administration of EOAZ could prevent and reverse the schizophrenia-like behavioral and neurochemical alterations induced by KET repeated administration. Hence, our primary outcome was evaluating the prevention or reversal of KET-induced behavioral alterations like positive, negative, and cognitive symptoms of schizophrenia by EOAZ and the presence of side effects associated with antipsychotic drug therapy, namely catalepsy, and weight gain. Our secondary outcome was determining the involvement of antioxidant, neurotrophic, and anti-inflammatory mechanisms in the effects of this oil in the hippocampus, a brain area related to schizophrenia neurobiology (Harrison 2004).

Materials and methods

Animals

We used adult male Swiss mice (25–30 g; 60 days old; 8 animals/group) housed in standard environmental conditions (22 ± 1 °C, humidity 60 ± 5 %, normal 12 h light: 12 h dark cycle) with free access to a standard commercial diet and with water freely available, following international recommendations (NIH 1996). The Animal Ethics
Committee of the Universidade Federal do Ceará (UFC) approved the experimental protocol (number 106/2014).

**Plant material**

The essential oil was extracted from the leaves of A. zerumbet (EOAZ), collected from the Medicinal Plants Garden of the Laboratory of Natural Products of the Universidade Federal do Ceará, Ceará State, Brazil, during January 2015. A voucher specimen of A. zerumbet is at Herbarium Prisco Bezerra (#10,858). According to the method described elsewhere (Craveiro et al. 1976), researchers from the Department of Organic and Inorganic Chemistry of the UFC isolated the essential oil. Briefly, freshly chopped plant leaves were placed in a glass flask, connected at one end to a glass vessel with water, and at the other end to a water-cooled condenser. The steam was percolated through the chopped plant leaves and collected in the condenser under boiling water. After condensation, the liquid phase with its solutes, called ‘aqueous extract’, was separated from an oily phase, the essential oil. Gas chromatography and mass spectrometry (Shimadzu QP5050 GCMS gas chromatograph, Shimadzu Corporation, Kyoto, Japan) was used to determine the oil composition. The constituents were identified by a computer library search based on their retention indices and visual interpretation of the mass spectra (Sparkman 2009). The main constituents identified in the EOAZ were (%): terpinene-4-ol, 25.7; 1,8-cineole, 24.61; γ-terpinene, 14.28. Supplementary Table 1 presents the major chemical constituents identified in EAOZ.

**Drugs**

The animals received oral doses of 100 or 200 mg/kg EOAZ dissolved in 2% tween 80 (Sigma-Aldrich, St. Louis, USA) or olanzapine (OLZ – 2 mg/kg - Zyprexa®, Elli Lilly, Brazil), used, here as a standard antipsychotic. Ketamine hydrochloride (KET - Ketalar®, Parke-Davis Lab, Brazil), at a dose of 20 mg/kg, was administered intraperitoneally (IP) to induce schizophrenia-like alterations. The dose of OLZ was calculated based on body surface area (BSA) as described elsewhere (Reagan-Shaw et al. 2008). Based on this calculation, 2 mg/kg OLZ in mice is equivalent to the human therapeutic dose of 10 mg/day OLZ. Previous studies on KET as a pharmacological animal model of schizophrenia guided KET dose choice (Monte et al. 2013). The doses of EOAZ were selected based on a previous study conducted by our research group evaluating the effects of this oil against animal models of schizophrenia (De Araújo et al. 2011).

**Experimental design**

We used two distinct protocols, namely prevention and reversal. In the prevention protocol, we aimed at mimicking the maintenance treatment phase of schizophrenia (Monte et al. 2013). In this protocol, the animals received a daily oral administration (gavage) of vehicle, EOAZ (100 or 200 mg/kg), or OLZ 2 mg/kg for 14 days. Between the 8th and 14th days of treatment, mice additionally received a daily intraperitoneal injection of KET or vehicle (also called the control group) 30 min after EOAZ. Hence, the prevention protocol comprised the following groups: control, EOAZ100 + SAL, EOAZ200 + SAL, SAL + KET, EOAZ100 + KET, EOAZ200 + KET, OLZ + KET. The reversal protocol simulated an acute treatment of psychotic episodes (da Silva Araújo et al. 2017; Monte et al. 2013). Each animal received one daily intraperitoneal injection of KET 20 mg/kg or vehicle for 14 days in this protocol. From the 8th day of treatment onwards, mice additionally received a daily oral administration (gavage) of vehicle, EOAZ (100 or 200 mg/kg), or OLZ 2 mg/kg, with a 30 min interval between drugs administration. The reversal protocol comprised the following groups: control, KET + EOAZ100, KET + EOAZ200, KET + KET, KET + EOAZ100, KET + EOAZ200, KET + OLZ. The atypical antipsychotic OLZ was chosen as standard treatment because the ketamine-induced model of schizophrenia is more responsive to atypical antipsychotics (Becker and Greckisch 2004).

Behavioral alterations related to schizophrenia symptoms, namely positive-, negative- and cognitive symptoms, were respectively evaluated by prepulse inhibition of the startle reflex (PPI), social interaction, and Y-maze task, on the 14th day of treatment, 30 min after the last drug administration. In addition, the catalepsy test and body weight gain evaluated extrapyramidal side effects and weight gain and significant side effects of antipsychotic treatment.

We divided the animals into two cohorts to avoid bias in the behavioral tests (i.e., reduce stress by the exposure to multiple tests). Thus, we submitted cohort 1 to catalepsy and PPI tests in this order and cohort 2 to the Y maze and social interaction test, respectively.

We estimated the groups’ sample size with the resource equation method that is based on the calculation of “E” (degree of freedom of the ANOVA) (Charan and Kantharia 2013). Our experiments used eight animals/groups divided into seven groups obtaining an E value of 49. According to this method, for robust sample size, the minimum value “E” should be 10.

We chose male animals to conduct this protocol since schizophrenia is a mental disorder with sex influence in its onset and symptoms’ severity (Aleman et al. 2003; Abel et al. 2010). In this regard, male subjects present earlier and more severe schizophrenia symptoms, such as cognitive and
negative, concerning females. Figure 1 presents a graphical representation of the study design.

**Behavioral determinations**

**Prepulse inhibition of the startle reflex**

PPI evaluates sensorimotor gating, an endophenotype of schizophrenia (Turetsky et al. 2007). PPI test was conducted in a startle chamber (Insight, São Paulo, Brazil), as described previously (Kinkead et al. 2006). In brief, the test was initiated with a 5 min acclimatization to the startle chamber in the presence of 65 dB background noise. Next, the animals received nine single 120 dB pulses (startle amplitude) and eighteen pulses preceded by 100 ms by a prepulse (PP) of 70-, 75- or 80 dB intensity. The level of PPI in each mouse was determined by expressing the PP + pulse startle amplitude as a percentage decrease from pulse-alone startle amplitude (P), according to the following formula: % PPI = 100 − [100 × (PP/P)]. The results are expressed as mean % PPI. We determined acoustic startle reactivity (ASR) by the mean amplitude of the nine trials of single pulses.

**Social interaction test**

The social interaction apparatus consisted of a Plexiglas box divided into three chambers [total cage size: 40.5 cm x 60 cm x 22 cm (height)] that communicate through a small opening where the animals can explore all three chambers. The social chamber had a same-sex and age strange animal. On the other side, the non-social chamber had an empty cage. Each mouse was allowed to explore the chambers for 5 min freely. Social interaction was defined as: (% time spent in the social chamber) – (% time spent in the opposite chamber) (Radyushkin et al. 2009).

**Y-maze test**

We evaluated spatial working memory by the spontaneous alternation performance in the Y-maze, which allows the evaluation of cognitive searching behavior (Maurice et al. 1996). A Y-maze apparatus made of black acrylic consisted of three arms with 42.5 cm (length), 14.5 cm (width), and 22.5 cm (height) mounted (120° between arms) to an equilateral triangular center compartment. Each mouse was placed at the end of one arm and freely moved through the three maze arms for 8 min. Raters blinded to the experimental groups registered the sequence of arms entries. The number of maximum alternations was the total number of arms entered minus 2. The percent alternation was calculated as (actual alternations/maximum alternations) X 100 (Yamada et al. 1996; Dall’Igna et al. 2007).

Fig. 1 Schematic representation of the experimental design. In the prevention protocol, from the 1st to the 7th-day, mice received daily oral administrations of EOAZ 100, 200 mg/kg, or OLZ 2 mg/kg, while from the 8th to 14th days further received intraperitoneal injections of KET 20 mg/kg. In the reversal protocol, from the 1st to the 7th-day, the animals received intraperitoneal injections of KET 20 mg/kg, while from the 8th to the 14th -day, they were further treated with EOAZ 100, 200 mg/kg, or OLZ 2 mg/kg. Abbreviations: SAL – saline; EOAZ – essential oil of Alpinia zerumbet; OLZ – olanzapine; KET – ketamine.
Evaluation of extrapyramidal side effects (EPSE) and weight gain

Catalepsy test

Catalepsy is the inability of an animal to correct an externally imposed posture. Catalepsy time was measured by placing each animal on a flat horizontal surface (15 cm long) and 5.5 cm above the surface level (Costall and Naylor 1974). The total length of time that the animal stayed on the bar without any voluntary movement was recorded.

Evaluation of weight gain

The animals were weighed on the 1st, 8th, and 14th days of each respective protocol to determine body weight gain. The body weight obtained on the 1st day was defined as 100 %, and the subsequent percent of weight alterations obtained on days 8 and 14 were calculated as a percent of increase or decrease concerning the initial weight. Therefore, results are expressed as mean % alterations ± SEM.

Brain oxidative stress determinations

Immediately after the last behavioral determination, the animals were euthanized by decapitation, and the hippocampi dissected for neurochemical assays. Samples were immediately frozen and stored at −80 °C until assayed.

Neurochemical parameters were evaluated in the control group and animals exposed to the ketamine-schizophrenia model and pretreated or post-treated with EOAZ or OLZ.

Reduced glutathione (GSH) Levels

We estimated endogenous defenses against oxidative stress by GSH levels. The method is based on Ellman’s reagent (DTNB) reaction, as described elsewhere (Sedlak and Lindsay 1968). For the determination of GSH levels, absorbance was set at 412 nm. Results are expressed as mg GSH/g wet tissue.

Nitrite levels

To assess alterations in nitric oxide (NO) production, we evaluated nitrite levels in hippocampal samples. NO was determined based on the Griess reaction (Green et al. 1981; Radenovic and Selakovic 2005), with absorbance set at 550 nm. The standard curve was prepared with several concentrations of NaNO₂ (ranging from 0.75 to 100 µM). Results are expressed as µM/g of protein.

Immune and neurotrophic enzymatic assay for IL-6 and BDNF

According to the manufacturers’ instructions, these parameters were determined in each sample by enzyme immunoassays (Mouse IL-6 DuoSet ELISA DY406-05 and Human/Mouse BDNF DuoSet ELISA DY248 from R&D Systems, Minneapolis, MN, USA). Results are expressed as pg/g wet tissue.

Statistical analysis

We used the Shapiro-Wilk test to verify the normality of the data. Repeated measures (RM) two-way ANOVA with Tukey post hoc test evaluated PPI results considering “PP intensities” (PP70, 75, and 80) as a within-groups factor and “drug treatment” (prevention and reversal groups) as a between-groups factor. For % weight gain, we used RM two-way ANOVA with “day of treatment” as a within-subjects factor and “drug treatment” as a between-subjects factor. Social interaction, Y maze, and catalepsy time were evaluated by regular two-way ANOVA followed by Tukey post hoc test, with “treatment protocol” and “drug treatment” as factors. Neurochemical parameters were evaluated by one-way ANOVA followed by the Tukey post hoc. The significance level was set at P ≤ 0.05. Prism 6 software® analyzed the data.

Results

Effects of EOAZ and OLZ against KET-induced schizophrenia-like symptoms

In the analysis of PPI results of the prevention protocol (Fig. 2A), we observed a significant main effect of “drug treatment” [F (6, 43) = 17.93, P < 0.0001]. KET repeated administration caused significant PPI deficits on PPs 70, 75, and 80 in relation to control (P < 0.0001). EOAZ100 (P < 0.0001), EOAZ200 (P = 0.0015), or OLZ (P < 0.0001) prevented KET-induced PPI deficit. Considering PPs 75 and 80, only EOAZ100 (P < 0.0001) or OLZ (P < 0.0001) prevented KET-induced alterations. In the reversal protocol (Fig. 2B), we observed a significant “PP intensities” vs. “drug treatment” interaction [F (12, 86) = 2.099, P = 0.0251]. In this protocol, KET also caused significant PPI deficits on PPs 70, 75, and 80 compared to control (P < 0.0001). Post-treatment with EOAZ100, 200 or OLZ significantly reversed KET-induced PPI deficits in all PP intensities evaluated (PP70: KET vs. KET + EOAZ100,
In the evaluation of working memory (Fig. 3B), there was a significant main effect of “drug treatment” [F (6, 85) = 15.93, P < 0.0001]. In both protocols, KET-treated mice showed working memory deficits compared to control (P < 0.0001). In the prevention protocol, EOAZ100 maintained working memory performance like control. In contrast, both EOAZ200 and OLZ maintained KET-induced working memory deficits (control vs. EOAZ200 + KET, P = 0.0041; control vs. OLZ + KET, P < 0.0001). Furthermore, KET + OLZ had working memory deficits in the reversal protocol compared to the control group (P < 0.0001), while KET + EOAZ100 or KET + EOAZ200 presented working memory performance like the control group.

Effects of EOAZ and OLZ in common side effects related to antipsychotic drug therapy

In the prevention protocol, catalepsy time increased in the groups treated with EOAZ200 alone or EOAZ200 + KET, while a marked 25-fold increase was observed in OLZ + KET compared to control (P < 0.0001). EOAZ200, KET + EOAZ100, or KET + EOAZ200 incremented
Effects of EOAZ against KET-induced hippocampal oxidative, neuroinflammatory, and neurotrophic alterations

As depicted in Fig. 5A, we observed a significant interaction between “treatment protocol” vs. “drug treatment” in the analysis of GSH hippocampal levels [F (4, 53) = 36.89, P < 0.0001]. Tukey test revealed a significant decrease in GSH levels in KET-treated mice from both prevention and reversal protocols compared to control (P < 0.0001). In the prevention protocol, only OLZ pretreatment significantly prevented GSH deficits induced by KET (P < 0.0001). Conversely, pretreatment with EOAZ100 or EOAZ200 maintained GSH deficits induced by KET (P < 0.0001, compared to control). In the reversal protocol, post-treatment with
EOAZ100, EOAZ200, or OLZ significantly reversed the decrease in GSH levels induced by KET (P < 0.0001).

In the evaluation of hippocampal nitrite levels (Fig. 5B), there was also a significant “treatment protocol” vs. “drug treatment” interaction [F (4, 60) = 15.53, P < 0.0001]. Tukey’s test revealed a marked 5-fold increase in nitrite levels in the KET-treated group from reversal protocol in relation to control (P < 0.0001). All treatments significantly reversed this KET-induced alteration in nitrite levels (P < 0.0001). We observed no hippocampal changes in nitrite levels in mice subjected to the prevention protocol.

Interleukin 6 levels (Fig. 6A) significantly increased after KET administration in both protocols when compared to control animals (P < 0.0001). Only EOAZ200 + KET maintained IL-6 levels like those of control (EOAZ200 + KET vs. KET, P < 0.0001). Conversely,
EOAZ100 + KET (P = 0.0026), or OLZ + KET (P = 0.01), despite causing a significant decrease in IL-6 levels when compared to KET, had increased levels of IL-6 when compared to control (P < 0.0001). In the reversal protocol, both doses of EOAZ reversed the increase in IL-6 induced by KET (P < 0.0001). KET + OLZ group had a slight and significant decrease in IL-6 levels when compared to KET (P = 0.0022), although significantly higher than control group (P < 0.0001) (One-way ANOVA - prevention protocol [F (4, 25) = 44.57, P < 0.0001]; reversal protocol [F (4, 29) = 88.14, P < 0.0001].

Considering BDNF levels, in the prevention protocol, we observed decreased levels of this neurotrophin in the groups’ KET, EOAZ200 + KET, or OLZ + KET when compared to control (P < 0.0001). Despite presenting decreased levels of BDNF when compared to control, the EOAZ100 + KET group had increased levels of this neurotrophin in relation to KET (P = 0.0074) (One-way ANOVA: [F (4, 27) = 55.28, P < 0.0001]). In the reversal protocol, the levels of BDNF were decreased in all groups in relation to control (KET vs. KET + EOAZ100 and KET + EOAZ200, P < 0.0001; KET vs. KET + OLZ, P = 0.0022). Nevertheless, KET + EOAZ100 or KET + OLZ groups presented increased BDNF when compared to KET (P < 0.0001) (One-way ANOVA: [F (4, 30) = 31.77, P < 0.0001].

**Discussion**

Here we add novel evidence for EOAZ antipsychotic effects by showing that it prevents and reverses behavioral alterations induced by KET repeated administration in mice that resemble schizophrenia symptoms by anti-inflammatory, antioxidative, and neurotrophic mechanisms. We also showed that the oil’s effects are quite like those of the atypical antipsychotic OLZ but devoid of some important side effects observed in antipsychotic drug therapy, namely weight gain, and catalepsy. Notably, only EOAZ, but not OLZ, prevented and reversed working memory deficits and turned KET-induced IL-6 increments to control levels. Hence, EOAZ seems to be a promising drug therapy for schizophrenia or a source of new compounds for investigation against this devastating disease.

Herbal and plant-derived medicines are recognized for their beneficial therapeutic effects with few adverse effects Edris 2007; Kennedy and Wightman 2011; Ben-Azu et al.
Regarding schizophrenia, some plant extracts presented promising efficacy in preclinical and some preliminary human trials, such as Melissa officinalis L (Melissa or Lemon balm) and Valeriana officinalis L (Valerian) (Rahmatullah et al. 2010; Ahmed and Kabidul Azam 2014; Dey et al. 2016). We have previously demonstrated the efficacy of the EOAZ in preventing hyperlocomotion induced by a single KET dose in mice. In this previous study, 200 mg/kg EOAZ induced sedative effects with no motor coordination impairment (De Araújo et al. 2011). Importantly, KET single administration causes psychotic alterations that resemble only positive symptoms of schizophrenia but not the syndrome (Chatterjee et al. 2011).

We used repeated KET administration to induce broader behavioral changes that simulate positive, negative, and cognitive symptoms of schizophrenia (Chatterjee et al. 2011). The KET model presents relevant face and predictive validity while lesser construct validity since it does not consider the multifactorial etiology of schizophrenia (Monte et al. 2013; Frohlich and Van Horn 2014). PPI is a neurophysiological endophenotype of schizophrenia, reflecting the ability to regulate sensory information (Braff and Light 2005). PPI is disrupted in mice repeatedly exposed to KET (Monte et al. 2013), and attenuated by atypical antipsychotics, for example, risperidone (Chatterjee et al. 2011), olanzapine (Ximenes et al. 2019), and clozapine (Vasconcelos et al. 2015).

Our results revealed that EOAZ, like OLZ, prevented and reversed PPI deficits induced by KET in both doses tested, but most efficiently at 100 mg/kg. These findings corroborated our previous evidence about EAOZ effects against schizophrenia-like psychotic symptoms in rodents (De Araújo et al. 2009, 2011). Cognitive deterioration is another major breakpoint of schizophrenia psychopathology. Combined with negative symptoms (for example, asociality, avolition, and anhedonia), these are the major causes of functional impairment and morbidity of this disorder (Bowie and Harvey 2006). Notably, current antipsychotic
drugs present a limited effect against negative and cognitive symptoms of schizophrenia (Burton 2006).

Here, EOAZ successfully prevented the emergence of social deficits induced by KET, i.e., prevented the emergence of negative symptoms. On the other hand, OLZ prevented and reversed sociability changes induced by this model.

Ketamine caused a marked working memory impairment in mice, which was not affected by OLZ. However, EOAZ 100 mg/kg had a modest effect in improving this cognitive alteration in both prevention and reversal protocols. Thus, to our knowledge, our results bring the first evidence about EAoz effects against working memory alterations induced by the KET-model of schizophrenia, which, despite the promising potential, should be confirmed by further studies evaluating other tasks and cognitive domains such as attention, cognitive flexibility, and reference memory.

Although initial reports have pointed to some advantages of atypical antipsychotics for treating cognitive symptoms, subsequent studies failed to significantly benefit these agents for cognition (Meltzer et al. 1999; Cuesta et al. 2001). Therefore, therapeutic strategies for working memory deficits in schizophrenia are demanding and of great interest.

The catalepsy test evaluates drug-induced EPSE in mice (Gobira et al. 2013). Both typical and atypical antipsychotics can induce catalepsy (Kapur et al. 2000). We observed that OLZ, in both prevention and reversal protocols, induced a marked cataleptic effect. In the prevention protocol, EOAZ 100 mg/kg caused no cataleptic alteration. In the reversal protocol, EOAZ increased catalepsy time in mice. EOAZ at the higher dose also increased catalepsy time in control conditions, which may be associated with the oil’s antagonistic dopaminergic activity (de Araújo et al. 2009). These results suggest that the oil presents less potential to cause adverse EPSE than OLZ. It deserves mention that EPSE is related to the striatal dopamine D2 receptor occupancy (Wadenberg et al. 2000).

In our protocol, as expected, OLZ caused a weight gain in both prevention and reversal treatments. Conversely, EOAZ caused no critical alterations in weight gain, mainly at a dose of 200 mg/kg. Atypical antipsychotics have severe metabolic effects, being weight gain being the most common of them. Antipsychotic-induced weight gain is also frequently accompanied by hypertension, insulin resistance, and hypertriglycerideremia (Elmslie et al. 2009). Olanzapine and clozapine are the antipsychotics associated with the highest amount of weight gain (Elmslie et al. 2009). Notably, EOAZ presents antihypertensive and antiatherogenic properties (Bezerra et al. 2000; Lahlou et al. 2002; De Araújo Pinho et al. 2005; Chompoo et al. 2012).

Lieberman and coworkers (Lieberman et al. 2018) proposed a unifying theory for hippocampal changes in the evolution of schizophrenia (Lieberman et al. 2018). Briefly, genetic and/or environmental factors promote dysregulation of glutamatergic neurotransmission beginning in the Cornus ammonis (CA1) region related to prodromal symptoms and initiation of psychosis. As the illness progresses, this pathological process expands to other regions of the hippocampal circuit and projects to other anatomic areas causing hippocampal neuropil and interneurons atrophy (Lieberman et al. 2018), another relevant alteration observed in schizophrenia (Osimo et al. 2019).

An altered redox state is a well-known neurobiological feature of schizophrenia (Bošković et al. 2011). In addition, oxidative stress seems to be an initial and final mechanism important to mediate neurodegeneration after several injury factors, such as neuroinflammation, glutamatergic dysfunction, and dopamine imbalance (Bitanihirwe and Woo 2011). In this context, atypical antipsychotics present marked antioxidant actions (Sadowska-Bartosz et al. 2016).

EOAZ, mainly at 200 mg/kg, successfully reversed the hippocampal depletion of endogenous GSH and increased nitrite levels caused by the KET model. Similarly, OLZ protected mice’s hippocampus against pro-oxidative changes. Interestingly, we observed increased nitrite levels in the hippocampus of mice exposed to the reversal protocol but not to the prevention protocol. We need to have in mind that in the reversal protocol, the animals receive fourteen injections of ketamine instead of seven injections in the prevention protocol. Indeed, increased nitric oxide synthase and nitrite levels are observed in brain areas of post mortem schizophrenic patients (Bernstein et al. 2001).

Interleukin-6 is the major cytokine in the central nervous system, being altered in major psychiatric disorders (Ertu et al. 2012). Chronic schizophrenic patients present increased levels of IL-6 in the cerebrospinal fluid (CSF) (Schwieler et al. 2015). Furthermore, a single IL-6 injection during pregnancy causes schizophrenia-like behavioral abnormalities in wild-type mice but not in IL-6 knockout mice (Smith et al. 2007). Together, these data posit IL-6 relevance for schizophrenia neurobiology (Ben-Azu et al. 2019).

We have previously shown that the KET model increased hippocampal IL-6 levels, followed by increased levels of other pro-inflammatory and pro-oxidative markers (lipid peroxidation, MPO activity, nitrosative stress) (Araújo et al. 2016). Here, we replicated our previous results showing a KET-induced marked rise in hippocampal IL-6 in both prevention and reversal protocols, which EOAZ efficiently counteracted. Notably, the effect of the oil was superior to OLZ.

Oxidative imbalance and immune disturbances compromise synaptic plasticity, causing neurodegeneration in schizophrenia. BDNF is a crucial mediator of several different neuron populations’ differentiation, survival, and plasticity (Zagrebelsky and Korte 2014). In schizophrenia,
a robust meta-analysis described moderately reduced peripheral BDNF levels in the serum and plasma of patients and recovered levels after antipsychotic therapy (Fernandes et al. 2011). In the KET model, mice seem to present reduced hippocampal BDNF (Fraga et al. 2013; Vasconcelos et al. 2015). However, another study also showed that repeated KET administration in mice did not cause any significant difference in brain BDNF expression (Gama et al. 2012). However, different KET doses used in these studies, protocol duration, animal age, and BDNF detection method (gene expression vs. protein detection) can explain these discrepancies.

In our results, the KET model caused a consistent reduction in hippocampal BDNF expression in both prevention and reversal protocols. In addition, EOAZ 100 mg/kg in both protocols significantly increased BDNF levels more efficiently than OLZ. Some constituents of EOAZ, namely 1,8-cineole, terpinene-4-ol, and caryophyllene, present neuroactive and neuroprotective properties. 1,8-cineole, also known as eucalyptol or cajeputol, is a monoterpenic ether present in many plants’ essential oils, known as eucalypt cajeputol. 1,8-cineole alters neural firing in the olfactory lobe and has antinociceptive action (Liapi et al. 2007). Terpinene-4-ol, in turn, showed CNS depressant and anticonvulsant activity in mice (Nóbrega et al. 2014; Sousa et al. 2015).

Caryophyllene is a phytocannabinoid with cannabinoid receptor type 2 (CB-2) agonist properties. Caryophyllene presents antinociceptive, anxiolytic, and antidepressant effects (Bahia et al. 2014; Aly et al. 2020; Hwang et al. 2020). Additionally, there is an ongoing clinical trial with beta-caryophyllene for schizophrenia treatment (application Nº EP13763464.8 A). These phytochemicals alone or combined can be responsible for EOAZ promising effect seen here but needs further evaluation.

The present study has some limitations. Firstly, we only evaluated oxidative, nitrigric, neuro-immune, and neurotrophic markers in mice treated with the studied drugs in the presence of KET and not when singly administered. This decision was taken based on the absence of apparent behavioral alterations related to schizophrenia in the groups treated with the drugs alone. Secondly, we did not measure alterations in dopamine, serotonin, GABA, and glutamate levels. Finally, we used only males and not females. Despite the greater severity of symptoms observed in men, some evidence indicates that females also present significant deficits (Kekesi et al. 2015) that need to be better understood.

Taken together, we observed a distinct profile of EOAZ doses in the protocols studied. In this regard, we observed that the lower dose of 100 mg/kg was overall more effective in preventing KET-induced behavioral alterations, including the manifestation of catalepsy. On the other hand, both doses effectively reversed KET-induced alterations, except the social deficit that EOAZ did not reverse. Furthermore, weight gain was prevented by both doses of the EOAZ in the reversal protocol. This result shows us that EOAZ 100 mg/kg dose is the best one to be translated to clinical studies since it seems to be absent of side effects (i.e., catalepsy and weight alterations).

Conclusions

Our results reveal the promising anti-schizophrenia action of the EOAZ. EOAZ counteracted the most behavioral alterations resembling schizophrenia symptoms induced by KET, with a more favorable side effect profile than the standard antipsychotic OLZ. Also, EOAZ protected mice’s hippocampus against the most KET-induced pro-oxidative changes and restored BDNF contents. Together, these findings provide broader preclinical evidence about using this plant oil as a valuable new therapeutic strategy for schizophrenia.

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Authors’ contributions FYRA, DFL, MOM, MEM – Designed the study
AJMCF, AMN, GVO, PXLG – treated the animals and performed behavioral tests
GSV, AJMCF, JC – Performed neurochemical determinations
DFL, DSM, FCFS – Performed statistical analysis of the data
DFL, DSM – constructed the graphics
FYRA, DSM, JC, AJMCF – Wrote the first draft of the manuscript
All authors approved the final version of the manuscript.

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Data availability Data will be made available under request.

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