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Attenuation of Anxiety Behaviours by Xylopic Acid in Mice and Zebrafish Models of Anxiety Disorder

Robert Peter Biney[1]*, Charles Kwaku Benneh[2], James Oppong Kyekyeku[3], Elvis Ofori Ameyaw[4], Eric Boakye-Gyasi[5], Eric Woode[5]

[1]Department of Pharmacology, University of Cape Coast, Cape Coast, Ghana
[2]Department of Pharmacology and Toxicology, University of Health and Allied Sciences, Ho, Ghana
[3]Department of Pharmaceutical Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[4]Department of Biomedical Sciences, University of Cape Coast, Cape Coast, Ghana
[5]Department of Pharmacology Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

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Corresponding Author:
E-mail : robert.biney@ucc.edu.gh
Mob.: +233 (0)332 18107

Abstract

Anxiety disorders affect people worldwide with disabling symptoms. Xylopic acid, an *ent*-kaurane diterpene, exerts central nervous system depressant, opioid receptor-mediated analgesic and anti-neuropathic pain effects. Agents acting as CNS depressants can ameliorate anxiety disorders hence, this study evaluates the anxiolytic potential of xylopic acid in mice and zebrafish. Xylopic acid was given orally at 3, 10 or 30 mg kg^{-1} to mice or 3, 10 or 30 µM to zebrafish. Anxiety was assessed in mice using open field (OFT), novelty-induced hypophagia (NIH), and elevated plus maze (EPM) models and in zebrafish using novel tank (NT) and scototaxis (ST) models. Additionally, xylopic acid's activity on anxiety induced by alcohol withdrawal was also evaluated. Xylopic acid at doses 3-30 mg kg^{-1} reduced latency to feeding in mice in the hyponeophagia test for anxiety and also significantly reduced thigmotaxis in the OFT at 30 mg kg^{-1} (P<0.001). All mice given xylopic acid significantly spent more time in the open arms of the EPM (P<0.001). At 10 µM xylopic acid-treated zebrafish exhibited significant (P<0.001) reduction in time spent at bottom of novel tank but it did not reduce scototaxis in the light-dark test. Furthermore, xylopic acid attenuated increased bottom dwelling induced by alcohol withdrawal in zebrafish. The doses of xylopic acid used did not impair locomotion in the chimney test for mice. These findings indicate anxiolytic-like properties of xylopic acid in mice and zebrafish models of anxiety disorder.

1 Introduction

Although anxiety is a common emotional activity, it can usually switch into an overdrive and result in a prolonged devastating psychiatric syndrome and reduce overall quality of life[1]. All over the world, anxiety is a common psychiatric disorder which leads to significant functional impairment[2]. The use of benzodiazepines has been the mainstay in anxiety management for several years[3] although with time, their continued use has led to significant untoward side effects including anterograde amnesia and psychological dependence which restrict their usage[4]. Newer anxiolytics like the selective serotonin reuptake inhibitors (SSRIs) are also limited by their sexual dysfunction adverse effect and slower commencement of therapeutic effect[5]. Anxiety that is comorbid with other diseases like depression and epilepsy is even more difficult to manage with the current regimens. These gaps in the treatment of anxiety disorders demands continued investigations for new chemical entities with better therapeutic and side effect profiles as replacements to treat anxiety disorders.

Kaurane diterpenes have been shown to exhibit central nervous system effects including anticonvulsant[6] and neuroprotective effects[7]. Xylopic acid is a kaurane diterpene that has been

isolated from plants in the Annonaceae family. It previously exhibited sedative[8] and central analgesic effects[9] as well as ameliorative effects against chemotherapy-induced neuropathic pain[10]. CNS-acting drugs may be useful in managing more than one CNS disorders[11, 12]. For example the anticonvulsant gabapentin, is used to treat neuropathic pain and anxiety whereas the antidepressant duloxetine is also used in chronic pain and anxiety management[13]. It is therefore possible that xylopic acid having shown anti-neuropathic pain and other CNS effects may exhibit additional anxiolytic effects. This contribution therefore explores the anxiolytic potential of xylopic acid in response to the continued search for novel pharmacological options for anxiety disorder management.

2 Materials and Methods

2.1 Animals

NMRI mice (20-25 g) were acquired from Noguchi Memorial Institute for Medical Research (NMIMR), Ghana. They were used for behavioural experiments only after a 14-day period of familiarization with the new standard laboratory environment. They were fed with mice pellet, (Agricare, Kumasi, Ghana) and drunk tap water *ad libitum*. Behavioural experimentations were conducted from 9:00 to 15:00 hours daily. In behavioral experiments each arena was dubbed with 70% $^{v}/_{v}$ ethanol soaked cloth after every mice's test to eradicate any olfactory signals that may have been left behind by the preceding mice. Experiments were carried out at preliminarily determined times of peak effect i. e. 120 minutes after xylopic acid or 60 minutes after diazepam administration.

In zebrafish experiments, 4 month-old adult *Danio rerio* (wild-type, short fin), 3 to 5 cm long were acquired (Aquarium Marshall, Accra, Ghana). They were kept in 30 L glass holding tanks at 3-5 fishes/L. The water was kept at 26±2°C and pH 7.0-8.0 with 30% of the water replaced daily. They were fed 8 hourly with commercial fish lakes (Aquafin Professionals, Guangzhou, China) under a 12/12 h light/dark cycle schedule.

Mice experiments were compliant with NIH Guidelines for the Care and Use of Laboratory Animals while the protocols for zebrafish experiments were compliant with the European Union recommendations for zebrafish experiments (EU Directive 2010-63-Experiments with zebrafish). Ethical approval was obtained from Department of Pharmacology Animal Ethics Committee.

2.2 Drugs and Chemicals

Desipramine, diazepam and pentylenetetrazole and were obtained from Sigma-Aldrich, St Louis, MO, USA.

2.3 Extraction and Characterization

Xylopic acid (Fig 1a) was extracted from the fruits (unripe and dried) of *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) as previously described by Woode and colleagues (2012)[14]. The isolated xylopic acid was purified by recrystalization with a reflex condenser. It was characterized and the purity confirmed by HPLC-HRESI-MS (Fig 1b). Full scan of the isolated xylopic acid in methanol was measured on an LTQ-Orbitrap spectrometer (Thermo Fisher, USA) (nominal mass resolving power 60,000 at *m/z* 400, scan rate of 1 Hz) with an HESI-II source and coupled to an Agilent 1200 HPLC system (Santa Clara, USA) which comprised a column oven, pump, an auto-sampler (injection volume 5 µL) and a PDA detector. HPLC profile was obtained on a Phenomenex (Torrance, USA) Luna C 18 (2) column (50×3 mm, 3 µm particle size) with formic acid (0.1%)+water (A) and methanol (B) gradient as the mobile phase (flow rate 350 µL/min).

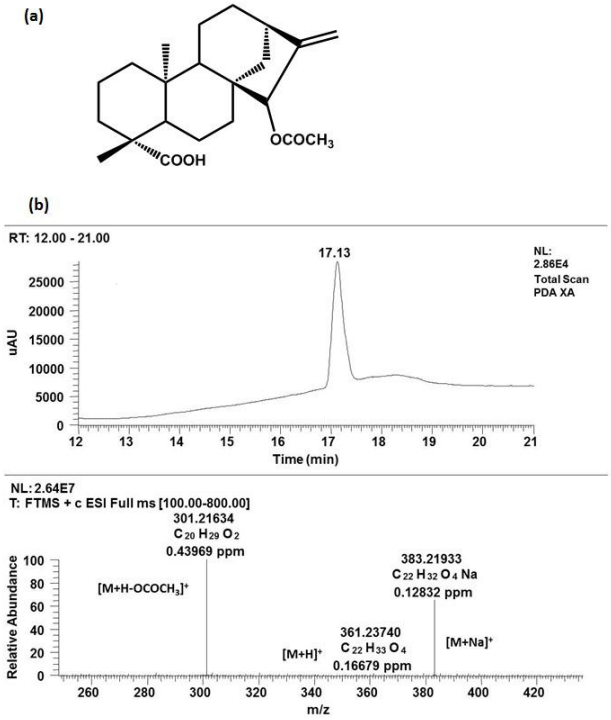

Fig 1 (a): Chemical structure of xylopic acid, (b) HPLC-HRESI-MS spectra of isolated xylopic acid

2.4 Murine Models of Anxiety

2.4.1 Open Field Test (OFT)

The OFT as shown in Kasture *et al.,* 2002 was used to assess anxiolytic effects[15]. Mice received either xylopic acid (XA) (3, 10, 30 mg kg^{-1} *p.o.*), or the anxiogenic agent; pentylenetetrazole (PTZ) (30 mg kg^{-1} i.p.) or diazepam (Dzp) (0.1, 0.3, 1 mg kg^{-1} i.p.). One twenty minutes after oral administration and 30 minutes after i.p., mice were allowed to freely explore a Plexiglas® arena (40 × 40 × 30 cm^3, ~350 lux) segmented on the floor into 16 equal squares. These squares were labeled either as (a) corner i.e. the four squares in the arena's corner, (b) periphery i.e. all the squares lining the walls of the arena, or (c) center i.e. the four squares of the innermost perimeter. Mice were held briefly in the hands one at a time by a male experimenter before each trial to minimize stress. They were

then placed in the center of the arena. Spontaneous activity in the zones was reordered for 5 minutes by the aid of a camera fixed ~0.1 m overhead. Frequency of entries and total time used in exploring the various zones were blindly scored by an experienced personnel with JWatcher™ (Available at http://www.jwatcher.ucla.edu/). Thigmotaxis was assessed by creating heat maps of the locomotion within the arena using Ethovision (Ethovision, version XT 10, Wageningen, Netherlands)

2.4.2 Elevated Plus Maze (EPM) Test

A modified EPM test was carried out in mice as earlier demonstrated in rats[16]. The model was built from Plexiglas® and comprised of two open arms (30 × 5 cm^2, with raised 0.5 cm rim to prevent falling) on opposite sides of two closed arms (30 × 5 × 30 cm^3) linked to a 5 × 5 cm^2 platform in the center of the apparatus. The model was then mounted on an 80 cm platform.

Mice received either XA (3, 10, 30 mg kg^{-1} *p.o.*), PTZ (30 mg kg^{-1} *i.p.*), DZP (0.1, 0.3, 1 mg kg^{-1} *i.p.*), or distilled water 10 ml kg^{-1} *p.o*. Thirty minutes after i.p. and 2 hours after *p.o.* administrations, they were introduced onto the center of apparatus facing an open arm. Total time used in exploring each of the arms in addition to risk assessment behaviours (unprotected and protected head dipping) was captured by the aid of a camera fixed ~50 cm overhead. These behaviours in the 5-minute test were independently scored by an experienced observer using JWatcher™. Ethovision Version XT 10 was used to evaluate the total distance trekked and also create activity heat maps of locomotion within the maze.

2.4.3 Hyponeophagia Test

The novelty-induced hyponephagia test described by Dulawa and colleagues was modified and used[17]. Mice in groups of ten (n=10) were kept in the usual mouse cages to acclimatize for 24 h without food but had water *ad libitum*. Twenty-four hours post food deprivation, each received either XA (3, 10, 30 mg kg^{-1} *p. o*), DZP (0.1, 0.3, 1 mg kg^{-1} i. p.) or distilled water 10 ml kg^{-1} *p. o*). Two hours after *p.o.* and 30 minutes after i.p. administration, they were gently taken individually to a novel cage (20 × 30 × 20 cm^3) containing weighed mice *chow,* held in a receptacle in the middle of the arena. Latency to feed was scored as time taken for the mouse to start feeding on the chow. Total food consumed in 5 minutes was measured as the difference in weight of *chow* before and after introducing the mice into the arena.

2.4.4 Chimney Test

Effect of xylopic acid on locomotor activity and neuromuscular co-ordination was assessed with chimney test as previously described[18]. Xylopic acid-treated mice (3, 10 30 mg kg^{-1}) were allowed to climb backwards in a vertical-placed transparent glass cylinder (2.8 cm × 30 cm). Motor impairment was recorded as inability to complete the challenge in 60 s.

2.5 Zebrafish Models of Anxiety

2.5.1 Novel Tank (NT) Test

This test was performed as earlier on described by Levine and colleagues with slight differences[19]. The apparatus is a slender glass chamber (15 × 10 × 25 cm^3) containing normal tank water (25°C) 18 cm deep. It is divided into three equal imaginary sections of 6 cm each (bottom, middle and top). Zebrafish were treated by immersion in 1 L tumblers with either XA (3, 10 or 30 µM), desipramine (DES) (10, 30 or 100 µM) or normal tank water. One hundred and twenty minutes post treatment, they were gently placed in the novel tank and recorded for 5 minutes with a camera. Total time spent in the various sections in addition to time taken to first enter the topmost section was independently scored with JWatcher™.

2.5.2 Scototaxis Test (Dark Preference Test)

The innate inclination of zebrafish to stay in dark areas was assessed with procedures elaborated by Holcombe *et al.,* 2013[20]. Zebrafish were treated with solutions of XA (3, 10, 30 µM), DES (10, 30, 100 µM) or normal tank water for 20 minutes before being introduced individually into the scototaxis apparatus (9 × 55 × 10 cm^3 with each half painted either white or black) containing water (25-26°C) 5 cm deep. Swim behaviour was recorded for 5 minutes and independently scored with JWatcher™ for total of time used in exploring the light or dark halves of the tank.

2.5.3 Alcohol Withdrawal-induced Anxiety

The activity of XA on anxiety induced after alcohol withdrawal was evaluated in zebrafish as per protocol previously published[20] but with slight modifications. To induce withdrawal anxiety, zebrafish were treated by immersion with ethanol (0.5 % $^{v}/_{v}$) for 30 minutes daily for 8 consecutive days followed by 8 consecutive ethanol-free days. Drug treatment started on the 16th day of experiment either XA (1, 3, 10 µg ml^{-1}), DZP (1.5 µg ml^{-1}) or distilled water for 30 minutes. This was repeated for 3 days as described above. Twenty four hours after the last drug treatment, the novel tank test was performed as elaborated above.

2.6 Data Analysis

Data was analyzed blindly of group treatments. Results indicate group mean ± SEM with $P<0.05$ deemed statistically significant. GraphPad Prism for Windows Version 6 (GraphPad Software, San Diego, USA) was used for the analyses. Differences in total time used in exploring various arenas in the OFT, EPM and NT tests were evaluated using 2-way ANOVA (treatment × time spent in arm or section). One-way ANOVA was used to analyze the other results. Where significant differences in treatment was

observed, Holm-Sidak *post hoc* test multiple comparisons was carried out.

Dose-responses relationship were computed with iterative curve fitting with the three parameter logistic nonlinear regression equation:

$$Y = \frac{a + (b - a)}{\left(1 + 10^{(LogED_{50} - X)}\right)}$$

Where, Y is the response (from bottom (a) to top (b) with a sigmoid profile and X is the logarithm of dose. The *F* test was employed to compare various ED_{50}s.

3 Results

3.1 Open Field Test

When mice were freely allowed to explore the novel brightly lit arena, there was an increase in total duration in the corner zone and a reverse scenario in the center zone as indicated by mice in the control group (Fig 2a, b). Xylopic acid treatment reduced this trend significantly at 30 mg kg^{-1} although less potent than diazepam (Fig 2c) ($F_{8, 60}$ = 33.66 P<0.001) as did DZP. The PTZ group showed increased activity in the corner and reduced total time in the center of the arena (Fig 2g).

Xylopic acid as well as diazepam dose-dependently increased exploration as shown by the increased total distance trekked (Fig 2h) ($F_{10, 44}$ = 5.683 P<0.001). Similarly, xylopic acid-treated mice exhibited reduced thigmotactic activity as revealed by the activity heat maps as little or no activity along the wall of the arena (Fig 2e).

3.2 Hyponeophagia Test

Xylopic acid significantly reversed novelty suppressed feeding as exhibited by an increase in weight of food consumed in the 5 min test period ($F_{3,35}$ = 4.923 P = 0.0169). It also reduced the latency to feeding at all doses tested ($F_{3,35}$ = 17.04 P < 0.001) (Fig 3a, b).

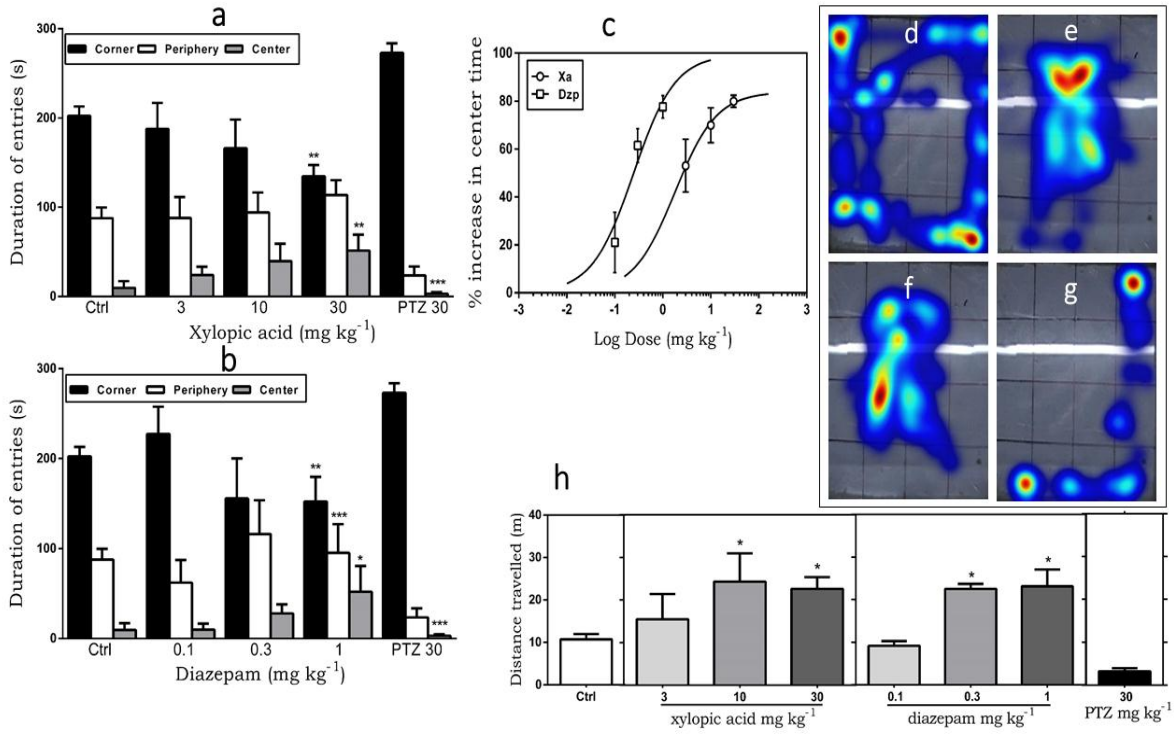

Fig 2: Xylopic acid displays anxiolytic effects in the open field test in mice

*(a, b) Effects of xylopic acid, diazepam and pentylenetetrazole in the open field test in mice. 1-way ANOVA and Holm-Sidak multiple comparison test. * P<0.05,** P<0.01, ***P< 0.001 compared to control*

(c) Dose-response curve indicates percentage increase in time spent in centre zone.

(d-g) Representative heat maps indicates activity of naïve (d) xylopic acid 30 mg/kg, (e) diazepam 0.3 mg/kg (f) or PTZ 30 mg/kg, (g) -treated mice in the open field arena.

(h) Effects of xylopic acid, diazepam and PTZ on distance travelled in the open field test.

3.3 Elevated Plus Maze Test

The EPM evoked anxiogenic responses in mice as exhibited by reduced open arm duration in control and PTZ-treated mice. This was however attenuated by both xylopic acid ($F_{6,58}$) = 6.48 P <0.001) and diazepam ($F_{6,58}$ = 7.495 P <0.001) treatments (Fig 4a, b). Heat maps revealed increased activity in open arms for xylopic acid-treated mice in contrast to control groups (Fig 4c-f). Xylopic acid reduced risk assessment behaviours similar to DZP. Both protected head dips and protected stretch-attend postures (SAPs) were minimized significantly by XA and DZP (Fig 4 g, h).

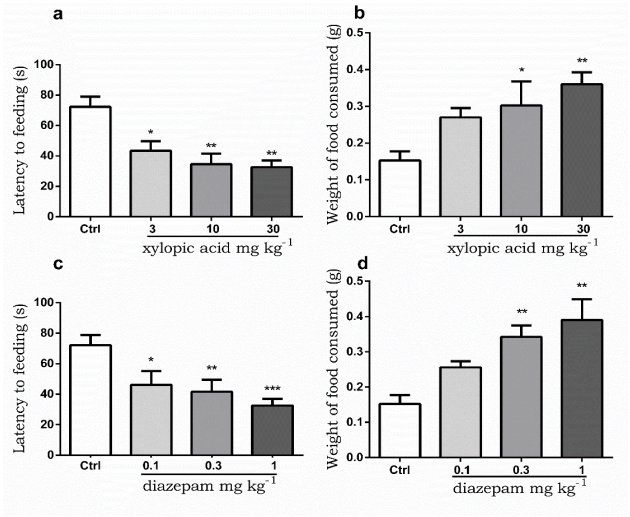

Fig 3*:* Xylopic acid reduced novelty-suppressed feeding in mice

*Effects of xylopic acid and diazepam on latency to feeding (a, c) and amount of food consumed (b, d) in the hyneophagia test. One-way ANOVA followed by Holm-Sidak multiple comparison test. Comparison to control * P<0.05, ** P<0.01, *** P<0.001.*

3.4 Chimney Test

Xylopic acid at all the doses tested did not impair neuromuscular coordination in mice. All mice completed the chimney test challenge under 60 s.

3.5 Novel Tank Test

In the NT test, naïve zebrafish demonstrated anxiety-like behaviours by exhibiting a significant increase in both latency to explore top section and the total duration spent at the lowest region of the tank. However, zebrafish administered with 30 µM XA exhibited decreased latency to explore the upper region ($F_{3, 39}$ = 1.329, P = 0.0236) (Fig 5a) in addition to spending more in the top region ($F_{7, 38}$ = 9.861, P < 0.001) (Fig 5b). Similar effect was observed in DES-treated zebrafish ($F_{3, 37}$ = 6.348, P <0.001).

3.6 Scototaxis Test

In the scototaxis test, the naïve zebrafish preferred dark regions. In contrast to desipramine that ameliorated this anxiety-related observation at 300 µM ($F_{7, 38}$ = 17.86, P < 0.001) (Fig 6), XA could not overturn this preference.

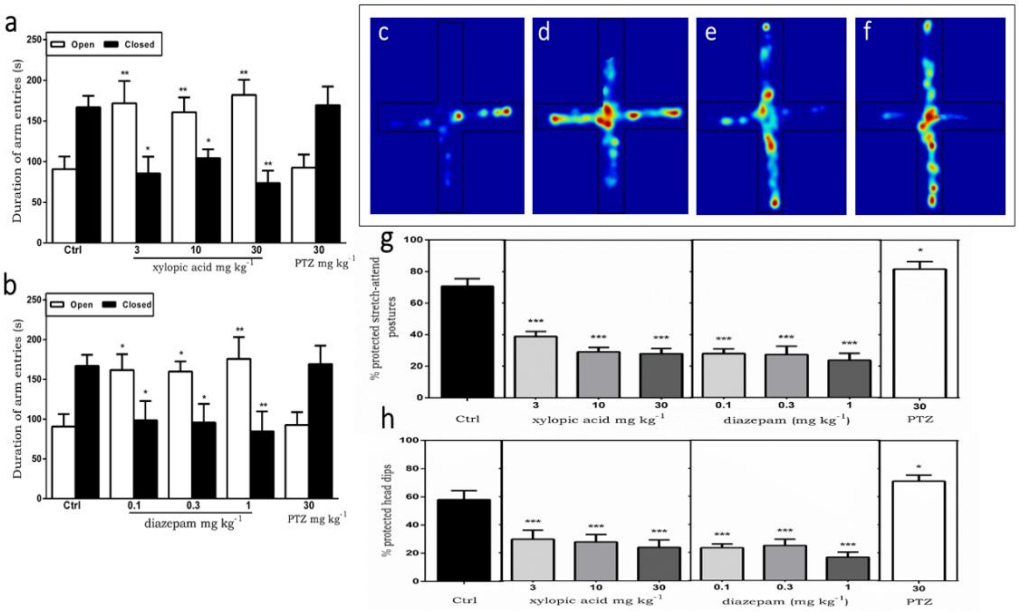

Fig 4: Xylopic acid reduces ethological endpoints of anxiety in EPM test in mice

(a, b) Effects of xylopic acid and diazepam on time spent in open and closed arms in the EPM. 2-way ANOVA and Holm-Sidak multiple comparison test are P<0.05, ** P<0.01 *** P< 0.001 compared to controls.*

(c-f) Representative heat maps of PTZ 30 mg kg^{-1} (c), naïve (d), xylopic acid 30 mg kg^{-1} (e) and DZP 1 mg kg^{-1} (f) treated mice in the EPM. Vertical arms represent open arms while and horizontal arms closed arms respectively.

(g, h) Xylopic acid (3, 10, 30 mg kg^{-1}) and diazepam (0.1, 0.3, 1 mg kg^{-1}) decreased but PTZ increased protected SAPs (g) and protected head dip (h) in a 5-minute EPM test.

3.7 Alcohol withdrawal-induced anxiety

Ethanol withdrawal evoked an anxiogenic response mirrored as the increase in bottom dwelling and increased latency to top region in untreated zebrafish in the control group (Fig 7). Xylopic acid however attenuated this resulting in increased time spent at the top and a converse decrease latency to top segment. Similar effects were observed in diazepam treated group.

4 Discussions

We have employed a battery-style methodology of different models of anxiety disorders in two species and present here, results that indicate xylopic acid, possesses anxiolytic-like effects.

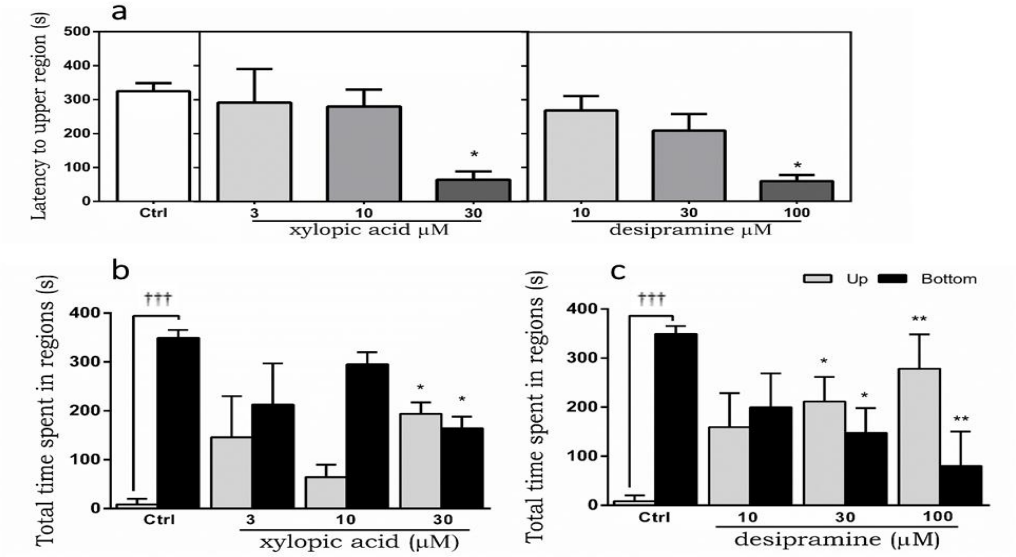

Fig 5: Anxiety phenotypes measured in XA-treated zebrafish in NT test

(a) Effect of XA and DES on latency into upper segment of the novel tank

(b) Effect of XA and DES on time spent in top and bottom regions of the novel tank. 2-way ANOVA and Holm-Sidak multiple comparison test. * P<0.05, ** P<0.01 compared to controls

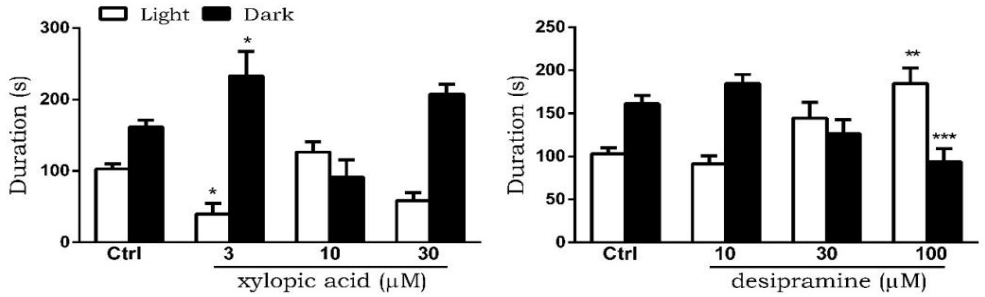

Fig 6: Xylopic acid does not reduces scototaxis in zebrafish

*Effects of XA (3, 10, 30 μM) and DES (10, 30, 100 μM) in the dark preference test. Data show mean ± SEM. 2-way ANOVA and Holm-Sidak post hoc test. Comparison to controls *** P<0.001, ** P<0.01, * P<0.05.*

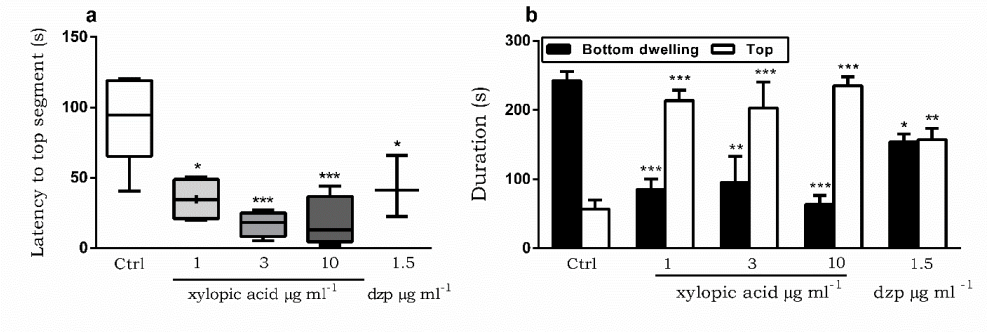

Fig 7: Xylopic acid ameliorates anxiety induced by alcohol withdrawal

(a) Box and whisker plot indicate latency into top region (median, 5th-95th percentile). 1-way ANOVA and Holm-Sidak post hoc.

*(b) Total duration in top and lower regions of the novel tank. 2-way ANOVA followed by Holm-Sidak post hoc test. Comparison to controls ** P<0.01, * P<0.05.*

In both open field and elevated plus maze test in mice, xylopic acid demonstrated appreciable anxiolytic-like effects. These tests have been employed successfully in the evaluation of novel chemical entities with potentials as chemotherapeutic agents in anxiety disorder management. They have proven to be valid and predictive animal models of anxiety disorders[21, 22] as similar behaviours have been recorded in humans when subjected similar environments[23]. The open field test for example takes

advantage rodents' innate dislike of for open and brightly lit arenas as opposed to much comfortable enclosed spaces. This vulnerability evokes an anxiogenic response when mice are placed in the open filed apparatus. The degree of anxiety can be measured by ethological behaviours such as thigmotaxis (snugging to the walls) and decreased duration of time used in exploring the "anxiety provoking" center of the OFT apparatus[22,24]. In this regard, efficacious anxiolytics calm this aversion and enhance the exploratory activity in anxiogenic zones[22]. Thus, the fact that both diazepam, an established anxiolytic and the investigational agent xylopic acid, enhanced total duration of exploration of the center zone in addition to decreasing thigmotaxis is an indication of possible anxiolytic-like effects. This suspicion is further corroborated by xylopic acid-treated mice increasing the average velocity and total distanced traveled in the OFT arena, an indication of increased exploration and for that matter increased overall anxiety of that animal.

To further check the anxiolytic-like properties of xylopic acid in the OFT, the novelty-induced hyponeophagia test was carried out. This test exploits a conflict between an anxiogenic provocation and hunger-induced approach behaviour[25,26]. The brightly lit and new environment in which the animal is placed evokes stressful and anxiogenic response. This model has also been employed extensively to evaluate anxiety reducing drugs. Additionally, hyponeophagia is also demonstrated in transgenic mice genetically manipulated to be spontaneously anxious[27]. Here again, xylopic acid ameliorated the anxiety fingerprint in this model of anxiety-like behavior and thus provides additional evidence to support a possible anxiolytic-like property.

Furthermore, elevated plus maze which is an approach-avoidance test was used to examine the anxiety-reducing properties of this investigational kaurane diterpene. The exploratory drive of rodents vis-à-vis an innate aversion of elevated environments is tested in this model which is predictive of possible anxiolytic-like properties[25, 28]. The effect of an investigational agent or experimental procedures on other risk assessment ethological behaviors such as stretch-attend postures and head dips can also be measured in the EPM to confirm if the agent or procedure produces an anxiolytic or anxiogenic response[29]. Xylopic acid-treated mice demonstrated increased exploration of the open arms, a very valid and popular parameter that assesses anxiety in the EPM[28] . It also increased risk assessment behaviors like unprotected stretch-attend postures and head dips as predicted in putative anxiolytics. These observations consolidate the anxiolytic-like properties of xylopic acid seen in the OFT and hyponeophagia models.

When experiments are conducted in just one specie during the investigation into the properties of any chemical entity, the results may be difficult to intemperate and extrapolate to other species because of inter-specie variations. Again, the differences in sensitivity mouse behavior in different anxiety tests during pharmacological manipulations can be a confounding factor when a battery of test are carried[30]. It was therefore imperative that to consolidate the observed anxiolytic-like properties of xylopic acid, the anxiolytic assessment should be carried out in more than one model organism. Hence the anxiolytic was additionally assed in the adult zebrafish.

The zebrafish has an evolutionary conserved neurocircuitry in addition to approximately 75% homology to the human genome[31]. It has analogous neurotransmitters, receptors and neurohormones to humans[32]. This has made it an attractive model organism for evaluating the properties of various agents on multifarious brain disorders in humans like anxiety disorders[33]. Even though it is comparatively a new model organism there have been several studies in which anxiety has been pharmacologically manipulated in zebrafish[34-36] thus making this model organism one of the most successful in various aspects biomedicine[37].

In the commonest zebrafish model of anxiety, the novel tank test, anxiety- like behavior in this model is demonstrated by decreased duration of exploration and frequency of entries into upper regions of the chamber[37, 38]. There is also enhanced freezing and erratic movement as well as a delayed latency to swim to the upper regions[33,39, 40]. Xylopic acid reversed this delayed latency to explore upper regions of the novel tank. It also as considerably minimized total duration of bottom dwelling. All these behaviors are suggestive of anxiolytic-like properties in adult zebrafish.

Even though xylopic acid could not alter the dark preference of adult zebrafish[39,41] as expected of most anxiolytics, this observation is not entirely suprising. Some anxiolytics such as citalopram as well as other 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2B} antagonists did not avert dark preference in adult zebrafish although they demonstrate anxiolytic behaviors in other models[42]. It has been hypothesized that other mechanisms may be significantly involved in the scototaxis model of anxiety in zebrafish[39] and therefore can be conveniently speculated that xylopic acid may not rely on such pathways in exhibiting the anxiolytic properties observed so far. This lack of anxiolytic-like response in the scototaxis test in zebrafish could be overlooked by the fact that, xylopic acid exhibited anxiolytic-like properties in another zebrafish model of anxiety induced by alcohol withdrawal. When regular consumption of alcohol is ceased in human[43], rodents[44] and zebrafish[45] withdrawal symptoms related to exaggerated anxiety is observed. In zebrafish models, there is enhanced bottom dwelling, erratic movement, and delayed latency to explore near-surface regions when alcohol exposure is abruptly withdrawn[46,47]. All these signatures of anxiety behaviors induced by alcohol withdrawal were ameliorated by xylopic acid which leads further credence to the fact that the kaurane diterpene, xylopic acid, has anxiolytic-like properties in mice and zebrafish.

5 Conclusion

These findings in mice and zebrafish and models of anxiety disorder demonstrate xylopic acid, a kaurane diterpene isolated from *Xylopia aethiopica* fruit extract possesses anxiolytic effects.

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7 Conflict of interest

None of the authors have any competing interest to declare with regards to information contained in this publication.

8 Author's contributions

RPB, CKB and JOK concieved, performed and analysed the experiments and drafted the manusrcipt. EOA, EBG and EW analysed the data and drafted the manuscript.

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