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Dose-Dependent Effects of Prolotherapy Dextrose on Neuronal Cells: A Systematic Review of in Vitro Evidence

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KEYWORDS

prolotherapy; dextrose; neuronal cells; neuroinflammation; hormesis

ABSTRACT:

Introduction

Dextrose prolotherapy is increasingly investigated for neuropathic conditions due to its potential regenerative and anti-inflammatory effects. However, the optimal concentration for neuronal applications remains unclear, as in vitro studies demonstrate both protective and cytotoxic effects depending on glucose levels.

Objective

To determine the optimal dextrose concentration for prolotherapy in neuronal models based on in vitro evidence.

Methods

A systematic review was conducted following PRISMA principles using a semantic search platform (2016–2026). A total of 1,000 articles were identified, 66 were screened by abstract, and 20 studies were included after full-text review. Eligible studies involved neuronal cell cultures exposed to defined glucose concentrations with quantitative outcomes, including cell viability, apoptosis, oxidative stress, and inflammatory markers.

Results

Moderate glucose supplementation (12.5–25 mM above baseline) demonstrated neuroprotective effects, particularly in TNF-α-induced neuronal injury models. A concentration of 12.5 mM improved cell survival and reduced reactive oxygen species, while 25 mM more effectively suppressed inflammatory mediators such as IL-6, IL-1β, NF-κB, and COX-2. In contrast, higher concentrations (>30–45 mM) consistently induced neurotoxicity, characterized by oxidative stress, mitochondrial dysfunction, apoptosis, and impaired neurite outgrowth. Glucose deprivation also reduced neuronal viability. Overall, a biphasic (hormetic) response was observed.

Conclusion

Dextrose exerts concentration-dependent effects on neuronal cells within a narrow therapeutic window. No universal optimal concentration can be defined, as outcomes depend on cellular context and experimental conditions. These findings highlight limitations in clinical translation and the need for further physiologically relevant studies.

1. Introduction

Prolotherapy using dextrose has been increasingly investigated as a therapeutic approach for chronic pain, including neuropathic conditions, due to its proposed regenerative and anti-inflammatory effects. Although clinical applications typically employ hypertonic dextrose solutions (5–25%), the underlying cellular mechanisms—particularly in neuronal tissues—remain incompletely understood. Emerging in vitro evidence suggests that glucose may exert concentration-dependent biological effects on neurons, ranging from metabolic support and anti-inflammatory signaling at moderate levels to cytotoxicity at higher concentrations.

Recent experimental studies using TNF-α-induced neuronal injury models have demonstrated that glucose supplementation in the range of 12.5–25 mM above baseline can restore cellular metabolism and reduce neuroinflammatory markers in SH-SY5Y neuroblastoma cells [1,2]. These effects are mediated through modulation of signaling pathways such as MAPK, Akt, and NF-κB, as well as reduction of reactive oxygen species (ROS). However, a large body of literature indicates that glucose concentrations exceeding approximately 30–45 mM induce neurotoxicity, characterized by oxidative stress, mitochondrial dysfunction, apoptosis, and impaired neurite outgrowth across multiple neuronal cell types, including PC12 cells, dorsal root ganglion neurons, and cortical neurons [3–7].

This apparent biphasic (hormetic) response highlights the importance of identifying an optimal therapeutic window for dextrose exposure in neuronal systems. Furthermore, the translational relevance of in vitro findings is complicated by differences in baseline glucose conditions, exposure duration, and tissue buffering between laboratory and clinical settings. Therefore, this study aims to systematically review and synthesize in vitro evidence to determine the optimal dextrose concentration for prolotherapy in neuronal models and to evaluate its potential clinical implications.

This review provides a novel synthesis of in vitro evidence by defining a mechanistic, dose-dependent framework for dextrose action in neuronal systems, highlighting a hormetic response that bridges metabolic regulation and neuroinflammatory modulation, and clarifying the translational gap between experimental concentrations and clinical prolotherapy practice.

2. Objectives

This study aimed to systematically identify the optimal dextrose concentration for prolotherapy in neuronal models based on in vitro evidence. Additionally, it sought to evaluate the dose–response relationship of glucose exposure on neuronal outcomes, including cell viability, apoptosis, oxidative stress, and inflammatory markers, and to assess the translational relevance of these findings for clinical applications

3. Methods

Study Design and Search Strategy

This study was conducted as a systematic review following general PRISMA principles. A comprehensive semantic literature search was performed using the Elicit platform, which aggregates data from multiple databases, including Semantic Scholar, OpenAlex, and PubMed-indexed literature, providing broad coverage that overlaps substantially with major biomedical indexing systems. The search query used was: "In vitro study: optimal dextrose concentration for prolotherapy in neurons" within 2016 until 2026. A total of 1,000 articles were retrieved and screened for relevance.

Eligibility Criteria

Studies were included based on the following criteria: (1) in vitro experimental design; (2) use of neuronal cell lines or primary neuronal cultures (e.g., SH-SY5Y, PC12, dorsal root ganglion neurons); (3) evaluation of dextrose/glucose as an intervention with clearly defined concentrations; (4) comparison of multiple concentrations; and (5) reporting of quantitative outcomes such as cell viability, apoptosis, oxidative stress, or inflammatory markers. Non-original articles, in vivo studies, and studies not involving neuronal cells were excluded.

Study Selection

Titles and abstracts were screened independently based on predefined criteria. Of the initial 1,000 records, 66 studies met the inclusion criteria for abstract screening. Following full-text evaluation, 20 studies were included in the final analysis. The main reasons for exclusion were lack of full text (n = 39) , duplication (n=1), and failure to meet inclusion criteria (n = 6). Selection explained in PRISMA Flow Diagram below

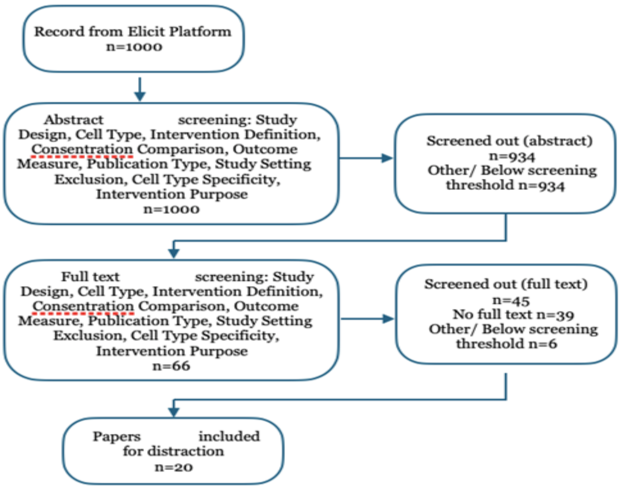
Data Extraction

Data extraction was performed systematically using predefined variables, including: (1) glucose concentrations tested; (2) neuronal cell type and species; (3) experimental conditions (e.g., inflammatory stimuli such as TNF-α); (4) outcome measures (cell viability, ROS production, inflammatory markers, apoptosis); and (5) identified optimal concentrations. Dose-response relationships and exposure durations were also recorded. Duplicate records were identified and consolidated during data extraction

Risk of Bias Assessment

The risk of bias of the included in vitro studies was assessed using an adapted methodological quality framework based on previous experimental study recommendations. The following domains were evaluated: (1) clarity of glucose concentration reporting, (2) appropriateness of control conditions, (3) completeness of outcome reporting, (4) consistency of exposure duration, and (5) presence of potential confounding factors (e.g., co-treatments or baseline variability). Each domain was rated as low risk, unclear risk, or high risk of bias.

Overall, most studies demonstrated low risk in reporting glucose concentrations and outcome measures. However, variability in exposure duration, baseline glucose conditions, and use of co-interventions introduced moderate heterogeneity and potential bias. In addition, the majority of studies lacked standardized experimental conditions, limiting comparability across studies.



Data Synthesis

The included studies were categorized into three groups: (1) glucose as a therapeutic intervention in neuroinflammation, (2) hyperglycemia-induced neurotoxicity, and (3) glucose deprivation models. A qualitative synthesis was performed to evaluate concentration-dependent effects and identify patterns of hormesis across studies.

4. Results

Characteristics of Included Studies

Twenty sources were identified and included in this review. The overall risk of bias across included studies was low to moderate, primarily due to variability in exposure conditions and confounding factors (Table 1)

Study	**Concentration Reporting**	**Control Condition**	**Outcome Reporting**	**Exposure Consistency**	**Confounding Factors**	**Overall Risk**
Cherng et al., 2023 [1]	Low	Low	Low	Low	Unclear	Low
Wu et al., 2022 [2]	Low	Low	Low	Low	Unclear	Low
Liu et al., 2019 [3]	Low	Low	Low	Unclear	Moderate	Moderate
Pereiro et al., 2018 [4]	Low	Low	Low	Unclear	Moderate	Moderate
Dedert et al., 2022 [5]	Low	Low	Low	Unclear	Moderate	Moderate
Cannas et al., 2025 [6]	Low	Low	Low	Low	Moderate	Moderate

Pham et al., 2022 [7]	Low	Low	Low	Unclear	Moderate	Moderate
Swain et al., 2025 [8]	Low	Low	Low	Low	Low	Low
Deardorff et al., 2018 [9]	Low	Low	Low	Unclear	Moderate	Moderate
Dedert et al., 2023 [10]	Low	Low	Low	Unclear	Moderate	Moderate
Yako et al., 2025 [11]	Low	Low	Low	Low	Moderate	Moderate
Dodson et al., 2022 [12]	Low	Low	Low	Low	Moderate	Moderate
Kravtsov et al., 2021 [13]	Low	Low	Low	Unclear	Moderate	Moderate
Baek et al., 2018 [14]	Low	Low	Low	Unclear	Moderate	Moderate
Zhang et al., 2024 [15]	Low	Low	Low	Unclear	Moderate	Moderate
Moloney et al., 2024 [16]	Low	Low	Low	Unclear	Moderate	Moderate
Salazar et al., 2021 [17]	Low	Low	Low	Unclear	Moderate	Moderate
Gonmanee et al., 2021 [18]	Low	Low	Moderate	Unclear	Moderate	Moderate
De Gregorio et al., 2020 [19]	Low	Low	Moderate	Unclear	Moderate	Moderate
Friedman et al., 2024 [20]	Low	Low	Moderate	Unclear	Moderate	Moderate

The studies varied considerably in their direct relevance to the research question of optimal dextrose concentration for neuronal prolotherapy. Only two studies explicitly investigated glucose supplementation as a therapeutic intervention in inflamed neuronal cells; the remainder examined either hyperglycemia-induced neurotoxicity, glucose deprivation/oxygen-glucose deprivation (OGD) models, or neuronal culture optimization with varying glucose levels. The (table 2) below summarizes all included studies.

Study	**Full Text Retrieved ?**	**Cell Model**	**Species**	**Glucose Concentrations Tested**	**Injury/Disease Model**	**Primary Research Focus**
J. Cherng et al., 2023	Yes	SH-SY5Y neuroblastoma [1]	Human [1]	3.125, 6.25, 12.5, 25 mM [1]	TNF-α-induced neuroinflammation [1]	Glucose as therapeutic agent for inflamed neurons [1]
Yung-Tsan Wu et al., 2022	Yes	SH-SY5Y neuroblastoma [2]	Human [2]	0–25 mM (optimal tested: 25 mM) [2]	TNF-α-induced neuroinflammation [2]	Glucose as anti-inflammatory neural injection [2]
Sarpras Swain et al., 2025	Yes	Primary hippocampal and cortical neurons [8]	Mouse (C57BL/6N Crl) [8]	5 mM vs. 25 mM [8]	None (culture optimization) [8]	Effect of culture glucose on neuronal metabolism [8]
Claudia Cannas et al., 2025	Yes	PC12 pheochromocytoma [6]	Rat [6]	25, 50, 100, 150 mM [6]	None [6]	Hyperglycemia-induced neurodegeneration [6]
Phillip M. Deardorff et al., 2018	Yes	Human induced neural stem cells (hiNSCs) [9]	Human [9]	25 (control), 35, 45, 55 mM [9]	Diabetic corneal neuropathy model [9]	3D corneal model of hyperglycemia [9]
Chunhong Liu et al., 2019	Yes	DRG neurons [3]	Rat (Wistar) [3]	25 (control), 45 mM [3]	High glucose-induced neurotoxicity [3]	IGF-1 protection against glucose toxicity [3]
Cass Dedert et al., 2022	Yes	Primary cortical neurons [5]	Rat (Sprague-Dawley) [5]	25 (control), 100 mM [5]	Hyperglycemic stress [5]	Progranulin effects on autophagy under high glucose [5]
Cass Dedert et al., 2023	Yes	Primary cortical neurons [10]	Mouse (C57BL/6) [11]	25 (control), 100 mM	Hyperglycemic stress	Progranulin via GSK3β signaling
Xandra Pereiro et al., 2018	Yes	Primary RGCs and Müller glia co-cultures [4]	Rat (Sprague-Dawley) [4]	10, 30 mM [4]	Hyperglycemia (diabetic retinopathy model) [4]	Dexamethasone protection against hyperglycemia [4]

Study	**Full Text Retrieved?**	**Cell Model**	**Species**	**Glucose Concentrations Tested**	**Injury/Disease Model**	**Primary Research Focus**
Vuong M Pham & Nitish V. Thakor, 2022	Yes	Primary sensory and motor neurons + Schwann cells [7]	Rat (Sprague Dawley) [7]	5.5 (control), 10, 30, 35, 45, 60 mM [7]	Diabetic neuropathy model [7]	Insulin effects on neurite extension and myelination [7]
Hideji Yako et al., 2025	Yes	ND7/23 hybrid cell line [11]	Mouse/Rat hybrid	5 (control), 60 mM	High glucose-induced neurite inhibition	Pyruvate administration for DPN
M. Dodson et al., 2022	Yes	Primary E18 cortical neurons [12]	Rat	0 (deprivation), 5 mM (control)	Electrophilic/oxidative stress (HNE)	Glucose utilization and mitochondrial response
A. Kravtsov et al., 2021	Yes	Cerebellar neurons [13]	Rat (Wistar)	0 (deprivation), 5.6 mM	Glucose deprivation	Deuterium reduction and neuroprotection
Ahreum Baek et al., 2018	Yes	Neuro-2a (N2a) differentiated with RA [14]	Mouse	Glucose-free (OGD) vs. standard	Ischemia/reperfusion (OGD/R)	Repetitive magnetic stimulation
Lu Zhang et al., 2024	Yes	Primary neural stem cells [15]	Rat (Sprague-Dawley)	Glucose-free (OGD) vs. complete media	Oxygen-glucose deprivation	CART peptide anti-apoptotic effects
Roisin A Moloney et al., 2024	Yes	Primary neurons and oligodendrocytes [16]	Guinea pig [17]	0 mM (OGD), 25 mM (sham) [17]	OGD (preterm birth model) [17]	Neurosteroid protection [17]
Katterine Salazar et al., 2021	Yes	Primary cortical neurons [17]	Rat (Sprague Dawley)	0 (no glucose), 25 mM	Glucose deprivation	Vitamin C recycling in neuronal differentiation
Thanasup Gonmanee et al., 2021	Yes	Human dental pulp stem cells (DPSCs) [18]	Human	Not tested	None [19]	Neurosphere formation and neuronal differentiation

Study	Full Text Retrieved?	Cell Model	Species	Glucose Concentrations Tested	Injury/Disease Model	Primary Research Focus
C. De Gregorio & F. Ezquer, 2020	Yes	DRG primary neurons from diabetic mice [19]	Mouse (BKS db/db)	Not tested	Diabetic neuropathy (T2DM)	Axonal regeneration defects in diabetic neurons
Timothy N. Friedman et al., 2024	Yes	DRG neurons [20]	Mouse (C57/BL6)	Not tested	Inflammatory conditioned media	Inflammation-driven neuronal plasticity

All 20 sources had full texts retrieved. The studies encompassed a range of neuronal cell types, including human neuroblastoma (SH-SY5Y), rat pheochromocytoma (PC12), primary dorsal root ganglion (DRG) neurons, primary cortical neurons, retinal ganglion cells (RGCs), neural stem cells, and hybrid cell lines. Species sources included human, mouse, rat, and guinea pig. Three studies did not test specific glucose concentrations as an experimental variable [18–20]. The glucose concentrations tested across studies ranged from 0 mM (glucose deprivation) to 150 mM, with exposure durations from 2 hours to 14 days.

Effects of Glucose Concentration on Neuronal Outcomes (Table 3)

Study	Concentration Range	Key Outcome Measure	Principal Finding	Optimal or Threshold Concentration	Statistical Significance
J. Cherng et al., 2023	3.125–25 mM [1]	Cell survival, ROS, CREB/JNK/p70S6K [1]	12.5 mM preserved cell survival and reduced ROS; increased CREB, JNK, p70S6K [1]	12.5 mM optimal for metabolic restoration and ROS scavenging [1]	$p < 0.01$ for CREB, JNK, p70S6K [1]
Yung-Tsan Wu et al., 2022	0–25 mM [2]	Cell viability, IL-6, IL-1β, NF-κB, COX-2 [2]	25 mM reduced IL-6, IL-1β, COX-2, NF-κB upregulation and restored metabolism [2]	25 mM optimal for anti-inflammatory and metabolic recovery [2]	$p < 0.05$ [2]

Study	Concentration Range	Key Outcome Measure	Principal Finding	Optimal or Threshold Concentration	Statistical Significance
Sarpras Swain et al., 2025	5 vs. 25 mM [8]	ATP pathways, mitochondrial content, inflammation [8]	25 mM biased neurons toward glycolysis (68.18%); 5 mM promoted balanced OXPHOS (92.26%); 25 mM increased inflammation [8]	5 mM recommended for physiological relevance [8]	Significant differences in ATP pathways and mitochondrial content [8]
Claudia Cannas et al., 2025	25–150 mM [6]	Cell viability, MDA, O2−, caspase-3/9, wound healing [6]	100–150 mM strongly reduced viability; MDA increased 6.5–8.8 fold; dose-dependent wound closure impairment [6]	Concentrations above 100 mM are clearly neurotoxic [6]	Significant at 100 and 150 mM [6]
Phillip M. Deardorff et al., 2018	25–45 mM [9]	Cell density, axon length/density, IL-1β, aldose reductase [9]	45 mM reduced cell density 1.8-fold; axon density dropped from 86.8 to 37.2 termini/mm^2; IL-1β upregulated [9]	45 mM is a critical damage threshold [9]	Significant between 45 mM and control [9]
Xandra Pereiro et al., 2018	10 vs. 30 mM [4]	RGC survival, cytokines (IL-6, IL-1β, TNFα) [4]	RGC survival dropped to 54% at 30 mM; IL-6 increased 172%; dexamethasone partially rescued survival to 79.53% [4]	30 mM is harmful; lower concentrations less detrimental [4]	$p < 0.05$ [4]
Vuong M Pham & Thakor, 2022	5.5–60 mM [7]	Cell viability, proliferation, neurite length, myelination [7]	Linear dose-dependent decline: dead cells rose from 1.28% to 8.61% at 60 mM; neurite length and myelination impaired [7]	No optimal high glucose; lower concentrations yielded better outcomes [7]	Higher glucose significantly impaired all outcomes [7]

Study	Concentration Range	Key Outcome Measure	Principal Finding	Optimal or Threshold Concentration	Statistical Significance
Cass Dedert et al., 2022	25 vs. 100 mM [5]	Cell viability, neurite outgrowth, AGE, mitochondrial complexes [5]	Viability dropped from 89.55% to 74.61% at 100 mM; neurites decreased from 9.067 to 7.325 [5]	100 mM confirmed as damaging [5]	Significant for viability and neurite count [5]
Cass Dedert et al., 2023	25 vs. 100 mM [10]	Cell viability, neurite quantity, mitochondrial potential, autophagy [10]	Viability dropped from 86.49% to 78.17% at 100 mM [10]	100 mM confirmed as damaging [10]	Significant for multiple outcomes [10]
Chunhong Liu et al., 2019	25 vs. 45 mM [3]	Neurite length, viability, apoptosis, ROS, ATF3 [3]	45 mM induced ROS, apoptosis, and ATF3 upregulation; IGF-1 reversed effects [3]	45 mM is neurotoxic [3]	Not detailed [3]
Hideji Yako et al., 2025	5 vs. 60 mM [11]	Cell viability, neurite length, ROS [11]	60 mM inhibited neurite length; 10 mM pyruvate fully restored length [11]	60 mM is neurotoxic; pyruvate at 10 mM restores function [11]	Significant [11]

The included studies can be stratified into three functional categories based on the glucose concentration ranges examined and the context in which glucose was applied: (1) therapeutic glucose supplementation after neuroinflammatory insult, (2) hyperglycemia-induced neurotoxicity, and (3) glucose deprivation models. The following (table 3) summarizes key quantitative outcomes across studies that reported dose-dependent neuronal responses.

Glucose as a Therapeutic Agent in Neuroinflammation

The two studies most directly relevant to neuronal prolotherapy — those by Cherng et al. (2023) and Wu et al. (2022) — employed the same cell model (SH-SY5Y human neuroblastoma) and the same inflammatory stimulus (TNF-α), but identified slightly different optimal concentrations. Cherng et al. found that 12.5 mM glucose was optimal for preserving apoptotic cell survival and reducing ROS production, with significant upregulation of anti-inflammatory signaling molecules CREB, JNK, and p70S6K ($p < 0.01$) [1]. Wu et al. identified 25 mM as the concentration that most effectively suppressed TNF-α-induced upregulation of IL-6, IL-1β, NF-κB, and COX-2 while restoring metabolic function [2]. Both studies used the same baseline culture medium (DMEM/F12 with 17.5 mM glucose) [1, 2], though the TNF-α exposure duration differed (24 hours vs. 16 hours) [1, 2], which may partially account for the divergent optimal concentrations. The glucose exposure duration in both studies was 24 hours [1, 2].

Notably, these two studies frame glucose supplementation not as a metabolic substrate but as a pharmacological agent with anti-inflammatory and antioxidant properties. Cherng et al. proposed that glucose acts through ROS scavenging and enhancement of MAPK family and Akt pathways [1], while Wu et al. emphasized the suppression of NF-κB-mediated inflammatory signaling [2].

Both studies acknowledged that SH-SY5Y neuroblastoma cells are cancer-derived and may not accurately represent normal peripheral neuronal responses [1, 2].

Hyperglycemia-Induced Neurotoxicity

The majority of included studies examined glucose at supraphysiological concentrations as a neurotoxic insult rather than a therapeutic agent. A consistent finding across these studies is that glucose concentrations above approximately 30–45 mM cause dose-dependent neuronal damage across multiple cell types and species.

In PC12 cells, glucose at 100–150 mM produced robust neurotoxicity, with MDA levels increasing 6.5–8.8 fold and wound closure rates declining dose-dependently (control 18% at 24 h vs. 8% at 100–150 mM) [6]. In the 3D corneal model using human induced neural stem cells, 45 mM glucose reduced cell density 1.8-fold and axon density from 86.8 to 37.2 termini/mm², while 35 mM showed less severe effects [9]. DRG neurons exposed to 45 mM glucose demonstrated ROS overproduction, apoptosis, and ATF3 upregulation [3]. Primary sensory and motor neurons showed a linear dose-dependent decline in viability, proliferation, neurite extension, and myelination across the 10–60 mM range [7]. RGC survival dropped to 54% at 30 mM glucose, accompanied by a 172% increase in IL-6 [4].

At very high concentrations (100 mM), primary cortical neurons showed viability reductions to 74.61–78.17% [5], with impaired autophagy flux and mitochondrial dysfunction that could be partially rescued by progranulin treatment [5]. These findings collectively establish a clear upper boundary for safe glucose exposure in neuronal systems.

Glucose Deprivation and Oxygen-Glucose Deprivation Models

Several studies examined the opposite end of the spectrum — glucose absence. Complete glucose deprivation increased neuronal death by 16% in cerebellar neurons [13] and reduced cortical neuron viability by 41.46% [17]. OGD models consistently demonstrated significant cytotoxicity in neurons and oligodendrocytes [14–16], confirming that glucose availability is essential for neuronal survival. The 2-deoxyglucose (2DG) study further demonstrated that pharmacological inhibition of glucose utilization decreased cell viability by approximately 55–65% in the presence of oxidative stress, whereas simple glucose deprivation had a comparatively minimal effect [12], suggesting that active glycolytic intermediates — rather than glucose per se — are critical for neuronal defense against electrophilic insult.

Culture Glucose and Physiological Relevance

Swain et al. (2025) provided important context for interpreting the prolotherapy-relevant studies. Standard neuronal culture media typically contain 25 mM glucose, yet brain extracellular glucose concentration is approximately 1–2 mM in vivo. Neurons cultured at 25 mM preferentially utilized glycolysis for 68.18% of ATP production, while those at 5 mM showed a more balanced reliance on oxidative phosphorylation (92.26% OXPHOS contribution) and greater mitochondrial reserve capacity [8]. Critically, the 25 mM condition also increased expression of inflammatory genes (Serpinb2 LFC = 6.679, Il12b LFC = 7.280) [8], suggesting that standard culture glucose levels may introduce a pro-inflammatory artifact. The authors recommended 5 mM glucose for neuronal cultures to better recapitulate physiological metabolism [8].

5. Discussion

The present review demonstrates that glucose exerts a concentration-dependent biphasic (hormetic) effect on neuronal cells, with moderate supplementation (12.5–25 mM above baseline) showing protective effects, while higher concentrations (>30–45 mM) consistently induce neurotoxicity. This apparent contradiction can be explained by differences in baseline cellular conditions. In inflamed or metabolically compromised neurons, such as TNF-α-induced models, additional glucose restores metabolic activity and activates anti-inflammatory signaling pathways, including MAPK and Akt, while suppressing NF-κB-mediated responses [1,2]. Conversely, in metabolically normal neurons, sustained exposure to high glucose concentrations promotes oxidative stress, mitochondrial dysfunction, and apoptosis, indicating that glucose acts as a context-dependent modulator rather than a universally beneficial agent.

The threshold for glucose-induced neurotoxicity is not uniform and varies depending on cell type and exposure duration. Neurotoxic effects have been observed at approximately 30 mM in retinal ganglion cells, 45 mM in dorsal root ganglion neurons, and higher concentrations in cortical neurons, suggesting differential vulnerability between peripheral and central neuronal populations [3–7]. This variability aligns with clinical observations in metabolic disorders, where peripheral neurons are more susceptible to glucose-related damage.

At the molecular level, the divergence between therapeutic and toxic effects reflects activation of distinct biochemical pathways. Moderate glucose concentrations enhance antioxidant capacity and cell survival signaling, whereas higher concentrations activate the polyol pathway via aldose reductase, increase reactive oxygen species (ROS) production, and trigger caspase-mediated

apoptosis and inflammatory cytokine release [3–6,9]. This mechanistic shift supports the concept of hormesis, in which biological responses depend on exposure intensity.

An important consideration is the influence of baseline culture conditions. In commonly used neuronal models such as SH-SY5Y cells, baseline glucose concentrations are already elevated (~17.5 mM), and the addition of 12.5–25 mM results in total concentrations overlapping with ranges reported as neurotoxic in other systems [4,7]. This suggests that the observed protective effects may depend on specific experimental contexts, particularly the presence of inflammatory stimuli, rather than representing a generalized neuroprotective property.

Furthermore, the glucose toxicity threshold appears to be modifiable by co-administered agents. Neurotrophic and pharmacological factors such as IGF-1, insulin, dexamethasone, and progranulin have been shown to attenuate glucose-induced neuronal damage through modulation of survival and anti-inflammatory pathways [3–5,7,10–11]. This finding has implications for prolotherapy strategies, where combination approaches may optimize therapeutic outcomes.

Despite these insights, the translational relevance of the findings remains limited. Most prolotherapy-related evidence is derived from a single cancer-derived neuronal cell line, and in vitro models lack key physiological features such as vascular clearance and tissue buffering. Additionally, continuous exposure conditions in vitro differ substantially from the transient and diluted exposure following clinical dextrose injection. Therefore, the concentrations identified in vitro (approximately 30–42.5 mM total) cannot be directly equated with clinical prolotherapy concentrations (5–25%).

From a clinical perspective, these findings suggest that the therapeutic effects of dextrose prolotherapy are unlikely to depend solely on absolute concentration, but rather on transient local exposure and the inflammatory microenvironment of the target tissue. Accordingly, optimizing prolotherapy protocols may require consideration of tissue-specific conditions and potential combination with neuroprotective agents to enhance efficacy while minimizing adverse effects.

Limitations

This review has several limitations. First, the majority of prolotherapy-relevant findings were derived from a single cancer-derived neuronal cell line (SH-SY5Y), which may not fully represent the biology of primary peripheral neurons. Second, all included studies were conducted in vitro, lacking key physiological factors such as vascular clearance, immune interactions, and extracellular matrix components, which may influence glucose distribution and cellular responses in vivo. Third, there was considerable heterogeneity in experimental conditions, including baseline glucose concentrations, exposure durations, and the presence of co-interventions, limiting direct comparability across studies. Finally, the absence of standardized methodological frameworks for in vitro risk of bias assessment may introduce subjectivity in quality evaluation.

Conclusion

This review demonstrates that glucose exerts a concentration-dependent biphasic effect on neuronal cells, with moderate supplementation (12.5–25 mM above baseline) providing metabolic and anti-inflammatory benefits, while higher concentrations (>30–45 mM) induce neurotoxicity. The findings indicate a narrow and context-dependent therapeutic window influenced by cellular condition, neuronal subtype, and exposure dynamics. Importantly, no direct dose equivalence can be established between in vitro concentrations and clinical prolotherapy formulations. Future studies using physiologically relevant models are required to define safe and effective translational parameters for dextrose-based therapies.

Taken together, this study reframes dextrose from a purely osmotic agent to a context-dependent metabolic modulator, emphasizing that its therapeutic and toxic effects are governed by a narrow, biologically sensitive concentration window. These findings provide a mechanistic basis for refining prolotherapy protocols beyond empirical concentration selection.

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