Scientia Pharmaceutica

Opinion

Crocin Modified Drugs for Neuronal Trans-Differentiation: A Future Regenerative Approach

Pratikshya Paudel [1] and Prabir Kumar Gharai [2,*]

[1] Department of Chemistry, Oklahoma State University, Stillwater, OK 74078, USA; pratikshya.paudel@okstate.edu
[2] Department of Animal and Food Sciences, Oklahoma State University, Stillwater, OK 74078, USA
* Correspondence: pkgiicb@gmail.com or pgharai@okstate.edu

Abstract

Neurodegeneration—driven by oxidative stress, chronic inflammation, and protein aggregation—underlies disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and stroke. Current pharmacological treatments are largely symptomatic and do not restore lost neural circuitry, motivating regenerative approaches. Mesenchymal stem cells (MSCs) provide neurotrophic and immunomodulatory benefits and can support synaptic repair, yet robust conversion into mature, electrophysiologically functional neurons remain challenging and often depends on complex inducer cocktails with translational limitations. Crocin, a saffron-derived carotenoid, is reported to enhance neurogenesis and neuroprotection in preclinical models through pathways including Wnt/β-catenin, Notch1, CREB/BDNF, and modulation of GSK-3β, while reducing apoptosis and inflammatory signaling. Here, we synthesize evidence supporting crocin's neuroprotective and proneurogenic activity and propose a testable hypothesis that crocin-based or crocin-modified formulations could be evaluated as adjuncts to guide MSC neuronal lineage commitment. Importantly, direct evidence that crocin alone can drive MSC trans-differentiation into fully functional neurons is currently insufficient; future work should define functional benchmarks (electrophysiology, synaptogenesis, and phenotypic stability) and rigorously validate safety, dosing, and delivery strategies for neuroregenerative translation.

Keywords: crocin; retinoic acid; human mesenchymal stem cells; neuronal trans-differentiation; Acetylcholinesterase; neuroregenerations

Check for updates

Academic Editor: Thierry Besson

Received: 18 November 2025
Revised: 24 December 2025
Accepted: 3 January 2026
Published: 8 January 2026

1. Introduction

1.1. Neurodegeneration: Pathophysiology and Unmet Clinical Need

Neurodegeneration relates to the gradual loss of neuronal structure and function within the central nervous system (CNS), leading to cellular death. It is the basis of many long-term, debilitating diseases, such as Alzheimer's, Parkinson's, Huntington's disease, and stroke [1]. These diseases all have the same basic pathological features, even though they show up in different ways in people. Common symptoms include cognitive decline, memory loss, slower processing speed, and trouble with speech and movement skills, all of which make life much worse. Neurodegeneration at the cellular and molecular levels often indicates an imbalance between reparative mechanisms and harmful processes, such as oxidative stress, chronic inflammation, and aberrant protein aggregation. In Alzheimer's disease, extracellular amyloid-β plaques and intracellular hyperphosphorylated tau tangles disrupt synaptic signaling, impair neuronal function, and accelerate cell death. Other neurodegenerative illnesses involve distinct proteins or processes, sometimes affected by disease-specific genetic factors. Excess reactive oxygen species (ROS) are a major cause of damage to neurons [2,3]. Neurons are especially vulnerable since they need a lot of energy, rely heavily on mitochondria, and have a limited ability to fight against oxidative stress. Oxidative stress that lasts for a long time causes mitochondria to stop working, lipids to break down, DNA to be damaged, and finally leads to cell death by apoptosis or necrosis. At the same time, long-lasting neuroinflammation makes the injury worse because microglia and astrocytes release pro-inflammatory cytokines when they are damaged or when protein aggregates form. This keeps the cycle of stress and degeneration going. Together, these mechanisms speed up the death of neurons and make it harder for the brain to repair or rebuild functional neural circuits.

Despite intensive research spanning several decades, effective disease-modifying therapies for neurodegenerative disorders remain elusive. Most medications just treat symptoms. For example, cholinesterase inhibitors can briefly improve memory and thinking in people with Alzheimer's disease [4], while dopaminergic medicines can lessen motor symptoms in those with Parkinson's disease [5]. But none of them stop neurons from dying or fix broken neural circuits. This difference has led to more interest in regenerative therapies, especially those that use stem cells to rebuild brain tissue and restore function.

1.2. Mesenchymal Stem Cells (MSCs) in Neuroregeneration: Promise and Limitations

Mesenchymal stem cells (MSCs) are particularly fascinating because of their biological characteristics and practical uses. MSCs are versatile stromal cells capable of differentiating into osteoblasts, chondrocytes, adipocytes, and, under some conditions, neuron-like cells. They can be readily obtained from sources such bone marrow, adipose tissue, and umbilical cord, and expanded in culture, facilitating therapeutic use. Their reduced immunogenicity also lowers the chance of rejection after transplantation. Along with lineage differentiation, MSCs have strong immunomodulatory and trophic effects that change the local environment by lowering harmful inflammation and encouraging the survival, protection, and repair of brain tissue. This makes them a complete way to help with functional recovery [6].

Recent preclinical studies reveal the methods via which MSCs provide benefits to the injured or degenerating CNS via multiple, synergistic routes. MSCs produce numerous neurotrophic factors, notably brain-derived neurotrophic factors (BDNF) and glial cell line-derived neurotrophic factors (GDNF), which support neuronal survival, augment synaptic plasticity, and encourage axonal regeneration [7]. They let out exosomes that have proteins, lipids, and microRNAs in them. These change how genes are expressed and how cells talk to each other, which helps protect neurons and heal tissue. At the same time, MSCs reduce maladaptive neuroinflammation by lowering the release of pro-inflammatory cytokines from activated microglia and astrocytes and increasing anti-inflammatory pathways. In combination, these actions reduce neuronal loss, stimulate intrinsic pathways, and improve functional outcomes in animal models of cerebral injury and neurodegeneration.

Even with these skills, there are still a lot of problems. A primary limitation is the difficulty in prompting MSCs to develop into completely mature, electrophysiologically active neurons that integrate into established brain networks. Although MSCs can have neuron-like morphologies and express neuronal markers under some conditions, authentic functional differentiation characterized by cells that can consistently generate action potentials and facilitate synaptic transmission remains unclear. To address this challenge, researchers frequently utilize combinations of small molecules or growth factors to generate neuronal phenotypes in vitro. Nevertheless, numerous approaches rely on poorly defined combinations that may pose safety risks or lead to incomplete and unstable neural characteristics. These inaccuracies and safety concerns highlight the necessity for more meticulously designed mechanistically informed differentiation procedures to direct MSCs into stable, functional neural lineages appropriate for therapeutic integration.

Future innovations are anticipated to emerge from the integration of MSC-based therapeutics with targeted molecular or genetic engineering to augment their neurogenic capacity and overall efficacy [8–10]. Strategies include changing the signaling pathways that control neuronal differentiation, such as Wnt/β-catenin, Notch, and CREB/BDNF, or genetically altering MSCs to make neurotrophic factors more active to improve survival, engraftment, and integration. At the same time, advanced biomaterial scaffolds and three-dimensional culture platforms that closely mimic the brain microenvironment by imitating the composition, architecture, and mechanical properties of the extracellular matrix can send signals that help MSCs grow, choose their lineage, and mature into functional neural phenotypes [11].

Again, the combination of donepezil with NSC transplantation yields synergistic advantages in Alzheimer's disease models by enhancing cognition, synaptic density, and the viability of transplanted cells. Donepezil seems to augment neurogenesis, facilitate neuronal differentiation, and safeguard engrafted cells in the adverse environment of the Alzheimer's disease brain. The therapeutic impact was partially associated with enhanced BDNF expression and cholinergic signaling, both of which facilitate synaptic plasticity and neural integration. Given that diminished neurogenesis and synaptic degeneration are fundamental characteristics of Alzheimer's disease, this integrated approach presents a possible therapy opportunity. These findings underscore the potential of combining pharmaceutical treatments with stem cell therapy to enhance outcomes in Alzheimer's disease [12].

2. Crocin and Retinoic Acid as Proneurogenic Modulators

2.1. Crocin: Source, Chemistry, and Neuroprotective Profile

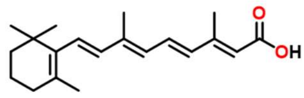
Saffron is obtained from the stigma of *Crocus sativus* L. and contains various bioactive substances, such as carotenoids like crocetin and its glycosylated form crocin, flavonoids such as quercetin and kaempferol, and monoterpenoids like safranal. Crocin is a water-soluble carotenoid that is found in a variety of forms with one or two sugar moieties, mainly gentiobiosyl and glycosyl groups and the most prevalent of which is trans-crocin-1 (digentiobiosyl ester). It has been proven to have therapeutic effects for multiple types of organ systems, including the neurological, cardiovascular, immunological, and endocrine systems [13].

2.2. Retinoic Acid: Canonical Differentiation Signal and Delivery Strategies

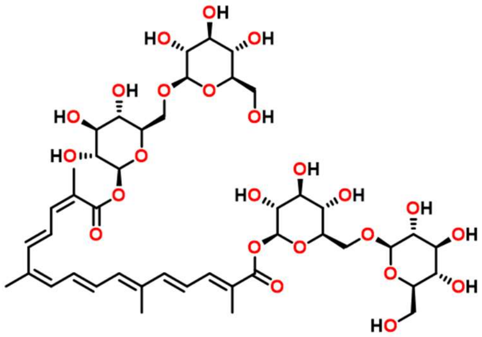
Retinoic acid (RA) induces redox-sensitive alterations that promote early neuronal differentiation in SH-SY5Y cells. In numerous proteins, it induces the reversible oxidation of cysteine residues and NOX2 dependent ROS generation. This redox shift underscores its critical function in neuronal development by affecting neuronal marker expression, neurite outgrowth, retinoic acid homeostasis, and cytoskeletal dynamics [14]. Retinoic acid in RA–PEI complex nanoparticles have a dual function: it creates stable nanostructures with polyethyleneimine and acts as a bioactive signal to direct stem cell destiny. Controlled release of RA under varying pH circumstances facilitates the effective stimulation of neuronal differentiation from embryonic stem cells, underscoring its therapeutic potential [15]. Retinoic acid promotes cellular differentiation across various lineages, including myoblasts, neuroblasts, and epithelial cells. In neuroblastoma models, it enhances neurite outgrowth and the expression of neural markers. This study demonstrates that retinoic acid-triazolyl derivatives amplify these effects, with certain molecules exhibiting superior neuronal differentiation capacity, indicating enhanced therapeutic prospects compared to retinoic acid alone [16]. Retinoic acid administered by nanoparticles promotes neurogenesis in subventricular zone stem cells. Upon internalization, these nanoparticles augment NeuN-positive neuronal populations and facilitate the development of functioning neurons that respond to depolarization and express the NMDA receptor subunit NR1. Consequently, retinoic acid serves as a crucial proneurogenic agent with therapeutic promise in cerebral [17]. Vitamin K, particularly in its MK-4 variant, exhibits neuroprotective and differentiation functions, which are amplified when combined with retinoic acid. The altered molecule stimulates nuclear receptors and mGluR1, facilitates neuronal development, traverses the blood–brain barrier, and converts into MK-4, underscoring its therapeutic promise for neurodegenerative disorders [18]. Retinoic acid facilitates neuronal development in SH-SY5Y cells by augmenting BDNF production, promoting neurite outgrowth, and elevating dopamine release. These results underscore its critical role in facilitating neurogenesis and functional development. Nevertheless, silver nanoparticles interfere with this mechanism by provoking oxidative stress and compromising mitochondrial activity [19]. Carotenoids and retinoids possess a fundamental resemblance, such as polyenes with conjugated double-bond structures, imparting unique optical and electrical properties crucial for biological activity. Both derive from the same biological family, as retinoids are metabolites of carotenoids via the activity of carotenoid-cleaving enzymes (CCEs). This structural relationship elucidates their concurrent biological importance. In photosynthetic organisms, carotenoids like lutein and zeaxanthin facilitate light absorption, energy transfer, and photoprotection, while simultaneously serving as antioxidants by neutralizing ROS. In both humans and animals, retinoids such as vitamin A and its derivatives (retinoic acid, retinal) utilize their polyene chains to facilitate vision, embryonic development, immunological response, and cellular homeostasis. Both groups function as protective agents against oxidative stress, thereby preserving tissue integrity and health.

2.3. Shared Structure–Activity Features of Carotenoids/Retinoids

A further similarity resides in their pharmacological and therapeutic potential. Carotenoids serve as dietary precursors of vitamin A, essential for averting deficiency-related problems, whereas synthetic retinoids are extensively researched for cancer treatment, metabolic disorders, and neurodegeneration. Furthermore, both families regulate genes via nuclear receptors (e.g., RAR, RXR), affecting cellular differentiation, survival, and death [20]. Retinoic acid and crocin exhibit activity via their conjugated polyene structures (Figure 1), facilitating redox modulation and gene expression regulation. Retinoic acid operates primarily through nuclear receptors (RAR/RXR), regulating the transcription of genes associated with cell differentiation, neurogenesis, and embryonic development. Crocin demonstrates activity via antioxidant effects, neutralizing reactive oxygen species and safeguarding lipids, proteins, and DNA. RA's structure-activity relationship depends on its carboxyl group for receptor binding and transcriptional control, whereas crocin's sugar moieties improve solubility and bioavailability. Both emphasize how the length of polyenes and their functional groups determine biological activity in development and neuroprotection.



Retinoic acid



Crocin

Figure 1. The chemical structures of Retinoic acid and Crocin.

3. Evidence for Crocin-Linked Neurogenesis and Pathway Modulation

3.1. Wnt/β-Catenin Activation and Adult Neurogenesis

Crocin has shown promising antidepressant effects that seem to be related to its capacity to control hippocampus neurogenesis and turn on the Wnt/β-catenin signaling pathway. In mice with chronic stress-induced depression, therapy with crocin improved behavioral symptoms, boosted the growth and maturation of hippocampal neurons, and improved synaptic plasticity. Experimental data corroborated that the suppression of Wnt/β-catenin signaling attenuated crocin's neurogenic and antidepressant properties, indicating that this pathway is essential for its efficacy. Interestingly, crocin helped neurons stay alive when they were under stress, but it did not change the regular rate at which neurons die and are replaced. This shows that crocin is selective and may be safer than typical antidepressants like fluoxetine. These results provide a molecular foundation for crocin's antidepressant properties and endorse its potential as an effective treatment for depression [21].

Crocin has been getting more attention for its neuroprotective and neurogenic characteristics. One of its main ways of working is by activating the Wnt/β-catenin signaling pathway [21], which speeds up the growth and maturation of neural progenitor cells and increases hippocampus neurogenesis. Research on mice has shown that crocin increases the number of $Ki67^{+}$ proliferating cells in the dentate gyrus, helps BrdU-labeled precursors turn into $NeuN^{+}$ mature neurons, and encourage changes in shape, such as more dendritic branching, spine density, and synaptic plasticity. These changes in structure help cognitive and behavioral models of depression and neurodegeneration get better at what they do [21].

3.2. CREB/BDNF and Pro-Survival Signaling

Crocin might protect neurons from damage and behavioral problems caused by alcohol by changing the CREB/BDNF and Akt/GSK pathways to lower oxidative stress, inflammation, and apoptosis [22]. Recent reports suggested that crocin enhances in vitro neurogenesis in human adipose-derived mesenchymal stem cells by regulating gene expression and increasing neural protein levels via Notch and CREB/BDNF signaling pathways [23]. Nonetheless, more in vivo and clinical investigations are required to examine alternative signaling systems and to ascertain if crocin alone, in the absence of neural inducers, can facilitate neurogenesis. Again, isolated Epidermal neural crest stem cells (EPI-NCSCs) were subjected to different concentrations of lithium carbonate, crocin, and their combination, subsequently evaluated for BDNF and GDNF expressions [24]. The findings suggested that although these agents may not significantly facilitate the generation of new neurons, they improve cell viability, reduce cell mortality, and promote neuronal differentiation by activating Wnt/MAPK, ERK, and CREB pathways, resulting in heightened expressions of neurotrophic factors such as BDNF and GDNF at the site of injury.

A recent study showed that 5% crocin-embedded PVA/gelatin nanofiber scaffolds, when mixed with β-carotene, greatly boosted the growth and metabolic activity of C6 cells and effectively pushed MSCs from human bone marrow to become neurons. SEM imaging and molecular data substantiate the increased expression of MAP-2 mRNA, Nestin, and proteins in human mesenchymal stem cells generated from bone marrow (hBMMSCs) after a duration of 10 days [25].

3.3. Notch1 Signaling and Post-Injury Neural Repair

In addition to activating Wnt/β-catenin, crocin also affects other signaling pathways that are important for protecting the nervous system. In models of ischemic injury, crocin was demonstrated to turn on Notch1 signaling [23,26], which helped endogenous neural stem cells grow and move around while also lowering apoptosis and neuroinflammation. This dual effect shows that crocin can both boost regenerative responses and lessen harm caused by disease.

4. GSK-3β as a Mechanistic Node for MSC Neuronal Commitment

A key part of how crocin works is that it controls glycogen synthase kinase-3β (GSK-3β) [27], a serine/threonine kinase that is found in large amounts in the central nervous system. GSK-3β is very important in pathogenic processes, such as tau hyperphosphorylation and amyloid precursor protein (APP) processing, which are both signs of Alzheimer's disease. Additionally, GSK-3β inhibits the Wnt/β-catenin pathway by promoting β-catenin degradation [28], which restricts neurogenesis. Inhibition of GSK-3β activity has demonstrated a reduction in tau phosphorylation, a decrease in amyloid-beta buildup, and an enhancement of adult hippocampus neurogenesis in both in vitro and in vivo models.

Synthetic GSK-3β inhibitors, including SG145C [29], can replicate similar results by stabilizing phosphorylated β-catenin and facilitating the trans-differentiation of mesenchymal stem cells into neuronal lineages. However, these drugs generally need to be given with growth hormones, which raises questions about their long-term safety. Crocin, on the other hand, is a natural alternative that has less side effects and may change many neurogenic and neuroprotective pathways at the same time.

5. In Silico Prioritization and Comparative Analyses

Crocin was given priority because it was one of the best binders in a high-throughput in silico screen of 313 phytochemicals against β-catenin (PDB:1JDH). Crocin had a binding energy of about −8.1 kcal/mol when tested with PyRx version 0.8/AutoDock Vina version 2.0 blind docking. This was better than the reference Wnt/β-catenin inhibitors IWP-4 (−7.1) and cardionogen-1 (−6.3), and it was the same as several other top "front-runners". Comparative ADMET profiling then put this potency in context by pointing out that crocin is not a good oral drug candidate because it violates Lipinski's rules multiple times. This suggests that crocin is more of a mechanistic lead or scaffold than a drug-like oral candidate. Its choice also shows that it is bioactive in plants and can be used in follow-up biochemical and cellular validation studies [30].

To validate this, Molecular docking was employed to predict the binding interactions and free binding energy (ΔG_B) of both crocin and retinoic acid with GSK3β. This was conducted to evaluate its potential as a competitive inhibitor of the natural pheromone. The three-dimensional structure of GSK3β was obtained from the RCSB Protein Data Bank

(PDB ID: 1Q4L) [29] to elucidate the molecular basis of ligand recognition. Employing the Merck Molecular Force Field (MMFF94), Avogadro42 (version 1.2.0) [31] constructed and energy-minimized the two ligands. The structures were subsequently saved in Mol2 format for future docking analysis. We employed CB-Dock (version 2), a web-based blind docking tool utilizing AutoDock Vina to identify potential binding cavities in the protein and to rank docking poses according to Vina scores, for our docking studies [32]. CB-Dock (version 2) and LigPlot+ (version 2.2.9) were utilized to examine and analyze the docked protein-ligand complexes. This facilitated a deeper understanding of the interactions between ligands and proteins, including hydrogen bonding and residue contacts within the binding pocket.

Molecular docking analyses utilized CB-Dock to predict the binding interactions and binding free energies (ΔG_B) of GSK3β with crocin and retinoic acid. For each ligand, the docking poses generated included five configurations. The pose that exhibited ligand binding within the largest predicted hydrophobic pocket, with binding energies of −8.6 kcal/mol for crocin and −8.3 kcal/mol for retinoic acid, was selected for comparison (Figure 2). The selected poses were utilized to compare ligand conformations, energetic profiles, and significant molecular interactions (Figure 2).

Crocin interacts primarily with residues Val61, Ile62, Gly63, Asn64, Gly65, Ser66, Phe67, Gly68, Val69, Val70, Tyr71, Ile84, Lys85, Lys86, Val87, Leu88, Gln89, Asp90, Arg92, Phe93, Lys94, Asn95, Arg96, Glu97, Leu98, Phe116, Ser118, Ser119, Gly120, Asp124, Glu125, Tyr127, Leu128, Asn129, Tyr134, Val135, Pro136, Glu137, Thr138, Tyr140, Arg141, Arg180, Asp181, Lys183, Pro184, Gln185, Asn186, Leu188, Asp200, Phe201, Gly202, Ser203, Ala204, Lys205, Val214, Tyr216, Ile217, Cys218, Ser219, Arg220, Tyr221, Tyr222, Arg223, and Gln265, whereas retinoic acid engages with residues Ile62, Gly63, Asn64, Gly65, Ser66, Val70, Gln72, Ala83, Ile84, Lys85, Glu97, Val110, Leu132, Asp133, Tyr134, Val135, Pro136, Glu137, Thr138, Tyr140, Arg141, Arg144, Lys183, Pro184, Gln185, Asn186, Leu188, Cys199, Asp200, Ser219, Arg220, Tyr221, and Tyr222 (Figure 2).

The Ligplot+ analysis indicates that Crocin interacts with GSK3β via 14 hydrogen bonds. The interacting residues include Asn64 (one hydrogen bond: 3.09Å), Lys94 (one hydrogen bond: 3.04Å), Asn95 (three hydrogen bonds: 2.96, 2.98, and 3.19Å), Arg96 (one hydrogen bond: 3.09Å), Glu97 (two hydrogen bonds: 2.95 and 3.32Å), Lys183 (two hydrogen bonds: 3.03 and 3.28Å), Asp200 (one hydrogen bond: 2.81Å), Ser219 (two hydrogen bonds: 2.88 and 3.07Å), and Arg220 (one hydrogen bond: 2.83Å). In contrast, retinoic acid does not form any hydrogen bonds with GSK3β (Figure 2C,D).

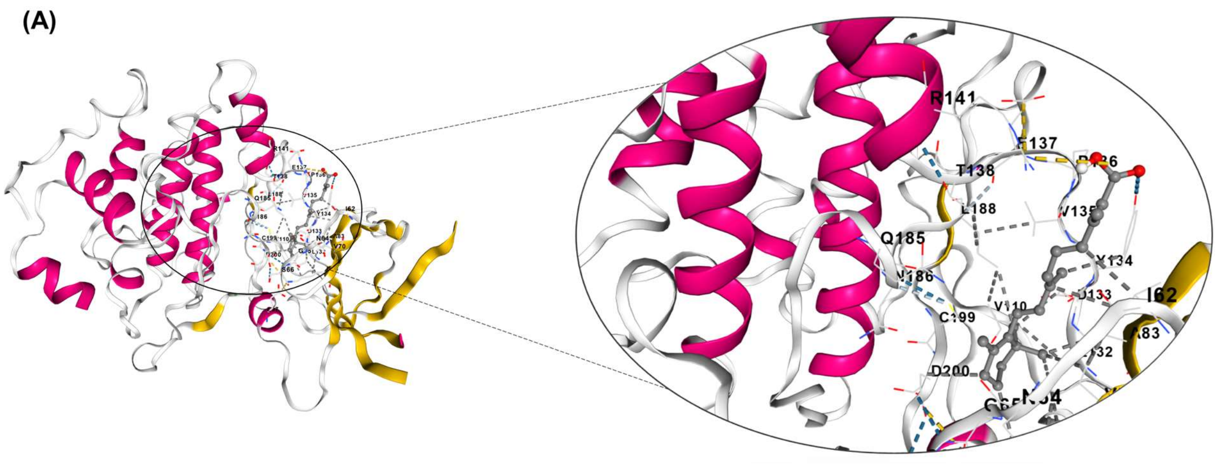


Figure 2. *Cont.*

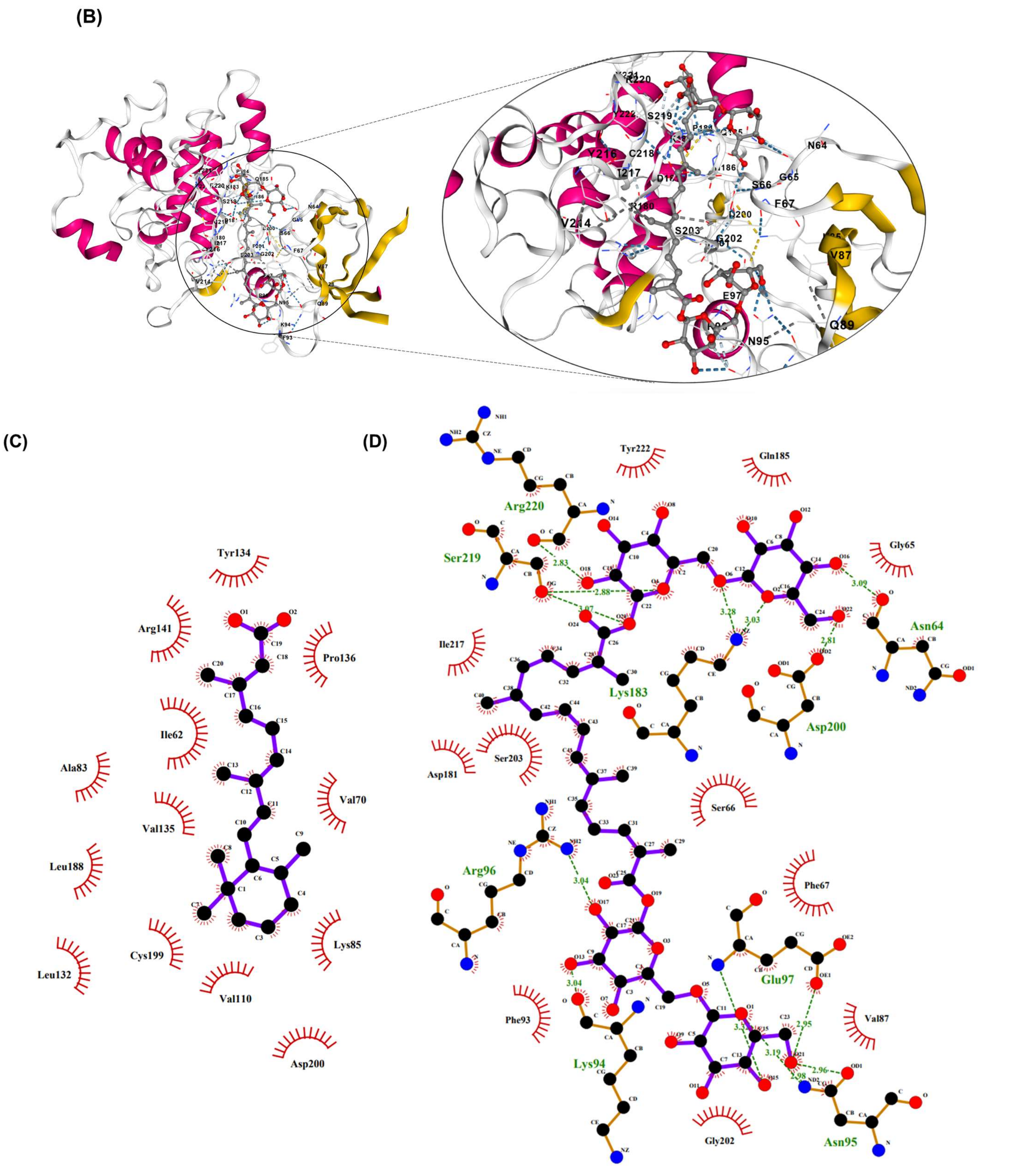


Figure 2. Molecular docking studies of retinoic acid and crocin with GSK-3β (PDB ID: 1Q4L). (**A**) Docked pose of retinoic acid within the GSK-3β binding pocket and Close-up view of retinoic acid-binding site within GSK-3β. (**B**) Docking pose of crocin within the same binding pocket and Close-up of the crocin-binding site within GSK-3β. (**C**) LigPlot representation showing 2D interaction of retinoic acid with GSK-3β. and (**D**) LigPlot representation showing 2D interaction of crocin with GSK-3β.

6. Working Model and Testable Hypothesis

While crocin has demonstrated the capacity to decrease GSK-3β activity and promote neurogenesis and neuronal survival, direct data supporting its role in facilitating the trans-differentiation of hMSCs into fully functional neurons is still insufficient. This signifies a significant and intriguing deficiency in the existing comprehension of crocin's neurogenic capabilities, presenting a valuable prospect for additional exploration. We hypothesize that crocin may promote neuronal lineage commitment in hMSCs would yield significant molecular insights and facilitate the advancement of stem cell-based treatments for neurodegenerative illnesses.

7. Translational Considerations

Crocin's beneficial biological features make it a good candidate for translational uses, in addition to its direct effects on cells. Crocin's noncytotoxic properties, bioavailability, and capacity to traverse the blood–brain barrier (BBB) [33] indicate that crocin or crocin-modified formulations may function as safe and efficacious adjuncts to stem cell therapies (Figure 2). These kinds of drugs could make differentiation regimens more useful for the body and less dependent on chemical inducers that might be harmful. To make the most of crocin's therapeutic potential, it will be important to optimize its distribution to neural progenitor populations in vivo while keeping effective concentrations.

Several human studies have investigated how crocin can be used in real life. The most common and well-tolerated dose is 30 mg/day (15 mg twice a day). These doses are a good way to see how crocin might be useful in a clinical setting. It is still not clear what the right doses are to cause trans-differentiation or neuroregenerative effects, but this dose seems to be safe. Specifically, pharmacokinetic characteristics, blood–brain barrier permeability, and tissue-level exposure pertinent to neurodegenerative disorders necessitate additional examination. Consequently, to ascertain whether the identified trans-differentiation effects occur at doses achievable within clinically acceptable dosage ranges, additional research must incorporate dose–response analyses in pertinent cellular and in vivo neurodegenerative models [34].

8. Future Directions and Experimental Benchmarks

Subsequent investigations ought to concentrate on the thorough validation of crocin-induced trans-differentiation in hMSCs, encompassing comprehensive characterization of neuronal marker expression, functional electrophysiology, and synaptic connections. Simultaneously, clarifying the exact signaling mechanisms that mediate crocin's effects, especially its interactions with the Wnt/β-catenin, Notch, CREB/BDNF, and GSK-3β pathways will establish a molecular foundation for informed treatment design. Moreover, investigations aimed at optimizing dosage, delivery methods, and combinatorial tactics utilizing biomaterials or supporting growth factors will be essential for the translation of these findings into therapeutically pertinent therapies.

Success in this area could lead to a new generation of crocin-based regenerative therapies that are safer and more in line with how the body works. These therapies could help repair damaged neural circuits, stop neurodegeneration, and bring back cognitive and motor function in people with neurodegenerative diseases. Crocin has the potential to be an important part of future treatment efforts since it connects natural neurogenic substances with stem cell technologies.

While crocin has demonstrated neuroprotective effects and has been associated with enhanced neurogenesis in neural progenitors and in vivo models, these findings do not directly establish neuronal trans-differentiation of human mesenchymal stem cells (hMSCs). Neural progenitors and MSCs represent distinct cellular states, and increased neurogenesis in endogenous neural lineages cannot be assumed to translate into MSC-to-neuron conversion. Therefore, we present the following schematic (Figure 3) as a conceptual framework that integrates published pathway-level evidence (e.g., Wnt/β-catenin, Notch1, CREB/BDNF, and GSK-3β modulation) and highlights a focused knowledge gap: whether crocin can reproducibly support hMSC neuronal commitment and maturation under defined conditions. This framework is intended to guide future experimental testing rather than to summarize demonstrated outcomes.

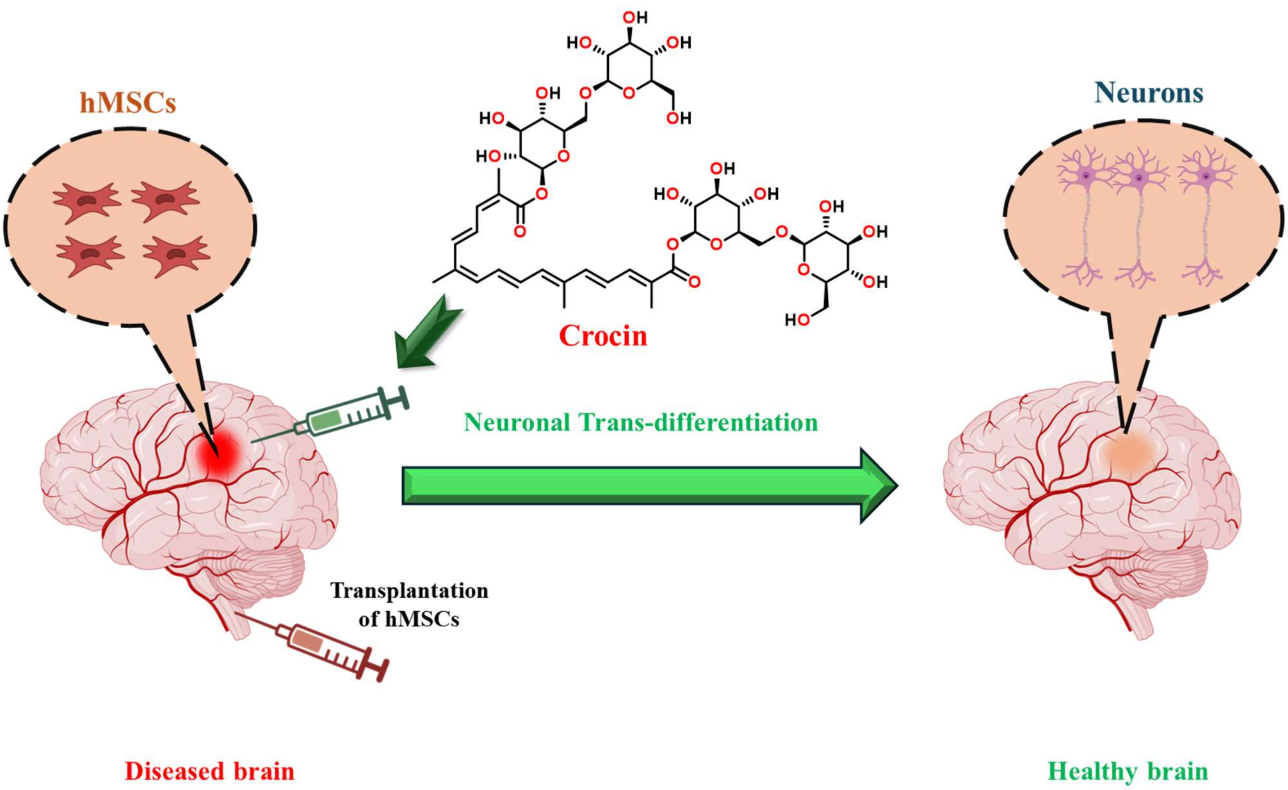


Figure 3. Conceptual schematic of hypothesized crocin-mediated signaling nodes that may support hMSC neuronal lineage commitment and maturation.

9. Conclusions

In summary, symptom-focused therapies do not adequately address neurodegenerative disorders, highlighting the necessity for regenerative strategies that restore functional neural circuitry. MSC-based interventions are appealing due to their trophic and immunomodulatory properties; however, the consistent production of mature, electrophysiologically functional neurons from MSCs continues to be a significant challenge. The literature consistently supports crocin's neuroprotective and proneurogenic effects in preclinical settings, including the modulation of Wnt/β-catenin, Notch1, CREB/BDNF-related signaling, and pathways associated with GSK-3β, a crucial negative regulator of canonical Wnt/β-catenin signaling, and crocin may promote hMSC-to-neuron trans-differentiation by blocking it. Strong binding of crocin to GSK3β (≈−8.6 kcal/mol) is predicted from our blind-docking data, which is somewhat better than retinoic acid (≈−8.3 kcal/mol). In line with stable pocket engagement, LigPlot+ shows a large amount of hydrogen bonding (14 H-bonds; for example, Asn64, Lys94/183, Asn95, Arg96, Glu97, Asp200, Ser219, and Arg220). Functionally, GSK3β inhibition would raise pro-neurogenic transcription, stabilize β-catenin, and direct hMSCs toward indicators of neuronal lineage commitment and maturation. Nevertheless, these data predominantly indicate the safeguarding of pre-existing neural cells or the augmentation of neurogenesis in neural progenitors, lacking direct evidence that crocin can facilitate functional MSC-to-neuron trans-differentiation.

Consequently, crocin ought to be regarded as a plausible, testable adjunct candidate rather than a confirmed trans-differentiation agent. Future research must (i) establish clear functional criteria for MSC-derived neurons (including action potentials and voltage-gated currents, synaptic activity, and phenotype stability following the cessation of inducers), (ii) differentiate the effects of crocin alone from those of combination systems (such as scaffolds, β-carotene, lithium, or other co-treatments), and (iii) refine dosing and delivery methods appropriate for in vivo application. If validated according to these criteria, crocin-based formulations may enhance safer, physiologically compatible differentiation protocols and fortify stem cell-mediated neuroregenerative therapy.

Author Contributions: P.P. and P.K.G. wrote the manuscript. P.K.G. conceived the idea and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: This opinion contains only molecular docking results. Data available on request due to restrictions.

Acknowledgments: Pratikshya Paudel and Prabir Kumar Gharai thank to Oklahoma State University.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Maqbool, M.; Mobashir, M.; Hoda, N. Pivotal Role of Glycogen Synthase Kinase-3: A Therapeutic Target for Alzheimer's Disease. *Eur. J. Med. Chem.* **2016**, *107*, 63–81. [CrossRef]
2. Biswas, K.; Alexander, K.; Francis, M.M. Reactive Oxygen Species: Angels and Demons in the Life of a Neuron. *NeuroSci* **2022**, *3*, 130–145. [CrossRef]
3. Singh, A.; Tiwari, V.; Roy, S. Multifaceted Role of Oxidative Stress in Neurological Disorders. *Mol. Biol. Rep.* **2025**, *52*, 640. [CrossRef]
4. Grossberg, G.T. Cholinesterase Inhibitors for the Treatment of Alzheimer's Disease: Getting on and Staying On. *Curr. Ther. Res. Clin. Exp.* **2003**, *64*, 216–235. [CrossRef] [PubMed]
5. Freitas, M.E.; Hess, C.W.; Fox, S.H. Motor Complications of Dopaminergic Medications in Parkinson's Disease. *Semin. Neurol.* **2017**, *37*, 147–157. [CrossRef]
6. Gupta, V.; Gharai, P.K.; Kar, C.; Garg, S.; Ghosh, S. Ratiometric Fluorescent Probe Promotes Trans-Differentiation of Human Mesenchymal Stem Cells to Neurons. *ACS Chem. Neurosci.* **2024**, *15*, 222–229. [CrossRef] [PubMed]
7. Gugliandolo, A.; Bramanti, P.; Mazzon, E. Mesenchymal Stem Cells in Multiple Sclerosis: Recent Evidence from Pre-Clinical to Clinical Studies. *Int. J. Mol. Sci.* **2020**, *21*, 8662. [CrossRef]
8. Paudel, P.; Kaur, R.; Gharai, P.K. Cell-Penetrating Peptoids: A Dual Functional Strategy for Upcoming Neuroregeneration Application. *ACS Chem. Neurosci.* **2025**, *16*, 3631–3633. [CrossRef]
9. Paudel, P.; Kumarchinchole, R.P.; Gharai, P.K. Can Oxytocin Neuropeptide Promote Human Mesenchymal Stem Cells to Neuron Conversion?: A Novel Approach in Future Neurotherapeutic Research. *ACS Chem. Neurosci.* **2025**, *16*, 2753–2755. [CrossRef]
10. Paudel, P.; Gharai, P.K. Dual-Function Fluorescent Probes for Neuronal Trans-Differentiation: A Promising Therapeutic Strategy in Neuroregenerative Research. *ACS Chem. Neurosci.* **2025**, *16*, 2561–2563. [CrossRef]
11. Yamanur, D.C.; Paudel, P.; Gharai, P.K. Sequence-Controlled Peptoid Hydrogels for Neuronal Trans-Differentiation: A Synthetic Biomaterials Approach in Future Neuroregenerative Research. *RSC Med. Chem.* **2025**, *16*, 5765–5769. [CrossRef]
12. Wu, C.-C.; Lee, Y.-K.; Tsai, J.-K.; Su, Y.-T.; Ho, Y.-C.; Chu, T.-H.; Chen, K.-T.; Chang, C.-L.; Chen, J.-S. Cholinesterase Inhibitor Reveals Synergistic Potential for Neural Stem Cell-Based Therapy in the 5xFAD Mouse Model of Alzheimer's Disease. *Biol. Targets Ther.* **2024**, *18*, 363–375. [CrossRef]
13. Boozari, M.; Hosseinzadeh, H. Crocin Molecular Signaling Pathways at a Glance: A Comprehensive Review. *Phytother. Res.* **2022**, *36*, 3859–3884. [CrossRef]
14. Brenig, K.; Grube, L.; Schwarzländer, M.; Köhrer, K.; Stühler, K.; Poschmann, G. The Proteomic Landscape of Cysteine Oxidation That Underpins Retinoic Acid-Induced Neuronal Differentiation. *J. Proteome Res.* **2020**, *19*, 1923–1940. [CrossRef] [PubMed]
15. Ku, B.; Kim, J.; Chung, B.H.; Chung, B.G. Retinoic Acid-Polyethyleneimine Complex Nanoparticles for Embryonic Stem Cell-Derived Neuronal Differentiation. *Langmuir* **2013**, *29*, 9857–9862. [CrossRef] [PubMed]

16. Lone, A.M.; Dar, N.J.; Hamid, A.; Shah, W.A.; Ahmad, M.; Bhat, B.A. Promise of Retinoic Acid-Triazolyl Derivatives in Promoting Differentiation of Neuroblastoma Cells. *ACS Chem. Neurosci.* **2016**, *7*, 82–89. [CrossRef] [PubMed]
17. Maia, J.; Santos, T.; Aday, S.; Agasse, F.; Cortes, L.; Malva, J.O.; Bernardino, L.; Ferreira, L. Controlling the Neuronal Differentiation of Stem Cells by the Intracellular Delivery of Retinoic Acid-Loaded Nanoparticles. *ACS Nano* **2011**, *5*, 97–106. [CrossRef]
18. Hirota, Y.; Sato, T.; Watanabe, R.; Takeda, K.; Sano, S.; Asano, S.; Shibahashi, Y.; Yasuda, Y.; Takagi, Y.; Yamashita, Y.; et al. A New Class of Vitamin K Analogues Containing the Side Chain of Retinoic Acid Have Enhanced Activity for Inducing Neuronal Differentiation. *ACS Chem. Neurosci.* **2025**, *16*, 2812–2828. [CrossRef]
19. Yamaguchi, S.; Isaka, R.; Sakahashi, Y.; Tsujino, H.; Haga, Y.; Higashisaka, K.; Tsutsumi, Y. Silver Nanoparticles Suppress Retinoic Acid-Induced Neuronal Differentiation in Human-Derived Neuroblastoma SH-SY5Y Cells. *ACS Appl. Nano Mater.* **2022**, *5*, 19025–19034. [CrossRef]
20. Álvarez, R.; Vaz, B.; Gronemeyer, H.; de Lera, Á.R. Functions, Therapeutic Applications, and Synthesis of Retinoids and Carotenoids. *Chem. Rev.* **2014**, *114*, 1–125. [CrossRef]
21. Tao, W.; Ruan, J.; Wu, R.; Zhao, M.; Zhao, T.; Qi, M.; Yau, S.S.Y.; Yao, G.; Zhang, H.; Hu, Y.; et al. A Natural Carotenoid Crocin Exerts Antidepressant Action by Promoting Adult Hippocampal Neurogenesis through Wnt/β-Catenin Signaling. *J. Adv. Res.* **2023**, *43*, 219–231. [CrossRef] [PubMed]
22. Motaghinejad, M.; Safari, S.; Feizipour, S.; Sadr, S. Crocin May Be Useful to Prevent or Treatment of Alcohol Induced Neurodegeneration and Neurobehavioral Sequels via Modulation of CREB/BDNF and Akt/GSK Signaling Pathway. *Med. Hypotheses* **2019**, *124*, 21–25. [CrossRef] [PubMed]
23. Vafaei, S.; Mirzaie, V.; Baghalishahi, M.; Mousanejad, E.; Nematollahi-Mahani, S.N. Effects of Crocin on the Enhancement of in Vitro Neurogenesis: Involvement of Notch and CREB/BDNF Signaling Pathways. *Iran. J. Basic Med. Sci.* **2024**, *27*, 914–922. [CrossRef] [PubMed]
24. Ahmadi, S.; Nabiuni, M.; Tahmaseb, M.; Amini, E. Enhanced Neural Differentiation of Epidermal Neural Crest Stem Cell by Synergistic Effect of Lithium Carbonate and Crocin on BDNF and GDNF Expression as Neurotrophic Factors. *Iran. J. Pharm. Res.* **2021**, *20*, 95–106. [CrossRef]
25. Asghari, N.; Irani, S.; Pezeshki-Moddaress, M.; Zandi, M.; Mohamadali, M. Neuronal Differentiation of Mesenchymal Stem Cells by Polyvinyl Alcohol/Gelatin/Crocin and Beta-Carotene. *Mol. Biol. Rep.* **2022**, *49*, 2999–3006. [CrossRef]
26. An, B.; Ma, Y.; Xu, Y.; Liu, X.; Zhang, X.; Zhang, J.; Yang, C. Crocin Regulates the Proliferation and Migration of Neural Stem Cells after Cerebral Ischemia by Activating the Notch1 Pathway. *Folia Neuropathol.* **2020**, *58*, 201–212. [CrossRef]
27. Karimi, H.; Mohamadian, M.; Azizi, P.; Ghasemi, P.; Karimi, M.; Layegh, T.; Rahmatkhah-Yazdi, M.; Vaseghi, S. Crocin Has a Greater Therapeutic Role in the Restoration of Behavioral Impairments Caused by Maternal Social Isolation in Adolescent than in Adult Offspring Probably through GSK-3beta Downregulation. *Learn. Motiv.* **2024**, *88*, 102060. [CrossRef]
28. Caspi, M.; Zilberberg, A.; Eldar-Finkelman, H.; Rosin-Arbesfeld, R. Nuclear GSK-3β Inhibits the Canonical Wnt Signalling Pathway in a β-Catenin Phosphorylation-Independent Manner. *Oncogene* **2008**, *27*, 3546–3555. [CrossRef]
29. Gupta, V.; Mahata, T.; Roy, R.; Gharai, P.K.; Jana, A.; Garg, S.; Ghosh, S. Discovery of Imidazole-Based GSK-3β Inhibitors for Transdifferentiation of Human Mesenchymal Stem Cells to Neurons: A Potential Single-Molecule Neurotherapeutic Foresight. *Front. Mol. Neurosci.* **2022**, *15*, 1002419. [CrossRef]
30. Egbuna, C.; Patrick-Iwuanyanwu, K.C.; Onyeike, E.N.; Uche, C.Z.; Ogoke, U.P.; Riaz, M.; Ibezim, E.N.; Khan, J.; Adedokun, K.A.; Imodoye, S.O.; et al. Wnt/β-Catenin Signaling Pathway Inhibitors, Glycyrrhizic Acid, Solanine, Polyphyllin I, Crocin, Hypericin, Tubeimoside-1, Diosmin, and Rutin in Medicinal Plants Have Better Binding Affinities and Anticancer Properties: Molecular Docking and ADMET Study. *Food Sci. Nutr.* **2023**, *11*, 4155–4169. [CrossRef]
31. Hanwell, M.D.; Curtis, D.E.; Lonie, D.C.; Vandermeersch, T.; Zurek, E.; Hutchison, G.R. Avogadro: An Advanced Semantic Chemical Editor, Visualization, and Analysis Platform. *J. Cheminform.* **2012**, *4*, 17. [CrossRef] [PubMed]
32. Liu, Y.; Grimm, M.; Dai, W.-T.; Hou, M.-C.; Xiao, Z.-X.; Cao, Y. CB-Dock: A Web Server for Cavity Detection-Guided Protein-Ligand Blind Docking. *Acta Pharmacol. Sin.* **2020**, *41*, 138–144. [CrossRef] [PubMed]
33. Ozogul, F.; Ahmad Nayik, G.; Carpena Rodríguez, M.; Yu, L.; Qin, H.; Faisal Manzoor, M. Crocin: Functional Characteristics, Extraction, Food Applications and Efficacy against Brain Related Disorders. *Front. Nutr.* **2022**, *9*, 1009807. [CrossRef] [PubMed]
34. Mehdizadeh, R.; Parizadeh, M.-R.; Khooei, A.-R.; Mehri, S.; Hosseinzadeh, H. Cardioprotective Effect of Saffron Extract and Safranal in Isoproterenol-Induced Myocardial Infarction in Wistar Rats. *Iran. J. Basic Med. Sci.* **2013**, *16*, 56–63.