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Research Paper

Inhibition of ketamine-induced hyperlocomotion in mice by the essential oil of *Alpinia zerumbet*: possible involvement of an antioxidant effect

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Abstract

Objectives The antipsychotic, hypnotic, myorelaxant and antioxidant effects of the essential oil of *Alpinia zerumbet* (EOAZ) were studied.

Methods EOAZ (50, 100 and 200 mg/kg i.p.) was administered once to mice for the determination of antipsychotic activity (evaluated by ketamine-induced hyperlocomotion), hypnotic activity (induced by sodium pentobarbital, 40 mg/kg i.p.), motor coordination (rotarod test), antioxidant effects (determination of lipid peroxidation and GSH levels), as well as alterations in nitric oxide levels (determination of nitrite content).

Key findings EOAZ at doses of 100 and 200 mg/kg prevented ketamine hyperlocomotion, as did haloperidol (0.2 mg/kg i.p). EOAZ at a dose of 200 mg/kg decreased sleep latency, while all doses increased sleeping time. There was no effect on motor coordination. The in-vitro antioxidant capacity of the oil caused a decrease in lipid peroxidation and increase in GSH levels. EOAZ also prevented the decrease in nitrite content caused by oxidative stress.

Conclusions The results suggest antipsychotic and antioxidant effects for the EOAZ that may have promising efficacy for the treatment of schizophrenia.

Keywords *Alpinia speciosa*; *Alpinia zerumbet*; antioxidant effect; essential oil; ketamine-induced hyperlocomotion

Introduction

Schizophrenia is a mental disorder characterized by 'positive' and 'negative' symptoms, and by less recognized cognitive deficits in executive functions, working memory and attention.[1] *N*-Methyl-D-aspartate (NMDA) receptor antagonists, such as ketamine and dissociative anaesthetics, are a class of compounds that produce a transient schizophrenia-like state in humans[2] and have been shown to produce hyperlocomotion, enhanced stereotyped behaviour, cognitive and sensorimotor gating deficits, and impaired social interactions in rodents.[3] Dopamine, which has long been considered important in the pathophysiology of schizophrenia, also appears to be a critical neurotransmitter mediating the effects of NMDA receptor antagonists.[4]

Several studies have shown that reactive oxygen species have an important role in the pathogenesis of many diseases, especially neurological and psychiatric ones.[5] Oxidative stress may be a common pathogenic mechanism underlying many major psychiatric disorders as the brain is comparatively vulnerable to oxidative damage. In this context, it has already been reported that free radicals are elevated in patients diagnosed with schizophrenia.[6]

Antipsychotic medications are also related to alterations in oxidative stress parameters. Some studies have reported increased lipid peroxidation in rats chronically treated with

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haloperidol but not in animals treated with atypical antipsychotics.[7] A recent study clearly demonstrated that oxidative stress damage occurs in patients with schizophrenia and one possible therapeutic solution is to use antioxidants.[8] For example, the association of *Ginkgo biloba* extract with classical haloperidol treatment results in better scores in the Scale for the Assessment of Positive and Negative Symptoms,[9] as well as enhanced antipsychotic efficacy and reduced extrapyramidal side-effects.[10] Also, the use of essential polyunsaturated fatty acids has been suggested, considering the dysregulation of membrane phospholipid metabolism throughout the body in patients with schizophrenia.[11]

Based on the benefits of *Ginkgo biloba* extract in the treatment of schizophrenia, the discovery of other plants with similar or greater activity may contribute to the clinical outcome of schizophrenia. *Alpinia zerumbet* (Pers.) B. L. Burtt from the Zingiberaceae family is usually cited in the literature with the binomial *Alpinia speciosa* K. Schum. This species is popularly known in Brazil as 'colônia' and it is used in traditional medicine and religious rituals. The major constituents present in its roots, leaves and stems are sesquiterpenoids and diterpenoids.[12]

Some effects of the essential oil of *A. zerumbet* leaves (EOAZ) have already been determined in animals, including arterial hypotension effects,[13] antinociceptive effects, probably involving the participation of opiate receptors,[14] and myorelaxant and antispasmodic effects.[15]

In phytotherapy, *A. zerumbet* is used to treat neuropsychiatric symptoms such as depression, stress and anxiety, but it is only recently that the central nervous system (CNS) effects of the essential oil from the plant leaves have been studied. Our research group showed a possible involvement of dopaminergic neurotransmission in the central actions of EOAZ, since a decrease in locomotor activity and attenuation of apomorphine-induced stereotypy behaviour were observed in mice treated with the essential oil at intraperitoneal doses of 50 and 100 mg/kg.[16] Inhalational administration of EOAZ (0.087 and 8.7 ppm) showed an anxiolytic-like activity in mice.[17,18]

Antioxidant compounds isolated from the rhizomes of *A. zerumbet* have shown greater activity than Trolox.[19] However, there is no evidence in the literature for the antioxidant activity of *A. zerumbet* leaves.

The EOAZ has possible antipsychotic effects as evidenced by the attenuation of apomorphine-induced stereotypy, a pharmacological model of schizophrenia that resembles mainly positive symptoms.[17] In the present study, we aimed to confirm our preliminary findings studying the effects of the EOAZ on ketamine-induced hyperlocomotion. This schizophrenia model has widespread acceptance as it may simulate other dimensions of the disorder, namely cognitive and negative symptoms.[20] Furthermore, we investigated possible CNS antioxidant effects of EOAZ, which might partly explain its antipsychotic activity.

Methods

Animals

Male Swiss mice (20–30 g) were housed in a temperature-controlled room (25 ± 1°C) under standard laboratory conditions, with free access to food and water and a 12-h light/dark cycle (lights on at 0630 h). Procedures were conducted in accordance with the Brazilian College of Animal Experimentation (COBEA) guidelines for the care and use of laboratory animals, as well as the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Betesda, MD, USA), in compliance with international laws and policies. The study was approved by the University Animal Ethics Committee (protocol no. 45/10).

Plant material

The essential oil was extracted from leaves of *A. zerumbet* collected in the Medicinal Plants Garden of the Laboratory of Natural Products of the Federal University of Ceará, Ceará State, Brazil, during December 2009. A voucher specimen of *A. zerumbet* was deposited at the Herbarium Prisco Bezerra (no. 10858), as identified by Dr Edson Paula Nunes and Dr Peres Martins. The isolation of the essential oil was carried out at the Department of Organic and Inorganic Chemistry of the Federal University of Ceará, according to the method described elsewhere.[21] 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 water was heated to boiling point, and the steam percolated through the chopped plant leaves and collected in the condenser. After condensation, the watery phase with its solutes, termed the 'hydrolate', was separated from an oily phase; the essential oil, which, when rediluted in water is termed the 'pseudo-hydrolate'. The composition of the EOAZ was determined by gas chromatography and mass spectrometry: 1,8-cineole, 20.57%; terpinen-4-ol, 19.39%; g-terpinene, 15.08%; sabinene, 9.68%; ρ-cimene, 8.54%; α-tujene, 6.35%; α-terpinene, 3.88%; β-pinene, 3.02%; limonene, 2.64%; α-pinene, 2.38%; terpinolene, 1.93%; β-mircene, 1.20%; *trans*-cariophilene, 1.11%; α-terpineol, 0.86%; not identified, 3.35%.

Drugs and administration schedule

The essential oil was emulsified with 2% Tween 80 (Sigma, St Louis, MO, USA) in distilled water. Ketamine (Sigma) was dissolved in saline. All solutions were freshly prepared before injection. Animals, 6–10 per group, were injected once with EOAZ (50, 100 and 200 mg/kg i.p.) or vehicle 30 min before the test, while ketamine (20 mg/kg i.p.) or saline were applied immediately before the beginning of behavioural tests. Haloperidol was used as a standard antipsychotic drug. For the ketamine-induced hyperlocomotion test the experimental groups were divided as follows: group 1, vehicle + saline (control); group 2, vehicle + ketamine; group 3, EOAZ 50 mg/kg i.p. + saline; group 4, EOAZ 100 mg/kg i.p. + saline; group 5, EOAZ 200 mg/kg i.p. + saline; group 6, haloperidol 0.2 mg/kg i.p. + saline; group 7, EOAZ 50 mg/kg i.p. + ketamine; group 8, EOAZ 100 mg/kg i.p. + ketamine; group 9, EOAZ 200 mg/kg i.p. + ketamine; group 10, haloperidol 0.2 mg/kg i.p. + ketamine.

Behavioural tests

Ketamine-induced hyperlocomotion

The behavioural effects produced by drug treatments were tested in an open field made of acrylic (transparent walls and

black floor, 30 cm × 30 cm × 20 cm) divided into nine squares of equal area. The testing room was illuminated with three 25-W bulbs placed around the open field and had a constant temperature of 25 ± 1°C. Immediately after the second injection (20 mg/kg ketamine or saline), the mice were placed in the centre of the open field and locomotor activity was recorded during 20 min. The test was performed during the light period between 0800 and 1200 h. After determination of locomotor activity, the mice that received only the EOAZ were placed on a rotarod apparatus to determine the effects of the essential oil on motor coordination.

Pentobarbital sleeping time

At 30 min after intraperitoneal administration of EOAZ (50, 100 and 200 mg/kg) or vehicle, all groups received sodium pentobarbital (40 mg/kg i.p.). The time between the injection to the loss of the righting reflex was recorded as the sleep latency, and the time elapsed between the loss and voluntary recovery of the righting reflex was recorded as the sleeping time.[22] Diazepam at a dose of 1 mg/kg was used as a standard sedative drug.

Rotarod test

After determination of locomotor activity mice treated with EOAZ (50, 100 and 200 mg/kg) and controls were placed with their four paws on a 2.5-cm diameter bar, 25 cm above the floor and the time of permanence on the bar was measured during 1 min for each animal. The rotating speed was 15 rev/min.[23]

In-vitro antioxidant activity

In-vitro antioxidant activity was assessed by measuring the inhibition of spontaneous lipoperoxidation of homogenates from the brains of mice (without cerebellum) in the presence of different concentrations of EOAZ equivalent to 25, 50 and 100 µg/ml. Vitamin E (100 µg/ml) used as a standard antioxidant. After 1 h incubation of previously frozen (–20°C for 24 h) brain homogenate at 37°C, the antioxidant activity was calculated for each concentration of the oil. The samples submitted to oxidative stress (i.e. frozen and thawed at 37°C) were used as positive controls. The samples frozen and not incubated for 1 h at 37°C were used as negative controls.

The same homogenates were used to assess the in-vitro effects of the EOAZ on nitric oxide (NO) production assessed by determination of nitrite levels and reduced glutathione (GSH) to evaluate defences against oxidative stress,[24] according to the following procedures.

Determination of lipid peroxidation

Lipid peroxide formation was analysed by measuring the thiobarbituric-acid reacting substances (TBARS) in the homogenates. The samples were briefly mixed with 50 mM potassium phosphate monobasic buffer (pH 7.4), and 63 µL of the homogenate was mixed with 100 µL of 35% perchloric acid. The samples were then centrifuged (3350*g*/10 min) and 150 µL of the supernatants was retrieved and mixed with 50 µL of thiobarbituric acid 1.2%, and heated in a boiling water bath for 30 min. After cooling, the lipid peroxidation was determined by absorbance at 535 nm and was expressed as µmol MDA/g tissue.

Determination of GSH levels

GSH levels were evaluated to estimate endogenous defences against oxidative stress. The method was based on Ellman's reagent (DTNB) reaction with free thiol groups. Striatum homogenates 10% (w/v) in EDTA 0.02 M were added to a 50% trichloroacetic acid solution. After centrifugation (1200*g*/15 min), the supernatant of homogenate was collected and the production levels of GSH were determined as described elsewhere.[25] Briefly, the samples were mixed with 0.4 M Tris-HCl buffer (pH 8.9) and 0.01 M DTNB. The GSH level was determined by the absorbance at 412 nm, calculated based on a standard glutathione curve and expressed as ng of GSH/g wet tissue.

Nitrite determination

For the assessment of nitrite, derived from NO, 100 µl of Griess reagent (1% sulfanilamide in 1% H_3PO_4/0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride/1% H_3PO_4/distilled water, 1 : 1 : 1 : 1) was added to 100 µl of brain homogenates or to 100 µl of $NaNO_2$ at concentrations ranging from 0.75 to 100 µM (standard curve). For the blanks, 100 µl of the Griess reagent was added to 100 µl of homogenate. The absorbance was measured with a plate reader at 560 nm. The standard curve was used for determination of nitrite concentrations in samples.[26]

Statistical analysis

All the results are expressed as mean ± SEM. Treated groups were compared with controls and differences were estimated by analysis of variance followed by Student-Newman-Keuls post-hoc test for multiple comparisons. In all comparisons, $P < 0.05$ was considered to indicate statistical significance.

Results

Inhibition of ketamine-induced hyperlocomotion by EOAZ

As expected, the animals treated with ketamine (20 mg/kg i.p.) showed an increase of 181% in the number of crossings as compared with control animals ($F(9,67) = 19.48$, $P < 0.001$). EOAZ (200 mg/kg) and haloperidol (0.2 mg/kg) alone decreased locomotor activity ($F(9,67) = 19.48$, $P < 0.05$) as compared with controls. Pretreatment with EOAZ (100 and 200 mg/kg) and haloperidol (0.2 mg/kg) prevented ketamine-induced hyperlocomotion ($F(9,67) = 19.48$, $P < 0.001$) (Figure 1).

Effects of EOAZ on sleeping time

According to Figure 2a EOAZ at the dose of 200 mg/kg decreased sleeping latency by 40%, an effect similar to that presented by diazepam ($F(4,33) = 8.726$, $P < 0.01$).

EOAZ at all doses studied increased the sleeping duration in a dose-dependent manner. The augmentation of sleeping duration seen at the dose of 200 mg/kg was 50% higher than that registered with diazepam (control *vs* EOAZ 50 mg/kg and EOAZ 100 mg/kg *vs* EOAZ 200 mg/kg ($F(4,31) = 18.35$,

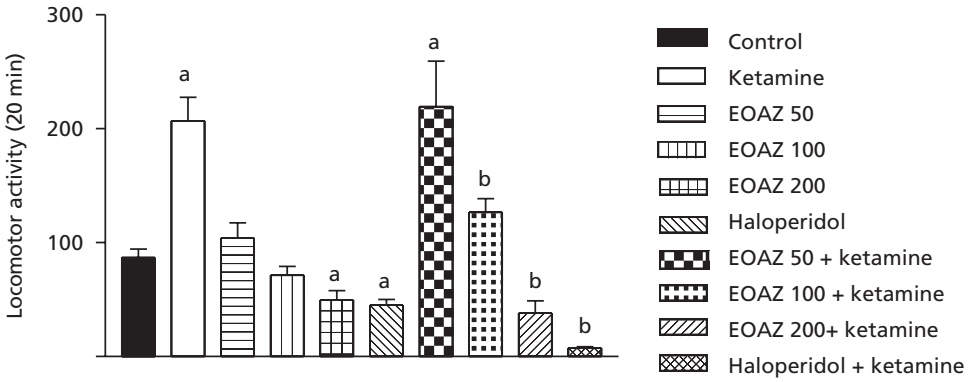

Figure 1 Effect of EOAZ on ketamine-induced hyperlocomotion. Mice were injected with the essential oil from *Alpinia zerumbet* (EOAZ 50, 100 and 200 mg/kg i.p.) or 0.2 mg/kg haloperidol alone or associated with 20 mg/kg ketamine (with a 30-min interval between drugs) and were immediately placed in an open field arena. Bars show locomotor activity ± SEM. $^{a,b}P < 0.05$, significant difference compared with control and ketamine, respectively (analysis of variance followed by Student-Newman-Keuls post-hoc test).

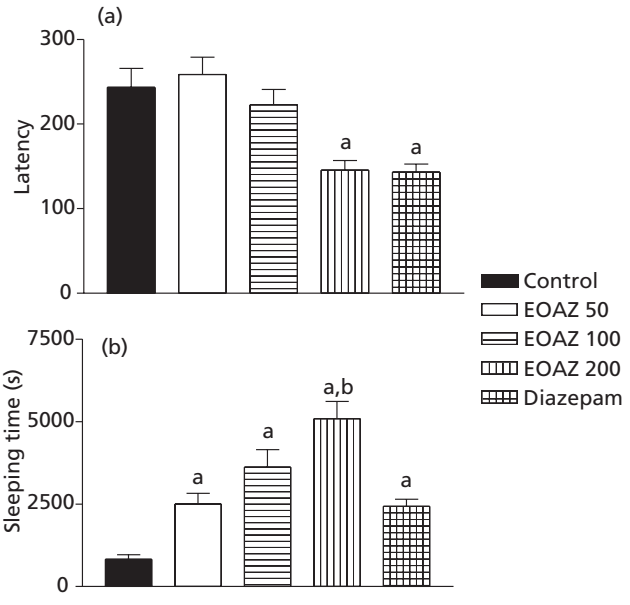

Figure 2 Effect of EOAZ on pentobarbital sleeping time. Groups of mice received vehicle, the essential oil of *Alpinia zerumbet* (EOAZ 50, 100 and 200 mg/kg) and diazepam (1 mg/kg). The parameters analysed were the latency time and the sleeping time. The results are presented as means ± SEM. $^{a,b}P < 0.05$ significantly different compared with control and 100 mg/kg EOAZ (analysis of variance and Student-Newman-Keuls post-hoc test).

$P < 0.05$); control *vs* EOAZ 100 mg/kg and control *vs* EOAZ 200 mg/kg ($F(4,31) = 18.35$, $P < 0.001$); control *vs* diazepam ($F(4,31) = 18.35$, $P < 0.01$)) (Figure 2b).

Effects of EOAZ on motor coordination

As shown in Figure 3 none of the EOAZ doses used (50, 100 and 200 mg/kg) altered motor coordination in mice ($F(3,35) = 0.06867$, not significant).

In-vitro antioxidant activity

Figure 4 shows that the MDA content was increased by 83% in the positive control compared with the negative control

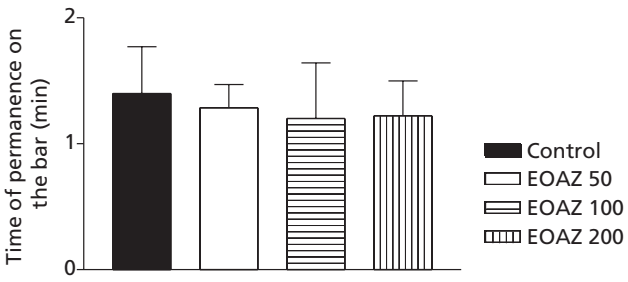

Figure 3 Effect of EOAZ on motor coordination. Groups of mice received vehicle and the essential oil of *Alpinia zerumbet* (EOAZ 50, 100 and 200 mg/kg). The parameter analysed was the time of permanence on the bar in the rotarod test. The results are presented as means ± SEM (analysis of variance and Student-Newman-Keuls post-hoc test).

($F(5,35) = 18.58$, $P < 0.001$). EOAZ at all concentrations studied (25, 50 and 100 μg/ml), significantly decreased lipid peroxidation by approximately 80% as compared with the positive control, and this effect was comparable with that presented by vitamin E which decreased MDA content by 84% compared with the positive control ($F(5,35) = 18.58$, $P < 0.001$).

In-vitro administration of EOAZ at a concentration of 50 and 100 μg/ml significantly increased GSH levels as compared with the positive control which decreased this parameter in relation to the negative control ($F(5,34) = 5.667$, $P < 0.05$). Vitamin E also increased the GSH content as compared with the positive and negative controls ($F(5,34) = 5.667$, $P < 0.001$) (Figure 5).

As can be seen in Figure 6, under our experimental conditions, the homogenates submitted to oxidative stress (positive control) had decreased nitrite content as compared with the negative control, while EOAZ at all doses studied and vitamin E returned this parameter to negative control levels (positive control *vs* negative control and positive control *vs* EOAZ 25 μg/ml ($F(5,42) = 4.043$, $P < 0.05$); positive control *vs* EOAZ 50 μg/ml, positive control *vs* EOAZ 100 μg/ml and positive control *vs* vitamin E ($F(5,42) = 4.043$, $P < 0.01$)).

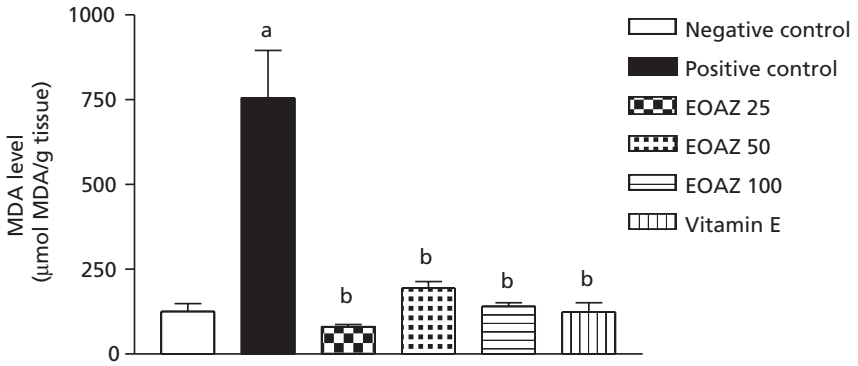

Figure 4 In-vitro effect of EOAZ on brain lipid peroxidation. Bars show malondialdehyde (MDA) levels ± SEM from whole-brain homogenates (without cerebellum) of mice submitted (positive control) or not (negative control) to oxidative stress, and from homogenates submitted to oxidative stress pre-incubated with the essential oil from *Alpinia zerumbet* (EOAZ 25, 50 and 100 µg/ml) or vitamin E (100 µg/ml). $^{a,b}P < 0.05$ significantly different compared with negative control and positive control, respectively (analysis of variance and Student-Newman-Keuls post-hoc test).

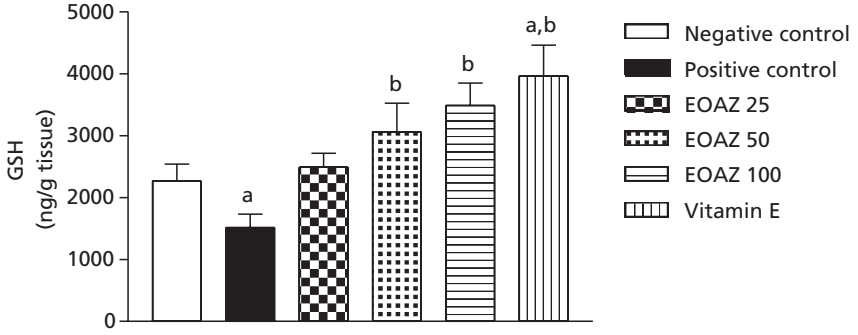

Figure 5 In-vitro effect of EOAZ on brain glutathione levels. Reduced glutathione (GSH) levels were determined after in-vitro administration of the essential oil from *Alpinia zerumbet* (EOAZ). Bars show GSH levels ± SEM from whole-brain homogenates (without cerebellum) of mice submitted (positive control) or not (negative control) to oxidative stress, and from homogenates submitted to oxidative stress pre-incubated with EOAZ (25, 50 and 100 µg/ml) or vitamin E (100 µg/ml). $^{a,b}P < 0.05$ significantly different compared with negative control and positive control, respectively (analysis of variance and Student-Newman-Keuls post-hoc test).

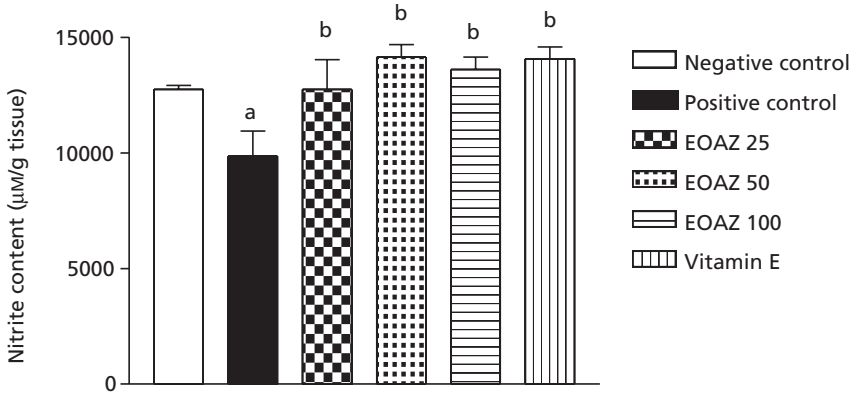

Figure 6 In-vitro effect of EOAZ on brain nitrite content. The nitrite content was determined after in-vitro administration of the essential oil from *Alpinia zerumbet* (EOAZ). Bars show nitrite levels ± SEM from whole-brain homogenates (without cerebellum) of mice submitted (positive control) or not (negative control) to oxidative stress, and from homogenates submitted to oxidative stress pre-incubated with EOAZ (25, 50 and 100 µg/ml) or vitamin E (100 µg/ml). $^{a,b}P < 0.05$ significantly different compared with negative control and positive control, respectively (analysis of variance and Student-Newman-Keuls post-hoc test).

Discussion

Pretreatment with EOAZ at concentrations of 100 and 200 mg/kg prevented ketamine-induced behavioural alterations. Drugs such as ketamine, phencyclidine and other similarly acting psychotomimetic compounds induced their unique behavioural effects by blocking neurotransmission at NMDA-type glutamate receptors.[27] The ability of these compounds to transiently reproduce key symptoms of schizophrenia by blocking NMDA receptors led to the concept that symptoms in schizophrenia may reflect underlying dysfunction or dysregulation of NMDA receptor-mediated neurotransmission. This model has been increasingly adopted and is now considered to be one of the useful models for both aetiological conceptualization of schizophrenia and new treatment development,[28] since the antipsychotics in current use best treat positive symptoms, while negative and cognitive symptoms remain a problem.

NMDA dysfunction may also account for both the impaired dopaminergic regulation and the impaired GABAergic neurotransmission that has been documented in schizophrenia. Deficits similar to those observed in schizophrenia are observed in normal volunteers undergoing ketamine infusion,[29] and in rodents treated subchronically[30] with NMDA receptor antagonists, suggesting that dopaminergic dysregulation in schizophrenia may be downstream of a primary deficit in NMDA function.

In a recent study, we showed that the intraperitoneal administration of EOAZ at doses of 50 and 100 mg/kg prevented the behaviours induced by apomorphine administration (climbing and sniffing) in a dose-dependent manner, strongly indicating the participation of dopamine receptors in the EOAZ mechanism of action and suggesting an antipsychotic activity (possible by a blockage of dopaminergic receptors), since it was effective in the prevention of apomorphine-induced stereotypy.[16]

The pentobarbital sleeping time test was used to confirm the depressant effects of the essential oil. A decrease in sleep latency and increase in sleeping time are classically related to CNS depressant drugs.[31] The present results showed that EOAZ significantly decreased the sleep latency (at a dose of 200 mg/kg), and prolonged sleeping time at all doses studied, suggesting a possible sedative effect of this oil. Previous research showed that oral administration of the hydroalcoholic extract of *A. zerumbet* to mice produced a prolongation of sleeping time over the dose range of 500–1000 mg/kg.[32] It is important to note that all antipsychotic medications are associated with an increased likelihood of sedation.[33] Atypical antipsychotics such as olanzapine in placebo-controlled trials induce sedation as one of the most common adverse events.[34] The intraperitoneal administration of haloperidol and pimozide (2.5 and 5.0 mg/kg) 10 min before pentobarbital sodium (60 mg/kg) injection in rats significantly increased the onset time and duration of sleep.[35]

A deficit in motor coordination would very likely affect performance in the behavioural tests. To test this, we determined the effects of EOAZ in the rotarod test, a classic animal model used to evaluate peripheral neuromuscular blockage. Our findings showed that the oil had no significant effect on motor coordination of the mice in this test. Benzodiazepine compounds show anxiolytic, sedative and muscle relaxation effects. These effects could involve facilitation by some inhibitory systems such as the GABAergic system. EOAZ did not show anxiolytic effects[16] or, as seen in the present study, muscle relaxation. Based on this, we can postulate that this oil does not act as a benzodiazepine but rather its effects are closer to those produced by antipsychotic drugs.

Under our experimental conditions the in-vitro administration of EOAZ was able to prevent lipid peroxidation, increase GSH levels and normalize nitrite content in whole-brain homogenates, thus showing an antioxidant effect. The brain is susceptible to oxidative damage since it is under very high oxygen tension and highly enriched in reactive oxygen species susceptible proteins, lipids and poor DNA repair. Indeed, an altered redox state is evident in some psychopathologies such as bipolar disorder,[36] anxiety and depression,[37] and schizophrenia.[38] In schizophrenia, a reduction in both reduced and oxidized glutathione, reduced superoxide dismutase, reduced catalase and glutathione peroxidase, as well as increased lipid peroxidation has been reported.[5]

Since oxidative stress is a possible mechanism involved in schizophrenia, the adjuvant use of antioxidants in the treatment of this mental illness may be very useful. Schizophrenia affects approximately one in a 100 individuals, of which about one-third respond fully to treatment, one-third incompletely and one-third not at all.[39] Part of the problem with the inadequate response to therapy may be due to the fact that drugs in current use were not designed to treat GSH deficiency[40] or oxidative stress, and the adjunctive use of antioxidants is not yet standard practice.[39]

Aberrations in NO signalling have been associated with schizophrenia in both clinical studies and animal models of the disorder.[41,42] A role for NO dysregulation is supported by recent findings showing increases in NO levels and NO-dependent increases in cGMP signalling in the prefrontal cortex following phencyclidine administration in rodents.[43] In our study, EOAZ was able to prevent the alteration induced by oxidative stress in whole-brain homogenates, suggesting that this effect can also contribute to its beneficial effects in animal models of schizophrenia. It is important to mention that the oil did not increase nitrite content but rather brought it back to normal levels (negative control levels).

The main constituents of EOAZ (1,8-cineole and terpinen-4-ol) have already been studied in relation to some biological activities. 1,8-Cineole, also known as eucalyptol or cajeputol, is a monoterpene ether, present in many plant essential oils. It has been reported to alter neural firing in certain areas of the olfactory lobe, and to have hypotensive, smooth muscle relaxant[44,45] and antinociceptive activity.[46] Terpinen-4-ol, a monoterpenoid alcohol and component of the essential oils of several aromatic plants, showed CNS depressant and anticonvulsant activity in mice.[47] Thus, some neuroactive constituents of the EOAZ might account for the antipsychotic and antioxidant effects described in the present study and may open new perspectives for further investigation.

Conclusions

This study demonstrated the antipsychotic activity of EOAZ using a pharmacological model of schizophrenia

(ketamine-induced hyperlocomotion) and also showed a sedative effect of the oil. This study corroborates our previous research.[16] EOAZ presented an important antioxidant effect *in vitro*, suggesting that it may improve the treatment of mental illness such as schizophrenia. Further studies are needed to determine if the antipsychotic effect of this oil involves dopaminergic and/or serotonergic neurotransmission since this is a common mechanism of antipsychotic drugs.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Bowie CR, Harvey PD. Cognitive deficits and functional outcome in schizophrenia. *Neuropsychiatr Dis Treat* 2006; 2: 531–536.
2. Krystal JH *et al*. NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: toward a paradigm shift in medication development. *Psychopharmacology (Berl)* 2003; 169: 215–233.
3. Sams-Dodd F. Effects of continuous d-amphetamine and phencyclidine administration on social behaviour, stereotyped behaviour, and locomotor activity in rats. *Neuropsychopharmacology* 1998; 19: 18–25.
4. Moghaddam B. Bringing order to the glutamate chaos in schizophrenia. *Neuron* 2003; 40: 881–884.
5. Ng F *et al*. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. *Int J Neuropsychopharmacol* 2008; 11: 851–876.
6. Yao JK *et al*. Oxidative damage and schizophrenia: an overview of the evidence and its therapeutic implications. *CNS Drugs* 2001; 15: 287–310.
7. Kropp S *et al*. Oxidative stress during treatment with first- and second-generation antipsychotics. *J Neuropsychiatry Clin Neurosci* 2005; 17: 227–231.
8. Padurariu M *et al*. Evaluation of antioxidant enzymes activities and lipid peroxidation in schizophrenic patients treated with typical and atypical antipsychotics. *Neurosci Lett* 2010; 479: 317–320.
9. Zhang XY *et al*. A double-blind, placebo-controlled trial of extract of *Ginkgo biloba* added to haloperidol in treatment-resistant patients with schizophrenia. *J Clin Psychiatry* 2001; 62: 878–883.
10. Zhang XY *et al*. The effect of extract of *Ginkgo biloba* added to haloperidol on superoxide dismutase in inpatients with chronic schizophrenia. *J Clin Psychopharmacol* 2001; 21: 85–88.
11. Mahadik SP, Evans DR. Is schizophrenia a metabolic brain disorder? Membrane phospholipid dysregulation and its therapeutic implications. *Psychiatr Clin North Am* 2003; 26: 85–102.
12. Hong X *et al*. Labdane diterpenes from *Alpinia zerumbet*. *Phytochemistry* 1996; 42: 149–151.
13. Lahlou S *et al*. Cardiovascular effects of the essential oil of *Alpinia zerumbet* leaves and its main constituent, terpinen-4-ol, in rats: role of the autonomic nervous system. *Planta Med* 2002; 68: 1097–1102.
14. de Araujo PF *et al*. Antinociceptive effects of the essential oil of *Alpinia zerumbet* on mice. *Phytomedicine* 2005; 12: 482–486.
15. Bezerra MA *et al*. Myorelaxant and antispasmodic effects of the essential oil of *Alpinia speciosa* on rat ileum. *Phytother Res* 2000; 14: 549–551.
16. de Araujo FY *et al*. Central nervous system effects of the essential oil of the leaves of *Alpinia zerumbet* in mice. *J Pharm Pharmacol* 2009; 61: 1521–1527.
17. Murakami S *et al*. Effects of the essential oil from leaves of *Alpinia zerumbet* on behavioral alterations in mice. *Nat Prod Commun* 2009; 4: 129–132.
18. Satou T *et al*. Anxiolytic effect and tissue distribution of inhaled *Alpinia zerumbet* essential oil in mice. *Nat Prod Commun* 2010; 5: 143–146.
19. Masuda T *et al*. Isolation and structure determination of new antioxidative ferulic acid glucoside esters from the rhizome of *Alpinia speciosa*, a Zingiberaceae plant used in Okinawan food culture. *J Agric Food Chem* 2000; 48: 1479–1484.
20. Neill JC *et al*. Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. *Pharmacol Ther* 2010; 128: 419–432.
21. Craveiro AA *et al*. A simple and inexpensive steam generator for essential oil extractions. *J Chem Educ* 1976; 53: 652.
22. Wambebe C, Osuide G. Influence of FLA-63 on pentobarbitone sleep in chicks and rats. *Clin Exp Pharmacol Physiol* 1983; 10: 555–566.
23. Dunham N, Miya T. A note on a simple apparatus for detecting neurological deficits in rats and mice. *J Am Pharm Assoc* 1957; 46: 208–209.
24. Auddy B *et al*. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. *J Ethnopharmacol* 2003; 84: 131–138.
25. Sedlak J, Hanus L. Changes of glutathione and protein bound SH-groups concentration in rat adrenals under acute and repeated stress. *Endocrinol Exp* 1982; 16: 103–109.
26. Green LC *et al*. Analysis of nitrate, nitrite, and [^{15}N]nitrate in biological fluids. *Anal Biochem* 1982; 126: 131–138.
27. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* 1991; 148: 1301–1308.
28. Javitt DC. Phenomenology, aetiology and treatment of schizophrenia. *Novartis Found Symp* 2008; 289: 4–16. discussion 7–22, 87–93.
29. Kegeles LS *et al*. Modulation of amphetamine-induced striatal dopamine release by ketamine in humans: implications for schizophrenia. *Biol Psychiatry* 2000; 48: 627–640.
30. Balla A *et al*. Subchronic continuous phencyclidine administration potentiates amphetamine-induced frontal cortex dopamine release. *Neuropsychopharmacology* 2003; 28: 34–44.
31. Willianson E *et al*. *Selection, Preparation and Pharmacological Evaluation of Plant Material*. Chichester: Wiley, 1996.
32. Mendonca VL *et al*. Pharmacological and toxicological evaluation of *Alpinia speciosa*. *Mem Inst Oswaldo Cruz* 1991; 86(Suppl 2): 93–97.
33. Muench J, Hamer AM. Adverse effects of antipsychotic medications. *Am Fam Physician* 2010; 81: 617–622.
34. McCormack PL. Olanzapine: in adolescents with schizophrenia or bipolar I disorder. *CNS Drugs* 2010; 24: 443–452.
35. Bhargava HN. Enhanced sensitivity to pentobarbital in haloperidol or pimozide treated mice and rats. *Res Commun Chem Pathol Pharmacol* 1980; 28: 267–284.
36. Berk M *et al*. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation; oxidative stress and neurotrophic factors. *Neurosci Biobehav Rev* 2011; 35: 804–817.
37. Hovatta I *et al*. Oxidative stress in anxiety and comorbid disorders. *Neurosci Res* 2010; 68: 261–275.

38. Moller M *et al*. Isolation rearing-induced deficits in sensorimotor gating and social interaction in rats are related to cortico-striatal oxidative stress, and reversed by sub-chronic clozapine administration. *Eur Neuropsychopharmacol* 2011; 21: 471–483.
39. Seybolt SE. Is it time to reassess alpha lipoic acid and niacinamide therapy in schizophrenia? *Med Hypotheses* 2010; 75: 572–575.
40. Carter CJ. Schizophrenia susceptibility genes converge on interlinked pathways related to glutamatergic transmission and long-term potentiation, oxidative stress and oligodendrocyte viability. *Schizophr Res* 2006; 86: 1–14.
41. Baba H *et al*. Expression of nNOS and soluble guanylate cyclase in schizophrenic brain. *Neuroreport* 2004; 15: 677–680.
42. Bird DC *et al*. Lack of phencyclidine-induced effects in mice with reduced neuronal nitric oxide synthase. *Psychopharmacology (Berl)* 2001; 155: 299–309.
43. Fejgin K *et al*. Nitric oxide signaling in the medial prefrontal cortex is involved in the biochemical and behavioral effects of phencyclidine. *Neuropsychopharmacology* 2008; 33: 1874–1883.
44. Nikitin ES, Balaban PM. Optical recording of odor-evoked responses in the olfactory brain of the naive and aversively trained terrestrial snails. *Learn Mem* 2000; 7: 422–432.
45. Lahlou S *et al*. Cardiovascular effects of 1,8-cineole, a terpenoid oxide present in many plant essential oils, in normotensive rats. *Can J Physiol Pharmacol* 2002; 80: 1125–1131.
46. Santos FA, Rao VS. Antiinflammatory and antinociceptive effects of 1,8-cineole a terpenoid oxide present in many plant essential oils. *Phytother Res* 2000; 14: 240–244.
47. de Sousa DP *et al*. Evaluation of the anticonvulsant activity of terpinen-4-ol. *Z Naturforsch [C]* 2009; 64: 1–5.