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RESEARCH

Role of GLP-1 receptors in the effect of hypericin on mouse neuroblastoma cell line (NB2a)

Hiperisinin fare nöroblastoma hücre hattı (NB2a) üzerindeki etkisinde GLP-1 reseptörlerinin rolü

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Abstract

Purpose: This study aimed to evaluate the possible neurotoxic effects of hypericin in mouse neuroblastoma cell culture, the potential neuroprotective effects against chlorpyrifos-induced neurotoxicity, and the role of the Glucagon-like peptide-1 (GLP-1) receptor in these effects.
Materials and Methods: The toxic effects of hypericin and the GLP-1 receptor antagonist exendin 9-39 were evaluated using the neurotoxicity screening test (NTT), Annexin V method, and Luminoskan test. The role of the GLP-1 receptor was assessed in the absence and presence of exendin 9-39.
Results: Hypericin significantly decreased cell viability at high concentrations of 30 and 100 μM to 70,47% and 93,30% respectively. The addition of 30 μM exendin 9-39 slightly but significantly increased viability for a 100 μM concentration of hypericin. Although hypericin increased total apoptosis at these doses, the increase was not significant. According to NTT results, hypericin showed a significant dose-dependent neurotoxic effect, starting at 0.1 μM, by inhibiting neurite outgrowth by up to 77,89%. Addition of exendin 9-39 significantly reversed this neurotoxic effect at high hypericin doses.
Conclusions: Hypericin decreased cell viability at high concentrations, which is likely related to its anticancer activity. The reversal of this effect by exendin 9-39 was interpreted as a possible contribution of GLP-1 receptors. The fact that hypericin inhibited neurite outgrowth even at low concentrations suggests it may have neurotoxic activity. Given the widespread use of St. John's Wort, whose active ingredient is hypericin, in the treatment of central nervous system disorders, further research is warranted, and its use is recommended with caution.

Keywords: Hypericin, exendin 9-39, NB2a, neurotoxicity

Öz

Amaç: Bu çalışmanın amacı, hiperisin'in fare nöroblastoma hücre kültürü üzerindeki olası nörotoksik etkilerini ve klorpirifos kaynaklı nörotoksisite üzerindeki olası nöroprotektif etkilerini değerlendirmek ve bu etkilerde Glukagon benzeri peptid-1 (GLP-1) reseptörünün rolünü araştırmaktır.
Gereç ve Yöntem: Hiperisin ve GLP-1 reseptör antagonisti eksendin 9-39'un toksik etkileri nörotoksisite tarama testi (NTT), Annexin V yöntemi ve Luminoskan testi kullanılarak değerlendirildi. GLP-1 reseptörünün rolü, eksendin 9-39'un yokluğunda ve varlığında değerlendirildi.
Bulgular: Hiperisin, 30 ve 100 μM gibi yüksek konsantrasyonlarda hücre canlılığını sırasıyla %70,47 ve %93,30 oranında önemli ölçüde azalttı. 30 μM eksendin 9-39 ilavesi, 100 μM hiperisin konsantrasyonunda canlılığı hafif ancak anlamlı bir şekilde artırdı. Hiperisin bu dozlarda toplam apoptozu artırmasına rağmen, artış anlamlı değildi. NTT sonuçlarına göre, hiperisin 0,1 μM'den başlayarak nörit büyümesini %77,89'a kadar inhibe ederek anlamlı doz bağımlı nörotoksik etki gösterdi. Eksendin 9-39 ilavesi, yüksek hiperisin dozlarında bu nörotoksik etkiyi önemli ölçüde tersine çevirdi.
Sonuç: Hiperisin, yüksek konsantrasyonlarda hücre canlılığını azaltmıştır ve bu durum muhtemelen antikanser aktivitesiyle ilişkilidir. Bu etkinin eksendin 9-39 tarafından tersine çevrilmesi, GLP-1 reseptörlerinin olası bir katkısı olarak yorumlanmıştır. Hiperisin'in düşük konsantrasyonlarda bile nörit büyümesini engellemesi, hiperisin'in potansiyel nörotoksik aktiviteye sahip olabileceğini düşündürmektedir. Etken maddesi hiperisin olan Sarı Kantaron'un merkezi sinir sistemi bozukluklarında yaygın kullanımı göz önüne alındığında, bu madde üzerinde daha fazla araştırma yapılması ve dikkatli kullanılması gerektiği ortaya çıkmaktadır.

Anahtar kelimeler: Hiperisin, eksendin 9-39, NB2a, nörotoksisite

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INTRODUCTION

Chronic neurodegenerative diseases are a group of diseases characterized by neuronal loss and progressive degeneration of different parts of the nervous system, which constitute a significant health problem with an increasing incidence rate worldwide[1,2]. Although the exact pathogenesis of these diseases has not yet been clearly elucidated, the role of a complex interaction of genetic, epigenetic, and environmental factors has been suggested[3]. Treatment of these diseases generally aims at relieving symptoms. Although short-term improvements may occur, disease progression cannot be prevented. Therefore, effective treatment options for these diseases are still under investigation to this day.

Hypericin, obtained from plants of the *Hypericum* genus, is widely used due to its antidepressant and anxiolytic effects[4]. Hypericin has been investigated for a long time due to its extensive pharmacological activities, such as anticancer, antibacterial, and antiviral properties[5-7]. In recent years, it has been reported that it may exhibit neuroprotective effects, reduce nitric oxide (NO) production, and decrease the expression levels of proinflammatory cytokines in hippocampal cells[8]. It has also been reported that hypericin can reverse cognitive dysfunction caused by amyloid β (Aβ) accumulation, a possible mechanism of Alzheimer's disease, and increase the levels of anti-inflammatory proteins[9]. Hypericin, Alzheimer's disease, among other neurodegenerative disorders, has been investigated as a potential therapeutic agent, which has also been proposed to possess antidiabetic properties[10]. On the other hand, some studies have also reported neurotoxic effects due to hypericin[11,12].

GLP-1 (Glucagon-like peptide-1) is a 37–amino acid peptide hormone belonging to the incretin family and is synthesized and secreted by enteroendocrine L cells of the distal small intestine. Its biological actions are mediated through the GLP-1 receptor (GLP-1R), a class B G protein–coupled receptor. Beyond its classical endocrine and paracrine roles, GLP-1 also exerts neuromodulatory functions, acting as a neurotransmitter within the central and peripheral nervous systems[13]. Physiologically, GLP-1 orchestrates key aspects of metabolic regulation. It modulates satiety and nutrient intake in the gastrointestinal tract, enhances β-cell proliferation and survival, and suppresses glucagon secretion from pancreatic α-cells[14,15]. The peptide possesses well-characterized antidiabetic properties, and reduced endogenous GLP-1 secretion has been consistently reported in individuals with type 2 diabetes mellitus (T2DM)[16].

In recent years, considerable attention has shifted toward the neuroprotective potential of GLP-1 receptor agonists, which are widely implemented in contemporary T2DM pharmacotherapy[17,18]. Notably, GLP-1 readily traverses the blood–brain barrier and modulates multiple molecular pathways implicated in neurodegeneration, including neuroinflammatory responses, mitochondrial integrity, oxidative stress, and cellular proliferation dynamics[19]. In preclinical models of Alzheimer's disease, GLP-1 analogs have been shown to ameliorate cognitive deficits, attenuate amyloid-β plaque burden, and reduce tau hyperphosphorylation, suggesting a promising therapeutic avenue for neurodegenerative disorders[20].

It has been reported that long-term exposure to chlorpyrifos (CP), an organophosphate pesticide, may increase the risk of developing chronic neurodegenerative diseases[21, 22]. The mild neurotoxicity model induced by CP in neuroblastoma cell cultures, which we preferred in our study and used to evaluate cell viability and neurite extension, is a model frequently used to demonstrate the neuroprotective effects of various agents[23].

This study aims to evaluate the possible neurotoxic and neuroprotective effects of hypericin in the mouse NB2a neuroblastoma cell line and to investigate the role of GLP-1 receptors in these effects.

MATERIALS AND METHODS

This study was conducted in the Cell Culture Laboratory of the Department of Medical Pharmacology, Manisa Celal Bayar University, Faculty of Medicine. All experimental protocols in this study were performed by the physicians named in the article. The study protocol was approved by the Manisa Celal Bayar University Faculty of Medicine Health Sciences Ethics Committee with a letter dated 06.10.2023 and numbered 633939.

Materials

Mouse NB2a neuroblastoma cancer cells were obtained from the European Collection of

Authenticated Cell Cultures (ECACC). All chemicals were obtained from Sigma (St. Louis, USA). All materials used in the culture medium were obtained from Falcon/Fred Baker (Runcorn, Cheshire, UK), Gentamicin (GentaR 20 mg ampoule, I. Ethem, Istanbul, Turkey).

Cell culture studies

Mouse neuroblastoma cells were grown in a culture medium (in a flask) with high glucose concentration Dulbecco's Modified Eagle's Medium (DMEM) containing 5% fetal calf serum, 5% horse serum, 1% penicillin/streptomycin solution (10000 U/10 mg) and 0.1% gentamicin (50 mg/ml) in an incubator at 37 °C and 5% CO_2.

Hypericin and exendin 9-39 were dissolved in sterile distilled water at a concentration of 100 μM, stored at -80 °C, and dissolved once before application. Hypericin was applied to the experimental groups at serial concentrations of 0.1, 0.3, 1, 3, 10, 30, and 100 μM to obtain a stepwise concentration-response profile, and exendin 9-39 was applied at concentrations of 3, 10, and 30 μM in this *in vitro* study, respectively. The literature on exendin-9-39 concentrations is quite limited, with only primary neuronal culture studies[24,25], not neuroblastoma. For this reason, concentrations that did not affect cell viability were preferred, as determined in our preliminary studies.

Cell viability and proliferation tests

CellTiter-Glo® luminescence cell viability measurement (Promega, Madison, WI) was performed in 2 stages to determine the effects of hypericin and exendin 9-39 on cell viability and proliferation, alone and after CP-induced neurotoxicity.

The wells were filled with 100 μl of proliferation medium containing 50,000 cells, and the cells were left to acclimate for 24 hours. Then, 0.1, 0.3, 1, 3, 10, 30, and 100 μM hypericin, and 3, 10, and 30 μM exendin-9-39 were added to the wells. Proliferation medium without hypericin or exendin 9-39 was used as a positive control, and medium without any cells or hypericin or exendin 9-39 was used as a negative control. In addition, in this experiment, neurotoxicity was induced by adding CP at 30 μM, and neuroprotective effects were evaluated using different concentrations of hypericin and exendin 9-39. This concentration of CP was found to be the lowest and most effective concentration that inhibited the neurite outgrowth in previous studies[23].

This method is based on the principle that the number of living cells depends on the amount of ATP present. The addition of CellTiter-Glo® substrate solution allows cell lysis and the visualization of a luminescent signal proportional to ATP levels. Cell viability was assessed by the activity of Ultra-Glo-luciferase, the enzyme that catalyzes the oxidation of luciferin in the presence of ATP released by metabolically active cells. The CellTiter-Glo® reagent mixture was added directly to the cells without removing the medium from the wells. The contents were mixed to induce lysis and left at room temperature to stabilize the luminescence signal. The plate was then read by a GloMax® 96 microplate luminometer (Promega, Madison, WI), and a dose-response curve was obtained according to the measured luminescence signal as a function of hypericin concentration. All applications were repeated three times, and the results were averaged.

Neurotoxicity screening test

To investigate the toxic effects of hypericin and exendin 9-39 on neuron culture, NB2a cells were placed in all wells at a density of 20,000 cells per well in 500 μl of proliferation medium and incubated for 24 hours. After 24 hours, except for the first three wells (negative control), the medium in all the remaining wells was removed, and differentiation medium was added. Except for the first three wells containing differentiation medium (positive control), hypericin was added at different concentrations of 0.1, 0.3, 1, 3, 10, 30, and 100 μM; exendin 9-39 was added at 3, 10, and 30 μM concentrations, one concentration in every three wells. The plates with the wells were placed in the incubator at 37 °C and 5% CO2 and incubated for 1 day. At the end of the incubation period, the cells were fixed with 4% formaldehyde in phosphate-buffered saline (PBS) and stained with 0.6% Coomassie Brilliant Blue. Neurite lengths were measured under a light microscope using ImageJ software by randomly selecting 10 fields per well and measuring 10 neurites per field. The results were expressed as percentages relative to the positive control group.

Effect of hypericin and exendin 9-39 on neurotoxicity induced by chlorpyrifos

NB2a cells were placed in 500 μl of proliferation

medium in all wells, with 20,000 cells per well. After 24 hours, proliferation medium was added to the first three wells (negative control). Medium was then withdrawn from all remaining wells, and neurotoxicity was induced by adding dibutyryl cAMP (1 mM), differentiation medium, and 30 μM CP to serum-free DMEM. CP was added to the wells at this concentration, and hypericin was added at 0.1, 0.3, 1, 3, 10, 30, and 100 μM; exendin 9-39 was added at 3, 10, and 30 μM, with one concentration per 3 wells. No substance was added to the three wells in the culture plate (positive control).

After 24 hours, the cells were fixed in PBS containing 4% formaldehyde, then kept in Coomassie Blue dye (0.6%). The stained samples were washed with PBS. Then, neurite lengths in 100 cells from 10 randomly selected areas were measured under a light microscope using the image analysis system. The measured neurite length values were expressed as a percentage of inhibition.

Apoptotic index

Apoptotic index was determined using the Muse Annexin V & Dead Cell Kit (Millipore, Billerica, MA, USA, MCH100105) according to the manufacturer's instructions.

In healthy cells, phosphatidylserine (PS), a membrane phospholipid, is localized to the inner leaflet of the plasma membrane. During early apoptosis, PS translocates to the outer leaflet of the membrane without compromising membrane integrity. Annexin V, a protein with high affinity for PS, can bind to externalized PS when conjugated to a fluorescent dye (e.g., FITC), making apoptotic cells detectable by flow cytometry. However, because Annexin V may also bind necrotic cells, propidium iodide (PI), a non-vital DNA-binding dye, is used as a secondary marker. Dual staining with Annexin V-FITC (green fluorescence) and PI (red fluorescence) allows for discrimination between live (Annexin V−/PI−), early apoptotic (Annexin V+/PI−), and late apoptotic or necrotic (Annexin V+/PI+) cells.

For the experiments, NB2a cells were seeded into each well of a 6-well plate at a density of 250,000 cells/well in 2 mL of proliferation medium and incubated for 24 hours. Subsequently, CP (30 μM) was added to induce neurotoxicity. Hypericin was applied at concentrations of 1, 3, 10, 30, and 100 μM, and exendin 9-39 at 3, 10, and 30 μM. After a 48-hour incubation period, as described by Hansel et al.[26], cells were washed with 1X DPBS (Gibco, 14190144), stained with 100 μL of Annexin V & Dead Cell Reagent, and incubated in the dark at room temperature for at least 20 minutes. Analysis was performed using the Muse® Cell Analyzer (Merck Millipore, Billerica, MA, USA). The apoptotic effects of hypericin (0.1, 0.3, 1, 3, 10, 30, and 100 μM) and exendin 9-39 (3, 10, and 30 μM) were evaluated. All experiments were performed in triplicate, and the results were expressed as the mean of three independent experiments.

Statistical analysis

The normality of the data distribution was assessed using the Kolmogorov-Smirnov test for cell viability, apoptosis, and neurite outgrowth experiments. Since the data were not normally distributed, the Kruskal-Wallis test was used for group comparisons, followed by the Mann-Whitney U test for pairwise analyses. Results are presented as mean ± standard error (SE), and p-values <0.05 were considered statistically significant.

RESULTS

NB2a cells were treated with increasing concentrations of hypericin and compared to the control group that did not receive any drug treatment. A statistically significant reduction in cell viability was observed at 10 μM hypericin, whereas exposure to 100 μM hypericin resulted in near-complete cell death (Figure 1).

No significant change in cell viability was observed upon administration of exendin 9-39 alone. However, in experiments designed to investigate the role of GLP-1 signaling in hypericin-induced cytotoxicity, exendin 9-39 treatment after high-dose hypericin exposure (30 and 100 μM) increased cell viability. This increase was statistically significant in the 100 μM hypericin-treated group (Figure 2).

Following propagation of NB2a cells in proliferation medium, the medium was replaced with differentiation medium supplemented with one mM dibutyryl cAMP to induce neurite outgrowth. Under these conditions, prominent neurite extensions were observed in the control group (Figure 3).

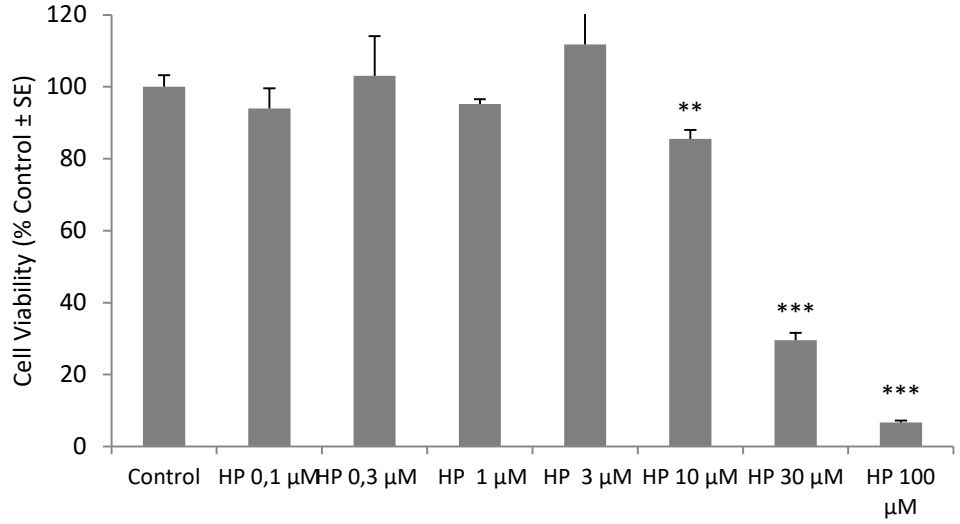


Figure 1. Effects of different hypericin concentrations on cell viability.

Significant difference compared to the control group, **p<0.01, ***p<0.001 HP; Hypericin.

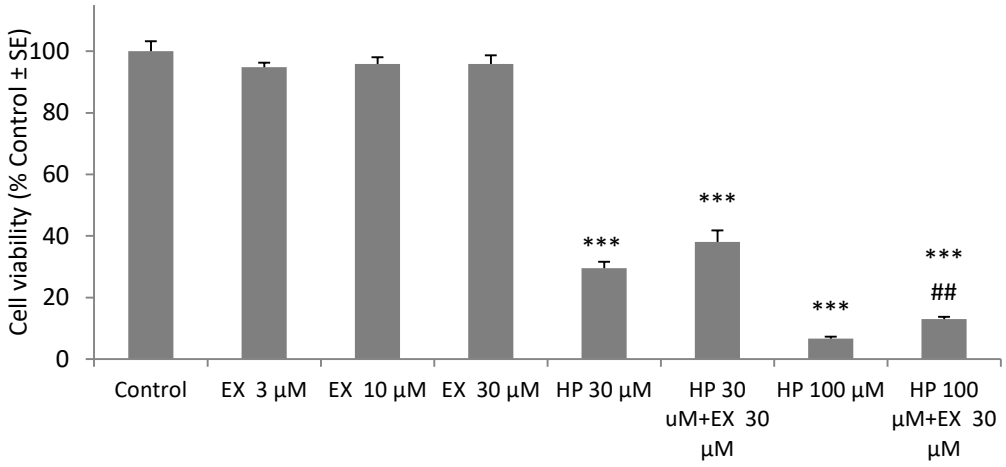


Figure 2. Effect of different exendin 9-39 concentrations on cell viability and observed effect of co-administration of 30 µM exendin 9-39 with 30 and 100 µM hypericin on cell viability.

Significant difference compared to the control group *** p<0.001, significant difference with exendin (30 µM) application, ## p<0.01. HP; Hypericin, EX; Exendin.

To investigate the potential neurotoxic (neurite-inhibitory) effect of hypericin, increasing concentrations of the compound were added to the differentiation medium, with one concentration applied to each of three wells. Starting from 0.1 µM, hypericin exhibited a dose-dependent neurotoxic effect, markedly inhibiting neurite outgrowth (Figure 4, Figure 5).

To assess the potential neuroprotective role of low-dose hypericin, cells were treated with 30 μM CP, which significantly impaired neurite extension in NB2a neuroblastoma cells. Co-administration with hypericin at increasing concentrations did not reverse the neurite-inhibitory effect of CP (Figure 6).

In experiments evaluating the potential neurotoxicity of exendin 9-39, no significant difference in neurite outgrowth was observed compared to the control. However, in experiments assessing the neuroprotective effect of exendin 9-39 against high-dose hypericin-induced neurotoxicity (30 and 100 μM), exendin 9-39 significantly reduced hypericin's neurite-inhibitory effects at both concentrations (Figure 7).

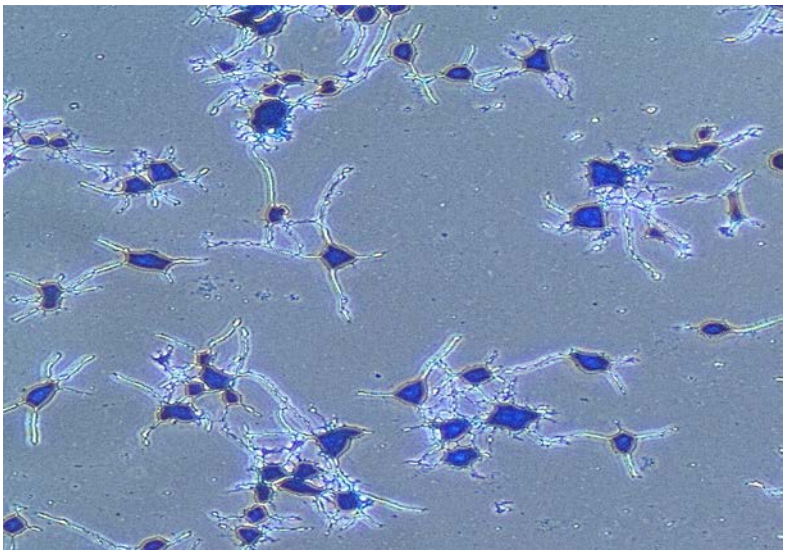


Figure 3. Image of NB2a culture cells differentiated with d-cAMP. Coomassie Blue staining, x400.

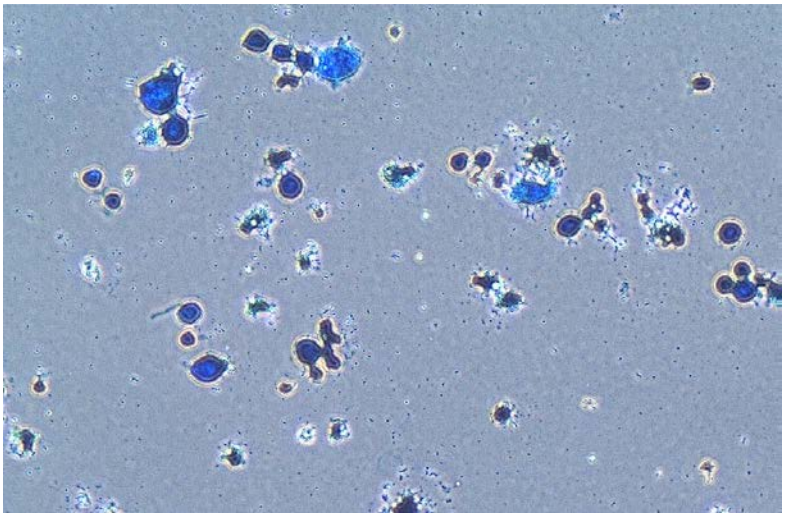


Figure 4. Inhibition of neurite outgrowth in NB2a cells in the presence of hypericin at a concentration of 100 μM, Coomassie Blue staining, x400.

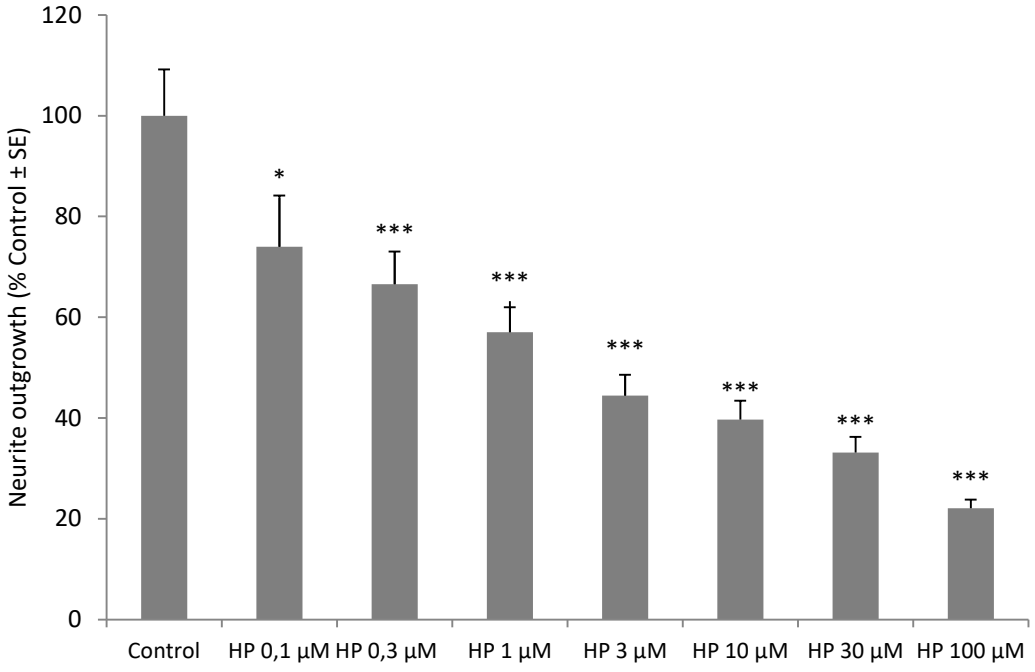


Figure 5. Effect of hypericin concentrations on neurite outgrowth in NB2a cells.

Significant difference compared to the control group, * p<0.05, *** p<0.001. HP; Hypericin**.**

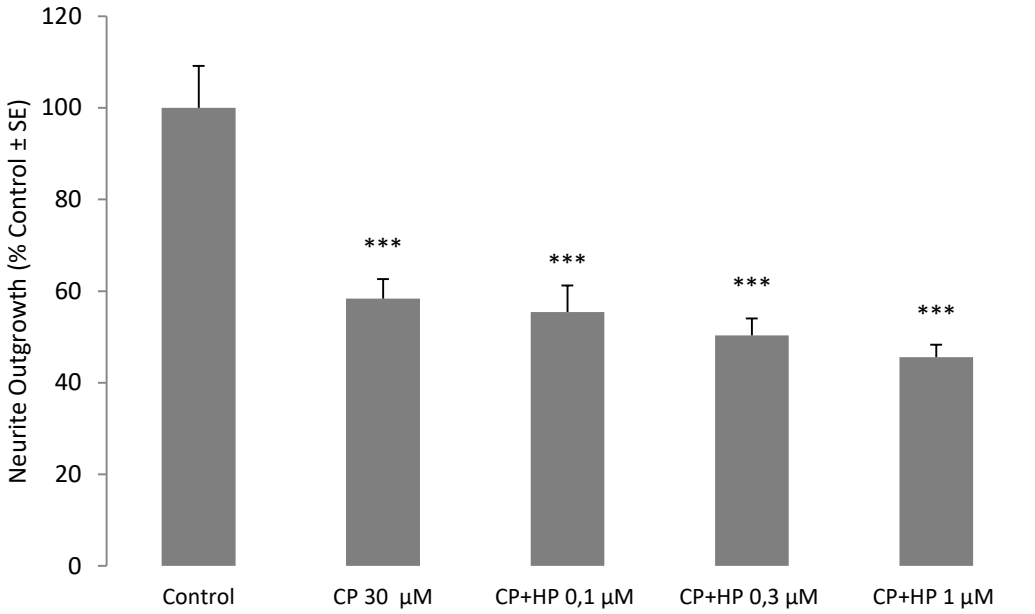


Figure 6. Effect of hypericin at different concentrations on neurite outgrowth inhibition induced by chlorpyrifos.

Significant difference compared to the control group, ***p<0.001, HP; Hypericin, CP; Chlorpyrifos.

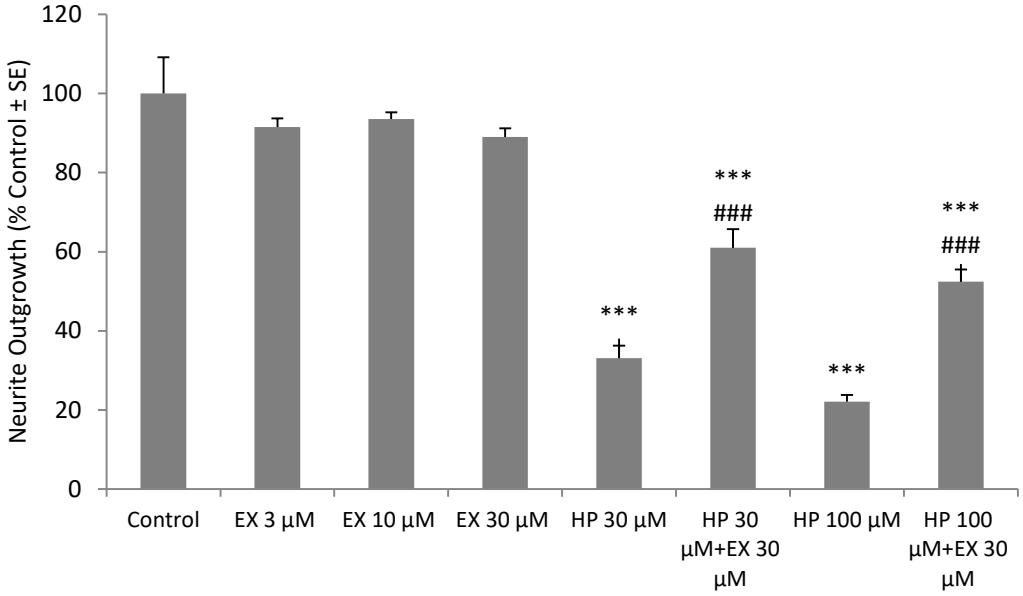


Figure 7. Effect of exendin 9-39 concentrations on neurite outgrowth and effects of 30 µM exendin application on neurite outgrowth when applied together with high concentration (30 and 100 µM) hypericin.

Significant difference compared to the control group *** $p<0.001$, considerable difference with exendin (30 µM) application, ### $p<0.001$. HP: Hypericin, EX: Exendin.

To evaluate potential apoptotic effects, NB2a cells were treated with CP (30 µM), exendin 9-39 (10 and 30 µM), and hypericin (10, 30, and 100 µM), and the number of apoptotic cells was assessed 48 hours after treatment in three independent replicates. While no effect of exendin 9-39 was observed, apoptotic index values were increased in CP and hypericin treatments. However, no statistically significant differences were found compared to the control group (data not shown).

DISCUSSION

Hypericin, obtained from *Hypericum perforatum* (St. John's Wort) and its extracts, is widely used in the treatment of psychiatric conditions such as depression and anxiety[27,28]. While its effects on the central nervous system (CNS) and its mechanisms of action have been partially elucidated, its potential protective or toxic effects on neuronal cells remain to be investigated. In this context, our study aimed to investigate the potential neurotoxic and neuroprotective effects of hypericin in NB2a neuroblastoma cells by assessing key parameters, including cell viability, apoptosis, and neurite outgrowth. Furthermore, we investigated whether GLP-1 receptors, known to have neuroprotective effects in various models, modulate hypericin's effects.

Our findings show that hypericin decreased cell viability in a dose-dependent manner ($p<0.001$). These results are consistent with the known cytotoxic potential of hypericin and suggest that the effects observed in NB2a cells, a neuroblastoma-derived line, may also reflect its anticancer properties. Similar cytotoxic and proapoptotic effects of hypericin have been reported in various cancer cell lines, including breast adenocarcinoma (MCF-7), human leukemia (HL-60 and U-937), glioma (U87), and colorectal cancer cells (HCT116 and SW620)[29-37]. In these studies, hypericin was shown to suppress protein kinase C activity and to induce apoptosis by increasing the expression of pro-apoptotic proteins such as Bax and caspase-3, while decreasing the expression of anti-apoptotic Bcl-2. Our apoptotic data, obtained across similar concentration ranges, support these findings and suggest that hypericin may

also have antiproliferative and cytotoxic effects on neuronal tumor cells.

In addition to their known role in glucose metabolism, GLP-1 receptors have also been suggested to play a role in various cancer models. Studies have shown that GLP-1 receptor agonists, such as exendin-4 and liraglutide, can reduce cancer cell proliferation and induce apoptosis, particularly in prostate and pancreatic cancer models[38-40]. In our study, partially consistent with these findings, co-administration of the GLP-1 receptor antagonist exendin 9-39 (30 μM) with high hypericin concentrations (30 and 100 μM) resulted in a slight but not statistically significant increase in cell viability. This may indicate a complex interaction between GLP-1 signaling and hypericin-induced cytotoxicity. Although this observation alone does not confirm a definitive antagonistic relationship, it does suggest that modulation of the GLP-1 pathway may mediate the biological effects of hypericin in neuronal cancer cells.

One of the most important findings of this study is that hypericin significantly inhibited neurite extension starting from the lowest concentration tested (0.1 μM), even at doses that did not significantly reduce overall cell viability. This suggested that hypericin may exert neurotoxic effects independent of its cytotoxic activity in neurite outgrowth experiments.

The growing body of literature indicates that hypericin may have both neuroprotective and neurotoxic effects on the nervous system. Due to its high light sensitivity, hypericin can generate reactive oxygen species (ROS) upon exposure to light, leading to oxidative stress. This process could potentially cause neuronal damage, as oxidative stress plays a central role in neurodegenerative conditions like Alzheimer's and Parkinson's diseases. In photodynamic therapy (PDT), an overproduction of ROS could further exacerbate oxidative stress, leading to neuronal cell death via mechanisms such as apoptosis, necrosis, or autophagy[11]. Moreover, hypericin has been shown to induce mitochondrial dysfunction by inhibiting or depleting key enzymes, such as succinate dehydrogenase and aconitase, as well as by promoting the detachment of hexokinase from the mitochondria, all of which may contribute to its neurotoxic effects[12].

On the other hand, several studies have also highlighted the potential neuroprotective effects of hypericin. For example, hypericin was reported to inhibit glutamate release in isolated nerve terminals from rat cerebral cortex, probably by suppressing voltage-gated $Ca2^{+}$ channels and MAP kinase signaling, thereby protecting against excitotoxicity[41]. In another study, treatment of cerebellar granule cells with low concentrations of hypericin activated the transcription factor NF-ϰB, which is involved in neuronal survival and anti-inflammatory responses[42]. Furthermore, a screening study evaluating acetylcholinesterase inhibition in various edible plants suggested that plants containing higher levels of hypericin showed stronger inhibitory capacity, indicating that this may play a role in preserving cholinergic function relevant to Alzheimer's disease[43]. However, in our study, hypericin failed to reverse neurite retraction induced by the neurotoxic pesticide chlorpyrifos, suggesting that the neuroprotective activity was absent in our model.

Numerous studies in the literature have demonstrated the neuroprotective effects of GLP-1 analogs in different *in vitro* and *in vivo* models. Exendin-4, for example, has been shown to reduce caspase activation and apoptotic cell death in neurons exposed to Aβ oligomers, a model relevant to Alzheimer's pathology[44]. Similarly, GLP-1 has been reported to mitigate methylglyoxal-induced toxicity, a metabolic stressor related to diabetic neuropathy[45] and in rodent models of diabetes-related cerebral ischemia and Parkinson's disease, GLP-1 receptor agonists improved neurological outcomes by suppressing oxidative stress and neuronal apoptosis[46- 49].

In contrast to these studies, our study demonstrated that the GLP-1 receptor antagonist exendin 9-39 significantly reversed hypericin-induced neurite retraction at high concentrations. This unexpected result implies that GLP-1 pathway activation may actually contribute to the neurotoxicity observed in this context, contrary to the widely accepted neuroprotective profile of GLP-1. Although this discrepancy is not yet fully explained, it is possible that partial reversal of hypericin-induced cell death by exendin 9-39 also contributes to this effect. Further studies are needed to elucidate the specific interactions between hypericin and GLP-1 receptor signaling in neuronal cells under both normal and stressed conditions.

The results of this study, conducted in NB2a cell culture, showed that hypericin significantly reduced cell viability at high concentrations. This effect is

suggested to be related to hypericin's anticancer activity, as described in previous studies. The fact that the GLP-1 antagonist exendin 9-39 reversed the decrease in cell viability caused by hypericin suggests that GLP-1 receptor activity may play a role in the anticancer activity of hypericin.

On the other hand, hypericin inhibited neurite extension even at low concentrations that did not alter cell viability. This data suggests that hypericin may have neurotoxic activity of its own. As a conclusion, while additional studies are needed to elucidate this toxic effect and the mechanisms mediating it, considering that St. John's Wort, whose active ingredient is considered hypericin, is widely used in central nervous system disorders such as depression and anxiety, caution is recommended.

Author Contributions: Concept/Design : FÖ, SG, EÖ; Data acquisition: ED, FÖ; Data analysis and interpretation: ED, FÖ, EÖ,. SG; Drafting manuscript: FÖ, EÖ; Critical revision of manuscript: EÖ, SG; Final approval and accountability: FÖ, ED, EÖ, SG; Technical or material support: ED, FÖ; Supervision: ED, EÖ, SG; Securing funding (if available): n/a.
Ethical Approval: Ethical approval was obtained from the Health Sciences Ethics Committee of Manisa Celal Bayar University Faculty of Medicine, with letter number 633939 dated 04.11.2023 and decision number 11.10.2023/20.478.486/2048.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support

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