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Research Article

NEUROPROTECTIVE EFFECT OF 7,3′-DIHYDROXYFLAVONE AGAINST PACLITAXEL-INDUCED NEUROTOXICITY IN SH-SY5Y NEUROBLASTOMA CELL LINE: AN *IN VITRO* MODEL

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ABSTRACT

Objectives: Paclitaxel (PT) induced peripheral neuropathy is associated with the neurotoxic effects of this chemotherapeutic drug. As a result, the need for natural substances that can mitigate neural toxicity is rising. This study investigated the Neuroprotective effects of 7,3′-dihydroxyflavone (7,3′-DHF) on PT-induced neurotoxicity in SH-SY5Y neuroblastoma cells.

Methods: The cells were exposed to a 1 μM concentration of PT, and the protective effects of various concentrations of 7,3′-DHF were evaluated. The reactive oxygen species (ROS) level in SH-SY5Y cells was measured using confocal fluorescence microscopy. The concentration of proinflammatory cytokines, interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) was determined by enzyme-linked immunosorbent assay.

Results: Treatment of SH-SY5Y cells with 1 μM PT significantly reduced cell viability to 19±4.8%. However, treatment with 7,3′-DHF in PT-exposed cells elevated SH-SY5Y cell viability in a dose-dependent manner, with viability reaching 55.34±3.8% and 83.93±4.1% at 7,3′-DHF concentrations of 75 μg/mL and 100 μg/mL, respectively. PT exposure elevated ROS levels in SH-SY5Y cells, but the presence of 7,3′-DHF reduced ROS levels against PT-induced toxicity. In addition, treatment with 7,3′-DHF lowered the increased levels of IL-6 and TNF-α induced by PT exposure.

Conclusion: 7,3′-DHF effectively preserved the viability of SH-SY5Y cells under PT-induced toxicity. This protective effect was mediated through the suppression of ROS production and the attenuation of proinflammatory cytokine release, highlighting its potential as a neuroprotective agent.

Keywords: 7,3′-Dihydroxyflavone, SH-SY5Y cells, Tumour necrosis factor-alpha, Interleukin-6.

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INTRODUCTION

Taxanes, which function as microtubule-stabilizing agents, are extensively employed in treating a wide spectrum of cancers such as breast, stomach, ovarian, bladder, prostate, lung, and melanoma [1,2]. Due to its wide range of applications, paclitaxel (PT) and other taxane-based treatments are administered to a significant number of cancer patients annually. Commonly used taxane formulations, such as taxol, PT liposome, docetaxel, nab-PT, and cabazitaxel are key components of chemotherapy regimens for these cancers [2]. The therapeutic action of PT involves binding to the α-tubulin and β-tubulin proteins in microtubules, promoting microtubule stability, activating the spindle checkpoint, halting the G2 or M phase of the cell cycle, and inducing cancer cell apoptosis [3,4].

A major non-hematologic side effect of Taxanes, particularly PT-induced peripheral neuropathy (PIPN) can interfere with treatment effectiveness by requiring dose reductions or early discontinuation. It is estimated that more than half of PT-treated patients develop neuropathy symptoms, with some experiencing long-lasting and irreversible effects that significantly reduce their quality of life [1,5-7]. Common symptoms include stocking-glove-like soreness, tingling, cold sensitivity, and numbness, with some patients also suffering from allodynia, where even light touch causes intense pain [5,8,9].

This is due to nerve fiber loss and activation of pain pathways, including transient receptor potential (TRP) channels and Mitogen-activated protein kinase (MAPK). In addition, the loss of intra-epidermal nerve fibers and activation of MAPK and TRP channels are believed to play roles in PIPN development [10]. Both oxidative stress and inflammation are central to this process; increased pain response, neuronal sensitization, and spontaneous neural discharge can result from elevated oxidative stress in peripheral sensory neurons [11,12]. Inflammation frequently occurs in PIPN, with Toll-like receptor 4 (TLR4)/nuclear factor kappa-B (NF-κB) (p65) signaling facilitating the production of proinflammatory factors that may harm neurons, inhibit synaptic transmission, and amplify neuronal responses to detrimental stimuli. Cell types include Schwann cells, satellite glial cells, immune cells, Merkel cells, and epidermal keratinocytes, along with central axon interactions with astrocytes and microglia, which are implicated in the condition's progression. Genetic predisposition further influences individual susceptibility to this complex neuropathic disorder [5,13-15].

Pharmacological strategies to prevent chemotherapy-induced peripheral neuropathy (CIPN) have so far met with limited success. Various agents, such as pregabalin, amitriptyline, acetyl-L-carnitine, Vitamin B, and calcium/magnesium infusions have been tested, but they often exhibit toxicity or provide only modest benefits in reducing neuropathic symptoms. Therapies such as glutathione, oxcarbazepine, glutamine, and venlafaxine have yielded mixed results across various studies, emphasizing the persistent challenge of effectively preventing CIPN in cancer patients receiving treatment [17].

Many *in vitro* and *in vivo* studies are currently being conducted to identify natural pharmacological agents that could prevent the neurotoxic effects of PT and reduce the incidence of PIPN. Among the agents under investigation are compounds such as daidzein, berbamine, bergapten, and carveol, each of which has shown potential in preclinical studies. Other herbal and natural compounds, including umbelliprenin, evodiamine, Ephedra herb extract, and Huangqi Guizhi Wuwu decoction are being studied for their neuroprotective effects against CIPN [16-21].

The search for effective, natural-based pharmacological options to prevent CIPN remains a crucial research focus to identify safe and accessible alternatives to safeguard nerve health during chemotherapy. In a recent previous study, the 7,3′-dihydroxyflavone (7,3′-DHF) has shown an anti-neuropathic effect against PIPN in mice [22]. Nevertheless, the chemical process by which DHF prevents PT-induced neuronal damage has not been clarified. This study investigated 7,3′-DHF's preventive properties against PT-induced neurotoxicity in the SH-SY5Y cell line. This study focused on key parameters such as cell viability, ROS production, and proinflammatory cytokines levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) to evaluate the potential neuroprotective properties of 7,3′-DHF.

METHODS

Materials

Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), and MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium) were sourced from HiMedia in Mumbai, India. The compounds PT, gabapentin (GP), and 7,3′-DHF were procured from Sigma-Aldrich, USA. 2′,7′-dichlorofluorescein diacetate (DCFH-DA), a fluorescent dye, was acquired from Life Technologies, Invitrogen, located in Carlsbad, California, USA. The enzyme-linked immunosorbent assay (ELISA) kits for TNF-α and IL-6 were purchased from RayBiotech in Georgia, USA.

Cell culture and treatments

The National Centre for Cell Science in Pune, India, is the source of the human neuroblastoma cell line SH-SY5Y. The cell line was cultivated in DMEM with 5% CO_2 and 10% FBS at 37°C.

Determination of cell viability using MTT assay

In a 96-well plate, roughly 2 × 10^4 SH-SY5Y cells were planted, and they were cultivated for 24 h. After that, the cells were exposed to varying amounts of 7,3′-DHF (10, 25, 50, 75, and 100 µg/mL) alongside 1 µM PT for another 24 h. For the positive control, cells were exposed to 1 mM GP in addition to 1 µM PT for 24 h. After the treatment period, the medium was removed, and then MTT was introduced to the cells at a dosage of 0.5 mg/mL in DMEM. The cells were then placed in a CO_2 incubator and treated with MTT for 1 h at 37°C. The MTT was taken out after the incubation period, and 100 µL of dimethyl sulfoxide was used to dissolve the dye crystals that had formed. At 570 nm, the absorbance was determined with an ELISA reader (Epoch2, BioTek Instruments, USA).

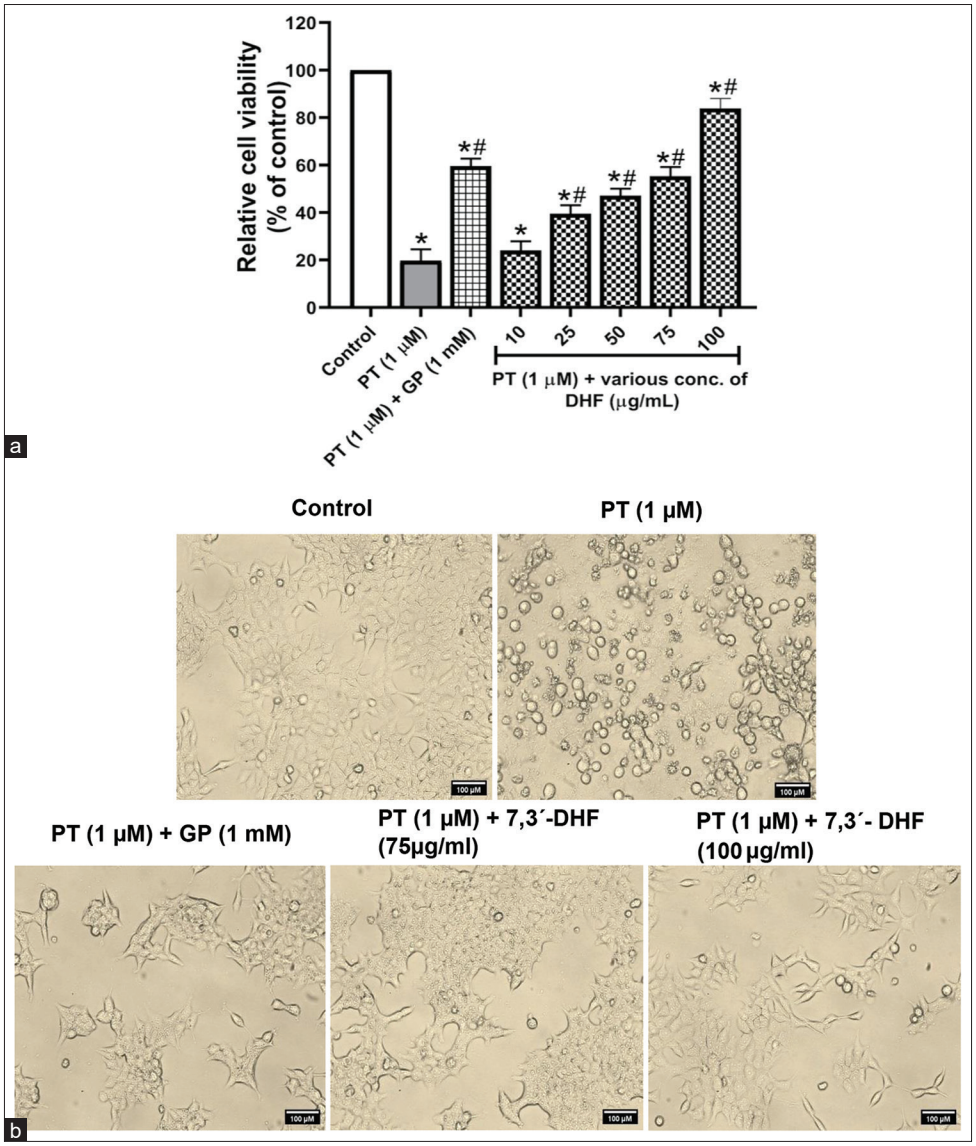

Fig. 1: (a) Viability of SH-SY5Y cells under different treatment conditions. (b) Bright-field microscopy image of cells from various groups. n=3; *p<0.05 versus control cells; #p<0.05 versus PT-exposed cells. PT: Paclitaxel, GP: Gabapentin, 7,3′-DHF: 7,3′-Dihydroxyflavone

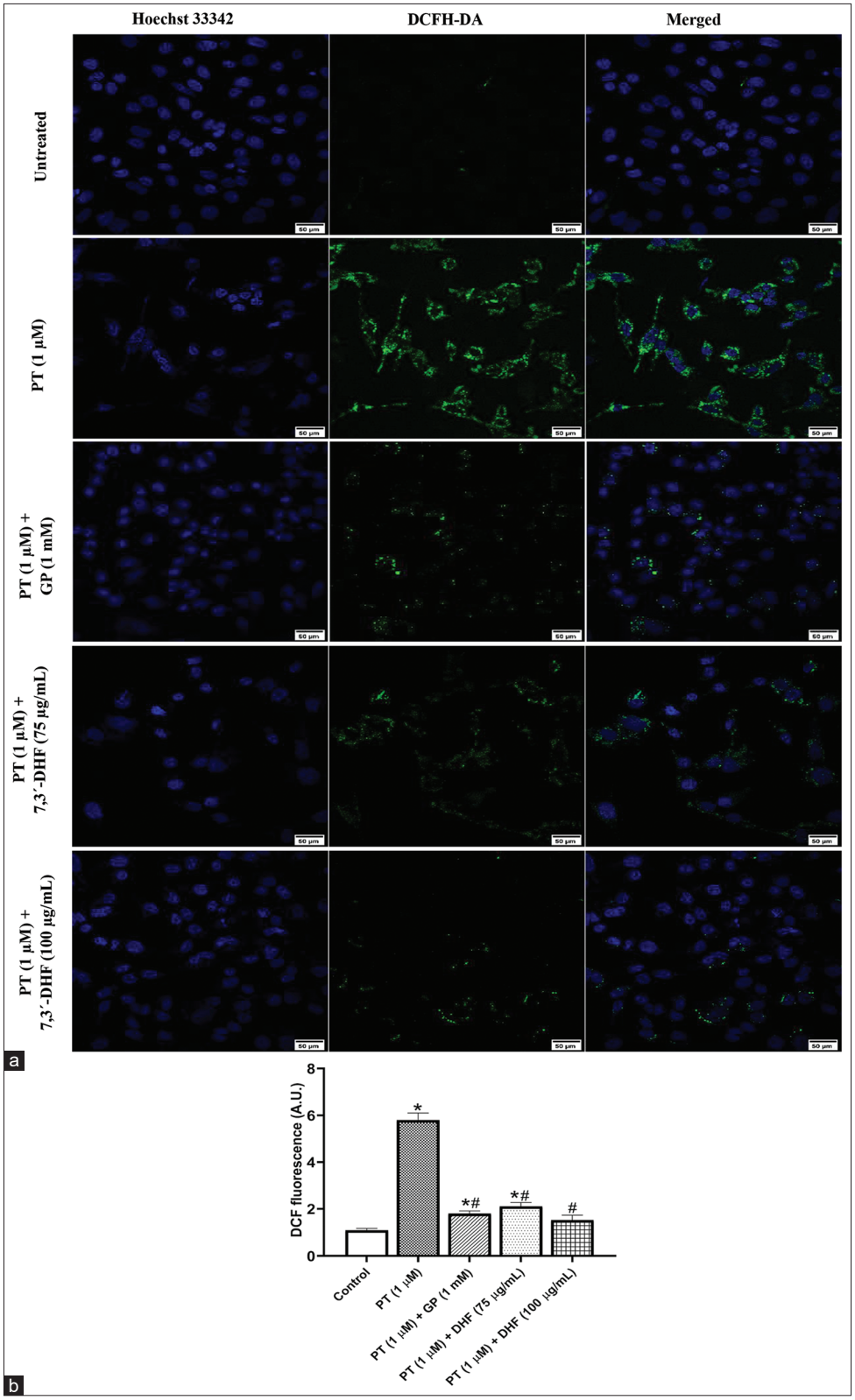

Fig. 2: Reactive oxygen species generation in SH-SY5Y cells. (a) Confocal microscopy image of SH-SY5Y cells stained with DCF and counter-stained with Hoechst 33342 from various groups. (b) The DCF fluorescence intensity measured cells using image J. PT: Paclitaxel, GP: Gabapentin, 7,3´-DHF: 7,3´-Dihydroxyflavone

Determination of intracellular ROS production by confocal microscopy

To measure intracellular ROS generation, cells were divided into five treatment groups: Control (no treatment), PT (1 μM), PT (1 μM)+GP (1 mM), PT (1 μM)+7,3´-DHF (75 μg/mL), and PT (1 μM)+7,3´-DHF (100 μg/mL), each for 12 h. The cells were rinsed with 1× phosphate-buffered saline (pH 7.2) after treatment, and the medium was taken out for fluorescence confocal imaging. After that, cells were incubated

with 10 μM DCFH-DA for 30 min in a dark environment using a CO_2 incubator. Then, cells were counterstained with Hoechst 33342, and fluorescence images were captured and analyzed using a confocal microscope (LSM710, Carl-Zeiss, Germany).

Measurement of IL-6 and TNF-α

The levels of TNF-α and IL-6 in the cell lysate were assessed utilizing an ELISA Kit, following the manufacturer's instructions. This involved incubating the lysate with pre-coated antibody wells, where the cytokines specifically bind to the antibodies. A secondary enzyme-linked antibody was then added, and after a substrate reaction, the color change was evaluated using a microplate reader to quantify the cytokine levels based on a standard curve.

Statistical analysis

All data were expressed using the mean±standard deviation (n=3). One-way analysis of variance and Tukey's multiple range tests for *post hoc* analysis were employed in the statistical analysis, which was performed using GraphPad Prism 8 software. A level of $p<0.05$ was used to determine statistical significance.

RESULTS

Effect of 7,3´-DHF on the viability of SH-SY5Y cells exposed to PT

When SH-SY5Y cells were treated with 1 μM PT, their cell viability dropped to 19±4.8% (Fig. 1a). In contrast, PT-exposed cells treated with 7,3´-DHF showed dose-dependent increases in SH-SY5Y cell viability. In particular, viability was determined to be 55.34±3.8% and 83.93±4.1% at 75 μg/mL and 100 μg/mL concentrations of 7,3´-DHF, respectively. The protective effect of DHF against PT-induced toxicity was comparable to that of the positive control, GP. Morphological observations under a bright-field microscope (Fig. 1b) showed healthy cell structure in the control group, while PT-exposed cells appeared rounded and lost normal morphology, indicating apoptosis. In contrast, cells treated with GP and 7,3´-DHF retained a morphology similar to that of the control group, suggesting protection against PT-induced damage.

Effect of 7,3´-DHF on ROS generation in PT-exposed SH-SY5Y cells

Confocal microscopy pictures of SH-SY5Y cells from various treatment groups are displayed in (Fig. 2a). DCF is used to show ROS, and Hoechst 33342 is used to counterstain the nuclei. Compared to control cells, PT-exposed cells showed noticeably more green fluorescence, which may indicate that increased intracellular ROS levels caused DCFH-DA to oxidize more. Conversely, cells exposed to PT and treated with GP and 7,3´-DHF had less green fluorescence, which is indicative of a reduction in ROS production. (Fig. 2b) displays the green fluorescence intensity quantification for each group, which was examined using the ImageJ program.

Effect of DHF on the concentration of inflammatory mediators in PT-exposed SH-SY5Y cells

Cytokine levels, such as IL-6 and TNF-α, were markedly elevated in SH-SY5Y cells exposed to PT (Fig. 3a and b). However, treatment with 7,3´-DHF at concentrations of 75 and 100 μg/mL reduced these inflammatory mediators compared to the PT-exposed group. The effects of 7,3´-DHF were comparable to those of the positive control, GP.

DISCUSSION

PT is recognized for its role in neuronal toxicity, specifically as a contributing element to PIPN. Among the various mechanisms proposed, an increase in ROS generation is considered a primary cause of PT-related neurotoxicity, which is possibly exaggerated by mitochondrial dysfunction [23-26]. In this study, PT exposure led to reduced SH-SY5Y cell viability, with ROS generation likely causing DNA fragmentation and triggering caspase-mediated apoptosis, observable as rounded cells under a brightfield microscope. Prior studies have also shown that exposure to PT can cause DNA damage, cleavage of the enzyme poly-(ADP-ribose)-polymerase, and caspase 7 activation [27].

Resveratrol, a naturally occurring non-flavonoid was able to preserve the SH-SY5Y cells against PT-induced neurotoxicity through the inhibition of these apoptosis activation [27]. The flavonoids such as icariin, flavanol, dimethoxy flavanol, 3-hydroxyflavone, and berberine have been reported to alleviate PIPN [28-31]. It has been shown in earlier research that 7,8-DHF shields HT22 cells from glutamate toxicity and SH-SY5Y cells from high-glucose toxicity. More recent research has reinforced these findings, showing that 7,8-DHF also protects against oxidative stress and excitotoxicity, highlighting its potential as a therapeutic strategy for neurodegenerative diseases [32,33,37]. When used *in vivo*, 7,8-DHF preserved neurons in a mouse model of Parkinson's disease caused by MPTP and in a rat model of ischemia-reperfusion brain injury [34,35]. Likewise, 7,3´-DHF has been shown to alleviate PIPN in mouse models [22]. Simultaneously, this present study shows that co-treatment of neuronal cells with 7,3´-DHF and PT exposure significantly lowers ROS production and associated cell death. These findings imply that 7,3´-DHF effectively counteracts oxidative stress, offering potential neuroprotective effects in conditions linked to oxidative damage.

In line with earlier findings, PT exposure increased proinflammatory cytokine levels of TNF-α and IL-6 in SH-SY5Y cells in addition to ROS formation [29]. PT activates nuclear factor-kappa B through TLR4 stimulation and upregulation of ERK/JNK signaling, leading to nuclear translocation of NF-κB, histone H4 acetylation, and transcription of proinflammatory proteins such as TNF-α, IL-1β, IL-6, and CX3CL1 [36]. In our study, 7,3´-DHF demonstrated a significant

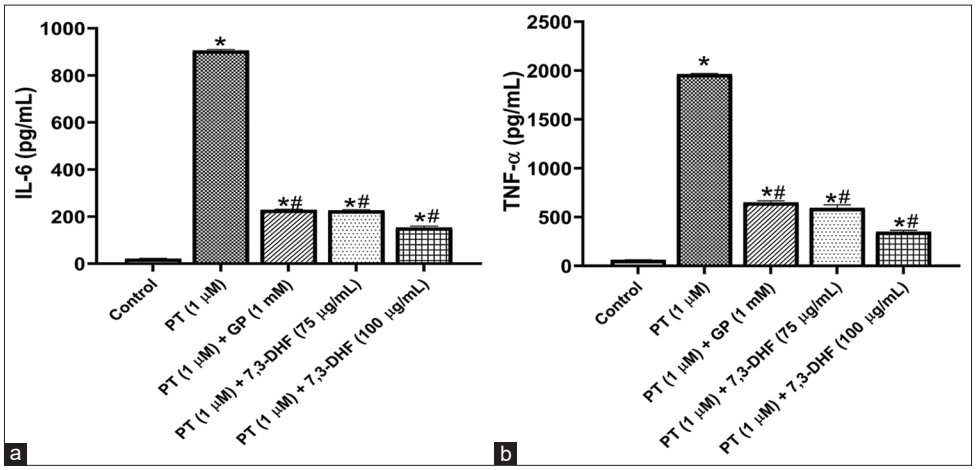

Fig. 3: Level of proinflammatory cytokines interleukin-6 (a) and tumor necrosis factor-alpha (b) in SH-SY5Y cells from various groups. *p<0.05 versus control; #p<0.05 versus PT-exposed cells. PT: Paclitaxel, GP: Gabapentin, 7,3´-DHF: 7,3´-Dihydroxyflavone

reduction in proinflammatory cytokine levels, which marks a critical observation. This suggests that 7,3′-DHF may exert its neuroprotective effects not only through antioxidant mechanisms but also by modulating the inflammatory response. In the case of neurodegenerative illnesses, where inflammation is a major factor in aggravating neuronal damage, the capacity to lower proinflammatory cytokines is very crucial. This finding highlights 7,3′-DHF as a promising compound for mitigating the inflammatory processes associated with neuronal toxicity [38-42].

While 7,8-DHF has been extensively studied for its neuroprotective effects, the potential of 7,3′-DHF in combating PT-induced neurotoxicity remains underexplored. In this investigation, we offer new insights into its protective benefits, showing that 7,3′-DHF efficiently reduces ROS formation and suppresses proinflammatory cytokine levels to ameliorate PT-induced neuronal injury. This implies that 7,3′-DHF may be a viable option for peripheral neuropathy neuroprotection. Further research is required to fully understand the molecular mechanisms behind its protective properties. Overall, 7,3′-DHF's neuroprotective qualities contribute significantly to PT-induced neuropathy reduction *in vivo*, underscoring its promise as a treatment for neurodegenerative diseases [22].

CONCLUSION

By lowering proinflammatory cytokine levels and oxidative stress, 7,3′-DHF successfully shields SH-SY5Y cells against PT-induced neuronal damage. These results imply that 7,3′-DHF might be a viable Neuroprotective drug that could be used to treat Neurodegenerative illnesses and Neuroinflammatory conditions.

ETHICS STATEMENT

This study was conducted following ethical guidelines, with no involvement of human or animal subjects. All experiments utilized commercially available cell lines and reagents. A waiver for ethical review was obtained from the Institutional Research Ethics Committee.

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AUTHOR'S CONTRIBUTION

Each author made an equal contribution to this work. The following contributions to the work are confirmed by the authors: Keerthana Vijayarajan conceptualized and designed the study; Kavitha Ramasamy analyzed the data and interpreted the findings; and Kranthi Karunai Kadal guided and prepared the draft manuscript. After reviewing the findings, each author gave their approval to the manuscript's final draft.

CONFLICTS OF INTEREST

No conflicts of interest are disclosed by the authors.

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Nil.

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