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MINIREVIEWS

Commentary: Discussing the antidepressant potential of silymarin

Claire E Manhard, Brandon Lucke-Wold

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Abstract

The therapeutic potential of diet, dietary supplements, herbal remedies, and nutraceuticals for treatment of depression and anxiety is being increasingly explored. In this commentary, we discuss the recent findings on the antidepressant potential of silymarin (SILY) in mice and present an alternative approach. We highlight the extensive research on another phytochemical, curcumin, for the treatment of depression and anxiety. Finally, we suggest a future application, which investigates the potential synergistic effects of combined treatment with SILY and curcumin for depression.

Key Words: Silymarin; Depression; Anxiety; Phytochemicals; Nutraceuticals; Curcumin

Core Tip: There are several reviews focused on the role of silymarin (SILY) in chronic diseases, however, there is a paucity of literature reviewing the potential antidepressant effects of SILY. This commentary serves as a discussion of the recent findings regarding the antioxidant, anti-inflammatory, and antidepressant-like potential of SILY in mice and as a catalyst for future discovery in phytochemistry.

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INTRODUCTION

The paper titled "Antidepressant-like potential of silymarin and silymarin -sertraline combination in mice: Highlighting effects on behavior, oxidative stress, and neuroin-

flammation" by Onaolapo *et al*[1] demonstrates the ability of silymarin (SILY) alone or in conjunction with oral sertraline to regulate behavior, oxidative stress, and neuroinflammation in dexamethasone (DEX)-induced depression in mice[2]. A summarization of this study's main findings regarding the effects of SILY alone can be found in Figure 1.

SILY, which is an active compound from the *Silybum marianum* L. Gaernt. herb, commonly called milk thistle, has antifibrotic, anti-inflammatory, and antioxidant properties[2-4]. Due to SILYs ability to prevent free radical formation and modify enzymes, its hepatoprotective effects have been well studied [5,6]. More recently, research has explored SILYs potential as a treatment for alcoholic liver cirrhosis[7]. Emerging evidence has suggested SILY's anti-viral[8], anti-cancer[9], anti-Alzheimer's disease[10], anti-Parkinson's disease[11], and anti-diabetic[12] therapeutic effects.

Depression, a complex neuropsychiatric disorder, is a leading cause of disability, a large contributor to global disease burden, and is associated with high suicidality[13]. One longstanding hypothesis for the development of depression is the "serotonin hypothesis"[14]. This hypothesis posits that serotonin (5-hydroxytyptamine) plays a critical role in the pathophysiology of depression[15]. Conventional treatment of depression with serotonin reuptake inhibitors (SSRIs) acts by blocking the reuptake of serotonin on the presynaptic neuron, thus increasing the amount of serotonin in the synaptic cleft and postsynaptic neuronal activity[16].

As the precise etiology of depression remains unknown, researchers have begun to investigate the potential benefits of mechanisms of action that go beyond neurotransmitter modulation such as the use of dietary supplements and herbal remedies[17,18]. Further, there is increasing evidence of the role of inflammation and oxidative stress in the pathogenesis of depression[19]. Several studies have demonstrated activated inflammatory pathways in patients with depression with increased acetylcholinesterase activity, malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-2, and IL-6, and decreased IL-10[20-26]. Interestingly, these inflammatory markers may also directly impact the release of serotonin and/or the production of serotonin[27,28]. Radical-induced oxidative stress has also been shown to be linked to depression, with increased superoxide dismutase (SOD) and decreased glutathione (GSH) peroxidase (GPx), GSH, and catalase in both nonclinical and clinical studies[20,29,30].

DISCUSSION OF ONAOLAPO ET AL

Given the association of inflammation and oxidative stress with depression, Onaolapo *et al*[1] sought to elucidate the potential antidepressant effect of SILY extract alone or in conjunction with a well-known SSRI, sertraline in an animal model. DEX, which was used as the mice model of depression, has been demonstrated to be effective at creating a low levels of corticosteroids in the brain and inducing depression and anxiety-like behaviors[31,32]. A total of 10 treatment groups were included: 1 vehicle control group, 1 DEX group, 1 oral sertraline group, 1 SILY 140 mg/kg group and 1 SILY 280 mg/kg group, 1 DEX and sertraline group, 1 DEX and SILY 140 mg/kg group, 1 DEX and SILY 280 mg/kg group, 1 DEX, sertraline, and SILY 140 mg/kg group, and 1 DEX, sertraline, and SILY 280 mg/kg group.

SILY's effects on body weight, activity, and memory

Onaolapo *et al*[1] found that SILY administered alone with DEX or with sertraline and DEX reversed the DEX-induced changes in mouse body weight, open field locomotor activity, rearing, and grooming. Body weight increased when mice were given SILY alone as compared to the control or sertraline. While SILY affected body weight, there was no significant change in food intake with SILY alone when compared to control, sertraline, or DEX. Interestingly, Guo *et al*[10] found that SILY administration in mice that were fed a high fat diet resulted in a decrease in body weight and a clinical trial by Momeni *et al*[33] revealed that treatment with SILY following cisplatin was able to significantly increase weight back to baseline. Onaolapo *et al*[1] findings in conjunction with results from Guo *et al*[10] and Momeni *et al*[33] suggest that SILY modulates body weight in a way that returns body weight back to a normal value.

Additionally, there was also a SILY concentration dependent increase in locomotor activity. Rearing activity in the SILY group was not significantly different from control; however, low dose SILY rearing activity was significantly greater than sertraline. With regards to self-grooming behavior, only the low dose of SILY resulted in a significant increase in self-grooming behaviors in comparison to control and sertraline. While there was a relatively unclear dose relationship between SILY alone and grooming behaviors, it is important to note that there was a significant attenuation of DEX-induced decreases when mice were administered SILY at both doses with DEX or SILY at both doses with sertraline and DEX. This corroborates Ashraf *et al*[34] findings that SILY administration at 200 mg/kg in chronically stressed mice resulted in a significant increase in grooming time.

Onaolapo *et al*[1] reported that spatial working memory, tested using the radial arm maze and Y maze, was increased in mice administered SILY. SILY administered alone at both concentrations increased working memory scores as compared to the vehicle control and sertraline, and SILY

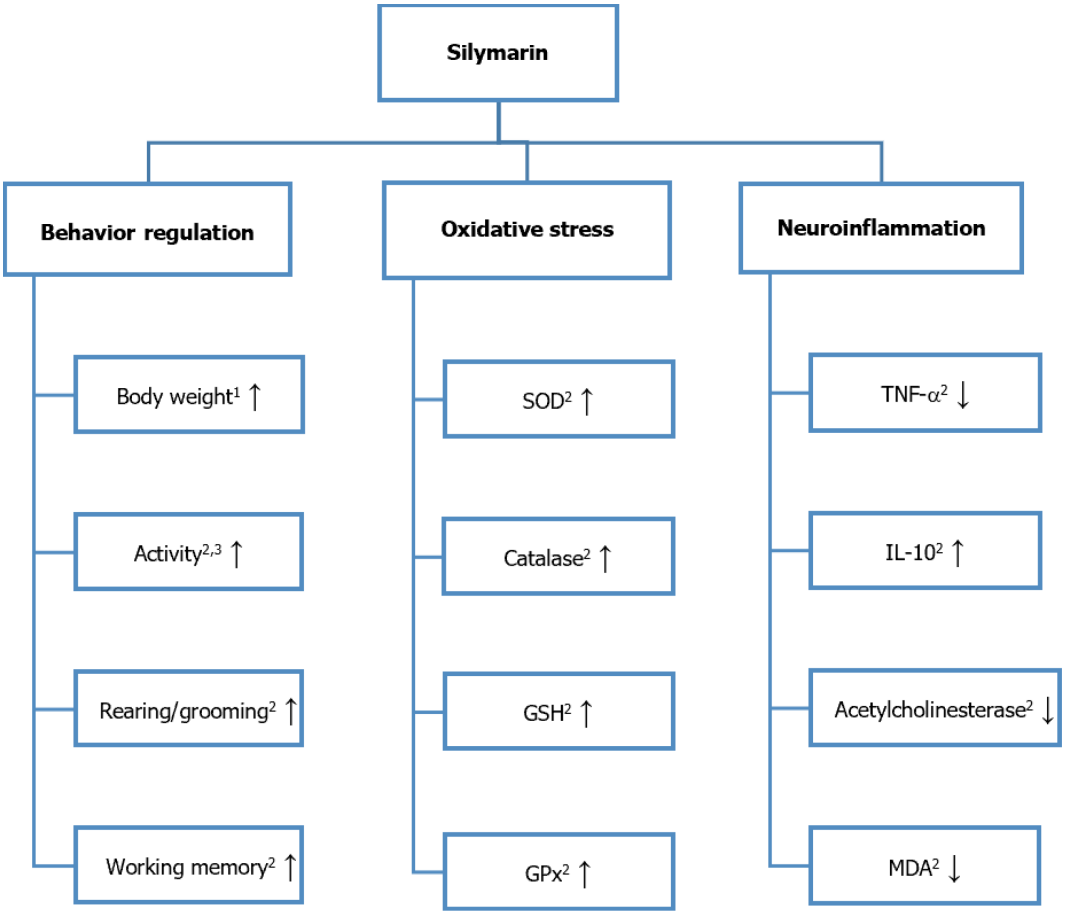

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Figure 1 Effects of silymarin alone on the pathogenesis and symptoms of depression in a study on dexamethasone-induced mice. [1]Indicates significant difference in the group administered silymarin 280 mg/kg only. [2]Indicates significant difference in both silymarin 140 mg/kg and 280 mg/kg. [3]Activity includes tail suspension and forced swim tests, elevated plus maze, and locomotor activity. ↑ Indicates a significant increase ($P < 0.05$) relative to dexamethasone-induced mice. ↓ Indicates a significant decrease ($P < 0.05$) relative to dexamethasone-induced mice. GPx: Glutathione peroxidase; GSH: Glutathione; IL-10: Interleukin-10; MDA: Malondialdehyde; SOD: Superoxide dismutase; TNF- α: Tumor necrosis factor-alpha.

administered with sertraline and DEX or alone with DEX attenuated the DEX-induced reduction in working memory. They also found that SILY was able to positively affect anxiety-related behaviors tested using the elevated plus maze. The results demonstrated that SILY alone increased the amount of time mice spent in the open arm and decreased the amount of time spent in the closed arm. SILY alone with DEX or with sertraline and DEX also counteracted the DEX-induced changes in time. El-Elimat *et al* [35] similarly demonstrated that SILY administration can prevent stress-induced memory impairments and improve anxiety-related behaviors in a rat model of post-traumatic stress disorder.

Results from the tail suspension and forced swim tests showed that SILY alone reduced the amount of immobility time significantly when compared to the vehicle control and sertraline groups. Further, SILY by itself or with sertraline attenuated the DEX-induced increases in immobility time in both tests. Lee *et al*[36] also assessed immobility time in forced swim tests in a rat model of stress and found a significant decrease in immobility time with administration of the main active ingredient in SILY.

SILY's effects on antioxidant status, inflammatory markers, and neuronal injury

Onaolapo *et al*[1] found mixed results for SILY's effect on serum antioxidant status. Serum SOD levels increased significantly with both SILY concentrations and with DEX and high dose SILY. A decrease in serum SOD levels was found for DEX, DEX with sertraline, and both concentrations of SILY administered with DEX and sertraline. These results are interesting given that multiple studies have found increased SOD in patients with major depressive disorder[29,37-39]. With regards to catalase, serum concentrations increased significantly with both concentrations of SILY and DEX administered with SILY 280 mg/kg when compared to the vehicle control. Surprisingly, DEX with sertraline and DEX with SILY 140 mg/kg and sertraline resulted in a significant decrease in catalase serum concentrations when compared to the vehicle control. GSH also increased significantly with both concentrations of SILY and with DEX and SILY 280 mg/kg and DEX with SILY 280 mg/kg and sertraline when comparing to the vehicle control. The final antioxidant status measure, GPx, was found to have increased significantly with both concentrations of SILY and decrease with DEX and DEX with

sertraline compared to the vehicle control. These mixed results put SILY's effect on antioxidant status into question. However, another study by Ashraf *et al*[34] investigated SILY's antioxidant potential in a chronically induced stress model of depression and found catalase, GSH, and SOD levels from both the hippocampus and cerebral cortex to be significantly greater in mice treated with SILY 200 mg/kg as compared to the control.

Inflammatory marker levels from the hippocampus and cerebral cortex were improved following SILY administration. TNF-α levels were lowered in mice administered SILY alone. Further, results showed SILY's ability to mitigate the severely lowered TNF-α levels observed in DEX mice. IL-10 levels were increased in mice administered SILY alone. Results also demonstrated SILY's ability to mitigate the decrease in IL-10 levels observed in DEX mice. Acetylcholinesterase activity was found to be significantly increased in DEX mice; however, this increase was again mitigated by SILY administration alone or in conjunction with sertraline. SILY at both concentrations also significantly reduced acetylcholinesterase activity as compared to the control and sertraline. Both concentrations of SILY were also found to significantly reduce the levels of MDA as compared to the control. SILY 280 mg/kg was also found to significantly reduce the levels of MDA when compared to sertraline, as well. As with the other inflammatory markers, SILY alone or with sertraline attenuated the increase in MDA levels observed with DEX administration. The levels of the brain antioxidant enzymes tested, GSH and GPx, were both increased with both concentrations of SILY when compared to control and to sertraline. Further, SILY alone or with sertraline also mitigated the decrease in GSH and GPx induced by DEX. Ashraf *et al*[34] similarly found improved levels of inflammatory markers in stressed mice, with significantly lowered IL-1β and TNF-α compared to the control. Thakare *et al*[40] also found significantly lowered IL-6, MDA, and TNF-α in stressed mice when compared to the control. Onaolapo *et al*[1] finally examined the morphology of the cerebral cortex and hippocampus. Cerebral cortex and hippocampal histomorphology demonstrated DEX-induced neuronal injury; however, when mice were administered DEX, sertraline, and SILY the histology appeared to have normalized suggesting SILY and sertraline's potential neuronal protective effects.

CONCLUSION

Overall, Onaolapo *et al*[1] conducted a well-designed study, which elucidated the effects of SILY on depressive and anxiety related behaviors, neuroinflammation, oxidative stress, and neuronal injury. The number of groups, randomization schemes, and the inclusion of 10 animals per group indicates strong experimental design. Results regarding its effects on oxidative stress were unclear, however, existing literature does suggest SILY's ability to modulate oxidative stress[34,40]. As the authors pointed out, the lack of assessment of glucocorticoid levels limits the study's ability to determine SILY's impact on the hypothalamus-pituitary-adrenocortical axis, which is implicated in depression and anxiety[41]. Further, the authors did not study SILY's effects on monoamine levels in the brain and failed to comment on the sex-dependent effects of SILY, which have been demonstrated in a couple of studies[42,43]. Lastly, as this is an animal study, the results cannot reliably predict SILY's effects in humans.

Alternative solution

Another widely studied phytochemical, curcumin, has both nonclinical and clinical evidence suggesting its antidepressant potential[44]. Curcumin is main component of the spice turmeric or *Curcuma longa,* and like SILY, has been shown to have anti-inflammatory, antioxidant, and neuroprotective activities [45,46]. The literature has demonstrated curcumin's ability to increase levels of monoamines in the central nervous system (CNS), inhibit glutamate release, decrease inflammation, improve oxidative stress, and attenuate symptoms associated with depression and anxiety[44].

A study by Bhutani *et al*[47] found that curcumin administration significantly reversed the increased depressive behaviors, low CNS monoamine levels, and increased monoamine oxidase activity observed in rats subjected to chronic unpredictable stress. Lin *et al*[48] showed curcumin's ability to inhibit glutamate release in the prefrontal cortex of rats through the suppression of presynaptic voltage-gated calcium channels. Given that glutamate has been demonstrated to be elevated in patients with depression, these results suggest that curcumin inhibited glutamate release may provide antidepressant effects. Curcumin has also been shown to decrease inflammatory markers in a rat model of depression. Fan *et al*[49] found that pretreatment with curcumin repressed inflammatory processes including microglia activation and overexpression of IL-1β, IL-6, and TNF-α which were induced by chronic unpredictable stress. Further, in a 2015 clinical trial, Yu *et al*[50] found that chronic supplementation with curcumin decreased inflammatory cytokines, IL-1β and TNF-α, and salivary cortisol in depressed patients when compared to placebo. Naqvi *et al*[51] showed in an animal model that curcumin administration also improved oxidative stress, which is associated with depression[20]. This study found that curcumin administration in mice exposed to unpredictable chronic mild stress resulted in a reduction in depression and anxiety symptoms, lipid peroxidation, and antioxidant enzymatic activity. Similarly, Moradi Vastegani *et al*[52] demonstrated that curcumin pretreatment in mice administered lipopolysaccharide (LPS) resulted in significantly increased activity of SOD and GPx enzymes. Further, it

attenuated the LPS-induced anxiety and depressive behavior.

Future directions

An interesting application of the results from Onaolapo *et al*[1] could be a study on the potential synergistic effects of SILY and curcumin for depression. The combination of SILY and curcumin has been tested in both human colorectal cancer cell lines and in radiation induced kidney injury in rats[53, 54]. One study by Montgomery *et al*[53] found that curcumin sensitized SILY's effects in human colorectal cancer cell lines. Another study found the combination of curcumin and SILY for treatment of radiation induced kidney injury in rats to be potentially more effective than curcumin or SILY alone [54]. Based on the anti-inflammatory and antioxidant properties of both SILY and curcumin alone for depression and anxiety, and the combined effects tested in other disease states, combination therapy with SILY and curcumin may be a promising treatment with greater antidepressant potential. Further, in patients for whom SSRIs are not effective or are not preferred, this alternative combination treatment may offer benefit.

FOOTNOTES

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ORIGINAL ARTICLE

Basic Study

In silico insight into Amurensinine - an N-Methyl-D-Aspartate receptor antagonist

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Abstract

BACKGROUND

Some isopavines can exhibit important biological activity in the treatment of neurological disorders since it is considered an antagonist of the specific N-methyl-D-Aspartate (NMDA) receptor. Amurensinine is an isopavine which still has few studies. In view of the potential of isopavines as NMDA receptor antagonists, theoretical studies using bioinformatics were carried out in order to investigate whether Amurensinine binds to the NMDA receptor and to analyze the receptor/Ligand complex. This data can contribute to understanding of the onset of neurological diseases and contribute to the planning of drugs for the treatment of neurological diseases involving the NMDA receptor.

AIM

To investigate the interaction of the antagonist Amurensinine on the GluN1A/GluN2B isoform of the NMDA receptor using bioinformatics.

METHODS

The three-dimen-sional structure of the GluN1A/GluN2B NMDA receptor was selected from the Protein Data Bank (PDB) - PDB: 4PE5, and the three-dimen-sional structure of Amurensinine (ligand) was designed and optimized using ACD/SchemsketchTM software. Prediction of the protonation state of Amurensinine at physiological pH was performed using MarvinSketch software (ChemAxon). Protonated and non-protonated Amurensin were prepared using AutoDock Tools 4 software and simulations were performed using Autodock Vina v.1.2.0. The receptor/Ligand complexes were analyzed using PyMol (Schrödinger, Inc) and BIOVIA Discovery Studio (Dassault Systemes) software. To evaluate the NMDA receptor/Amurensinine complex and validate the molecular

docking, simulations using NMDA receptor and Ifenprodil antagonist were performed under the same conditions. Ifenprodil was also designed, optimized and protonated, under the same conditions as Amurensinine.

RESULTS

Molecular docking simulations showed that both non-protonated and protonated Amurensinine bind to the amino terminal domain (ATD) domain of the GluN1A/GluN2B NMDA receptor with significant affinity energy, -7.9 Kcal/mol and -8.1 Kcal/mol, respectively. The NMDA receptor/non-protonated Amurensinine complex was stabilized by 15 bonds, while the NMDA receptor/protonated Amurensinine complex was stabilized by less than half, 6 bonds. Despite the difference in the number of bonds, the variation in bond length and the average bond length values are similar in both complexes. The complex formed by the NMDA receptor and Ifenprodil showed an affinity energy of -8.2 Kcal/mol, a value very close to that obtained for the NMDA receptor/Amurensinine complex. Molecular docking between Ifenprodil and the GluN1A/GluN2B NMDA receptor demonstrated that this antagonist interacts with the ATD of the receptor, which validates the simulations performed with Amurensinine.

CONCLUSION

Amurensinine binds to the NMDA receptor on ATD, similar to Ifenprodil, and the affinity energy is closer. These data suggest that Amurensinine could behave as a receptor inhibitor, indicating that this compound may have a potential biological application, which should be evaluated by *in vitro* and preclinical assays.

Key Words: Amurensinine; Bioinformatics analysis; Isopavines; Molecular docking; N-methyl-D-Aspartate receptor

Core Tip: Amurensinine binds to a region of the amino terminal domain on the N-methyl-D-Aspartate receptor and the interaction is stabilized mainly by covalent bonds, which confer an affinity energy of significant value to the receptor/Ligand complex. The interaction between Amurensinine and the receptor, which is involved in neurological diseases, suggests that this isopavine may interfere with its function, so it may have therapeutic potential in this area.

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INTRODUCTION

Ion receptors are voltage-dependent ion channels which, when activated by an electrical potential difference, lead to the influx and/or efflux of ions. These receptors are classified into three main families: The N-Methyl-D-Aspartate (NMDA) receptors, the alpha-amino-3-hydroxy-methyl-5-4-isoxazolpropionic receptors and, the Kainate receptors[1].

Studies show that hyperactivation, inhibition or dysfunction of these receptors are related to the development of several diseases such as schizophrenia, stroke, Alzheimer disease[2], anti-NMDA receptor encephalitis[3] and depression[4]. Alterations in the NMDA receptor may also be associated with neural disorders such as epileptic aphasia and mental retardation[5,6].

During the resting potential, the NMDA receptor presents its subunits joined together[7-10] and its ion channel is blocked by Mg^{2+} ion. Depolarization of the postsynaptic membrane potential[1] removes the Mg^{2+} ion from the receptor channel entrance[11,12] and thus, the agonists Glycine and Glutamate are able to bind to the GluN1[13-16] and GluN2 subunits of the NMDA receptor, respectively[14,16].

The channel only returns to its normal state after the ligand binding domain (LBD) gap opens allowing the agonist to dissociate from the active site. The opening of the gap may be caused due to the binding of the agonist itself to the binding site; this triggers a conformational change that decreases the sensitivity of the active site[17-22].

Excitotoxicity is caused by excessive stimulation by neurotransmitters which can lead to cell damage or death. It may occur in events that characterize a central nervous system trauma, an ischemic or

hemorrhagic condition, in which cells are deprived of energy to maintain ionic homeostasis[23]. The excess of Glutamate in the medium facilitates the activation of the NMDA receptor which allows the flux of Ca^{2+} ions into the cell. This intracellular Ca^{2+} accumulation can cause an osmotic swelling, lysis and cell death[23], activating enzymes such as proteases, phospholipases, and endonucleases, which can damage cell structures such as membranes and DNA itself.

In this situation, mitochondria are also harmed, because they are unable to buffer this excess of Ca^{2+}, resulting in the formation of reactive oxygen species[23]. Neurons, which are positively charged (depolarized), promote the unblocking of the ion channel caused by Mg^{2+}, facilitating a greater influx of ions through the channel, making it difficult to reestablish ionic homeostasis[24,25].

Allosteric modulators that bind to the amino terminal domain (ATD) are able to regulate the probability of receptor ion channel opening and its rate of closure, such as Zn^{2+} which binds to the GluN2A and GluN2B subunits, Ifenprodil that binds to GluN2B, and polyamines that bind to GluN2B [26-28]. LBD also has binding sites for agonist or antagonist allosteric modulators, capable of controlling ion channel opening[29,30].

Ifenprodil is a non-competitive antagonist that partially binds to the ATD of the GluN2B subunit of the NMDA receptor - where the Glutamate agonist binding site is located, and can inhibit its activity by up to 90% showing higher efficiency at GluN1/GluN2B compared to GluN1/GluN2A/GluN1/GluN2B [31-33].

Isopavines are alkaloids derived from plants of the genus *Papaver*[34-37] and are classified as benzopyridine isoquinolines[37] that have in common the tetrahydroisoquinoline in the central region of their structures[38,39]. Isopavines are considered non-competitive and specific NMDA receptor ion channel antagonists or blockers[39]. Studies indicate that isopavines may exhibit important biological activity in the treatment of neurological disorders such as Down syndrome, Alzheimer, Huntington's disease, amyotrophic lateral sclerosis, senile dementia, stroke, epilepsy, and olivo-ponto-cerebellar atrophy[38,39]. Amurensinine is an isopavine that has been found in *Meconopsis* species[40] and it has been identified in *Papaver alpinum*, *Papaver tatricum*, *Papaver pyrenaicum*, *Papaver suaveolens* and some varieties of *Papaver nudicaule*[34-37].

Molecular docking is a computational method used to predict possible sites of interaction of a ligand in a receptor, as well as the affinity of the interaction between receptor-ligand, the conformation of the receptor-ligand complex and the nature of the chemical bonds between the receptor and the ligand in order to define the stability of the interaction. This method has great importance for the development of new drugs, because it allows you to optimize the design of a drug, to find a candidate drug by virtual scanning in databases[41].

Therefore, molecular docking simulations between Amurensinine and the NMDA receptor were performed in order to evaluate the receptor/Ligand complex, and thereby provide data to support future research on neurological diseases involving this receptor.

MATERIALS AND METHODS

Receptor and ligands

This study was performed with the GluN1A/GluN2B isoform of the NMDA receptor. The native mutation-free three-dimensional structure of the human GluN1A/GluN2B NMDA receptor is not available on the structure databases, thus the structure of the receptor from a mammal used in preclinical trials - *Rattus norvegicus* - was selected. The three-dimensional structure of the GluN1A/GluN2B NMDA receptor was selected from the Protein Data Bank (PDB) (www.rcsb.org) - PDB 4PE5. Three-dimensional structure of Amurensinine (ligand) was designed and optimized using ACD/Schemsketch™ software. The protonation state prediction of the Amurensinine at physiological pH 7.4 was performed using the MarvinSketch software (ChemAxon). Molecular docking simulations were performed with Amurensinine in its protonated and non-protonated states.

Molecular docking and data analyses

Molecular docking was performed using the flexible ligand - rigid receptor methodology[42]. Simulations were performed with the B-chain of the GluN2B subunit of the GluN1A/GluN2B NMDA receptor, where the ATD - domain in which the binding site of the antagonist Ifenprodil, is located in human receptor. For the execution of molecular docking, the AutoDock Tools 4[43] and Autodock Vina v.1.2.0.[44] software was used. In these software the steps for the preparation of molecules were performed, the torsion points of ligand were detected, its torsion angles were calculated and Grid box dimensions were determined and the command was run by AutoDock Vina. Data were analyzed using PyMol (Schrödinger, Inc) and BIOVIA Discovery Studio (Dassault Systemes) software.

Molecular docking validation

To validate and analyze the NMDA receptor/Amurensinine complex, the molecular docking was performed with the Ifenprodil antagonist (Figure 1 under the same conditions. Ifenprodil was also designed, optimized and protonated as well as Amurensinine. Ifenprodil was docked on the ATD of the

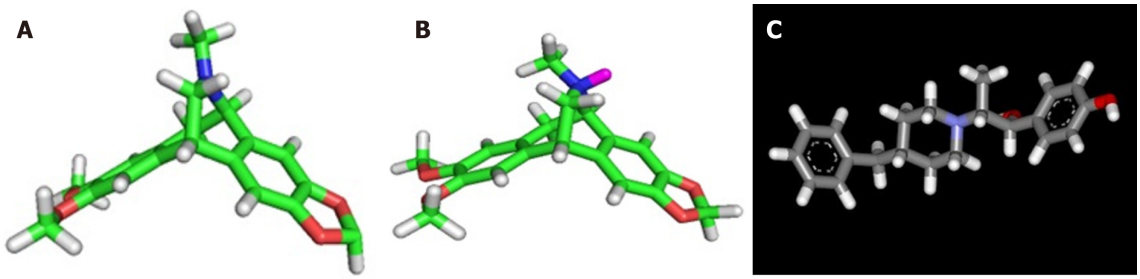

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Figure 1 Structures of Amurensinine and Ifenprodil. A: Amurensinine in the non-protonated state; B: Amurensinine in the protonated state at pH 7.4; C: Ifenprodil. These structures were designed using ACD/Schemsketch software.

GluN1A/GluN2B NMDA receptor (PDB 4PE5).

RESULTS

At physiological pH (pH = 7.4) it was observed that 68.5% of Amurensinine was in its non-protonated state and 31.5% was in the protonated one; both states show structural differences (Figure 1). Molecular docking showed that Amurensinine binds to the NMDA receptor; both protonated and non-protonated Amurensinine binds to the ATD of the GluN1A/GluN2B NMDA receptor with significant affinity energy, -7.9 Kcal/mol and -8.1 Kcal/mol, respectively. Despite the similar affinity energy values, the non-protonated Amurensinine makes 15 bonds with regions of the ATD, while the protonated compound makes only six bonds (Table 1). However, the variation in bond lengths and the average of these lengths in both the non-protonated Amurensinine/NMDA receptor complex (2.9-5.4 Å; average = 4.23) and the protonated Amurensinine/NMDA receptor complex (2.0-5.7 Å; average = 3.90) are similar. Upon coupling of Ifenprodil to ATD, an energy affinity = -8.2 Kcal/mol was obtained, a value very close to that obtained for protonated Amurensinine/GluN1A/GluN2B NMDA receptor complex; even the same number of bonds was observed (six bonds) (2.6-4.9 Å; average = 3.67) as shown in Table 2; however, the geometry of the bonds is different (Figure 2). This result is consistent, since the structures of Amurensinine and Ifenprodil are similar, but not identical (Figure 3). The interaction between Ifenprodil and ATD on NMDA receptor validates the molecular docking of Amurensinine and confers reliability to the data.

DISCUSSION

Neurological diseases are an important cause of morbidity and mortality and loss of quality of life. Their pathophysiology is complex and multifactorial, since it involves genetic and environmental factors, resulting in different clinical manifestations in patients with the same neurological damage[45].

Elucidated mechanisms involved in the onset of neuronal diseases include hyperactivation, inhibition and dysfunction of the NMDA receptor, which is involved in schizophrenia, stroke, Alzheimer[2], anti-NMDAR encephalitis[3], depression[4], mental retardation and epileptic aphasia[5,6]. This receptor is an ion channel composed of four subunits and has three families - GluN1, GluN2 (A-D isoforms) and GluN3 (A-B isoforms)[46]. Each receptor subunit is composed of two extracellular domains - LBD and ATD, an intracellular carboxy terminal domain (CTD) and a transmembrane domain (TMD), where the ion channel is located[7,16,39,47-49].

Therefore, compounds which regulate the opening and closing speed of the ion channel by binding to ATD, are promising for research into the treatment of neurological diseases[39,49]. *In silico*, the Amurensinine binds to the ATD of the GluN1A/GluN2B NMDA receptor with significant affinity. The affinity is measured by affinity energy which, when it is lower than -6.0 kcal/mol, indicates interaction in a biological environment[50]. This data is important once it indicates the possibility of interaction of the Amurensinine with the target receptor inside the organism. Ifenprodil is known to bind to the GluN2B subunit of the human NMDA receptor[32]; by binding in the same region of the NMDA receptor from *Rattus norvegicus* as Amurensinine, it is suggested that this isopavine may also interact with the human receptor. In addition, the low value of affinity energy points out the stability of the Amurensinine/NMDA receptor complex, and consequently indicates chances of pharmacological effectiveness. However, these assumptions need to be tested experimentally, *in vitro* and *in vivo*.

NMDA receptors normally function at physiological pH - neutral[44]; in cases of brain damage or neural disease, this pH may decrease and the environment becomes acidic[44,45]. The interaction of Amurensinine with the NMDA receptor in both protonated and non-protonated states, with similar

Table 1 Chemical bonds of the GluN1A/GluN2B N-methyl-D-Aspartate receptor/Amurensinine complex

Non protonated Amurensinine: Receptor[1]	Distance (Å)	Bond type
Aromatic ring (A): ARG347	4.1	π-alky[1]
Aromatic ring (A): TYR 287	4.8	π-π T-shaped
Aromatic ring (B): LYS361	2.9	Hydrogen bond
Aromatic ring (B): LYS361	4.8	π-alky[1]
Aromatic ring (B): PRO360	5.4	π-alky[1]
C-12: ARG347	4.2	Alky[1]
C-12: LEU349	5.2	Alky[1]
C-12: TYR287	5.1	π-alky[1]
C-22: ASP348	3.6	Carbon-hydrogen bond
C-9: PHE146	3.4	Carbon-hydrogen bond
Cycloheptane: LYS361	4.8	Alky[1]
Cyclopentane: LYS361	3.3	Hydrogen bond
Cyclopentane: PRO360	5	π-alky[1]
N-1: HIS359	3.3	Hydrogen bond
O-3: ASP295	3.5	Hydrogen bond
Protonated Amurensinine: Receptor*	Distance (Å)	Bond type
Aromatic ring (B): ASP477	4.4	π-anion
Aromatic ring (B): TRP166	5.7	Alky[1]
CH-19: TYR476	3.1	Carbon-hydrogen bond
Cyclopentane: VAL390	4.9	π- alky[1]
NH-1: ASP477	2	Carbon-hydrogen bond
O1: ASP165	3.3	Hydrogen bond

[1]Atom: Residue.
Amino acid residues are shown in the three-letter code and the number represents the position of the residue in the polypeptide chain. (A) and (B) indicate the different aromatic rings of Amurensinine.

Table 2 Chemical bonds in the GLUN1A/GLUN2B N-methyl-D-Aspartate receptor/Ifenprodil complex

Ifenprodil: Receptor[1]	Distance (Å)	Bond type
Aromatic ring: ARG393	3.8	π-sigma
Aromatic ring: VAL390	4.6	Alky[1]
Aromatic ring: LYS454	4.9	π- alky[1]
N-1: TYR164	3.5	Hydrogen bond
OH-2: THR255	2.6	Hydrogen bond
OH-1: ASP477	2.6	Hydrogen bond

[1]Atom: Residue.
Amino acid residues are shown in the three-letter code and the number represents the position of the residue in the polypeptide chain.

affinities, indicate that the pH and, consequently, the protonation state of this compound, do not influence its interaction. Thus, neurological disorders that alter the H+ concentration in the environment will not have a significant influence on the modulating activity of Amurensinine on the receptor.

An opposite result was observed with Endobain E and the NMDA receptor. This compound is an endogenous brain factor that acts as an inhibitor of Na/K ATPase and, as a modulator of the NMDA receptor by binding to the inner surface of the channel, decreasing the affinity of receptor ligands.

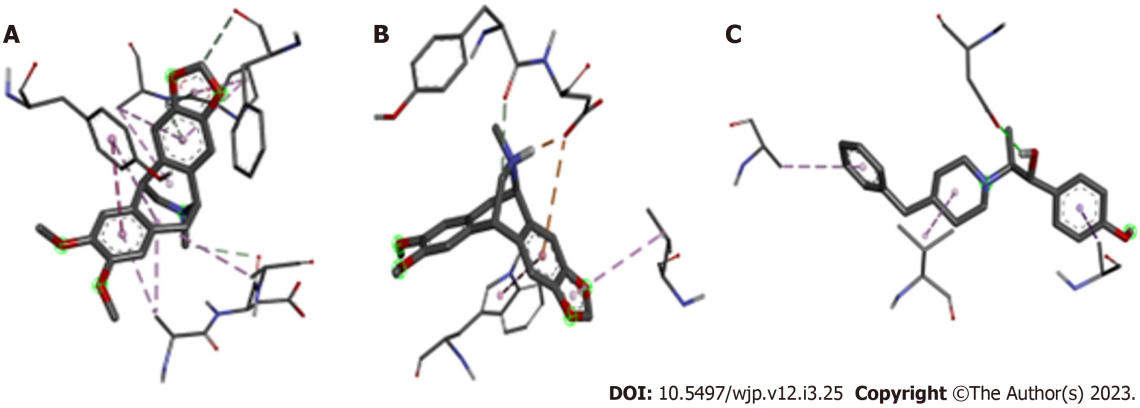

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Figure 2 Binding geometry of antagonists to the amino terminal domain on the GluN1A/GluN2B N-methyl-D-Aspartate receptor. A: Non-protonated Amurensinine; B: Protonated Amurensinine; C: Ifenprodil. The structures in thick sticks are the antagonists; the thin sticks are the amino acid residues of the receptor. Chemical bonds are represented by dashed lines. These structures were designed using ACD/Schemsketch software.

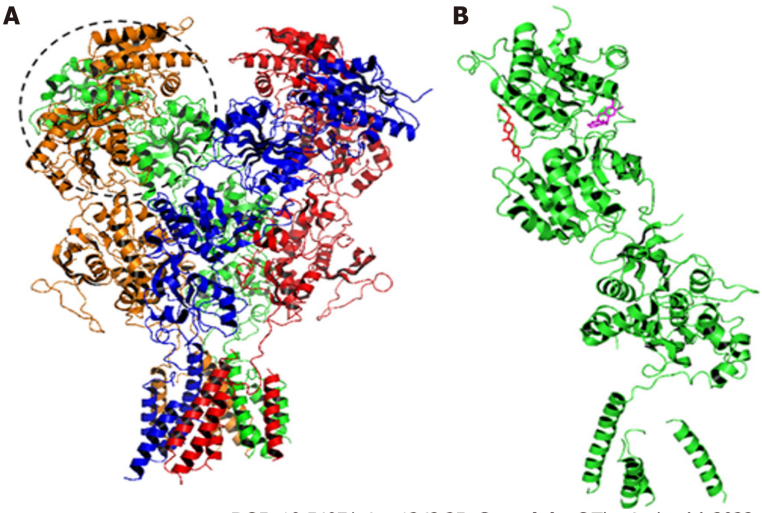

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Figure 3 Structure of the GluN1A/GluN2B N-methyl-D-Aspartate receptor. A: The dashed circle indicates the amino terminal domain (ATD). A-Chain: Orange; B-Chain: Green; C-Chain: Red; D-Chain: Blue. In the B-Chain are located the binding sites for Amurensinine and Ifenprodil; B: B-Chain with Ifenprodil (red) and non-protonated Amurensinine (pink) bound to the ATD. These structures were designed using ACD/Schemsketch software.

Under conditions of cerebral ischemia, Endobain E has its activity optimized due to the acidic environment of the ischemic brain (pH 6.5, 90% receptor inhibition). In a more alkaline environment, its activity is reduced (pH 7.4-8, 25% receptor inhibition)[51].

In general, proteins such as NMDA receptor-forming ones, have their structural conformation and, consequently, their activity directly influenced by pH. The sudden change in H+ concentration induces ionization of the residues, conferring an excessively negative or positive charge, which can lead to intramolecular repulsion, exposure of hydrophobic regions, and loss of function[52]. On the other hand, pH variation can also expose previously hidden sites, optimizing the receptor-ligand interaction, as occurs with Endobain E. Regarding Amurensinine, it is inferred that its binding site is not in a region of the receptor that undergo major conformational changes with pH variation of the medium and that this site is capable of binding both the protonated and non-protonated states of this isopavine.

The binding strength, also called intermolecular interaction, occurs between covalently interacting compounds and determines the effectiveness of the receptor-ligand interaction[53]. Moreover, the bond type that molecules establish with each other directly influences their physicochemical properties, such as solubility and boiling point, and their final conformation. In enzymes the type of contact established is able to alter their activity, strength, selectivity and the conformation of the enzyme-substrate complex [54]. Thus, predicting the strength and type of interaction between receptor and ligand is important to understand the mechanism of action of the ligand, which may be a drug candidate.

Amurensinine in protonated state interacts with the receptor through six chemical bonds of which the majority (83%) are covalent bonds. Amurensinine in the non-protonated state made 15 chemical bonds with the receptor, with a predominance of covalent bonds, and even with the large difference in the

number of bonds, the affinity energy was quite similar for both receptor/Ligand complexes. Possibly, a contributing factor to the similarity of affinities is the shorter bond lengths that stabilize the protonated Amurensinine/receptor complex.

In this regard, Medeiros[55] cites the physicochemical characteristics of the interaction of antagonistic compounds, such as Ifenprodil, with the ATD in the GluN2B subunit of the NMDA receptor. This compound, despite having a strong interaction with the receptor, has little selectivity as it also binds to the Alpha-1 and 5HT receptors, leading to impairments in motor function and a drop in blood pressure. However, its use is of fundamental importance as a parameter for studying compounds that interact differently with the binding site, such as MK-22, EVT-101, and Amurensinine[55].

CONCLUSION

Amurensinine, in both protonated and non-protonated states, binds to the ATD region of the GluN2B subunit of the murine GluN1A/GluN2B NMDA receptor, *in silico*, forming complexes stabilized mainly by covalent bonds. The affinity energy of the complexes is significantly low and indicates, in addition to probability of interaction in the physiological environment, the stability of the complexes. The structural and receptor interaction similarity between Amurensinine and Ifenprodil suggest that this isopavine could behave as a receptor inhibitor; therefore, this compound could present potential biological application, which needs to be evaluated by *in vitro* and *in vivo* assays.

ARTICLE HIGHLIGHTS

Research background

Isopavines are alkaloids derived from plants of the genus *Papaver* that have biological activity for the treatment of neurological disorders. Amurensinine is a little-studied isopavine whose activity in relation to neurological disorders has not been studied. Research on this isopavine may contribute new information about its action.

Research motivation

The research motivation was the scarce literature on the subject. There are few studies in health care using isopavines; similarly, studies on Amurensinine are quite scarce. Therefore, we studied Amurensinine *in silico* to verify its preliminary therapeutic potential.

Research objectives

To study, *in silico*, the interaction between Amurensinine and the N-methyl-D-Aspartate (NMDA) receptor, which is involved in the onset of neurological disorders.

Research methods

In this study we used molecular docking, a standardized bioinformatics methodology for analyzing chemical interactions between receptors and ligands.

Research results

The research results indicated that Amurensinine can interact with the NMDA receptor with high affinity, and that its protonation state does not significantly interfere with this affinity.

Research conclusions

The results of the research were satisfactory, as Amurensinine was able to bind to the tNMDA receptor. The occurrence of interaction indicates that this isopavine may interfere with the activity of the receptor. Since it binds with similar affinity and to the same subunit as the antagonist Ifenprodil, it may act as an inhibitor of the receptor. However, *in vitro* and *in vivo* studies are needed to affirm this.

Research perspectives

The research perspective is to conduct further *in silico* analyses with Amurensinine and other receptors involved in the onset of neurological diseases to further evaluate the potential of this isopavine for health care.

FOOTNOTES

Author contributions: Façanha Wendel C performed the experiments and analyzed the results; Hapuque Oliveira

Alencar Q and Viana Vieira R wrote the manuscript; Teixeira KN interpreted the data, performed the critical analysis of the results and coordinated the study; All authors approved the final version of the manuscript.

Institutional review board statement: The study was conducted *in silico* and involved neither humans nor animals, so it was not necessary Institutional review board statement.

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Data sharing statement: The study was conducted only in a computational environment and the data and three-dimensional structures used are available in public online databases.

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