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Antidepressant-like effect of *Albizia zygia* root extract in murine models

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Abstract:
Background: *Albizia zygia* (DC.) J.F. Macbr. (Leguminosae) has been used to treat mental disorders in traditional African medicine. Nonetheless, there is limited scientific evidence to justify its present use. The aim of this study was to evaluate the antidepressant activity of the hydroethanolic extract of *A. zygia* roots (AZE) in murine models.
Methods: AZE was evaluated in the tail suspension test, forced swim test, and the repeated open-space swim test of depression. In order to elucidate the mechanisms of action, the activity of AZE was re-evaluated after treating mice with selective inhibitors of monoamine biosynthesis. The potential of AZE to influence spontaneous locomotion was also examined.
Results: AZE (100–1000 mg/kg, p.o.) reduced the immobility time of mice in the tail suspension and forced swim tests (at least $p < 0.05$). In the repeated open-space swim test, AZE reduced the immobility time (at least $p < 0.05$) while concomitantly increasing the distance swam by mice ($p < 0.01$). However, the antidepressant-like activity of AZE was attenuated by α-methyl-*para*-tyrosine and reserpine ($p < 0.0001$) but not *para*-chlorophenylalanine.
Conclusions: The results of this study indicate that AZE possesses antidepressant-like properties and support the traditional use of AZE for the treatment of depression.

Keywords: α-methyl-*para*-tyrosine, forced swim, open-space swim, *para*-chlorophenylalanine, reserpine, tail suspension

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Introduction

Depression is a common mental disorder characterised by feelings of sadness, low self-worth and guilt, disturbed sleep and/or appetite, lack of motivation, disinterest in pleasurable activities, and recurrent suicidal thoughts [1]. Depression is an important cause of disability worldwide and accounts for 4.3% of the global disease burden [2]. Furthermore, up to two-thirds of patients suffering from major depression are refractory to their first antidepressant drug [3], and the reported benefit of antidepressant medications over placebo is only modest and does not always achieve clinical significance [4], [5]. Also, depressive disorders are highly comorbid with other psychiatric disorders like anxiety and psychosis [6]. Hence, there is on-going search for novel medicines with better efficacy and tolerability. Medicinal plants are a rich source of biodiversity for potential drug discovery. One such plant is *Albizia zygia* (DC.) J.F. Macbr. (Leguminosae). *Albizia zygia* is a medium-sized gum-producing tree up to 25 m high and is widely distributed across Africa [7], [8]. *Albizia zygia* is used in traditional African medicine to treat several diseases including fever, malaria, diarrhoea, and oedema [7], [8]. More importantly, *A. zygia* roots are also used in the treatment of insanity [9]. Previous pharmacological studies have demonstrated the anti-pyretic, anti-nociceptive [10], anti-oedemic, anti-oxidant [11], and anti-psychotic activities of *A. zygia* roots [12]. In acute toxicity studies, no mortality was observed in mice with oral doses up to 5000 mg/kg of the hydroethanolic root extract [10] and stem bark extract [13].

The phytochemical constituents of *A. zygia* have been studied extensively, with several flavonoids, saponins, and glycosides reported [14], [15], [16], [17], [18]. Recently, four triterpenoid saponins, namely coriarioside A, lebbeckoside A, and zygiaosides A and B, were isolated from the hydroethanolic root extract of *A. zygia* [15].

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The zygiaosides A and B were found to exhibit apoptotic effects on the A431 human epidermoid cancer cell lines [15].

On the basis of the traditional use of *A. zygia* roots in the treatment of mental disorders [9], as well as our recent findings of the antipsychotic activity of same [12], we designed this study to evaluate the antidepressant properties of the hydroethanolic root extract of *A. zygia* in murine models for antidepressants.

Materials and methods

Collection of plant material

Albizia zygia roots were collected on the campus of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana (6°40 31.8 N 1°34 44.1 W) in January 2015. The roots were authenticated by Dr. G.H. Sam, a botanist in the Department of Herbal Medicine, KNUST, where a voucher specimen (KNUST/H/M/2016/R001) has been deposited for reference.

Extraction

The extract was prepared as described in our recent work [12]. The roots were cleaned, dried under shade for 14 days, and milled into a coarse powder. The powder (1 kg) was extracted by maceration (27–28 °C) in 5 L of 70% (v/v) ethanol for 5 days. The supernatant was subsequently filtered and concentrated at 60 °C in a rotary evaporator (R-210, Buchi, Switzerland) to obtain a brown-coloured, semi-solid extract. A yield of 9.03% (w/w) was obtained, which was stored at 4 °C until use. The extract is hereafter referred to as *A. zygia* extract (AZE).

High-performance liquid chromatography (HPLC) characterisation

For quality control purposes, a chromatographic fingerprint of the extract was obtained using a chromatography system (model Flexar; Perkin Elmer, MA, USA) equipped with an auto sampler, pumps, and a photodiode array detector. The sample was prepared by dissolving the extract in methanol (50 mg/mL) and separated on a 300 mm × 5 mm, 5 μm particle, Phenomenex C18 column with an injection volume of 20 μL. Gradient elution was performed with a mobile phase of water and methanol in a linear gradient of 1–100% methanol over 40 min. The flow rate was kept constant at 1 mL/min with detection at 205 and 278 nm.

Drugs

The following drugs were used: α-methyl-*para*-tyrosine (AMPT), *para*-chlorophenylalanine (PCPA), reserpine (RES), and caffeine, all from Sigma-Aldrich (St. Louis, MO, USA); fluoxetine (FLX) (Medreich Plc, Feltham, UK); diazepam (Intas, Gujarat, India); and imipramine (IMI) (Almus Pharmaceuticals, Woburn, UK).

AZE, FLX, IMI, caffeine, and diazepam were suspended in 2% (w/v) tragacanth mucilage. RES and AMPT were dissolved in normal saline; 2% tragacanth mucilage was prepared as described earlier [10]. PCPA was prepared according to the method of Bapna and Dandiya [19]: after dissolving with a few drops of 3 N NaOH and diluting with distilled water, the solution was adjusted to pH 9 with 0.1 N HCl. All drugs were freshly prepared before use and administered in a volume of 10 mL/kg body weight. The doses of AZE and the other drugs were selected based on pilot experiments in our laboratory and data from the literature [20], [21], [22], [23], [24], [25].

Animals

Male ICR mice weighing 25–30 g (6–7 weeks old at the time of experiments) were purchased from the Centre for Plant Medicine Research (CPMR), Mampong-Akuapim, Ghana, and housed at the vivarium of the Department of Pharmacology, KNUST (temperature 24–28 °C; relative humidity 60–70%; 12 h light-dark cycle). The mice were housed in colonies with unrestricted access to water and commercial feed (Agricare Ltd., Kumasi, Ghana) and acclimatised to laboratory conditions for 7–10 days before the experiments. A total of 235 mice were used in this study. All procedures in this study were in compliance with the principles regarding the protection of

animals used for scientific purposes (Directive 2010/63/EU). Ethical approval for the study was granted by the Department of Pharmacology Ethics Committee, KNUST.

Tail suspension test

The tail suspension test was carried out as described by Steru et al. [26] with slight modifications. Sixty experimentally naive mice were randomly assigned to 10 groups (n = 6) and treated with either AZE (100, 300 or 1000 mg/kg, p.o.), the antidepressant drugs FLX and IMI (3, 10, or 30 mg/kg, p.o. each), or the vehicle (2% tragacanth mucilage; 10 mL/kg, p.o). Sixty minutes after treatment with the respective drugs, the mice were suspended by their tails with an adhesive tape on an aluminium rod (1 cm diameter) fixed 40 cm above the bench, and video-recorded for 6 min with a camera. The duration of immobility over the last 4 min of the test session was blindly scored by a trained observer using the JWatcher® software (University of California, Los Angeles, CA, USA, and Macquarie University, Sydney, Australia. http://www.jwatcher.ucla.edu/).

Forced swim test

Immediately after the tail suspension test detailed above, the same mice were subjected to the forced swim test, as outlined by Porsolt et al. [27]. Each mouse was gently placed in cylindrical Perspex tanks (diameter: 12 cm; height: 18 cm) containing water (26–27 °C) at a depth of 10 cm and recorded for 6 min by a video camera suspended above the tanks. The duration of immobility over the last 4 min of the test session was obtained by analysing the videos with the ANY-maze® Video Tracking System v5.28 (Stoelting Co., Wood Dale, IL, USA).

Repeated open- space swim test

The repeated open-space swim test by Stone et al. [28] was slightly modified to investigate the chronic antidepressant effects of the extract. The experimental schedule is illustrated in Figure 1. The test was conducted in rectangular plastic tanks (43 × 24 × 23 cm; $l \times b \times h$) with a video camera suspended above to record the mice movement. Each tank was filled with tepid water (32–34 °C) to a depth of 13 cm. Fifty experimentally naive mice were made to swim for 15 min each day for six consecutive days (days 1–6) to induce chronic immobility. The water was changed after every fourth swim session, and no special procedures were used to dry or warm the animals. On day 6, the mice were matched based on the duration of immobility (sixth swim) into seven groups (n = 7). From day 8 to day 22, the mice were treated once daily with either AZE (100, 300, or 1000 mg/kg, p.o.), FLX (3, 10, or 30 mg/kg, p.o.), or the vehicle (2% tragacanth mucilage; 10 mL/kg, p.o). Swim sessions were conducted and recorded on days 11, 15, 18, and 22. The videos were later analysed for the duration of immobility and distance travelled with the ANY-maze® software.

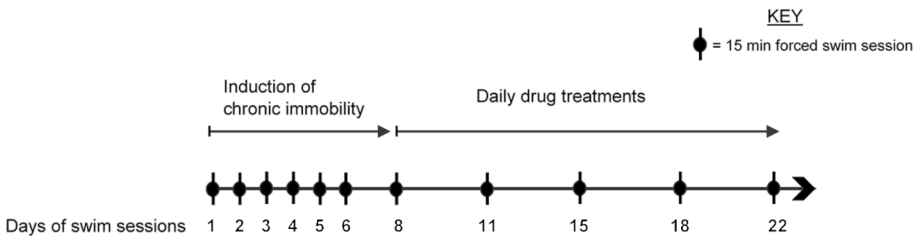

Figure 1: Experimental schedule for the repeated open-space swim test.

Elucidation of possible mechanisms of antidepressant activity

Catecholaminergic mechanisms

A possible role of catecholaminergic mechanisms in the antidepressant effects of AZE was investigated by depleting the catecholamines with RES and AMPT [25]. Sixty experimentally naive mice were randomly assigned to four sets of 15 mice each (Sets I–IV). In order to deplete fresh cytoplasmic stores of the catecholamines, AMPT (400 mg/kg, i.p.) was administered to mice (Set I) 4 h prior to testing. Next, to deplete vesicular monoamine stores, RES (1 mg/kg, s.c.) was administered to the mice (Set II) 24 h prior to testing. Again, mice (Set III) were

treated with RES (1 mg/kg, s.c., 24 h prior to testing) and AMPT (400 mg/kg, i.p., 4 h prior to testing) in order to reduce both vesicular and cytoplasmic stores of the catecholamines. Lastly, the control mice (Set IV) were treated with normal saline (10 mL/kg, i.p.). Thereafter, each set was subdivided into three groups (n = 5) and treated with either AZE (1000 mg/kg, p.o.), IMI (20 mg/kg, p.o.), or vehicle (2% tragacanth mucilage; 10 mL/kg, p.o) 1 h before testing. Afterwards, the tail suspension test was conducted and the duration of immobility over the 6-min test session was quantified as described above.

Serotonergic mechanisms

A potential role of serotonergic mechanisms in the antidepressant actions of AZE was also investigated. Thirty experimentally naive mice were randomly allocated to two sets of 15 mice each. To selectively deplete serotonin, one set of mice was treated with PCPA (300 mg/kg, i.p.) every 24 h for 3 days with the last dose administered 20 h prior to the behavioural test [21]. The other set of mice was treated with normal saline (10 mL/kg, i.p.) on the same schedule. Both sets were subdivided into three groups (n = 5) and given either AZE (1000 mg/kg, p.o.), FLX (20 mg/kg, p.o.), or the vehicle (2% tragacanth mucilage; 10 mL/kg, p.o.) 1 h before testing. Afterwards, the tail suspension test was conducted and the duration of immobility over the 6-min test session was quantified as described above.

Spontaneous locomotion

The open field test [29] was conducted to examine the influence of the extract on spontaneous locomotor activity. The set-up consisted of a video camera suspended above an acrylic chamber ($40 \times 40 \times 30\ cm^3$; $l \times b \times h$) to record the mice's movement. Thirty-five experimentally naive mice (n = 5) were allocated to the treatment groups as follows: AZE (30, 100, 300, or 1000 mg/kg, p.o.), caffeine (18 mg/kg, p.o.), diazepam (6 mg/kg, p.o.), or the vehicle (2% tragacanth mucilage; 10 mL/kg, p.o). Sixty minutes after drug administration, the mice were placed in the acrylic chamber in turn, and recordings were made for 30 min each. The acrylic chamber was cleaned thoroughly with ethanol (20% v/v) to remove any residual odour before each test session. Subsequently, the videos were analysed using the ANY-maze® system to obtain the distance covered by the mice.

Analysis of data

GraphPad Prism version 6.01 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses in this study. Two-factor repeated-measures analysis of variance (RM-ANOVA) with *treatment* as the main factor and *time* as the repeat factor was used to analyse the time course curves. The areas under the curve (AUCs) were also determined to assess the general treatment effect. The immobility time in the monoamine depletion tests were analysed by two-factor (*treatment* × *pre-treatment*) ANOVA complemented by Sidak's multiple comparisons test. All other data were analysed by one-way ANOVA followed by Dunnett's *post hoc* test. A p-value less than 0.05 was considered statistically significant.

Results

Characterisation of plant extract

Triplicate HPLC chromatograms of the extract at 205 and 278 nm are shown in Figure 2A and B, respectively.

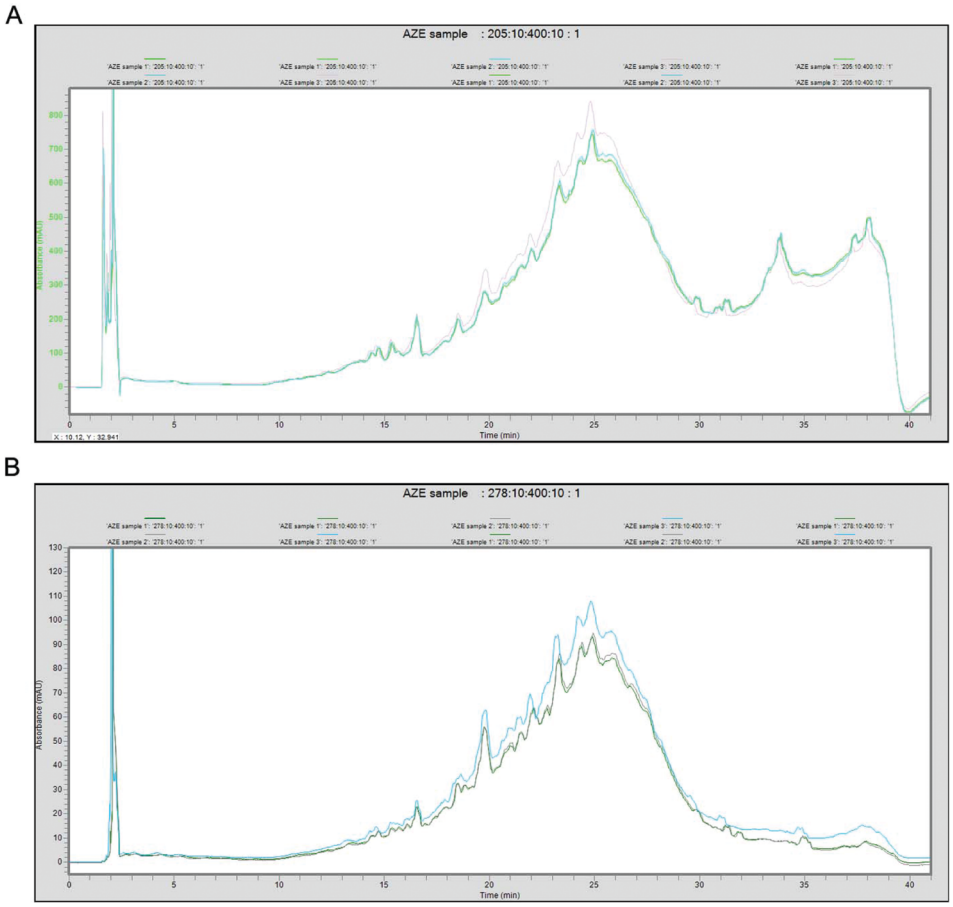

Figure 2: Triplicate HPLC chromatograms of AZE detected at (A) 205 nm and (B) 278 nm.

Tail suspension test

Figure 3 shows the effects of AZE, FLX, and IMI on the immobility time of mice in TST. ANOVA revealed that the immobility time was affected by AZE ($F_{3,19}$ = 3.381, p = 0.0397; Figure 3A), FLX ($F_{3,18}$ = 6.332, p = 0.0040; Figure 3B), and IMI ($F_{3,18}$ = 9.038, p = 0.0007; Figure 3C). Dunnett's *post hoc* test showed that AZE reduced the immobility time at 300 mg/kg (p = 0.0327) and 1000 mg/kg (p = 0.0437). Both standard drugs FLX and IMI reduced immobility at all doses tested (at least p < 0.05, Figure 3B, C).

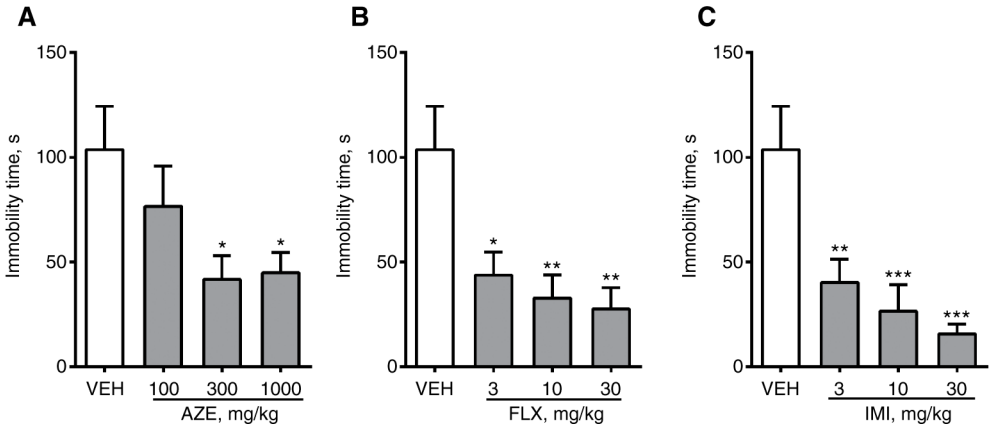

Figure 3: Effects of AZE (100–1000 mg/kg.), FLX (3–30 mg/kg), and IMI (3–30 mg/kg) on the immobility time in the tail suspension test.
*p < 0.05, **p < 0.01, ***p < 0.001 versus vehicle group (one-way ANOVA; Dunnett's *post hoc* test). Data are expressed as mean ± SEM (n = 6).

Forced swim test

Figure 4 shows the effects of AZE, FLX, and IMI on the immobility time of mice in FST. ANOVA showed that the immobility time was affected by AZE ($F_{3,20}$ = 6.609, p = 0.0028; Figure 4A), FLX ($F_{3,20}$ = 7.828, p = 0.0012, Figure 4B), and IMI ($F_{3,20}$ = 6.930, p = 0.0022, Figure 4C). Follow-up analysis with Dunnett's *post hoc* test indicated that only the highest dose of AZE (1000 mg/kg) significantly reduced the immobility time (p = 0.0437). On the other hand, both FLX and IMI exhibited significant effects at 10 and 30 mg/kg (at least p < 0.05).

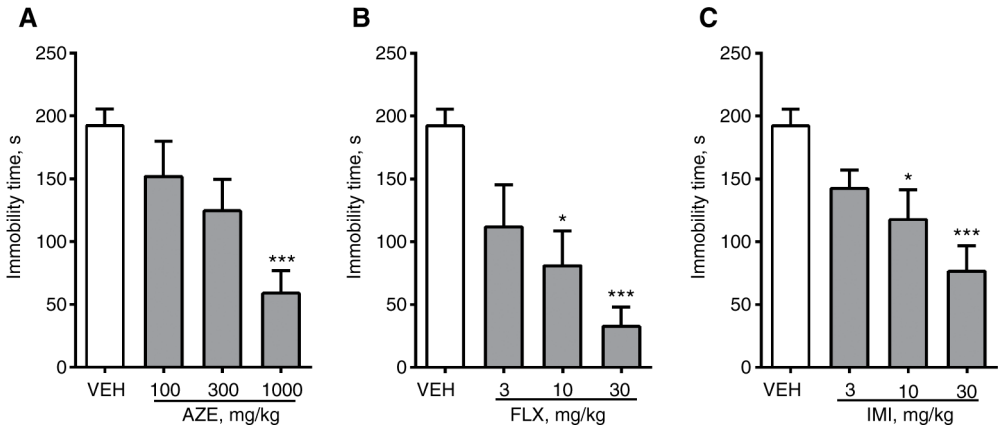

Figure 4: Effects of AZE (100–1000 mg/kg), FLX (3–30 mg/kg), and IMI (3–30 mg/kg) on the immobility time in the forced swim test.
*p < 0.05, ***p < 0.001 versus vehicle group (one-way ANOVA; Dunnett's *post hoc* test). Data are expressed as mean ± SEM (n = 6).

Repeated open-space swim test

Figure 5 shows the effects of AZE and FLX on the immobility time of mice in ROSST. As shown in the time course curves (Figure 5A, C), repeated swimming for 6 days resulted in an increase in the immobility time, which was affected by AZE (100–1000 mg/kg) and FLX (3–30 mg/kg). Considering the AUCs, ANOVA showed that AZE ($F_{3,19}$ = 4.878, p = 0.0111; Figure 5B) and FLX ($F_{3,19}$ = 15.67, p < 0.0001; Figure 5D) influenced the immobility time. Subsequent analysis of the AUCs by Dunnett's *post hoc* test revealed that AZE reduced immobility at a dose of 300 mg/kg (p = 0.0036) while FLX was effective at 10 mg/kg (p = 0.0035) and 30 mg/kg (p < 0.0001).

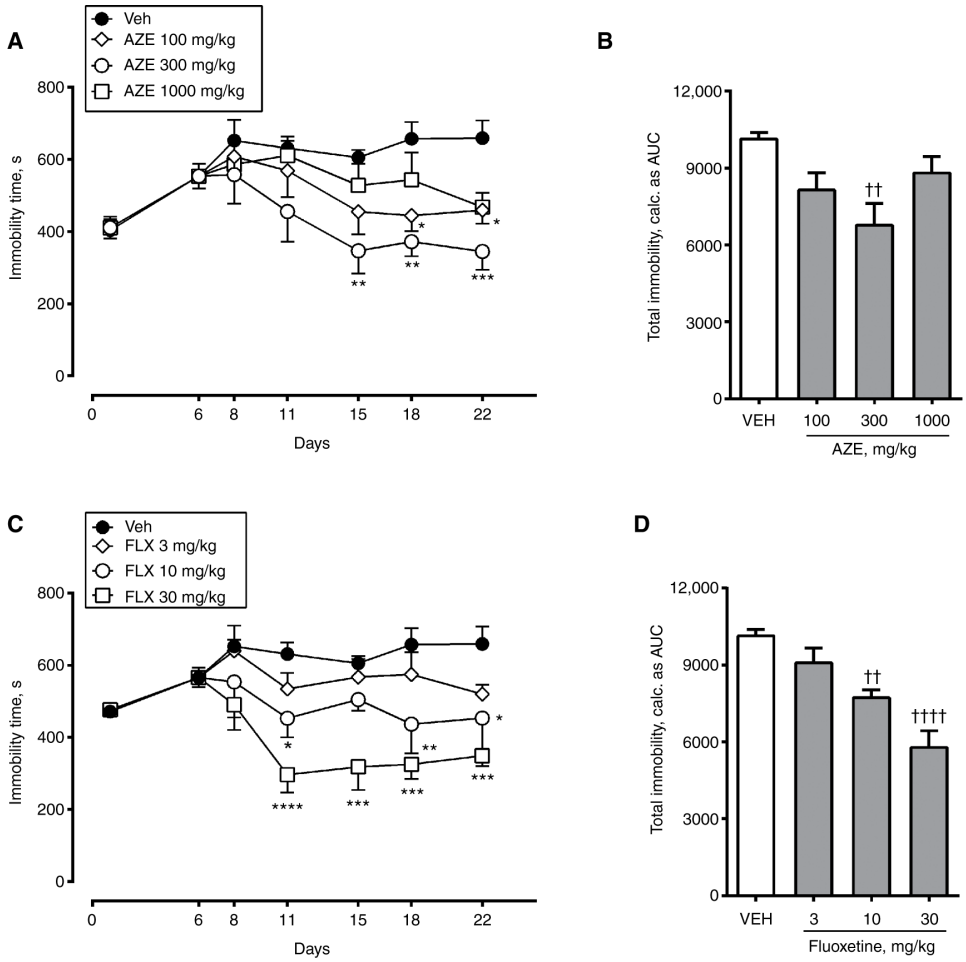

Figure 5: Effects of AZE (100–1000 mg/kg) and FLX (3–30 mg/kg) on immobility time in the repeated open-space swim test: time course curves (A, C) and total immobility calculated as AUCs (B, D).
*p < 0.05, **p < 0.01, ***P < 0.001, ****p < 0.0001 versus the vehicle group (two-way RM-ANOVA; Dunnett's *post hoc* test). ††p < 0.01, ††††p < 0.0001 versus the vehicle group (one-way ANOVA; Dunnett's *post hoc* test). Data are expressed as mean ± SEM (n = 6).

The effects of AZE and FLX on the distance swam by mice in the repeated open-space swim test can be seen in Figure 6. In parallel to the immobility time, there was a corresponding increase in the distance swam after 6 days of repeated swimming (Figure 6A, C), which was affected by AZE (100–1000 mg/kg) and FLX (3–30 mg/kg). Analysis of the resulting AUCs by ANOVA showed that AZE ($F_{3,19}$ = 4.878, p = 0.0111; Figure 6B) and FLX ($F_{3,19}$ = 15.67, p < 0.0001; Figure 6D) affected the distance swam. AZE increased the distance swam at doses of 300 mg/kg (p = 0.0004) and 1000 mg/kg (p = 0.0285). Similarly, FLX increased the distance swam at doses of 10 mg/kg (p = 0.0023) and 30 mg/kg (p < 0.0001).

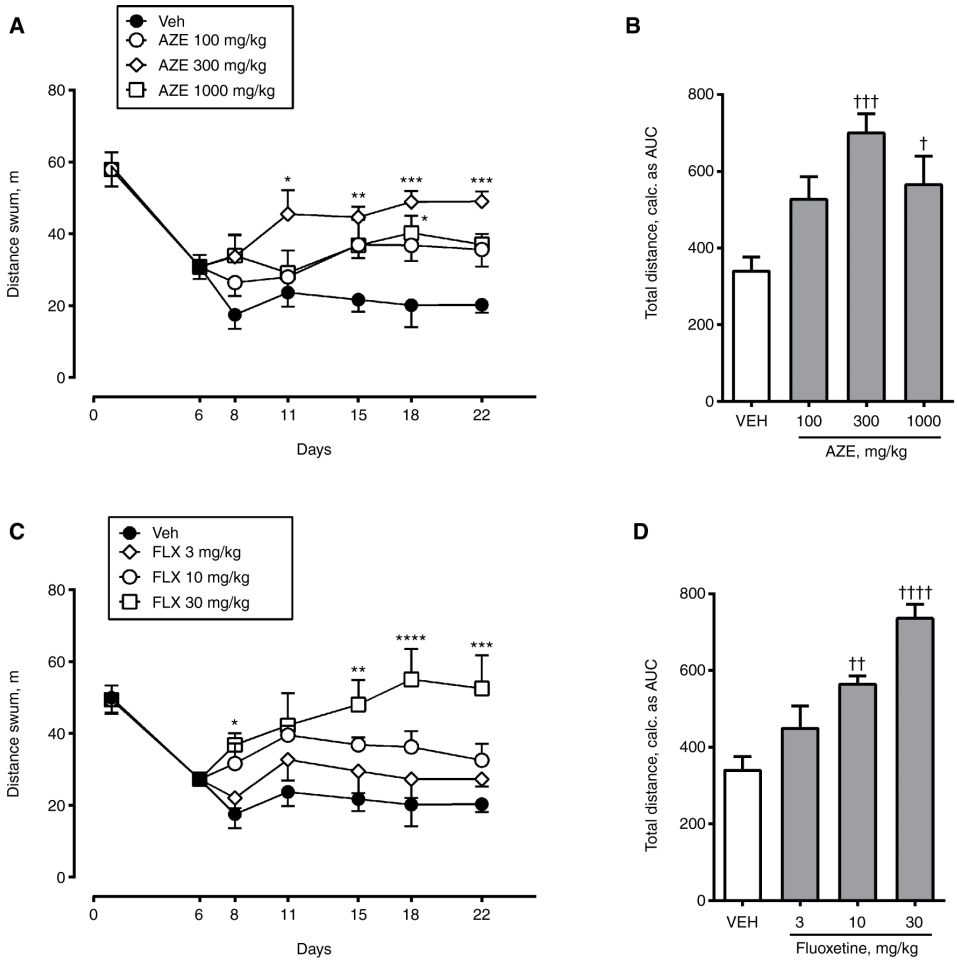

Figure 6: Effects of AZE (100–1000 mg/kg) and FLX (3–30 mg/kg) on the distance swam in the repeated open-space swim test: time course curves (A, C) and total distance calculated as AUCs (B, D).
*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 versus the vehicle group (two-way RM-ANOVA; Dunnett's *post hoc* test).
†p < 0.05, ††p < 0.01, †††p < 0.001, ††††p < 0.0001 versus the vehicle group (one-way ANOVA; Dunnett's *post hoc* test). Data are expressed as mean ± SEM (n = 6).

Investigation of potential antidepressant mechanisms

Figure 7 shows the effect of PCPA, AMPT, and RES on the antidepressant-like effects of AZE, FLX, and IMI in TST. Pre-treatment of mice with AMPT (400 mg/kg) reversed the antidepressant-like effects of AZE (p = 0.0009, Figure 7A) and IMI (p = 0.0029, Figure 7A).

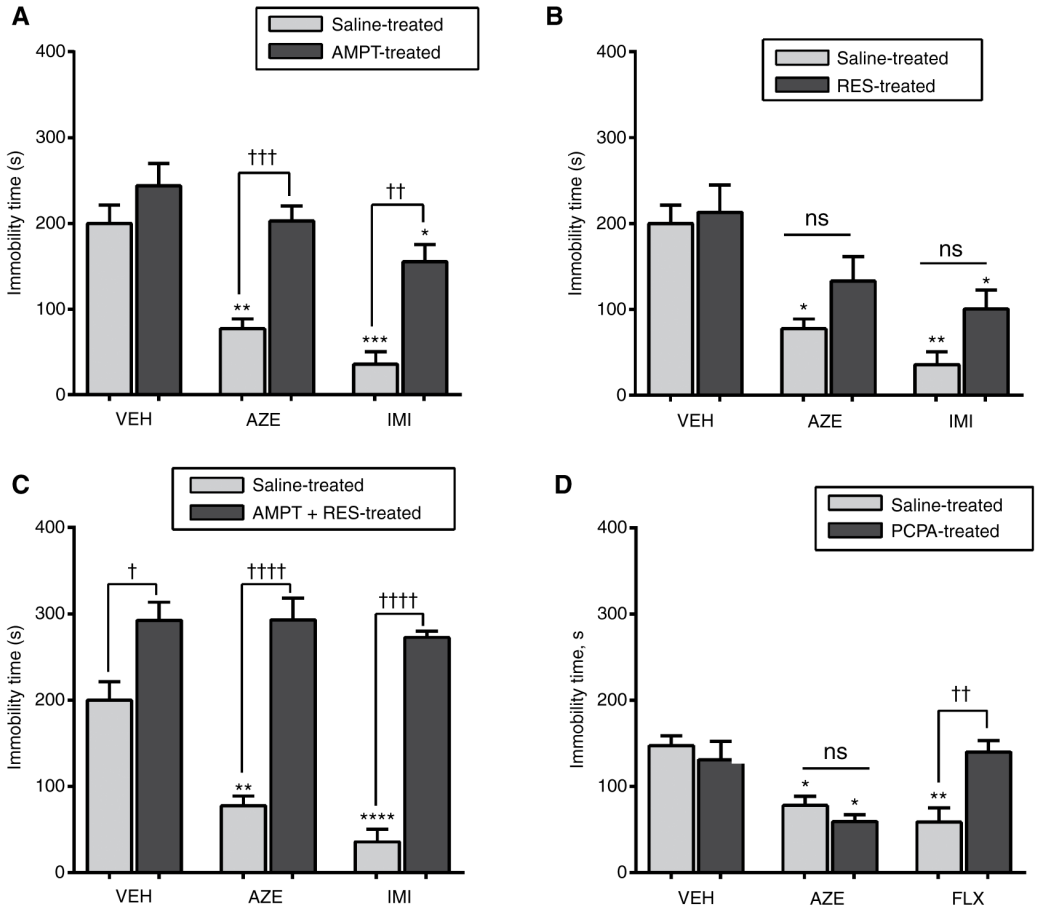

Figure 7: Effects of (A) AMPT, (B) RES, (C) RES + AMPT, and (D) PCPA on the antidepressant-like effects of AZE (1000 mg/kg), IMI (20 mg/kg), and FLX (20 mg/kg) in the tail suspension test.
*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 versus the corresponding vehicle controls. †p < 0.05, ††p < 0.01, †††p < 0.001, ††††p < 0.0001 versus pre-treated group (two-way ANOVA; Sidak's multiple comparisons test). Data are expressed as mean ± SEM (n = 5) (ns, non-significant).

Also, in mice pre-treated with RES (1 mg/kg), the antidepressant-like effects of AZE and IMI were attenuated, although these changes were not significant (p > 0.05, Figure 7B). Pre-treatment with RES and AMPT completely abolished the antidepressant-like effects of AZE and IMI (both p < 0.0001, Figure 7C).

Lastly, in mice pre-treated with PCPA, the antidepressant-like effect of FLX was abolished (p = 0.0076; Figure 7D). However, the antidepressant-like effect of AZE persisted after serotonin depletion by PCPA (Figure 7D).

Open field test

Table 1 shows the effect of AZE (30–1000 mg/kg) on spontaneous locomotion in the open field test. AZE showed no psychostimulatory effects, as indicated by no increase in the distance travelled. However, AZE at 300 mg/kg decreased the distance travelled. Caffeine (18 mg/kg) increased the distance travelled, while diazepam (6 mg/kg) decreased it.

Table 1: Effect of AZE (30–1000 mg/kg), caffeine (18 mg/kg), and diazepam (6 mg/kg) on the distance travelled by mice in the open field test.

Treatment, mg/kg	Total distance travelled, m	p-Value
Vehicle	20.85 ± 4.07	–
AZE 30	16.00 ± 0.62	0.7002
AZE 100	16.88 ± 3.32	0.8373
AZE 300	8.86 ± 0.88*	0.0334
AZE 1000	18.37 ± 1.97	0.9765
Diazepam 6	7.57 ± 0.42*	0.0435
Caffeine 18	32.60 ± 4.68*	0.0380

Data are expressed as mean ± SEM (n = 5) (one-way ANOVA; Dunnett's *post hoc* test). *p<0.05

Discussion

The present study examined the antidepressant property of AZE in several tests for antidepressant activity: the forced swim test, the tail suspension test, and the repeated open-space swim test. In summary, there was a significant reduction in immobility in all three tests and an increase in distance swam in the repeated open-space swim test. This observed antidepressant activity of AZE was abolished by pre-treatment with AMPT and RES but not PCPA. These data suggest that AZE possesses an antidepressant-like property, which is likely mediated by catecholaminergic mechanisms.

First, AZE was evaluated in two acute tests for antidepressants, namely the tail suspension test and the forced swim test. These two tests are widely used pre-clinical tests for antidepressant activity screening because they are easy to use, are reliable, and have good predictive validity [30], [31], [32]. In both tests, a rodent is subjected to a stressful, inescapable situation, and after an initial period of escape-oriented behaviours, assumes an immobile posture [32]. This immobility reflects a failure to persist in escape-directed movements, i.e. behavioural despair, and it has been shown that most antidepressants reverse this immobility [33]. In this study, AZE reduced the immobility time of mice, similar to the standard antidepressants FLX and IMI. These results suggest an antidepressant-like effect of AZE, because a diminution in immobility in these tests is predictive of antidepressant activity [32]. The effect of AZE in the forced swim test was weaker than in the tail suspension test: only a dose of 1000 mg/kg was active. However, this was not surprising since it has been noted that the tail suspension test exhibits a different spectrum of pharmacological sensitivity than the forced swim test [31].

On basis of the foregoing results, the repeated open-space swim test was conducted to evaluate the antidepressant effect of chronic AZE treatment. This test permits investigators to evaluate the effect of antidepressant drugs on chronic immobility. In this test, mice are forced to swim daily for 6 days, which results in a progressive increase in immobility duration over swim sessions, and a corresponding reduction in distance swam [28], [34]. These changes persist for several weeks and are reversed by antidepressants [28], [34]. The repeated open-space swim test offers two key advantages. First, it is sensitive to chronic but not acute antidepressant treatment in contrast to the forced swim test and tail suspension test [34]. Thus, the repeated open-space swim test better reflects depression pharmacotherapy in humans where there is a therapeutic lag of several weeks of treatment before benefits are seen [35]. Second, the repeated open-space swim test enables researchers to track the time course of drug action over several swim sessions, which is not possible in the acute tests [28]. Once again, AZE showed a significant antidepressant effect by decreasing immobility while concomitantly increasing the distance swam. This buttresses the results from the forced swim and tail suspension tests. A similar antidepressant effect was seen with FLX, consistent with previous studies [22], [28].

Although behavioural despair tests such as the forced swim test and tail suspension test have high predictive validity, they are unable to distinguish central stimulants, which also decrease immobility, from genuine antidepressants [36]. For instance, psychomotor stimulants such as caffeine and amphetamine generate misleading positive results in the forced swim test [37]. In light of this possibility, we also examined the effect of AZE on spontaneous locomotion in the open field test. The results obtained indicated that the anti-immobility effect of AZE is not associated with enhanced locomotor activity. This finding is consistent with a previous report [10], where AZE did not affect motor coordination in the rotarod test, up to a dose of 300 mg/kg. Taken together, these data suggest that the reduction in the immobility time in the forced swim test and tail suspension test is unlikely due to a psychomotor stimulant effect. There was, however, an interesting finding of decreased locomotor activity with AZE at the dose of 300 mg/kg. The mechanism of this effect is not immediately apparent and needs to be assessed in future studies.

Interestingly, these results are at variance with those of Amoateng and colleagues [38] who did not detect any antidepressant effect of an ethanolic leaf extract of *A. zygia* at the same doses employed in this study. This may be due to the different bioactive constituents present in the leaves and roots, since it has previously been shown that different parts of the same plant may contain different phytochemical constituents [39], [40]. Nonetheless, this discrepancy highlights the importance of reproducible characterisation of plant extracts in ethno-pharmacological studies to permit comparisons between labs. In this direction, therefore, we analysed AZE by HPLC to produce a fingerprint for quality control purposes.

Finally, an attempt was made to elucidate the underlying mechanisms involved in the antidepressant actions of AZE. The monoamine theory of depression, which has now become textbook knowledge, posits that depression results from a deficit of noradrenaline and serotonin in the brain [35]. Although there are several classes of antidepressants, their general mode of action is by enhancing monoaminergic transmission in the brain [41]. Consequently, prior depletion of neurotransmitters has been used as a strategy for assessing the importance

of various neurotransmitters to the actions of antidepressant drugs [25]. Therefore, the antidepressant activity of AZE was re-evaluated in the tail suspension test after pre-treatment with selective inhibitors of monoamine biosynthesis, namely RES, AMPT, and PCPA. RES is an irreversible inhibitor of the vesicular monoamine transporter (VMAT 2) and depletes vesicular stores of monoamines, whereas AMPT depletes neuronal stores of the catecholamines, dopamine, and noradrenaline by inhibiting their synthesis [21], [42]. PCPA is an inhibitor of tryptophan hydroxylase, the rate-limiting enzyme in the biosynthesis of serotonin [25].

AMPT pre-treatment reversed the antidepressant-like effects of AZE and IMI, suggesting the possible role of the catecholamines in the antidepressant effect of AZE. Unlike that of IMI, the antidepressant-like activity of AZE was completely lost. This result indicates that cytosolic stores of the catecholamines are crucial for the antidepressant-like effect of AZE. The finding in the case of IMI was expected since IMI inhibits the re-uptake of both noradrenaline and serotonin [43] and AMPT does not affect serotonin synthesis.

Also, RES pre-treatment mildly attenuated the antidepressant-like effects of AZE and IMI. Here too, the effect of AZE was abolished unlike that of IMI which persisted. This result suggests that vesicular release of monoamines is involved in the antidepressant-like effect of AZE since RES depletes vesicular stores of monoamines. Consistent with the first two results, co-administration of AMPT and RES completely abolished the antidepressant-like effects of IMI and AZE. This supports the hypothesis that both cytosolic and vesicular stores of the catecholamines are vital for the antidepressant actions of AZE. Lastly, depletion of serotonin by PCPA abolished the antidepressant actions of the selective serotonin re-uptake inhibitor FLX but not AZE, indicating that serotonergic mechanisms are not vital to the antidepressant-like actions of AZE.

Conclusions

The findings of this study indicate that *A. zygia* roots possess antidepressant-like properties, which are mediated, at least in part, by catecholaminergic mechanisms. This supports the traditional use of *A. zygia* roots for the treatment of depression.

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