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Xylopia aethiopica fruit extract exhibits antidepressant-like effect via interaction with serotonergic neurotransmission in mice

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Desipramine (PubChem CID: 2995): Fluoxetine (PubChem CID: 3386)
l-arginine (PubChem CID: 6322)
N_ω-nitro- l-arginine methyl ester (PubChem CID: 135193)
Noradrenaline (PubChem CID: 439260)
Reserpine (PubChem CID: 5770)

ABSTRACT

Ethnopharmacological relevance: *Xylopia aethiopica* has been used traditionally to treat some central nervous system disorders including epilepsy.
Aim of the study: Despite the central analgesic and sedative effects, there is little evidence for its traditional use for CNS disorders. This study thus assessed the antidepressant potential of *Xylopia aethiopica* ethanolic fruit extract (XAE).
Material and methods: Antidepressant effect was assessed in the forced swim test (FST) and tail suspension test (TST) models in mice. The role of monoamines in the antidepressant effects of XAE was evaluated by selective depletion of serotonin and noradrenaline, whereas involvement of NMDA/nitric oxide was assessed with NMDA receptor co-modulators; D-serine and D-cycloserine and NOS inhibitor, L-NAME.
Results: *Xylopia aethiopica* (30, 100, 300 mg kg^{-1}) dose dependently reduced immobility in both FST and TST. The reduced immobility was reversed after 5-hydroxytryptamine (5-HT) depletion with tryptophan hydroxylase inhibitor—*p*-chlorophenylalanine (*p*CPA) and after monoamine depletion with vesicular monoamine transporter inhibitor—reserpine. The observed antidepressant effect was not affected by catecholamine depletion with the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine (AMPT). Similarly XAE did not potentiate the toxicity of a sub-lethal dose of noradrenaline. XAE had a synergistic effect with the glycine$_B$ receptor partial agonist, D-cycloserine and nitric oxide synthase inhibitor, L-NAME. However established antidepressant effects of XAE were abolished by NMDA and NOS activation with D-serine and L-arginine.
Conclusion: This study shows that *Xylopia aethiopica* has antidepressant potential largely due to effects on 5-HT neurotransmission with possible glutamatergic effect through the glycine$_B$ co-binding site and nitric oxide synthase inhibition.

1. Introduction

Depression is a chronic debilitating disease with wavering symptomatology that affects over 120 million people globally (Poleszak et al., 2011). The WHO estimates that depression would have the highest burden of disease by the year 2030 (Kirsch et al., 2008; Cohn et al., 2012). In the USA, direct and indirect cost due to depression run up an estimated 83 billion dollars a year and similar economic implications exists in other countries (Greenberg et al., 2003). In West Africa, Ghanaian university students have the highest prevalence of depression in the sub-region (Asante and Andoh-Arthur, 2015). Although strides have been made in the search for antidepressants in the last few decades, the search for newer antidepressant is still relevant due to sub-optimal efficacies with clinical effects of current therapies not differing much from placebos (Khan et al., 2000; Kirsch et al., 2002). Over 40% of patients are refractory to current treatment whereas most antidepressants have slower onset of action (Rosenzweig-Lipson et al., 2007; Kirsch et al., 2008). Thus the race for better antidepressants is still relevant.

Xylopia aethiopica (Annonaceae) is a common spice in West Africa. It has been used traditionally for several disorders including neurological diseases like epilepsy (Souza and Dossa, 1988), inflammatory disorders: bronchitis, haemorrhoids and rheumatism (Igwe et al., 2003) and painful conditions such as neuralgia and lumbago. It has been shown to have anti-inflammatory effects (Obiri and Osafo, 2013), central analgesic (Ameyaw et al., 2014, Woode et al., 2013), sedative (Biney et al., 2014) and anticonvulsant effects (Okoye et al., 2013). The antiproliferative effects against human cervical cancer cells has been also reported

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(Choumessi et al., 2012) in addition to protective effect on γ radiation-induced liver and kidney damage (Adaramoye et al., 2010) while Woode et al. (2011) have also evaluated its effect on reproductive functions.

It is recognized that certain drugs that have been successful in the management of one neurological disorder have also found translational use in the management of other CNS disorders (O'Connor and Dworkin, 2009; Whiteside et al., 2010). For example, tricyclic antidepressants (TCAs) and the serotonin and noradrenaline reuptake inhibitor duloxetine is used as a first-line drug in managing neuropathic disorders like diabetic neuropathy and other chronic painful disorders such as fibromyalgia (O'Connor and Dworkin, 2009) while the anti-epileptic lamotrigine have also been used to manage mood and affective disorders (Ettinger and Argoff, 2007). Thus, the central analgesic and anticonvulsant effects of *Xylopia aethiopica* could suggest possible effects in other neurologic disorders like depression.

Activation of immune cells in the brain by induced inflammatory cytokines disturbs neuroendocrine function, neurotransmitter metabolism and neural plasticity leading to development of depression (Anisman et al., 2008; Dantzer et al., 2008; Raison et al., 2010). Recognizing significant nexus between inflammation and neurological disorders, the reported anti-inflammatory effects of *Xylopia aethiopica* as well as its CNS effects, this study evaluates the antidepressant potential of *Xylopia aethiopica* fruit extract and possible mechanisms underlining this.

2. Materials and methods

2.1. Animals

Swiss Webster mice (20–25 g, 8–10 weeks) were obtained from Noguchi Memorial Institute of Medical Research (NMIMR), University of Ghana, Accra and housed in the vivarium of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST), for acclimatization until they were used. Animals were housed 10 mice per cage with soft wood shavings as bedding in a 12/12 h day/night cycle. Food (normal mice *chow*: Agricare Ltd, Kumasi, Ghana) and water (normal tap water) was *ad libitum*. All experiments were carried out in accordance with NIH Guidelines for the Care and Use of Laboratory Animals with ethical approval from the Department of Pharmacology Animal Ethics Committee.

2.2. Extract preparation

Fresh unripe fruits of *Xylopia aethiopica* were harvested from KNUST Botanic Gardens (06° 41′6.38″ N; 01° 33′ 44.34″ W) in December 2013. Its authenticity was confirmed by comparison to voucher specimen (FP/09/77) at Department of Herbal Medicine, KNUST. It was shade-dried, milled and then 2 kg was cold macerated with 70% (v/v) ethanol for 72 h. The extract obtained was concentrated to a semisolid brownish mass (yield 32.5%). An HPLC fingerprint of the extract (Fig. 1) was obtained to characterize the extract as previously described by Adosraku and Kyekyeku (2011).

2.3. Forced swim test

The forced swim test was performed as outlined by Porsolt et al. (1977) and slightly modified. Animals (n=8) received either orally *Xylopia aethiopica* extract (XAE) 30, 100 or 300 mg kg^{-1}, fluoxetine (FLX) 3, 10 or 30 mg kg^{-1}, desipramine (DES) 3, 10 or 30 mg kg^{-1} or distilled water 10 ml kg^{-1}. Based on preliminarily determined time of peak effect (TPE), behavioural experiments were conducted after 120 min for XAE and 60 min for DES, FLX and distilled water post drug treatment. Mice were gently placed in identical cylindrical plastic tanks (25 cm high, 10 cm internal diameter) containing water (23 ± 1 °C) 15 cm deep and allowed to swim for six minutes recording with a camera suspended 80 cm above the tanks. Duration of escape oriented behaviours (climbing and swimming) and immobility over the last four minutes of the test were quantified by an experienced observer blinded to all treatment groups using the public domain software JWatcher Version 1.0™ (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia. Available at ⟨http://www.jwatcher.ucla.edu/⟩).

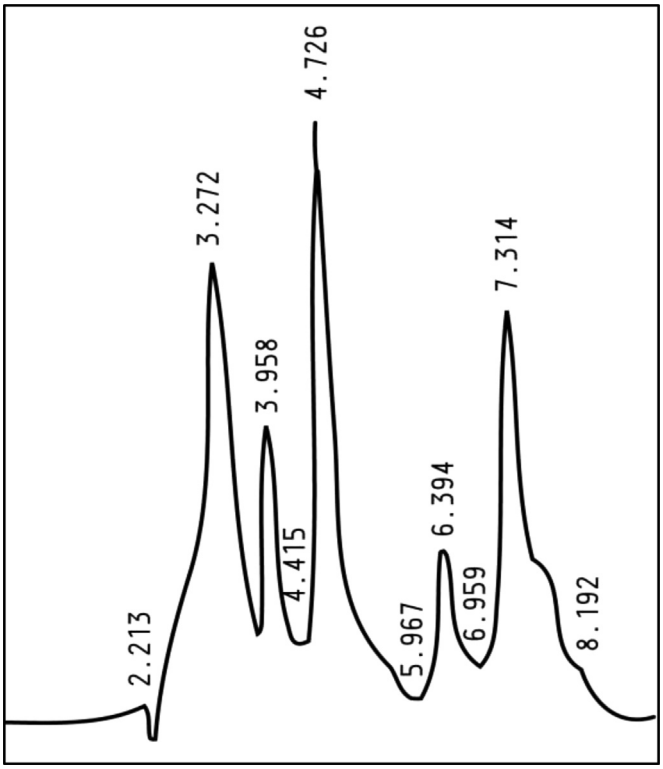

Fig. 1. HPLC fingerprint of ethanolic fruit extract of Xylopia aethiopica. Mobile phase: methanol and water (9:1) eluted isocratically at 0.5 ml min^{-1} and absorbance of eluent was monitored at 206 nm.

2.4. Tail suspension test

Tail suspension test as earlier described was employed (Steru et al., 1985). Randomly-grouped Swiss mice (n=8) received XAE 30, 100 or 300 mg kg^{-1}, fluoxetine 3, 10 or 30 mg kg^{-1}, desipramine 3, 10 or 30 mg kg^{-1} or vehicle 10 ml kg^{-1}. At time of peak effect, they were individually suspended at their tail (1 cm from the tip) with an adhesive tape on a horizontal bar raised 52 cm from a table top. Duration of escape-oriented behaviours (pedaling, curling and swinging) and immobility were recorded with a camera for 6 min and quantified with JWatcher™ by an experienced observer blinded to all treatment groups. Mice that climbed on their tail were gently pulled down and the test continued.

2.5. Effect of monoamines

The possible involvement of monoamines in the observed antidepressant-like effects of XAE was assessed by inhibition of storage or synthesis of monoamines based on previous work by O'Leary and colleagues (O'Leary et al., 2007). To deplete both cytoplasmic pools and vesicular stores of 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine (DA), mice were assigned

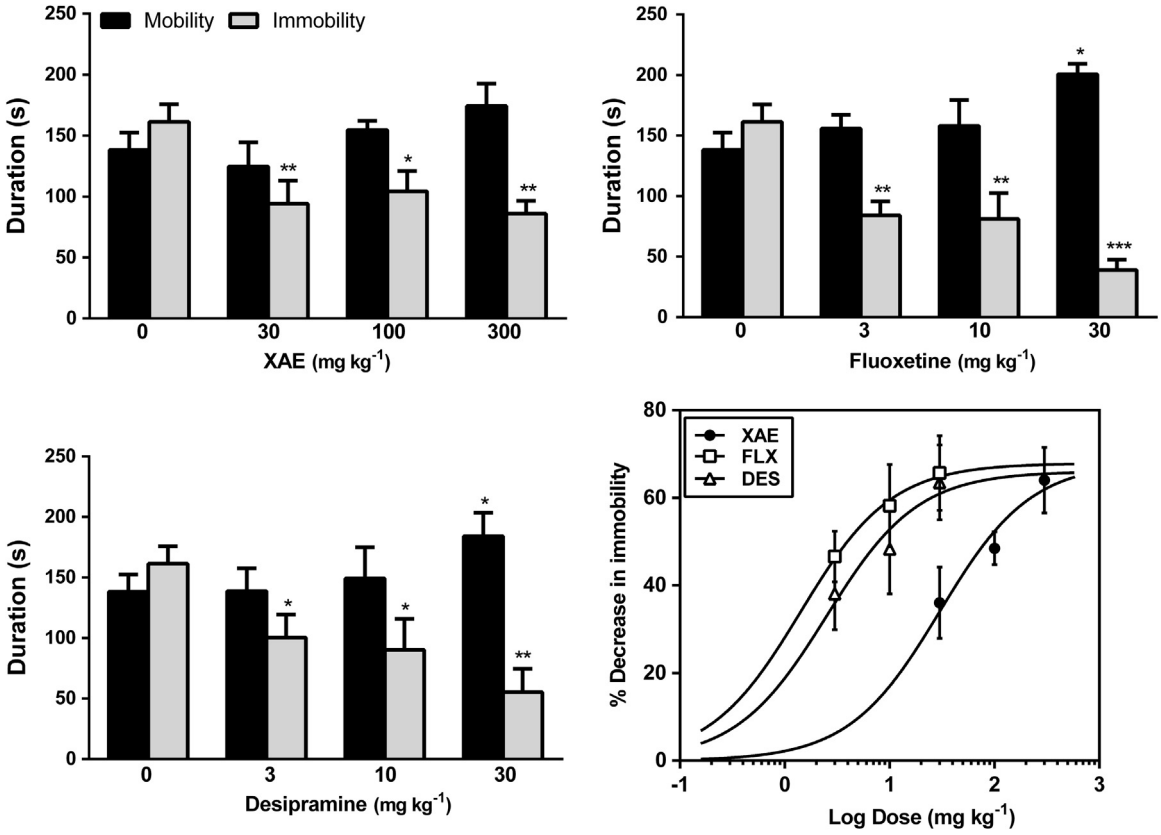

Fig. 2. *Xylopia aethiopica* exhibits antidepressant-like effects in the forced swim test. Effect of XAE (30, 100, 300 mg kg^{-1}), fluoxetine (3, 10, 30 mg kg $^{-1}$) and desipramine (3–30 mg kg^{-1}) in the forced swim test.*$P < 0.05$ **$P < 0.01$ ***$P < 0.001$ Inset: Dose response curves for XAE, FLX and DES in the forced swim test.

to two groups. One group was pretreated with a single dose of the vesicular monoamine transporter (VMAT) inhibitor, reserpine, (1 mg kg^{-1}) and the other 10 ml kg^{-1} distilled water. Eighteen hours after pretreatment both naïve and reserpine-treated groups received the vehicle or equipotent doses of XAE (100 mg kg^{-1}), fluoxetine (10 mg kg^{-1}) or desipramine (10 mg kg^{-1}) before behavioural testing in the forced swim test.

2.6. Effect of 5-hydroxytrptamine

The role of 5-hydroxytryptamine in the antidepressant effect of XAE was evaluated by selective inhibition of 5-hydroxytryptamine synthesis. Mice were randomly assigned to two groups. One group received a tryptophan hydroxylase inhibitor, *p*-chlorophenylalanine, (*p*CPA) 300 mg kg^{-1} i. p. daily for 3 consecutive days and the other 10 ml kg^{-1} normal saline. Twenty hours after the last pretreatment, both naïve and treated groups (n=8 each) received distilled water or equipotent doses of XAE (100 mg kg^{-1}), fluoxetine (10 mg kg^{-1}) or desipramine (10 mg kg^{-1}). One hour post drug treatments, behavioural effects were assessed in the forced swim test. The behaviours were recorded and quantified as described above.

2.7. Involvement of catecholamines

The influence of catecholamines was assessed by selective depletion of noradrenaline and dopamine with a single dose of a tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine (AMPT) 100 mg kg^{-1} i. p. Mice were assigned to two groups (untreated and treated) and received equipotent doses of either XAE (100 mg kg^{-1}), fluoxetine (10 mg kg^{-1}) or desipramine (10 mg kg^{-1}) alone (untreated) or in combination with AMPT (treated) (n=8 each) given 4 h earlier followed by behavioural assessment in the FST earlier described.

In another experiment, mice (n=10) (25–30 g) were treated with either XAE 30, 100, 300 mg kg^{-1}, desipramine 3, 10, 30 mg kg^{-1} or distilled water 10 ml kg^{-1} *p. o*. One hour post treatments, all animals received a sub-lethal dose of the catecholamine noradrenaline (3 mg kg^{-1} i. p.). They were then observed 6 h continuously and subsequently twelve hourly for 48 h for death.

2.8. Involvement of glutamatergic transmission

Glutamatergic involvement was assessed as described in Poleszak et al. (2011). Mice were randomly assigned to two groups (untreated and treated) and received either a sub-effective dose of XAE (10 mg kg^{-1}), FLX (3 mg kg^{-1}) or DES (3 mg kg^{-1}) alone (untreated) or in combination with D-cycloserine (2.5 mg kg^{-1} i. p.) (n=8). In a similar paradigm, mice either received orally, XAE 100 mg kg^{-1}, fluoxetine 30 mg kg^{-1} or desipramine 30 mg kg^{-1} alone or in combination with D-serine (320 mg kg^{-1} i. p.) (n=8). Behavioural effects were assessed in the FST in each instance, one hour later as described above.

2.9. Involvement of nitric oxide

Mice were randomly assigned to two groups (untreated and treated) and received a sub-effective dose of XAE (10 mg kg^{-1}), FLX (3 mg kg^{-1}) or DES (3 mg kg^{-1}) alone (untreated) or in combination with L-NAME (20 mg kg^{-1} i. p.). Similarly, in another experiment, mice (n=8) either received *p. o.* XAE 100 mg kg^{-1}, fluoxetine 30 mg kg^{-1} or desipramine 30 mg kg^{-1} alone or in combination with L-arginine (750 mg kg^{-1} i. p.). Behavioural effects were assessed in the FST in either instance one hour later as described above.

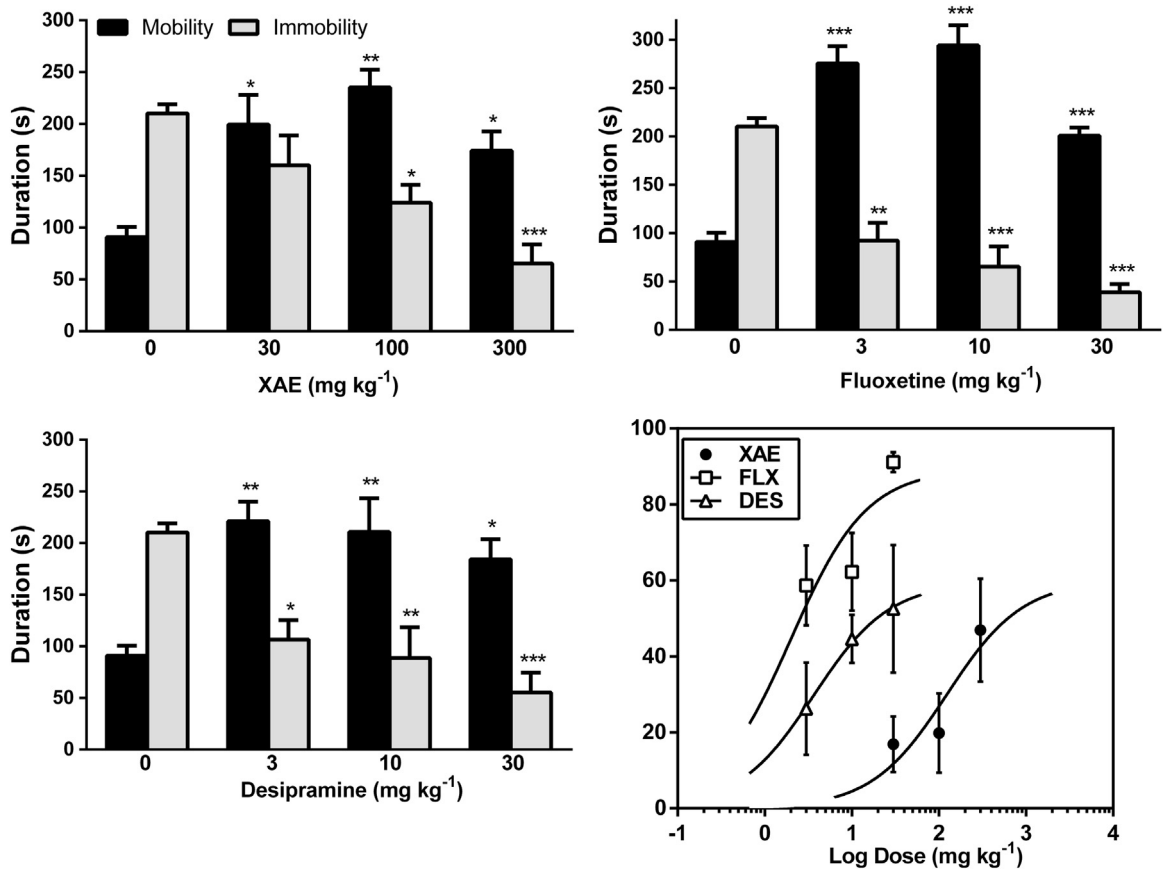

Fig. 3. *Xylopia aethiopica* exhibits antidepressant-like effects in the tail suspension test. Effect of XAE (30-300 mg kg^{-1}), fluoxetine (3–30 mg kg^{-1}) and desipramine (3–30 mg kg^{-1}) in the tail suspension test. $^{*}P<0.05$ $^{**}P<0.01$ $^{***}P<0.001$. Inset: Dose-response curves showing percentage reduction in immobility in TST. Data points are mean ± SEM of n=7 mice.

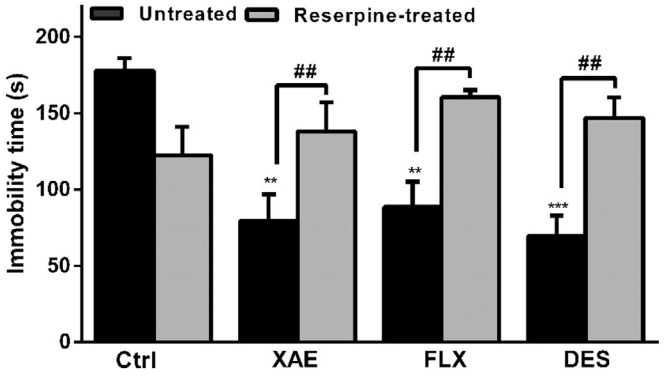

Fig. 4. Reserpine pretreatment reversed established antidepressant effects of XAE. Effects of XAE 100 mg kg^{-1}, fluoxetine 10 mg kg^{-1}and desipramine 10 mg kg^{-1} with or without reserpine (1 mg kg^{-1}) on immobility time in the FST. Compared to reserpine control *** $P<0.001$,** $P<0.01$ comparison within groups (extract/drug +/− reserpine) ##P <0.01.

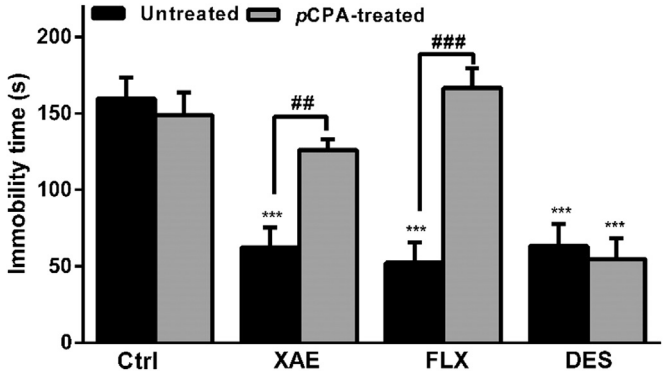

Fig. 5. *p*CPA reversed antidepressant effects of XAE and fluoxetine but not desipramine. Effect of 5-HT depletion with *p*CPA on duration of immobility in mice treated with XAE, fluoxetine and desipramine. Compared to *p*CPA control *** $P<0.001$, comparison within groups (extract/drug +/− *p*CPA) ## $P<0.01$, ### $P<0.001$.

2.10. Open field test

The open field test as described by Kasture et al. (2002) was carried out to rule out the confounding effect of a psychostimulant. Mice in groups (n=7) received either XAE (10, 30, 100 mg kg^{-1} *p.o.*) diazepam (Dzp) (0.1 mg kg^{-1} i.p.) or distilled water 10 ml kg^{-1}. Thirty minutes after i.p. and 120 min after oral treatments, mice were placed in a brightly lit (~350lx) Plexiglas® arena (40 ×40 ×30 cm^3) segmented on the floor into 16 equal squares and zoned as corner—one of the four corner squares, periphery—the squares along the walls and center—the four inner squares. Mice were individually handled prior to each evaluation to reduce handling-related stress, placed in the center of the arena and allowed to explore *a bene placito*. Their behaviour was recorded with a camera over 5 min and the total distance travelled quantified using the Behaviour Collect software.

2.11. Data analysis

All results are presented as mean ± SEM. Except otherwise noted, data was analyzed using one-way analysis of variance (ANOVA). When ANOVA was significant, multiple comparisons

between treatments was performed using Holm-Sidak *post hoc* test. Data on potentiation of noradrenaline toxicity was analyzed as survival curves and curves compared with Mantel-Cox test. Dose-responses curves are constructed by iterative curve fitting with the following nonlinear regression (three parameter logistic) equation:

$$Y = \frac{a + (b - a)}{\left(1 + 10^{(LogED50 - X)}\right)}$$

Where, X is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to b (the top) with a sigmoid shape. The fitted midpoints (ED_{50}s) of the curves were compared statistically using *F* test. GraphPad Prism for Windows Version 6 (GraphPad Software, San Diego, USA) was used for all statistical analyses.

3. Results

3.1. Forced swim test

Acute treatment with XAE (30, 100, 300 mg kg^{-1}) in mice produced significant reduction in duration of immobility at all doses tested with an increase in escape-oriented behaviours observed at 300 mg kg^{-1} ($F_{3,\ 48}$=35.18, p= <0.001) (Fig. 2). Dose response comparison showed XAE had similar efficacy (E_{max}=68.11 ± 9.04%) as fluoxetine (E_{max}=67.88 ± 8.67%) and desipramine (E_{max}=66.03 ± 11.36%) ($F_{3,\ 57}$=2.80).

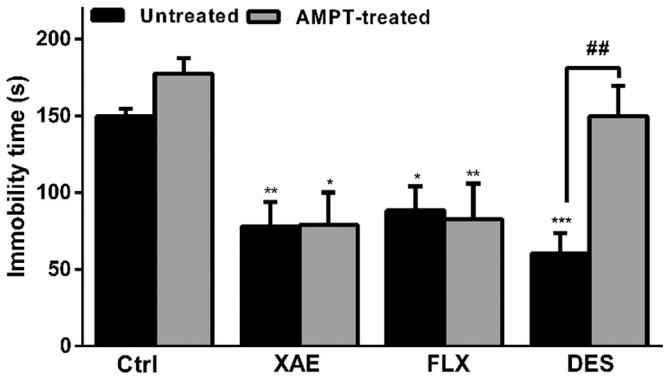

Fig. 6. AMPT reversed antidepressant effects of desipramine but not XAE and fluoxetine. Effect of catecholamine depletion with AMPA on duration of immobility in mice treated with XAE, fluoxetine and desipramine. Compared to AMPT control *** $P<0.001$, ** $P<0.01$, ** $P<0.05$, comparison within groups (extract/drug +/− AMPT) ## $P<0.01$.

3.2. Tail suspension test

XAE significantly and dose-dependently reduced the duration of immobility reaching a maximum of 68.87% reduction at 300 mg kg^{-1} ($F_{3,\ 32}$=15.17, $P<0.001$). It also increased escape-oriented behaviour in a non-dose dependent fashion (Fig. 3). The reference antidepressants; fluoxetine and desipramine also reduced immobility significantly and produced maximal reductions of 73.71% and 81.56% respectively ($F_{3,\ 32}$=53.83, $P<0.001$ and $F_{3,\ 32}$=16.21, $P<0.001$).

3.3. Effect of monoamine depletion on antidepressant-like effects on XAE

Pretreatment with vesicular monoamine transporter (VMAT) inhibitor, reserpine (1 mg kg^{-1}) abolished antidepressant effect of equipotent doses of XAE (100 mg kg^{-1}), fluoxetine (10 mg kg^{-1}) and desipramine (10 mg kg^{-1}) in the forced swim test (Fig. 4).

3.4. Effect of selective inhibition of 5-hydroxytryptamine synthesis

Pre-exposure to tryptophan hydroxylase inhibitor; *p*-chlorophenylalanine (*p*CPA) (300 mg kg^{-1} *p. o.* daily for 3 days) did not affect baseline immobility in control group but significantly reversed the observed antidepressant effect of XAE and fluoxetine. The effect of desipramine persisted in the presence of 5-hydroxytryptamine depletion (Fig. 5).

3.5. Effect of catecholamine depletion

Reduced immobility of equipotent doses of XAE and fluoxetine-treated mice persisted after noradrenaline depletion with α-methyl-*p*-tyrosine (AMPT). AMPT pretreatment however reversed antidepressant effects of desipramine (Fig. 6).

3.6. Potentiation of noradrenaline toxicity

Injection of sub lethal noradrenaline (3 mg kg^{-1}) exacerbated toxicity of desipramine leading to death but did not have any significant effect on XAE-treated mice at all doses tested over a forty-eight-hour period of observation (Fig. 7).

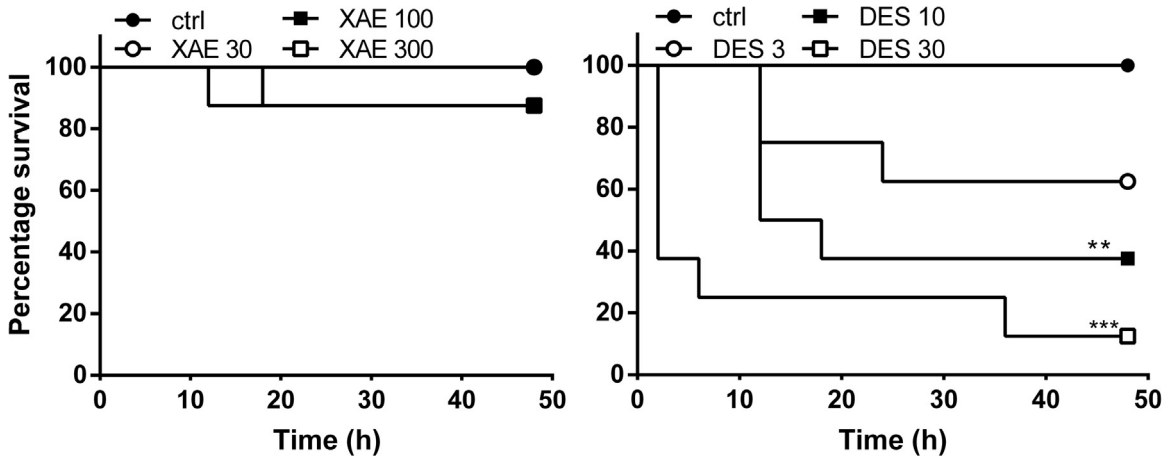

Fig. 7. XAE does not potentiate noradrenaline toxicity in mice. Effect of XAE and desipramine on the toxicity of noradrenalin in a Kaplan-Meier survival analysis over a 48 h period. ** $P<0.01$,*** $P<0.001$ Comparison of media survival hours to control in Mantel-Cox test.

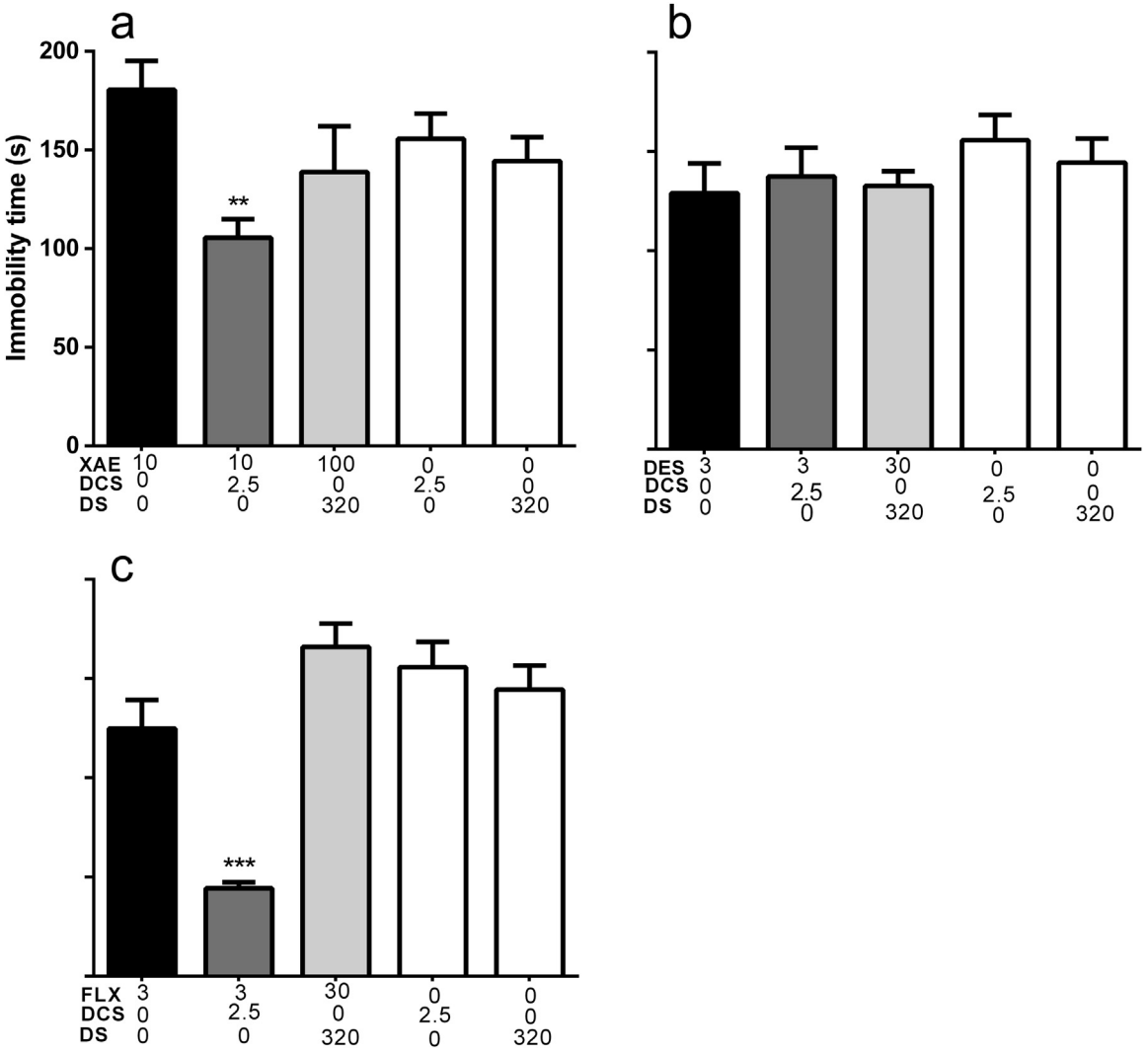

Fig. 8. Antagonizing glutamate transmission enhanced antidepressant effects of XAE and fluoxetine but not desipramine. Effects of Co-administration of D-cycloserine (DCS) (2.5 mg kg^{-1}) or D-serine (DS) (320 mg kg^{-1}) and XAE, fluoxetine or desipramine on immobility time. ** $P < 0.01$, *** $P < 0.001$ compared to XAE, FLX or DES alone treatment respectively.

3.7. Involvement of glutamatergic neurotransmission

Glycine$_B$ partial agonist, D-cycloserine (2.5 mg kg^{-1}) co-administration produced a synergistic antidepressant effect with XAE and fluoxetine. It did not however affect the antidepressant effects of desipramine. However the established antidepressant effects of XAE and fluoxetine were reversed when co-administered with the glycine$_B$ full agonist, D-serine (320 mg kg^{-1}) (Fig. 8).

3.8. Involvement of nitric oxide system

Pretreatment with N_ω-Nitro-L-arginine methyl ester (L-NAME) potentiated the antidepressant-like effect of sub-effective dose *Xylopia aethiopica* extract (10 mg kg^{-1}) in the forced swim test significantly ($F_{1,\ 69} = 68.54$ $P < 0.0001$). Similarly, antidepressant effect of fluoxetine but not desipramine was also potentiated. The established antidepressant-like effect of XAE and fluoxetine was significantly reversed after pretreatment the L-arginine (Fig. 9) although L-arginine itself did not have any effect on immobility.

3.9. Open field test

The extract did not exhibit psychostimulatory effects as indicted by no significant increase in distance travelled by XAE-treated mice compared to naïve animals in the 5-min open field test (Table 1).

4. Discussion

Depression is a chronic disorder that exhibits fluctuating course of symptoms (Poleszak et al., 2011). It is characterized by strong feelings of despair, hopelessness, lethargy, anorexia, suicidal thoughts and anhedonia (reduced response to pleasurable stimuli) according to the Diagnostic and Statistical Manual for mental disorders (American Psychiatric Association, 2013).

When rodents are subjected to an inescapable stressful condition, they assume an immobile posture after an initial pursuit of escape. This immobile posture has been hypothesized as behavioural despair (Porsolt et al., 1977). The period of immobility, measured in antidepressant tests in animals have good predictive value in screening antidepressant effects of drugs. The tail suspension test (Steru et al., 1985) and forced swim tests (Porsolt et al., 1977) are popular methods of evaluating antidepressants with appreciable reliability and predictive validity (Bourin et al., 2005; Cryan and Holmes, 2005; Petit-Demouliere et al., 2005). Drug-treated subjects are placed in an inescapable situation and

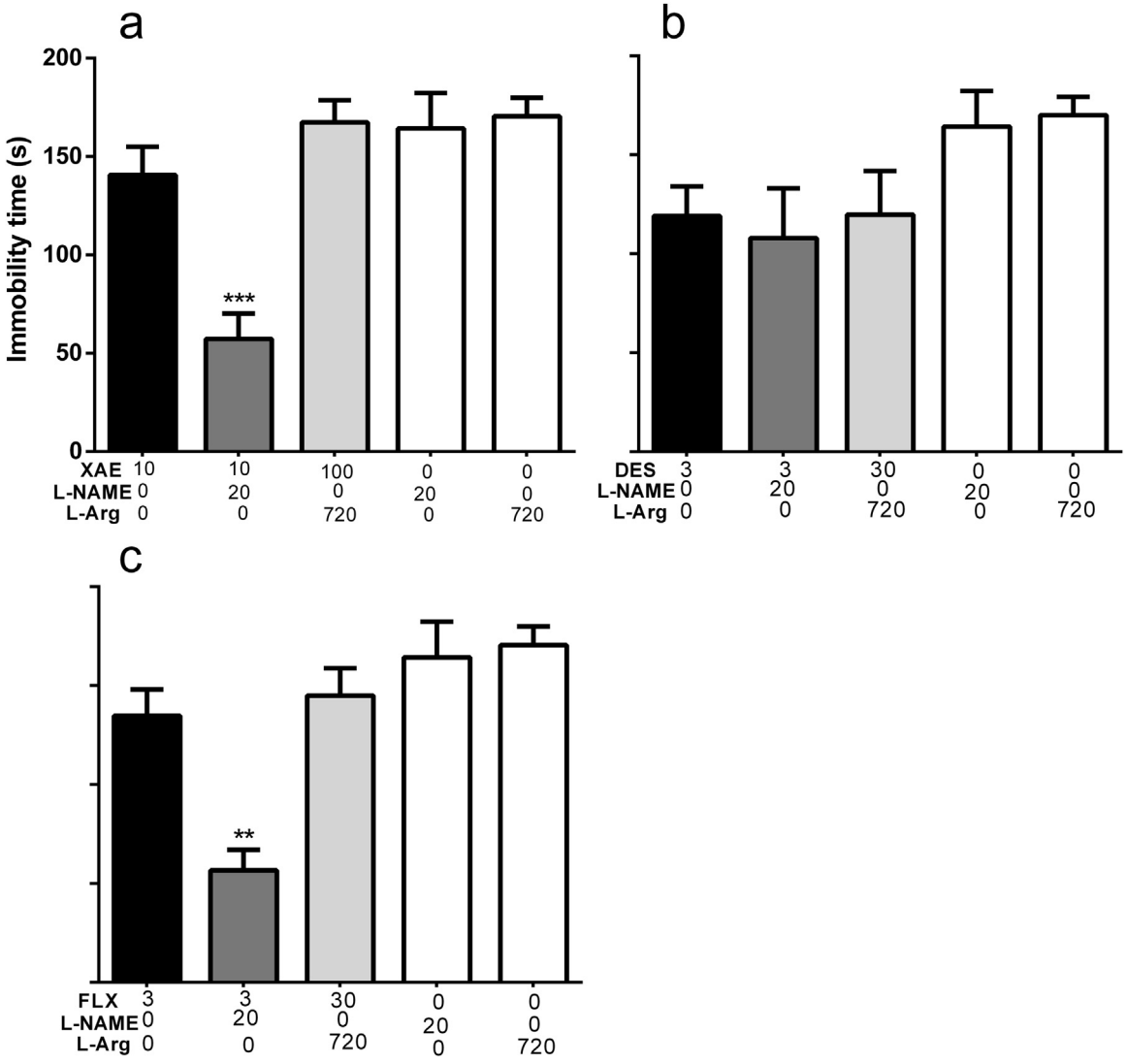

Fig. 9. Potentiation of antidepressant-like effects of XAE (a) and fluoxetine (c) by pretreatment with L-NAME (20 mg kg^{-1}) and reversal of antidepressant-like effects after pretreatment with L-arginine (750 mg kg^{-1}). ** $P < 0.01$,*** $P < 0.001$ compared to XAE, FLX or DES alone treatment respectively.

Table 1
Total distance travelled by XAE-treated mice compared to naïve in the open field test.

Treatment	Distance travelled (m)
Naïve	16.94 ± 1.46
XAE 10 mg kg^{-1}	10.91 ± 0.89*
XAE 30 mg kg^{-1}	13.67 ± 1.36
XAE 100 mg kg^{-1}	17.34 ± 1.52

* *$P < 0.05$ compared to naïve mice.*

reduction in immobility serves as the antidepressant-like signature.

Acute administration of *Xylopia aethiopica* fruit extract showed antidepressant-like effects by reducing significantly, duration of immobility in both the tail suspension and forced swim tests with a converse increase in escape-oriented behaviours. In both FST and TST in mice, acute treatment with antidepressants reverse the immobility readout produced in each test (Cryan and Holmes, 2005). Generally the extract showed better antidepressant effect in the forced swim test than the tail suspension test. This is not surprising as different mechanisms underlie these two models of depression hence drugs may interact with these mechanisms differently (Whiteside et al., 2010). For example the tail suspension test is more susceptible to the sedative effects of 5-HT $_{1A}$ agonist hence produce less reduction in immobility as compared to the forced swim test (Castagné et al., 2011) and we have shown previously the sedative potential of the extract (Biney et al., 2014).

The theory of monoamine dysregulation accounting for mood and affective disorders has achieved almost textbook truism (Merens et al., 2007; Meyer et al., 2006). Several groups have confirmed that selective depletion of neurotransmitters results in the abolishing of antidepressant effects of known antidepressants. Therefore if a drug is mediating its antidepressant effects by modulating 5-HT, then by depleting 5-HT, the antidepressant effects will be abolished (O'Leary et al., 2007; Ruhé et al., 2007). Depletion of neuronal and vesicular stores of monoamines resulted in the abolishing of the antidepressant effects of XAE, fluoxetine and desipramine implicating a role of monoamines in the observed antidepressant effects of these drugs. However, selective depletion of 5-hydroxytryptamine with *p*-chlorophenylalanine (*p*CPA), a tryptophan hydroxylase inhibitor, abolished antidepressant effects of XAE and the selective serotonin reuptake inhibitor; fluoxetine but not the noradrenaline reuptake inhibitor, desipramine. Inhibition of noradrenaline synthesis by AMPT did not affect antidepressant effect of the extract and

fluoxetine but the antidepressant effect of desipramine was expectedly reversed because of the predominance of noradrenergic system in its antidepressant effects (O'Leary et al., 2007). These indicate a predominance of the serotonergic system over the noradrenergic system in the observed antidepressant effects of XAE. Further evidence is adduced by the lack nordrenaline toxicity potentiation by XAE as would be expected by nordrenaline-based antidepressants.

In recent years, there have been results that point to a dysfunction of the excitatory amino acid glutamate and its receptors in the mechanisms that underpins the altered neuroplasticity associated with depression (Sanacora et al., 2012; Drewniany et al., 2015; Wang et al., 2015). It has been suggested that NMDA receptor antagonism produces a quicker onset of antidepressant effect which is the main challenge with conventional antidepressant therapy (Machado-Vieira et al., 2009; Belozertseva et al., 2007) NMDA receptor activation also leads to down stream activation of *n*NOS that produces nitric oxide which mediates the neurotoxic effects of glutamate (Pehrson and Sanchez, 2013; Joca and Guimarães, 2006). Thus a role of nitric oxide in glutamate neurotransmission and subsequent effectiveness of nitric oxide synthase inhibitors in depression is also established (Harkin et al., 2004; Rosa et al., 2003). 5-hydroxytryptamine has inhibitory role on glutamate transmission through activation of 5-HT_{2A} and 5-HT_7 receptors (Pehrson and Sanchez, 2013) therefore drugs that enhance 5-HT transmission produce syngestic effect with other modulators of glutamate transmission. Inhibition of *n*NOS with N_{ω}-nitro-L-arginine methyl ester (L-NAME) potentiates antidepressant effects of serotonin but not noradrenaline-targetted antidepressants while NO substrate L-arginine reverses this antidepressant effect (Rosa et al., 2003). This was observed with the extract and fluoxetine as expected but not desipramine. Similar observation was made by interrupting glutamate transmission through the gylcine$_B$ co-modulatory site using glycine$_B$ functional antagonist, D-cycloserine, with reversal of established antidepressant effects of XAE and fluoxetine but not desipramine in the presence glycine$_B$ agonist D-serine. Thus the syngestic effects of the extract and glutamate transmission inhibitors gives further credence to the predominance of serotonin in the observed antidepressant effects of the *Xylopia. aethiopica* ethanolic fruit extract. The fact that enhancement of glutamate transmission both through nitric oxide synthase and glycine co-binding site of NMDA receptor reversed antidepressant effect of the extract also suggest a possible role glutamate transmission in the observed antidepressant effect of the *Xylopia aethiopica* extract.

5. Conclusion

These results indicate that acute exposure of mice to *Xylopia aethiopica* fruit extract exhibits antidepressant-like effects predominantly through interactions with serotonergic neurotransmission.

Conflict of Interest

The authors declare no conflict of interests.

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