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COMMENTARY

On *Gelsemium* and Complementary and Alternative Medicine (CAM) in Anxiety and Experimental Neurology

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ABSTRACT

A recent discussion expanded the debate about the experimental research on *Gelsemium* in anxiety. Herbal medicine is widely used in anxiety and mood disorders, often with contradictory evidence, although some authors are yet prompted to promote their full introduction in pharmacology as a promising therapy. Complementary and alternative medicine (CAM) in anxiety is particularly appreciated by individual healthcare, but deserves further investigation, as many critical issues have been recently raised. Comments about the ability of negligible doses of *Gelsemium* hydroalcoholic extracts to affect gene expression were recently reported.

Keywords: *Gelsemium*; Gelsemine; GABA receptors; Behaviour; Anxiety

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INTRODUCTION

Herbal medicine is widely used in anxiety and mood disorders, often with contradictory evidence [1], although some authors are yet prompted to promote their full introduction in pharmacology as a promising therapy [2, 3]. Complementary and alternative medicine (CAM) in anxiety is particularly appreciated by individual healthcare [4], but deserves further investigation, as many critical issues have been recently raised. [5, 6]. As a matter of fact, a recent discussion expanded the debate about the experimental research on *Gelsemium* in anxiety [7–11]. This Commentary tries to elucidate major issues causing this debate by addressing the numerous aspects raised in comments published elsewhere in the literature.

The anxiolytic property of *Gelsemium* plant has been extensively reviewed [11–14]. Raw alcoholic extracts from *Gelsemium sempervirens* showed the ability to modify the response of mice in behavioural tests and reduce anxiety [15]. In this research, the anxiolytic property related to *Gelsemium* extracts has been quite exclusively associated with the alkaloid gelsemine [13, 15, 16]; yet, *Gelsemium* plants

contain many further alkaloids with anxiolytic potential [12], thus suggesting that the anti-anxiety activity of *Gelsemium sempervirens* may come indifferently from gelsemine, koumine, or gelsevirine or a complex mixture of several active alkaloids [13]. Actually, plants from the genus *Gelsemium* are considered a source of potential anxiolytic substances [12]. This means that experimental neuroscience based on the possible use of *Gelsemium* as a CAM therapy for anxiety shows many difficulties in highlighting a single active principle accounting for the presumptive evidence of efficacy observed in in vitro and animal models. The current debate on *Gelsemium* and anxiety includes the many issues exemplified in Table 1, where bias, comments, and replies to comments are thoroughly summarized. A comprehensive neuropharmacology of *Gelsemium* should take into consideration any aspect coming from issues described within the reported table.

Most of articles dealing with *Gelsemium* in anxiety pertain to CAM therapy. A Pubmed/Medline search of the MESH term *Gelsemium* allowed us to retrieve 121 papers from 1945 to date, of which 83 dealt with *Gelsemium* in herbal medicine and CAM. The excellent journal Psychopharmacology published at least two papers about *Gelsemium* in homeopathy [15, 17], showing either a cataleptogenic or anxiolytic action by *Gelsemium* 30cH, i.e. a theoretical gelsemine concentration less than 6×10^{-60} mol/L [15]. In this circumstance, it should be quite difficult to associate any neurologic effect whatsoever with any active molecule present in serially diluted extracts from the *Gelsemium* plant. Moreover, comments were raised about the presence of ponderable, significant moles of ethanol added as a co-solvent with water [9, 10, 18, 19]. While a *Gelsemium* 30CH might have negligible traces of possible active principles, its ethanol content would be within the range 0.5–1.0 mM [18], an occurrence that raised comments about the active molecule in the observed and reported effects [15, 18–21]. These issues prompted this author to address the debate about *Gelsemium* in the following step-points.

This article is based on previously conducted studies, and does not involve any new studies of human or animal subjects performed by the author.

ACTIVE PRINCIPLES, SOLVENT, AND MECHANISM OF ACTION

Alcoholic raw extracts from plants contain alkaloids and other molecules that may interfere with a plain interpretation of the pharmacology of active principles, due to the complex interaction, either synergistic or competitive, existing between different substances in the raw mixture [11]. Particularly, gelsemine has been recently associated with a well-defined neuro-pharmacological mechanism related with anxiety. It modulates anxiety in laboratory animals at a sub-micromolar dose range, and in fact, gelsemine doses from 10^{-6} to 10^{-10} M induce an anxiolytic action in rats in the elevated plus-maze test [13]. Gelsemine is a *Gelsemium* derived alkaloid sharing a chemical and functional kinship with strychnine [22]. In rat spinal cords, gelsemine showed an additive effect with glycine in increasing the production of the neurosteroid allopregnenolone (3α,5α-tetraidroprogesterone or 3α-idrossi-5α-pregnan-20-one, 3α,5α-THP), which in turn should increase anxiety, due to an increased hippocampal expression of α4βδ GABAA receptors [23, 24]. 3α,5α-THP is a positive

Table 1 Fundamental issues in the research about *Gelsemium* and anxiety

Topic	Issues and bias	References	(A) Comments and (B) replies in:
Active principles	Gelsemine (3-ethenyl-1-methyl-2,3,3a,7,8,8a-hexahydro-1 h,5 h-spiro[3,8,5-(ethane[1, 1, 2]triyl)oxepino[4,5-b]pyrrole-4,3′-indol]-2′(1′h)-one) was the only active principle described in the behavioural research	[15, 21, 40]	A [9] B [10]
	• BIAS 1. *Gelsemium* plant extracts contain other alkaloids with anxiolytic activity (e.g. koumine, gelsevirine)		
	• BIAS 2. The anxiolytic activity of *Gelsemium* alcoholic extracts was not further dissected in order to identify one or more coworking active principles;		
	• BIAS 3. Gelsemine was supposed to be the only active principle working in *Gelsemium* extracts on the simple basis of previous in vitro pharmacological evidence		
	• BIAS 4. Adverse effects not evaluated		
Solvent and test samples	*Gelsemium* extracts were used as a 30 % EtOH/water mixture and further diluted with EtOH and water to have test samples	[14–16, 20, 21]	A [8, 9, 18] B [10, 19]
	• BIAS 5. Refs [15, 16, 20, 21] started from a raw EtOH/H2O *Gelsemium* extract containing 30 % alcohol (∼50 mM EtOH), then a 1:100 dilution into water with 30 % EtOH and a 1.100 dilution into water (1:10,000) followed. Final dilution contained ∼500 μM EtOH, still biologically active		
	• BIAS 6. Ethanol is effective on gene expression at molar concentrations as low as 250 μM		
Experimental setting (a): animal model	Animal models: mouse	[15, 16]	A [8, 9] B [10]
	• BIAS 7. Behavioural tests were performed on mice and in vitro confirmatory cellular tests on humans		
	• BIAS 8. Behavioural tests performed did not include specific tests on anxiety, depression, sedation		
	• BIAS 9. Operators treating animals performed behavioural tests		
Experimental setting (b): in vitro cell model	In vitro cell model: human neuroblastoma cell line	[20, 21]	A [18] B [19]
	• BIAS 10. Criticism in gene expression performing and interpretation		

Table 1 continued

Topic	Issues and bias	References	(A) Comments and (B) replies in:
Pharmacological interpretation	Associated exclusively with gelsemine and considering the allopregnenolone/GABAR pathway • BIAS 11. The anxiolytic activity of *Gelsemium* may derive from other alkaloids besides gelsemine • BIAS 12. Gelsemine can be anxiolytic through a GlyR/GlyT1-mediated mechanism Dilutions and ponderal chemistry. Solvents • BIAS 13. Pharmacological interpretation may be hindered by diluted test solutions with negligible amounts of active principles • BIAS 14. Ethanol amount is much more higher than any *Gelsemium* derived active principles in tested solutions besides *Gelsemium* 2CH	[15]	A [8, 18] B [19]
Statistics	Statistics with pooling data • BIAS 15. Pooling data projected to retrieve positive evaluation of the mechanism • BIAS 16. Blinded confounders with the same operator in treating and performing test with animals	[15]	A [8] B [10]

modulator of GABAA receptors and may cause anxiogenic and adverse mood effects in particular circumstances involving steroid withdrawal [25]. The effect of 3α,5α-THP on GABAA receptors is particularly complex in neuroscience and depends on the many factors related to chronic stress, the expression level of the GABA receptor α4 subunit, the direction of chloride-mediated ionic fluxes created by these target receptors, leading also to a downregulation or dampening in the benzodiazepine ability to modulate this mechanism [8, 25]. This should suggest that, at least in animal models, the anxiolytic action attributed to gelsemine may be actually caused by other mechanisms, and more caution is requested about a presumptive 3α,5α-THP/GABA relationship with anxiolytic effects. Interestingly, recent reports on the effect of hydroalcoholic extracts from *Gelsemium sempervirens* on mouse behaviour showed a marked insensitivity of mice to diazepam [26]. In this circumstance, criticism was raised about setting and evaluation of mice stress response in behavioural tests [8, 9]. Furthermore, other alkaloids contained in *Gelsemium* plants, such as koumine, have been associated with a 3α,5α-THP/GABA receptor signaling [26].

Yet, the anxiolytic activity exerted by *Gelsemium* might be caused by many further mechanisms. Many *Gelsemium*-derived alkaloids, such as kuomine and gelsenicine [26, 27] exert a nociceptive effect. Particularly, gelsemine acts on chronic pain through the

activation of spinal α3 glycin receptors (GlyR) [22]. This should suggest that the anxiolytic activity associated with *Gelsemium* may not directly come from GlyR activation, but most probably from the contribution of activated GlyR on the anziolytic activity of glycine transporter inhibitors [28]. Therefore, it is very difficult to highlight the neurological mechanism by which *Gelsemium* exerts its anxiolytic activity, when *Gelsemium* extract is used within a micromolar-millimolar range. Furthermore, *Gelsemium* contains a lot of molecules with sedative, anti-depressant activity [8], for which it is very difficult to ascertain an anxiolytic activity only by widely used, not properly suited behavioural tests [8, 9, 15]. In this perspective, other components contained in *Gelsemium*-derived test solutions such as ethanol, may be significantly involved [8]. Describing a comprehensible overview of the anxiolytic activity of *Gelsemium* extracts, is hampered also by the recent observation that flavonoids, which are present in the *Gelsemium* plant, may exert an anxiolytic action [29]. Furthermore, the involvement of the GABAergic system in anxiety models is yet controversial, because anxiogenic/anxiolytic activity on GABAergic systems may be modulated by different types of orexins [30]. This strongly suggests that the interpretation of *Gelsemium* anxiolytic activity by involving a single, defined mechanism [15] may be reductive.

A recent behavioural research on ICR-CD1 mice used an hydroalcoholic extract of *Gelsemium sempervirens*, which was serially diluted to reach negligible concentrations of potentially bio-active molecules [15]. ICR-CD1 mice are not particularly suited for behavioural tests compared to the more considered C57BL6 J mouse [31]. A large number of the laboratory mice sold and used by investigators around the world are considered to be outbred or random-bred. Popular stocks of such mice in the US include CD-1 (Charles River Breeding Laboratories), Swiss Webster (Taconic Farms), and ICR, and NIH Swiss (both from Harlan Sprague–Dawley). Outbred mice are used for the same reasons as F_1 hybrids—they exhibit hybrid vigor with long life spans, high disease resistance, early fertility, large and frequent litters, low neonatal mortality, rapid growth, and large size. However, unlike F_1 hybrids, outbred mice are genetically undefined. Nevertheless, outbred mice are bought and used in large numbers simply because they are less expensive than any of the genetically defined strains. These animals are widely used for behavioural tests. Behavioural tests most commonly used, such as the light dark box test (LDBT) or open field test (OFT), should evaluate time spent at light, without hiding into a small pitch dark hole or walking on the centre of an empty arena, as a measure of stress lacking or anxiety absence for tested animals [15], yet these tests are used also to evaluate sedation, fear-related stress and depression [9] and are much less specifically used for anxiety research than others [8].

Test solutions of *Gelsemium* alcoholic extracts were made by diluting 1:100 solutions starting from a raw material containing 30 % or about 50 mM ethanol [15, 20, 21]. Concentration of gelsemine, a major component of *Gelsemium* extract, was calculated as low as 6.5×10^{-4} M in the fresh hydroalcoholic raw extract, then diluted 1:100 (6.5×10^{-6} M) in 30 % ethanol (49.93 mmol/L) and significant evidence reported for tested solutions containing an estimated concentration of 6.5×10^{-20} M gelsemine and 4.99×10^{-4} M ethanol [15, 20, 21]. While the final concentration of ethanol (EtOH) at the so-called *Gelsemium* 2CH should be as low as

5×10^{-6} M and gelsemine calculated as 6.5×10^{-8} M as 2CH means a final dilution 1:10,000, the authors made 1CH (1:100) in 30 % ethanol (50.5 mM EtOH) and 2CH into water (0.505 mM EtOH, i.e. 5.05×10^{-4} M EtOH) [15, 20, 21]. Therefore, in *Gelsemium* 2CH, the ratio EtOH/gelsemine was about 10,000:1 [15, 20]. Because any further dilution was made with this approach, this ratio was particularly higher for EtOH with respect to *Gelsemium* at 9CH and even more at 30CH. Comments raised about this alkaloid/ethanol disproportion, which suggested a preponderant role from ethanol respect to *Gelsemium* components in modifying mice behaviour in a LDBT and OFT [15], also highlighted why the evidence was scarcely reproducible [9, 32]. The authors claimed their results as promising and explained *Gelsemium* ability to reduce anxiety in mice by an anxiolytic effect attributed to gelsemine and 3α,5α-THP [15]. In their paper, the minimal effective concentration of gelsemine, estimated by the iterative dilution process from 6.5×10^{-4} M, was much lower the concentration reported in recent studies [13, 15].

The same research group recently showed that diluted hydroalcoholic extracts of *Gelsemium* were able to affect gene transcription in human neuroblastoma models [19–21]. They reported the same gelsemine concentration previously shown [20] and a slight reduction in a microarray gene expression model on human SH-SY5Y neuroblastoma cell line with an estimated concentration of gelsemine as low as 6.5×10^{-9} M, hence within ranges previously reported for rats [13, 21]. A cognate paper, published in a niche journal in CAM research, confirmed the effect of this gelsemine dosage, but highlighted also a significant effect, though slight, with doses decisively much lower than 6 nM gelsemine [20]. In both papers, a diluted hydroalcoholic extract of *Gelsemium* downregulated the expression of 49/56 [21] or 45/55 [20] genes in SH-SYS5 neuroblastoma. Published comments addressed the issue that the effect observed on gene expression might be brought up by EtOH carry-over in the test solution, due to the predominant presence of EtOH respect to any molecule of the starting *Gelsemium* extract [18]. No gene particularly involved in the neurological mechanism underlying the molecular action of *Gelsemium* alkaloids was up- or downregulated in the experimental research [18, 20, 21].

FURTHER COMMENTS AND CONCLUSIVE REMARKS

Comments about the ability of negligible doses of *Gelsemium* hydroalcoholic extracts to affect gene expression were recently reported [18]. In an attempt to highlight possible conclusive remarks on the research about *Gelsemium*, I will introduce these fundamental concerns.

1. A more general observation of these studies showed that *Gelsemium* extract did not undergo a thorough chemical analysis of its composition, but many authors attributed any result to the exclusive effect of gelsemine [15, 20, 21]. *Gelsemium sempervirens* extracts contain alkaloids with an in toto anxiolytic activity, yet a chemical definition of these components needs to be performed [14]. Other oxindole alkaloids contained in *Gelsemium* extracts besides gelsemine exhibit a neurotropic action [12]. New alkaloids are being discovered and a complex interaction between *Gelsemium* alkaloids and their metabolites may affect significantly the biological

response of an organism to plant extracts [33, 34].

2. Tested solutions contain a sizable molar fraction of ethanol, with respect to *Gelsemium* extract [13, 18]. The analytical evaluation of gelsemine reported in [21] should refer to the calculation indicated in [15], as the authors did not perform and subsequently describe the concentration of gelsemine in the alcoholic raw extract used for the experiment on gene expression [18, 19]. According to this assumption, a *Gelsemium* hydroalcoholic extract containing as low as 6.5×10^{-22} M gelsemine and 5.0×10^{-4} M ethanol downregulated the expression of bombesin-like receptor 3 (BRS3) and gastrin-releasing peptide receptor (GRPR) genes in SH-SY5Y cells [20]. Aside from the consideration about which genes are possibly expressed by neuroblastoma in a genomic microarray and how much they are involved in a behavioural mechanism [18], *Gelsemium* hydroalcoholic extracts appeared to fundamentally affect genes involved in olfactory and tumor-related mechanism [20, 35, 36], while changes in genes related to neurological mechanism involved in anxiety were not reported [18, 19] This evidence apparently discourages the promising role of *Gelsemium* used in CAM in modulating genes associated with neuroscience and mood disorders. This apparently aspecific activity by even nanomolar concentrations of gelsemine in *Gelsemium* might be related also to effects coming from other *Gelsemium* components, their complex interaction or even to alcoholic solvent, past reports have outlined a role for bombesin-like peptides in the neurological control of ethanol toxicity [37, 38], so EtOH in test solutions cannot be excluded from any comment about *Gelsemium* effect. The authors incubated SH-SY5Y cells with *Gelsemium* hydroalcoholic extracts for 24 h [20, 21]; ethanol itself may exhibit an activity on neuroblastoma cells at the estimated concentration of this research for that time course [39].
3. While the gross bulk of evidence in this research regards effects with micromolar-nanomolar concentrations of *Gelsemium* alkaloids [12], the lowest doses should raise criticism for an intrinsic difficulty in chemical analysis and for ethanol-related bias. The molar mass of ethanol solvent in tested solutions approaching a theoretical concentration of active principles lower than 1 attomole/L, is at least 16 orders of magnitude higher. As ethanol is a bioactive molecule at relatively low doses [18], its activity on gene expression may be highly complex and unpredictable, both in treated and control cells, so hindering a reliable statistical evaluation of the assay system.
4. Some authors recently reported that "Perhaps we need to remind that normally, when one performs a study of dose–response and the concentration in the highest dose and the dilution factor are known, there is no way to determine the concentration of substances in all successive dilutions…" [19]. This suggests that the way *Gelsemium* in CAM approaches mood disorders and anxiety needs this standpoint to be addressed and further elucidated.

CONCLUSIONS

While increasing evidence reports the anxiolytic activity of *Gelsemium* [1–6, 11, 13,

14], research on its active principles needs further investigation. A complex panoply of anti-depressant, sedative, anxiolytic, neurotropic, nociceptive, and mood modifier molecules represents the herbal potential of the *Gelsemium* plant. In this respect, CAMs using *Gelsemium* should reappraise experimental reports about test setting and molecular models, in order to reduce misinterpretations, comments and bias about the pharmacological interpretation of *Gelsemium* activity.

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