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ANXIOLYTIC ACTIVITY OF LAVENDER ESSENTIAL OIL IN THE ELEVATED PLUS MAZE MODEL

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Abstract

Lavender essential oil is widely used as a calming aroma, and rodent behavioural tests have been used to ask whether the reputation is deserved. This narrative review summarises published work on the anxiolytic activity of the oil, with emphasis on the elevated plus maze, and reports no new experimental data. The maze is described together with the factors that change its readouts, followed by the chemistry of the oil, the behavioural findings from inhalation and systemic dosing, proposed mechanisms and the clinical context. Most reports point towards anxiolytic-like effects, partly through serotonergic and olfactory routes rather than the benzodiazepine site. Direct plus-maze data are limited, and oil composition, dose and locomotor controls are often poorly reported.

1. INTRODUCTION

Anxiety disorders are among the most common psychiatric conditions worldwide, and the drugs used to treat them have well-known drawbacks. Benzodiazepines act quickly but cause sedation, tolerance and dependence, while selective serotonin reuptake inhibitors (SSRIs) take weeks to work and can briefly worsen nervousness at the start of treatment [1, 2]. The search for better-tolerated anxiolytics has been long and, by most assessments, disappointing, with very few mechanistically new agents reaching the clinic over several decades [2]. Against that background, plant-derived products with a long record of folk use have attracted steady interest, partly because patients already self-medicate with them and partly because some have been tested in controlled trials [3, 4].

Lavender is probably the best-known example. Essential oil distilled from the

flowering tops of *Lavandula angustifolia* has been used for centuries as a calming aroma, in baths, on pillows and in massage, and it is a standard ingredient of aromatherapy practice [3, 5]. Its reputation rests on a mixture of tradition, small clinical studies and a respectable body of animal work. The animal work matters because it allows the oil to be tested without the expectation effects that complicate aroma trials in people, and because it makes it possible to ask which constituents and which receptor systems are involved [6, 7, 8].

This review looks at what has been published on the anxiolytic activity of lavender essential oil when it is assessed in the elevated plus maze (EPM), the most widely used rodent test of anxiety-like behaviour. It reports no new experimental data. The aim is to describe how the maze is run and read, what the oil contains, what other investigators have found after inhalation or systemic dosing, how those effects have been explained, and where the evidence is weaker than the enthusiasm around it sometimes suggests [9, 10, 11]. Particular attention is given to design details that change the answer, such as route of administration, dose, strain, handling and the control of locomotor activity [12, 13, 14].

A word on terminology helps. In this literature, lavender oil, lavender essential oil and lavender aroma are sometimes used interchangeably, although they describe different exposures. Inhaled oil reaches the animal through the olfactory epithelium and, in part, through the lungs, whereas oil given by injection or by mouth is absorbed and distributed like any other lipophilic drug. A standardised capsule preparation such as Silexan is a defined, quality-controlled oil, and findings from it should not be assumed to transfer to a commercial fragrance oil of unknown composition [15, 16]. Reading the papers with that distinction in mind removes many apparent contradictions between them.

2. THE ELEVATED PLUS MAZE

The elevated plus maze was introduced for rats by Pellow and colleagues in 1985 and adapted for mice by Lister two years later [17, 18]. It exploits a conflict. Rodents are drawn to explore novel spaces but avoid open, brightly lit, elevated areas, so a plus-shaped platform with two enclosed and two open arms sets curiosity against fear. Anxiolytic drugs such as diazepam shift behaviour towards the open arms, increasing the proportion of entries and the time spent there, while anxiogenic compounds do the reverse [17, 18]. The apparatus needs no training or food deprivation, a session lasts five minutes, and the readouts are simple to score by hand or by video tracking.

Validation rested on pharmacology as well as behaviour. In the original work, open-arm avoidance was accompanied by raised plasma corticosterone and by freezing and defecation, and benzodiazepines reversed these signs [17]. Later reviews have confirmed that the test detects classical anxiolytics acting at the benzodiazepine site of the $GABA_A$ receptor, although it responds less reliably to drugs from other classes, and results with buspirone, for instance, have been inconsistent [13, 19, 20]. The test therefore has good predictive validity for one mechanism and uncertain validity for others, a point that matters when the compound under study is a multicomponent oil whose actions may not involve the benzodiazepine site at all.

What is scored, and how, shapes the conclusions. The two headline measures are the percentage of open-arm entries and the percentage of time on the open arms, both expressed relative to total arm activity so that an animal that simply moves a lot is not mistaken for a less anxious one [9, 21]. Closed-arm entries are commonly reported as a rough index of general locomotion [9]. Ethological measures, including head dipping, stretched-attend postures and rearing, add information about risk assessment and can reveal drug effects that the conventional indices miss [22]. Many lavender papers report only the conventional indices, which limits what can be said about mechanism.

The test is also sensitive to conditions that have nothing to do with the drug. Light intensity, the height of the maze, the time of day, the sex and strain of the animal and the handling history of the animals can all move open-arm exposure by a large margin [12, 13,

14]. Mouse strains differ markedly: some inbred lines are so anxious that open-arm exposure is close to floor, whereas others show ceiling levels, and either extreme hides a drug effect [23]. Prior experience of the maze is another trap, because a second exposure changes behaviour and often abolishes benzodiazepine sensitivity, so repeated testing of the same animal is rarely appropriate [12, 22]. Animal studies that omit randomisation and blinding have been reported to show larger apparent effects, so these details matter when methods are compared [24].

Distinguishing a true anxiolytic effect from a nonspecific change in activity is the central interpretive problem. A compound that sedates will reduce all arm entries, including those into the open arms, and may look anxiogenic, while a stimulant can increase open-arm entries simply because the animal covers more ground. For this reason the usual advice is to treat open-arm percentages as meaningful only when total entries or closed-arm entries are unchanged [9, 12]. In the lavender literature, where sedation is a documented property of the oil and of linalool, this advice is not a technicality: a lower level of locomotion could account for part of the reported change in behaviour [6, 25].

For these reasons the plus maze is best treated as one component of a battery. Open-field activity, the light-dark box, the social interaction test and conflict procedures each capture a slightly different facet of anxiety-like behaviour, and a compound that is active in several of them is more convincing than one active in a single test [19, 20, 26]. Lavender studies that combine the maze with at least one other paradigm, and with a locomotor control, give far more interpretable results than those that rely on the maze alone, and the better designed inhalation experiments have generally followed that route [25, 27, 28].

Practicalities of setting up the maze deserve attention in any comparison between laboratories. Mouse mazes typically have arms in the region of 25 to 35 cm long and 5 cm wide, raised some 40 to 50 cm above the floor, with enclosed arms bounded by walls around 15 cm high, while rat mazes are larger. Lighting in the room is commonly kept dim to moderate, the animal is placed in the central square facing an open or closed arm, and the maze is cleaned between animals to remove odour cues. That last step matters especially when the test substance is itself an odorant, since residual lavender scent on the platform could influence the next animal [9, 21].

3. COMPOSITION OF LAVENDER OIL

Lavender essential oil is obtained by steam distillation of the flowering spikes of *Lavandula angustifolia*, and its composition is dominated by two monoterpenoids, linalool and linalyl acetate, which together usually account for well over half of the oil. Smaller amounts of lavandulyl acetate, terpinen-4-ol, 1,8-cineole, camphor, borneol and several sesquiterpenes complete the profile [3, 8]. The proportions vary with cultivar, harvest time, geographical origin and distillation conditions, and pharmacopoeial and trade standards set ranges for the main constituents instead of fixed values [3, 29]. Two oils sold under the same name can therefore differ in ways that affect pharmacology.

Several different *Lavandula* species and hybrids reach the market, which complicates matters further. Lavandin (*Lavandula* × *intermedia*) is cheaper and richer in camphor and 1,8-cineole, and spike lavender (*Lavandula latifolia*) is higher still in those compounds, so substituting one for another changes the chemical signature of the oil [3, 29]. Adulteration with synthetic linalool or linalyl acetate has also been reported for commercial samples. Papers that identify the botanical source, give a gas chromatography (GC) profile of the batch actually used and state the supplier are far easier to interpret and to repeat than those that mention only lavender oil [8, 29].

Linalool has received the most attention as the putative active principle. Given by inhalation, it reduced locomotion in mice in a dose-related way, and at suitable exposures it produced anxiolytic-like effects without obvious motor impairment [25, 27]. Linalool oxide, a related monoterpenoid found in smaller amounts in some oils, was also active

in mouse anxiety models [30]. Anticonflict testing in mice, with fractionation of the oil, pointed to linalool as one of the active constituents [31]. Earlier neurochemical work had already linked linalool to the glutamatergic system, so the interest was not new [32]. Which compound carries the effect plainly depends on the model and the dose.

Absorption deserves a mention because the route changes exposure. Linalool is lipophilic, small and volatile, so inhaled material crosses the nasal and pulmonary mucosa readily, whereas oil given by mouth or by injection is absorbed and then metabolised in the liver, where linalool undergoes oxidation and conjugation [8, 29]. High or repeated dosing raises questions about enzyme induction and interaction with other central depressants, though formal pharmacokinetic work on the whole oil is thin. Studies that measured plasma or brain concentrations of the constituents alongside behaviour remain rare, which leaves the behavioural doses difficult to compare with human exposures [8, 25].

Analytical control is straightforward in principle. Gas chromatography with flame ionisation or mass spectrometric detection resolves the main components within a single run, and retention indices allow identification against reference libraries. Pharmacopoeial monographs for lavender oil specify ranges for linalool, linalyl acetate, lavandulyl acetate and terpinen-4-ol, together with limits for camphor and 1,8-cineole that help to exclude lavandin and spike lavender [3, 29]. Chiral analysis can go further, since natural linalool is predominantly a single enantiomer whereas synthetic material is racemic, which provides a check on adulteration. Few behavioural papers report any of this, even though the information is inexpensive to obtain [8, 29].

Because the oil is a mixture, combined effects are to be expected. Linalool and linalyl acetate may act additively, and minor constituents such as terpinen-4-ol, cineole and camphor have their own central or sensory activity [3, 8]. Whole-oil studies and single-compound studies answer different questions: the former ask whether the product works, the latter ask why. Papers that move straight from a positive result with the oil to a statement about linalool, without testing the isolated compound at a matching dose, go further than their data allow, and the reverse inference from a single compound to a whole oil is equally unsafe [27, 31, 33].

Odour itself may contribute to behavioural effects, independently of any pharmacological action in the brain. Rodents have a keen sense of smell and respond to novel odours with changes in exploration, which is why anosmic animals are such a useful control. In mice made anosmic, the anxiolytic-like effect of inhaled linalool was lost, which suggests that signalling through the olfactory system carries much of the effect, with any systemic action playing a smaller part [33]. Earlier inhalation work in mice had similarly shown that a sedative response to lavender oil and linalool could be recorded without any injection at all [6].

4. REPORTED FINDINGS

The published plus-maze studies of lavender oil are few in number but varied in design, and the main groups of findings are collected in table 1, together with the closest related behavioural tests. Inhalation is the most popular route and has produced the most consistent signals in mice, rats and gerbils, whereas systemic dosing has been explored in a smaller number of reports [7, 10, 28]. The entries in the table are grouped by the kind of evidence they provide, and the distribution of entries across those groups is shown in fig. 1. Direct plus-maze reports are fewer than the general reputation of the oil would suggest, and much of the supporting evidence comes from neighbouring tests.

The most direct plus-maze evidence comes from experiments in the Mongolian gerbil, in which exposure to lavender odour was associated with an anxiolytic-like pattern of open-arm behaviour [10]. Gerbils are not the usual choice, and their behaviour in the maze is not identical to that of rats and mice, so the result is best read as supportive rather than definitive. The study is nevertheless useful because it used the odour as the manipulated variable, which is how lavender is used in practice. It also shows how an olfactory stimulus can be introduced into the test without handling or injection stress, itself a

recognised source of variability in the maze [10, 12].

Table 1: Selected published behavioural findings for lavender oil and its constituents

Exposure and model	Reported finding	Ref.
Inhaled lavender odour Mongolian gerbil, plus maze	Anxiolytic-like pattern of open-arm behaviour during odour exposure compared with controls	[10]
Inhaled lavender oil Rat, open field	Behaviour shifted in a direction consistent with lower anxiety; ambulation also changed	[28]
Inhaled lavender oil Mouse anxiety tests	Anxiolytic-like effect not blocked by flumazenil but reduced by a 5-HT1A antagonist	[7]
Inhaled linalool Mouse, social interaction	Anxiolytic-like activity and reduced aggression at exposures that left activity largely intact	[27]
Inhaled linalool Mouse, locomotion	Dose-related sedation and reduced motor activity	[25]
Linalool oxide Mouse anxiety models	Anxiolytic-like effects after inhalation	[30]
Linalool odour Mouse, anosmic control	Anxiolytic-like effect lost in anosmic mice and not reversed by flumazenil	[33]
Lavender oil fractions Mouse conflict test	Anticonflict action with linalool among the active constituents	[31]
Lavender oil and linalool Mouse motility	Reduced motility, including caffeine-induced hyperactivity, after inhalation	[6]
Oral Silexan Human trials	Lower anxiety scores than placebo; similar to lorazepam in one trial and paroxetine in another	[15, 34, 35]

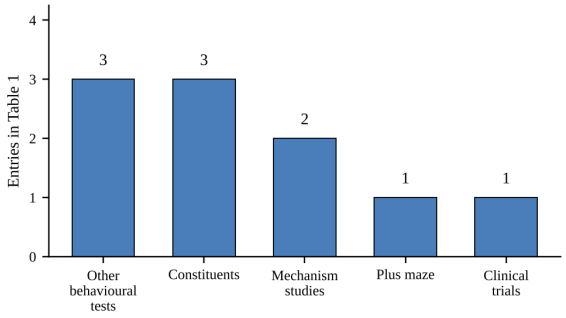


Fig. 1: Number of entries in table 1 by type of evidence

In rats, inhaled lavender oil altered behaviour in the open field in a direction consistent with reduced anxiety, and the authors compared the pattern with that produced by a classical anxiolytic [28]. The same report is a reminder of how hard it is to separate anxiety from activity, since ambulation and rearing changed as well. A reduction in anxiety-related behaviour that comes with sedation is a different thing from a selective anxiolytic effect, and the interpretation therefore depends on whether locomotor measures are reported alongside the anxiety indices [9, 28]. Few of the lavender papers publish both in a form that allows the reader to decide.

Work in mice has probed the mechanism more directly. Chioca and colleagues exposed mice to vaporised lavender oil and recorded anxiolytic-like behaviour that was not prevented by the benzodiazepine antagonist flumazenil but was reduced by a 5-HT_{1A} receptor antagonist [7]. Linck and colleagues, using inhaled linalool, found a sedative response in one study and, at other exposures, anxiolytic-like activity and reduced aggression in social-interaction testing [25, 27]. A conflict procedure in mice, with fractions of the oil, gave a comparable signal [31]. As a group, the direction of effect is consistent even though the individual tests differ.

Older inhalation work helped to establish the field. Buchbauer and colleagues showed that inhaled lavender oil and linalool reduced the motility of mice, including the hyperactivity produced by caffeine, which fits a sedative component [6]. More recently, linalool odour was reported to produce anxiolytic-like effects in mice that were lost in anosmic animals and were not reversed by flumazenil [33], and linalool oxide had anxiolytic-like activity in several mouse models [30]. These studies lean on odour as a route to the brain, a feature that makes the maze, with its dependence on an unconditioned reaction to novelty, a reasonable but far from perfect tool.

Better-designed studies share a few features. They state the species of lavender and the supplier, they include a vehicle or neutral-odour control and often a reference

drug such as diazepam, they report locomotor measures with the anxiety indices, and they use more than one exposure level so that a dose-response pattern can be judged [7, 27, 28]. Studies without randomisation and blinding have tended to report larger effects, and texts on experimental design stress adequate group sizes and random allocation [24, 36]. Studies meeting this standard remain the exception in the lavender field.

Reading these reports side by side, two cautions emerge. First, the exposure is rarely quantified. Concentrations are given as millilitres of oil on a pad or per hour of vaporisation, and the air concentration actually reached by the animal is almost never measured, so dose-response relationships between laboratories cannot be compared [7, 25, 28]. Second, the literature is small enough that no formal meta-analysis of rodent plus-maze data is possible, and positive findings are more likely to be published than negative ones [11, 24]. The consistent direction of effect is encouraging, but it falls short of firm evidence.

A detail that rarely receives comment is the choice of vehicle and control. When oil is injected, it must be emulsified in saline with a surfactant or dissolved in a carrier oil, and the vehicle itself can alter behaviour. When oil is inhaled, the control group needs to be exposed to an equally strong but neutral odour, otherwise the comparison confounds lavender with the presence of any strong smell. Several papers use water or untreated air as the only control, a design that makes it difficult to say whether the effect belongs to lavender specifically or to novel odour in general [9, 24, 36].

5. PROPOSED MECHANISMS

The first mechanistic question is whether lavender acts like a benzodiazepine. In vitro, linalool and some perfume constituents potentiated GABA-activated currents in recombinant receptors expressed in oocytes, which gave early support for a GABAergic action [37]. In the whole animal the picture is less clear. Flumazenil failed to block the anxiolytic-like effect of inhaled lavender oil or linalool odour in mice, which argues against a mechanism at the benzodiazepine site, although a modulatory action at other sites on the $GABA_A$ receptor cannot be excluded [7, 33, 37]. The oil does not appear to behave as a simple benzodiazepine mimic.

A serotonergic contribution has better in vivo support. The effect of inhaled lavender oil in mice was reduced by an antagonist of the $5\text{-}HT_{1A}$ receptor, the target of buspirone, and in binding experiments the oil displaced ligands from the serotonin transporter (SERT) [7, 8]. That fits with the clinical observation that standardised lavender oil, unlike a benzodiazepine, does not appear to produce marked sedation or withdrawal problems in trials [15, 35]. The two lines of evidence come from different laboratories and different preparations, so they should be treated as converging hints and not as proof of a single serotonergic mechanism.

Glutamatergic and ion-channel mechanisms have also been suggested. Linalool interfered with glutamate binding and release in rat cortical preparations, and the whole oil was reported to bind to the NMDA receptor in vitro [8, 32]. Such effects would not necessarily show up as a plus-maze signal, but they could account for sedative, anticonvulsant and anticonflict actions seen in other tests [6, 31, 32]. There is also an older literature on smooth-muscle relaxation by lavender oil, suggesting a nonspecific membrane effect at high concentrations that may not be relevant to behaviour at the doses reached through inhalation [38]. Separating specific from nonspecific actions remains an open task.

The olfactory route offers a different kind of explanation. Odour molecules bind receptors in the nasal epithelium, and the olfactory bulb projects to the amygdala, piriform cortex and hippocampus, structures involved in emotional processing. If lavender works partly as a learned or innate odour cue, the effect would depend on intact olfaction and not on blood levels, which is the pattern reported for linalool in anosmic mice [33]. Smell-mediated effects would also be sensitive to previous exposure, concentration and context, which could explain some inconsistency between studies and the difficulty of reproducing aroma effects with injected oil [6, 8, 33].

None of these mechanisms excludes the others, and the available data do not make it possible to rank them. A reasonable working view is that lavender oil has several weak actions, some pharmacological and some sensory, which add up to a modest anxiolytic-like effect at tolerable exposures. That view would also fit the clinical pattern, where the oil gives benefits comparable to some conventional drugs in short trials but without the sharp, rapid sedation of a benzodiazepine [34, 35]. Experiments that combine receptor antagonists, anosmia and isolated constituents in the same animal strain would give a clearer answer than further single-test reports.

Receptor-binding screens deserve a cautious reading. Binding to a transporter or receptor in a membrane preparation, usually at micromolar concentrations of oil, shows that an interaction is possible but says little about whether brain concentrations after inhalation ever reach that level. Without measured tissue concentrations, a binding result is a hypothesis generator. The same applies to patch-clamp results from oocytes, where concentrations can be set freely and the surrounding network of neurons is absent. Experiments that tie in vitro concentrations to the plasma or brain levels reached during behavioural testing would add real weight to the mechanistic claims [8, 37].

6. CLINICAL CONTEXT AND LIMITATIONS

The strongest human data concern Silexan, an oral capsule of lavender oil with a defined composition. In a trial of subsyndromal anxiety, the preparation reduced anxiety scores more than placebo [15]. In generalised anxiety disorder (GAD), a six-week comparison with low-dose lorazepam found similar improvement on the Hamilton anxiety scale, and a later study found it comparable to paroxetine and superior to placebo [34, 35]. A further trial in people with restlessness and disturbed sleep reported benefit over placebo [16]. These studies are industry-supported and short, but they give the oil a clinical foundation that most herbal anxiolytics lack.

Dose and formulation matter when moving from the bench to the bedside. The trials cited above used a fixed oral dose of the order of 80 mg of oil daily, in a capsule designed to avoid the reflux and aftertaste that unprotected lavender oil produces, and a 160 mg dose was also tested [15, 35]. Such doses are far removed from inhalation of a vaporised oil by a mouse in a chamber, and neither is much like a few drops placed on a pillow. The clinical findings therefore support the idea that lavender oil has anxiolytic potential, not that every route and product works equally well, and they give no direct validation of the plus-maze results [16, 34, 39].

Reviews of the clinical literature are more guarded than the individual trials. A systematic review of randomised trials of lavender in anxiety concluded that the evidence was promising for some preparations but limited by the small number of trials and by methodological weaknesses, and a general review of its effects on the nervous system came to a similar view [5, 11]. A network meta-analysis of oral Silexan reported favourable effects against placebo [39]. Guideline groups for anxiety disorders have continued to centre treatment on psychological therapy and on SSRI or related medicines, with lavender at most a candidate for further study [1].

Safety evidence is reassuring in some respects and incomplete in others. In the oral trials, adverse events were mostly gastrointestinal and often described as lavender aftertaste or belching, and withdrawal or dependence problems were not reported [15, 35]. Topical and aromatic exposure is less benign for some people, with allergic contact dermatitis described for lavender oil and its oxidation products [29]. A case series suggested an association between repeated topical use of lavender and tea tree oils and prepubertal gynaecomastia in boys, with endocrine activity of the oils shown in cell assays, although a causal link cannot be firmly established [40].

Several limitations apply to the preclinical evidence as a whole. The oils tested are poorly characterised, doses are expressed in ways that cannot be compared, and exposure by inhalation is seldom measured [8, 25, 29]. Most reports test males, often in a single

strain, and few state randomisation, blinding or sample-size justification, which are recognised weaknesses of animal research in general [24, 36]. Locomotor activity is not always controlled, and the plus maze may be a poor detector of the serotonergic and olfactory actions suggested for the oil [13, 20]. The step from rodent behaviour to a human anxiety disorder is large, a concern that applies to the field of anxiolytic discovery generally [2].

Future work can address most of these gaps. Studies should name the plant species and chemotype, report a GC profile, and measure the concentration of the main constituents in air and, ideally, in blood and brain [8, 29]. Designs should include a neutral odour control, a positive control such as diazepam, anosmic animals and both sexes, with scoring of ethological measures along with the conventional indices [9, 22, 41]. Combining the plus maze with the light-dark box or social interaction would help to separate anxiolytic from sedative effects [19, 26, 27]. If these steps were taken, the field would be in a far better position to explain, and perhaps to improve on, the calming effect of lavender.

A final practical point concerns the way results are discussed. Aroma research attracts a popular audience, and a positive rodent finding is easily presented as proof that lavender treats anxiety. The chain of inference is long: from an unconditioned avoidance behaviour in a mouse, to a pharmacological mechanism, to a clinical syndrome that is characterised by worry and autonomic arousal. Each link is weaker than the last. Honest discussion of the plus-maze results should therefore stay with what the test can show, namely a change in exploratory behaviour under conflict, and should leave claims about treatment to properly designed human trials [2, 19, 20].

7. CONCLUSION

The literature reviewed here suggests that lavender essential oil has anxiolytic-like activity in rodents, most clearly after inhalation, and that its effect is probably the sum of several modest actions rather than a single benzodiazepine-like mechanism [7, 10, 33]. Linalool is an important contributor, but linalyl acetate and minor constituents may add to the effect, and odour itself seems to carry part of the response [27, 30, 31]. Direct plus-maze data are thinner than the reputation of the oil would imply, and much of the supporting evidence comes from related tests.

The strongest message for readers concerns design. Botanical identity, chemical profile, exposure measurement, neutral odour controls, locomotor measures and reporting standards all determine whether a positive result is believable [24, 29, 36]. Clinical trials of standardised oral preparations give a more solid foundation than animal work alone, though they cannot be transferred automatically to aroma products [11, 15, 34]. Better characterised oils and better controlled mazes would make the next round of studies far more informative than another set of single-dose reports.

8. ABBREVIATIONS

EPM-Elevated plus maze, GABA-Gamma-aminobutyric acid, GAD-Generalised anxiety disorder, GC-Gas chromatography, 5-HT-5-Hydroxytryptamine (serotonin), NMDA-N-methyl-D-aspartate, SERT-Serotonin transporter, SSRI-Selective serotonin reuptake inhibitor

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10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

12. REFERENCES

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