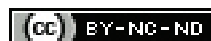


Ameliorating Effect of 7,3'-Dihydroxyflavone in Paclitaxel Induced Neurotoxicity: An In-silico and In-vitro Study

G KRITHIGA¹, KAVITHA RAMASAMY², S RAMYA³



ABSTRACT

Introduction: Paclitaxel is an antineoplastic agent belonging to taxane group. It stabilises microtubule structure and one of its major dose limiting side-effects is neurotoxicity. Flavones are promising compound to alleviate paclitaxel induced neurotoxicity as it has anti-nociceptive, anti-inflammatory effect, antioxidant and neuroprotective effect.

Aim: To evaluate the neuroprotective property of 7,3'-dihydroxyflavone (7,3'-DHF) against Paclitaxel induced neurotoxicity in SH-SY5Y neuroblastoma cell line through a combined in-silico and in-vitro approach with a focus on its ability to modulate ion channels.

Materials and Methods: The present in-vitro study employing human neuroblastoma cell line SH-SY5Y was conducted at Sri Ramachandra Medical College and Research Institute, Chennai, Tamil Nadu, India, from April to July 2024. Molecular docking was performed against Cav3.2, Nav1.7 and Kv7.2 protein targets using auto dock tools 1.5.7, followed by Molecular Dynamics (MD) analysis using PyMOL version 2.0. Flow cytometry was done to assess antioxidant effect of 7,3'-DHF by free radical scavenging activity. Reverse Transcription Polymerase Chain Reaction (RT-PCR) was done to study gene expression of

CACNA1H gene associated with paclitaxel induced peripheral neuropathy.

Results: Molecular docking scores of 7,3'-DHF with target was -6.66 kcal/mol for Nav1.7, -6.56 kcal/mol for Cav3.2 and -6.66 kcal/mol for Kv7.2. In MD, Root Mean Square Deviation (RMSD) stabilised around 5 Å for both Cav 3.2 and Kv 7.2 after 50 ns. Molecular Mechanics/Generalised Born Surface Area (MM/GBSA) binding free energy (ΔG_{Bind}) values were -62.2 kcal/mol for Cav 3.2 and -66.1 kcal/mol for Kv 7.2. Flow cytometry showed reduced Reactive Oxygen Species (ROS) generation in cells treated with 7,3'-dihydroxyflavone in dose dependent manner. Gene expression showed that 7,3'-dihydroxyflavone down regulates the CACNA1H gene that encodes Cav 3.2 which is associated with paclitaxel induced peripheral neuropathy.

Conclusion: This study adds evidence to the significant neuroprotective potential of 7,3'-dihydroxyflavone against paclitaxel-induced neurotoxicity, demonstrated through in-silico and in-vitro approaches and identifies it as one of the promising therapeutic candidates for mitigating neurotoxicity in paclitaxel-treatment. However further confirmation through in vivo study is needed.

Keywords: Flow cytometry, Free radical scavenging, Ion channels, Neuroprotective, SH-SY5Y neuroblastoma cell line

INTRODUCTION

Paclitaxel is obtained from the bark of the western yew *Taxus brevifolia* [1]. Solid tumours like lung, breast, ovary, stomach, prostate, head and neck are frequently treated with taxanes [2]. Paclitaxel works by attaching to polymerised tubulins (α and β) and stabilising microtubules, which stops the cell cycle at G_2 phase [3]. By binding to mitochondrial β -tubulin, taxanes also increase calcium efflux and activate the mitochondrial Permeability Transition Pore (mPTP) which damages the mitochondria [4-6]. These occurrences cause necrosis and apoptosis in cancer cells.

Its increased use in cancer treatment has raised concern due to peripheral neuropathy, a dose limiting side-effect [7]. Studies have shown that the response to oxidative stress is impaired by paclitaxel treatment and can result in peripheral neuropathy [8,9]. It can cause predominantly numbness, paraesthesia and burning pain in a glove and stocking distribution [7]. Current treatment options for Chemotherapy Induced Peripheral Neuropathy (CIPN) include gabapentin, pregabalin, opioids, antidepressants like duloxetine [10] and are frequently only partially successful [11]. It is also associated with significant side-effects including sedation, dizziness, dependence and gastrointestinal disturbances [12]. To overcome these limitations there is an increased need to identify a new therapeutic option that is more targeted for the treatment of CIPN.

Neurotoxicity due to paclitaxel use can involve both central and peripheral nerves [13]. Preclinical and clinical research has examined

the effectiveness of antioxidant molecules as neuroprotective measures to stop peripheral neuropathy from developing [14].

Vitamin E and Glutathione have been investigated as adjuvant treatments to alleviate CIPN but they lack proof of their effectiveness in clinical trials [15,16]. Amifostine [17], glutamine and acetyl L-carnitine are other neuroprotective therapies that have also been tried but have limited effectiveness [18]. Flavones are becoming recognised as essential components of the human diet that have a number of health advantages. According to studies, flavones have ability to ameliorate inflammation [19], reduces generation of ROSs, combat infections as well as chemopreventive properties [20-22]. The naturally occurring flavonoid 7,3'-dihydroxyflavone (DHF) has drawn interest due to its anti-inflammatory effect [23] and models of neuropathic pain, such as chemotherapy-induced peripheral neuropathy, have been used to investigate its possible therapeutic use as a neuroprotective agent [24].

CACNA1H gene encodes for Cav 3.2 T type calcium channel which is involved in peripheral neuropathy caused by paclitaxel [25]. Allodynia and hyperalgesia due to paclitaxel [26] are neuropathic manifestations that cause the patient great discomfort, drastically reduce their quality of life and increase the likelihood that they may stop their chemotherapy regimen. This gene is also involved in multiple disorders like childhood absence seizure [27], autism spectrum disorder [28] and amyotrophic lateral sclerosis [29]. Hence, gene expression was done to study the effect of 7,3'-dihydroxyflavone in altering the expression of CACNA1H gene.

Although drugs like gabapentinoids, antidepressants are used for CIPN, there is no approved treatment so far [30]. This study is a part of larger study [31] and here the neuroprotective effect of 7,3'-dihydroxyflavone against paclitaxel induced neurotoxicity and its role in modulating ion channels involved in CIPN were assessed.

MATERIALS AND METHODS

An in-vitro study employing human neuroblastoma cell line SH-SY5Y was conducted at Sri Ramachandra Medical College and Research Institute, Chennai, Tamil Nadu, India, from (April-July 2024). The study proposal was reviewed and approved by Institutional Ethical Committee (CSP-MED/24/JAN/97/01).

The study employed triple-cloned SH-SY5Y cell lines, derived from SK N-SH neuroblastoma cell line. The cell line was not directly derived from humans or animals but was cloned and cultivated from a pre-existing line. It was procured from the National Centre for Cell Science (NCCS) in Pune, Maharashtra. Each experiment was performed in triplicate. Gabapentin is used in prevention of Paclitaxel induced neuropathies widely [32,33]. So Gabapentin was chosen as standard to compare the neuroprotective effect of 7,3'-DHF in this study. From the result of the MTT assay in previous study done by Vijayarajan K et al., the concentrations of 7,3'-DHF showing maximum cell viability (75 µg/mL and 100 µg/mL) in comparison to the positive control (Gabapentin) were taken for further assays in this study [31]. CACNA1H gene is responsible for pain perception and regulates neuronal excitability [34,35]. Hence, modulation of the gene after treatment with 7,3'-DHF was also studied.

Study Procedure

Maintenance of cell lines: The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) a high glucose media with 10% Foetal Bovine Serum (FBS) and 1% antibiotic-antimycotic solution. It was placed in a CO₂ incubator at 37°C containing 5% CO₂, 18% O₂ and was sub-cultured every three days.

In-Silico study: The three-dimensional structure of 7,3'-dihydroxyflavone was retrieved from PubChem database. The 3-Dimensional (3D) protein structures of Nav 1.7, Cav 3.2 and Kv 7.2 were retrieved from Protein Data Bank (PDB). Proteins and ligands were meticulously prepared and intermolecular interactions predicted using Auto Dock Tools 1.5.7. Of these targets the docking scores were almost equal for all three targets but Kv-7.2 is a vital target for neurological illnesses because it regulates neuronal excitability [36]. Cav-3.2, a component of T-type calcium channels, is linked to neural communication, chronic pain [37] and epilepsy [38]. Hence, Cav 3.2 and Kv 7.2 were considered for Molecular Dynamics (MD) analysis.

RMSD and Root Mean Square Fluctuation (RMSF) of all the complexes were observed to predict the strength of the intermolecular interactions and conformational changes. The interaction of the complex was analysed using PyMOL Version 2.0 and Discovery Studio Visualiser.

ROS generation by flow cytometry: After being cultivated in a 6-well plate, the cells were maintained at 37°C for 24 hours in a CO₂ incubator. After aspirating spent medium, Phosphate-Buffered Saline (PBS) was used to wash the cells. The cells were now treated according to the defined groups: untreated control, toxicity group (1 µM paclitaxel), positive control (1 µM paclitaxel+gabapentin), test Group I (1 µM paclitaxel+75 µg/mL 7,3'-DHF) and test Group II (1 µM paclitaxel+100 µg/mL 7,3'-DHF), followed by incubation for 24 hours. A 250 µL of trypsin-Ethylenediamine Tetraacetic Acid (EDTA) solution was added to each well and cells were incubated at 37°C for three to four minutes.

The cells were harvested directly into polystyrene tubes. The supernatant was carefully removed from the tubes after they had been centrifuged at 300×g for five minutes at 25°C. The cells were washed twice with PBS and the H2DCFDA stock solution (4 mM)

was diluted in Dulbecco's Phosphate Buffered Saline (DPBS) to create a 10 µM working solution. The cells were suspended in the H2DCFDA working solution and then incubated at 37°C for 30 minutes without being exposed to light. The cells were resuspended in DPBS after being centrifuged to extract any unbound dye. A flow cytometer with 488 nm excitation and detection at 535 nm was used to analyse the samples.

Gene expression: Cells were seeded in a 6-well plate at a density of 0.5×10⁶ cells/2 mL and incubated at 37°C in a CO₂ incubator for 24 hours, after which the spent medium was aspirated and the cells were washed with 1 mL of 1×PBS. Cells were then treated according to the defined groups: untreated control, toxicity group (1 µM paclitaxel), positive control (1 µM paclitaxel+gabapentin), test Group I (1 µM paclitaxel+75 µg/mL 7,3'-DHF) and test Group II (1 µM paclitaxel+100 µg/mL 7,3'-DHF), followed by incubation for 24 hours and then cultured for further 24 hours. Trypsin-EDTA was employed to harvest the cells, spent media was collected and centrifugation was performed for five minutes. The Qiagen RNeasy kit was used to isolate the RNA and was then treated with DNase to eliminate genomic DNA and evaluated with Nanodrop (260/280 ratio: 1.8-2). The IScript cDNA synthesis kit was used to accomplish cDNA synthesis with oligo dT and random hexamer primers.

Gradient PCR optimised the annealing temperature at 59°C for all primers. qRT-PCR using SYBR Green was conducted on the QuantStudio3 system. Relative gene expression was determined using the ΔΔCt method, adjusted to beta-actin, and fold changes were computed using E^{-ΔΔCt}. Fold changes >1 indicated upregulation, while <1 indicated downregulation [39]. Results were analysed and interpreted using QuantStudio3 software.

STATISTICAL ANALYSIS

GraphPad Prism 8 (One-way ANOVA) and Tukey's post-hoc test was used for statistical analysis. Fold changes were calculated and interpreted using QuantStudio3 software. The p-value of less than 0.05 was considered significant. Mean±SD was used for expressing data.

RESULTS

In-Silico

Molecular docking score of 7,3'-DHF with Nav 1.7, Cav 3.2 and Kv 7.2 is given in [Table/Fig-1]. According to the docking analysis, 7,3'-dihydroxyflavone has significant interactions with all targets and shows favourable binding affinities with all three ion channels. The compound showed a docking score of -6.66 kcal/mol with Nav1.7 and Kv7.2, and -6.56 kcal/mol with Cav3.2 channels.

Protein	Ligand	Dock score (kcal/mol)
Nav-1.7	7,3'-dihydroxyflavone	-6.66
Cav-3.2	7,3'-dihydroxyflavone	-6.56
Kv-7.2	7,3'-dihydroxyflavone	-6.66

[Table/Fig-1]: Docking score of Nav-1.7, Cav-3.2 and Kv-7.2 with 7,3'-DHF.

The binding energies of 7,3'-DHF with all three targets were quite low, indicating strong interactions between 7,3'-DHF and the active site amino acids of the proteins as mentioned in [Table/Fig-2a].

Protein	G Bind	Coulomb	Covalent	H-bond	Lipo	vdW
Cav-3.2	-62.2	-19.9	0.3	-0.5	-27.4	-32.0
Kv-7.2	-66.1	-9.1	1.2	-0.2	-27.5	-32.8

[Table/Fig-2a]: MMGBSA binding free energy of 7,3'-DHF with Cav-3.2 and Kv-7.2 protein complex (in kcal/mol).

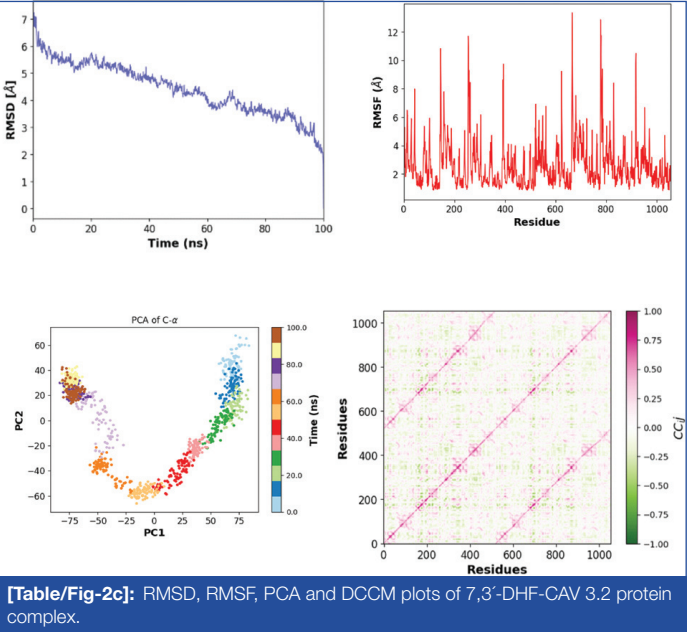
This suggests a high affinity of the molecule for the target proteins. Furthermore, the intermolecular interactions observed during docking are consistent across active site residues, as summarised in [Table/Fig-2b], highlighting the strong binding nature of 7,3'-DHF

with the targets. Kv-7.2 and Cav-3.2 were found to be the most intriguing options for molecular dynamics research [40,41] and hence considered for dynamics study.

Protein	Hydrogen bonding interactions		Hydrophobic interactions	
	Dock	MD	Dock	MD
Nav-1.7	Tyr1755 (3.1), Ile1756 (1.9), Ile1759 (2.6), Asn1461 (3.6), Leu964 (2.4), Leu398 (1.9)	-	Ile1756 (3.8), Ile1759 (3.5), Leu398 (4.5), Ile1457 (4.4)	-
Cav-3.2	Phe1802 (2.3), Ser1805 (2.1), Gln1848 (2.0), Asn412 (1.8)	Phe1756 (2.8), Phe408 (3.1), Ser407 (3.2), Ile400 (3.3), Phe1802 (2.4)	Leu1851 (5.0)	Phe408 (3.8), Ile 403 (4.9)
Kv-7.2	Phe305 (2.0), Gly239 (3.2), Phe240 (2.4), Trp236 (2.7), Ala309 (2.0)	Phe305 (3.5), Phe240 (3.0), Trp236 (3.2), Gly239 (2.8), Trp218 (3.1), Leu221 (2.3)	Phe305 (4.1), Trp236 (4.2), Pro308 (3.4)	Trp236 (3.9), Ala309 (4.4)

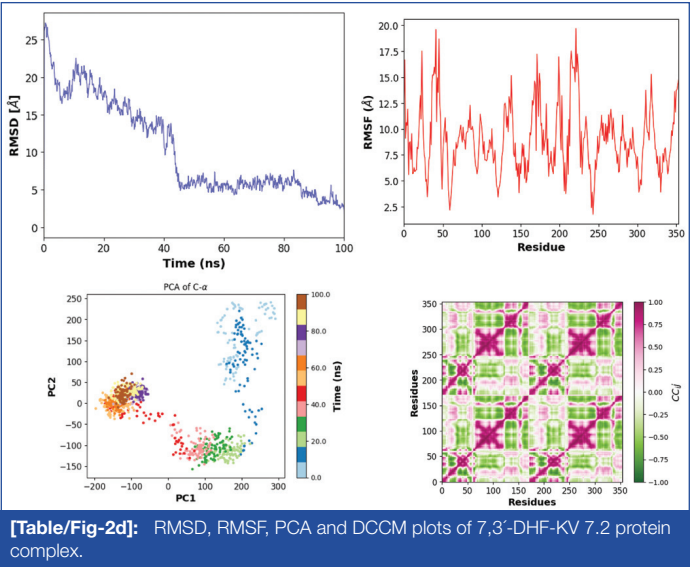
[Table/Fig-2b]: Intermolecular interactions of Nav-1.7, Cav-3.2 and Kv-7.2 with 7,3'-DHF (in Å).

Cav 3.2: MD simulation reveals that the RMSD of 7,3'-DHF-Cav 3.2 protein complex decreased below 5 Å after 50 ns indicating enhanced structural stability [Table/Fig-2c]. The RMSF values were high indicating significant structural modifications up to 100 ns [Table/Fig-2c]. The Radius of gyration (Rg) showed that the complex achieved compactness after 50 ns. The PCA plot revealed large-scale motion up to 70 ns, transitioning to more stable, slower motion thereafter, denoting high stability post-70 ns [Table/Fig-2c]. Furthermore, the Dynamic Cross-Correlation Matrix (DCCM) analysis showed neutral and pairwise correlations among residues, emphasising strong intermolecular interactions [Table/Fig-2c]. Secondary structure analysis also confirmed substantial conformational changes during the simulation, yet the molecule's active site binding remained consistent. The binding free energy analysis supported the inhibitory potential, showing low van der Waals, electrostatic and lipophilic interaction energy values. Therefore, 7,3'-DHF demonstrates strong binding and inhibitory potential for Cav 3.2.



Kv 7.2: The molecular docking and MD simulation results showed 7,3'-DHF binds effectively to the active site of Kv 7.2 protein, similar to its interaction with Cav 3.2. The RMSD stabilises around 5 Å after 50 ns, comparable to Cav 3.2, though it achieves stability earlier in the simulation [Table/Fig-2d]. The RMSF values indicate

significant structural changes for Kv 7.2, as seen with Cav 3.2, but these changes do not disrupt the molecule's binding [Table/Fig-2d]. Compactness, reflected in the radius of gyration (Rg), was achieved at a similar 50 ns point. However the DCCM analysis showed a negative correlation of residues for Kv 7.2, in contrast to the neutral and pairwise correlations seen in Cav 3.2, suggesting distinct interaction dynamics.



Flow Cytometry

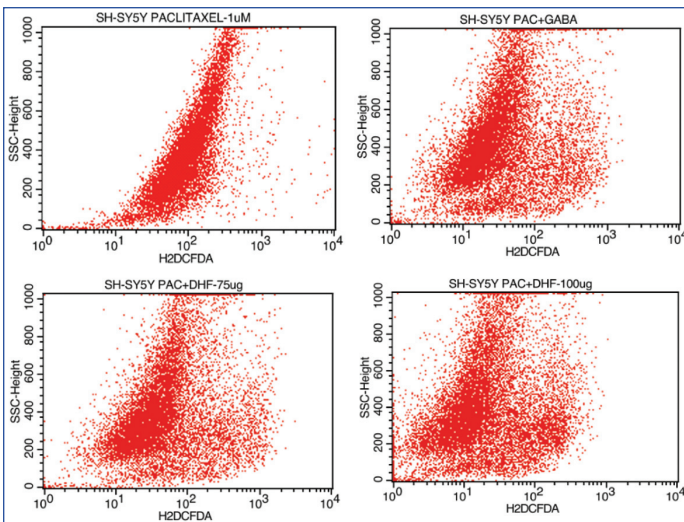
The effect of 7,3'-DHF and Paclitaxel (PT) treatments on the generation of ROS in SH-SY5Y cells were demonstrated by flow cytometry. Control group [Table/Fig-3a] shows baseline ROS levels with minimal cellular stress and granularity changes. Gabapentin treatment demonstrated reduction in ROS levels [Table/Fig-3b]. ROS levels were reduced in a dose-dependent manner by PT with 7,3'-DHF 75 µg (34.33% cells expressed DCF intensity) and 100 µg (23.69% cells expressed DCF intensity) [Table/Fig-3a]. The bar graph shows the percentage of cells expressing DCF fluorescence, indicating ROS levels [Table/Fig-3c] and treatment with 7,3'-DHF was statistically significant compared to paclitaxel group ($p < 0.01$). These results highlight the potential of 7,3'-DHF as an effective antioxidant to counteract PT-induced ROS generation, suggesting its protective role in reducing oxidative damage and cellular stress in a concentration-dependent manner.

Culture condition	% cells expressed DCF intensity
Untreated	0.05
Paclitaxel-1uM	81.14
PT+Gabapentin-1mM	24.7
PT+7,3'DHF-75ug	34.33
PT+7,3'DHF-100ug	23.69

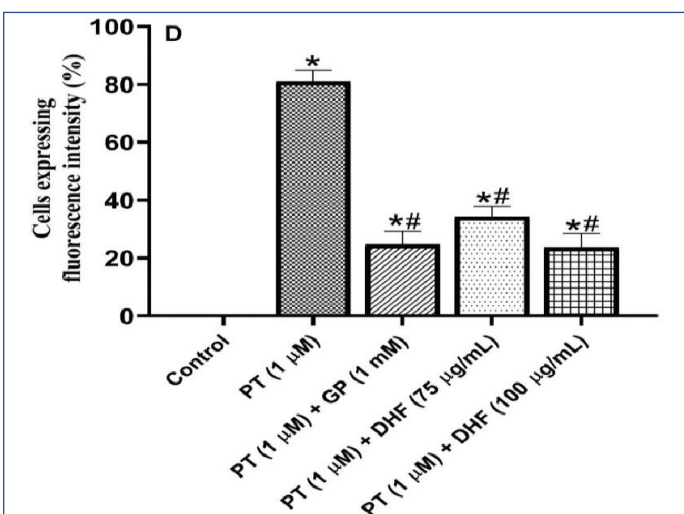
[Table/Fig-3a]: Quantification of ROS generation in paclitaxel-treated cells co-treated with positive control (Gabapentin)/7,3'-DHF assessed by flow cytometry.

Gene Expression

Quantitative analysis of gene expression across treatment conditions revealed significant changes in response to Paclitaxel and co-treatment interventions. Gene expression levels are reported as fold changes relative to the untreated control (set to 1.00 ± 0.10). Treatment with Paclitaxel (1 µM) significantly increased gene expression to 1.85 ± 0.06 compared to the untreated group ($p < 0.0001$). Co-treatment with GABA moderately reduced the expression to 1.25 ± 0.05 , which was not statistically significant when compared to the untreated control ($p = 0.0602$), but was significantly different from paclitaxel alone ($p = 0.0001$) [Table/Fig-4a,b]. Co-treatment with 7,3'-DHF at 75 µg significantly lowered gene expression to 1.59 ± 0.16 compared to paclitaxel alone ($p = 0.0494$), and remained significantly elevated



[Table/Fig-3b]: Histogram showing ROS generation using flow cytometry from various groups. The x-axis represents the fluorescence intensity of H2DCFDA, a marker for ROS and y-axis (SSC-height) indicates cell granularity or internal complexity.



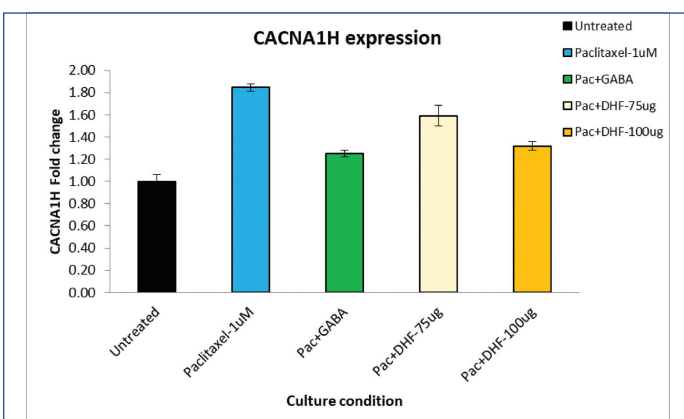
[Table/Fig-3c]: The percentage of cells expressing DCF measured using flow cytometry.

* $p < 0.05$ vs. control; # $p < 0.05$ vs. PT-exposed cells. PT: Paclitaxel; GP: Gabapentin; 7,3'-DHF: 7,3'-dihydroxyflavone

Culture condition	Fold changes
Untreated	1.00±0.10
Paclitaxel-1 μ M	1.85±0.06
PT+GABA	1.25±0.05
PT+7,3'-DHF-75 μ g	1.59±0.16
PT+7,3'-DHF-100 μ g	1.32±0.07

[Table/Fig-4a]: Relative mRNA Expression Levels of CACNA1H gene in SH-SY5Y cells under various treatment conditions.

Data are presented as mean±standard error of the mean (SEM)



[Table/Fig-4b]: mRNA levels of CACNA1H in SH-SY5Y cells from various groups.

Expression levels are normalised to untreated control (set as 1.00)

relative to the untreated control ($p=0.0002$). At 100 μ g, 7,3'-DHF further reduced expression to 1.32 ± 0.07 , showing statistical significance compared to paclitaxel ($p=0.0004$) and untreated ($p=0.0153$). When comparing co-treatments, 7,3'-DHF was significantly more effective than GABA ($p=0.0104$), while 7,3'-DHF was not significantly different from GABA ($p=0.895$). Notably, 7,3'-DHF showed a modest but significant reduction in expression compared to 7,3'-DHF ($p=0.0406$). These findings suggest that both GABA and 7,3'-DHF attenuate paclitaxel-induced gene expression, with 7,3'-DHF showing a dose-dependent effect.

DISCUSSION

Many flavones have been studied to demonstrate beneficial effect in CIPN and offer neuroprotective effect in various neurological conditions [42]. Previously the action of 7,3'-DHF on GABAA, KATP channels and adenosine receptors were studied [24]. This study has demonstrated the binding affinity of 7,3'-DHF on Cav3.2, Kv 7.2 and its neuroprotective role in Paclitaxel induced neurotoxicity using in-silico and in-vitro analysis.

A 7,3'-DHF has a substantial binding affinity and stability with Cav3.2 and Kv7.2, two important ion channels linked to neuropathic pain, according to molecular docking and dynamics studies. Its potential as a dual inhibitor of these channels is further supported by the robust intermolecular interactions and low binding free energy values by molecular dynamics.

In the previous study conducted by Vijayarajan K et al., the strong antioxidant potential of 7,3'-DHF using the MTT assay, where it significantly preserved cell viability under oxidative stress conditions [31]. Building upon those findings, the current investigation further elucidates the neuroprotective role of 7,3'-DHF by evaluating its effect on intracellular ROS generation and its ability to modulate sodium, potassium and calcium ion channels in a paclitaxel-treated SH-SY5Y neuroblastoma cell model. Paclitaxel is well-documented to induce oxidative stress by disrupting mitochondrial function, leading to excessive production of ROS, which contributes to neuronal damage and peripheral neuropathy [8,9]. Flow cytometry analysis using ROS-sensitive fluorescent probes revealed a marked increase in intracellular ROS levels following paclitaxel treatment. Interestingly, co-treatment with 7,3'-DHF led to a significant attenuation of this ROS surge, suggesting a direct antioxidant effect or an indirect role in enhancing cellular antioxidant defences.

This ROS-scavenging property of 7,3'-DHF may be attributed to its hydroxyl functional groups capable of donating hydrogen atoms to neutralise free radicals, thereby interrupting the chain reactions of oxidative damage. Structurally similar flavones are known to modulate endogenous antioxidant pathways such as Nrf2/HO-1 signalling, which warrants further mechanistic exploration of 7,3'-DHF [43]. These findings are consistent with other studies that support the neuroprotective and redox-modulating effects of flavonoids in models of chemotherapy-induced neurotoxicity [24,44] and highlights the therapeutic potential of 7,3'-DHF in mitigating such effects.

Studies have shown Paclitaxel-induced alterations in mitochondrial structure are linked to an increase in calcium-mediated neuronal excitability and medications that decrease calcium availability should counteract the negative effects [45,46]. In animal models, intrathecal administration of calcium-reducing agents such as TMB-8, Quin-2, EGTA, and EGTA-AM significantly alleviated mechanical allodynia and hyperalgesia induced by paclitaxel. These treatments did not affect pain responses in control animals, suggesting a specific effect on chemotherapy-induced neuropathic pain [47]. A recent study conducted by Son DB et al., showed that Decursin alleviated mechanical allodynia and hyperalgesia by decreased intracellular calcium levels, thereby mitigating neuronal excitability and pain in a mouse model of paclitaxel-induced neuropathic pain [48]. This

suggests agents that alter the calcium levels can be tried to alleviate CIPN. Gene expression in this study provides evidence that 7,3'-DHF supports its function in reducing calcium dysregulation linked to neurotoxicity by downregulating the expression of CACNA1H almost equal to the positive control group treated with Gabapentin. Thus, 7,3'-DHF may serve as a promising candidate for further investigation and can be considered for in vivo studies to evaluate its potential in alleviating paclitaxel-induced neuropathic pain.

Limitation(s)

In-vivo study was not done which might have helped in better understanding of the effects in complex biological system. The study hypothesises mechanisms such as antioxidant activity and ion channel modulation, but deeper exploration of signalling pathways (e.g., Nrf2, MAPK) was not carried out due to time constraint.

CONCLUSION(S)

In conclusion, paclitaxel-induced peripheral neuropathy remains a significant clinical challenge, with current treatment options offering limited efficacy and being associated with notable side-effects. Based on our study findings, 7,3'-DHF shows potential as a treatment option for CIPN. By targeting key ion channels such as Cav3.2 and Kv7.2, 7,3'-DHF demonstrates its potential to be considered for neuroprotection via in-silico and in-vitro studies. Further *in-vivo* research and clinical trials are necessary to confirm the efficacy, safety and neuroprotective potential of 7,3'-DHF in a therapeutic context. If successful, 7,3'-DHF could offer a novel and targeted approach to alleviating CIPN.

Acknowledgement

We would like to express our gratitude to the Department of Pharmacology, Sri Ramachandra Medical College and Research Institute for providing the facilities to complete this research.

Authors' contribution: All authors contributed equally to this work. The authors confirm contribution to the paper as follows: Study conception and design by GK; Data analysis and interpretation of results by KR; Draft manuscript guidance and preparation by RS. All authors reviewed the results and approved the final version of the manuscript.

REFERENCES

- [1] Tong Y, Luo YF, Gao W. Biosynthesis of paclitaxel using synthetic biology. *Phytochem Rev*. 2022;21(3):863-77.
- [2] Sharifi-Rad J, Quispe C, Patra JK, Singh YD, Panda MK, Das G, et al. Paclitaxel: Application in modern oncology and nanomedicine-based cancer therapy. *Oxid Med Cell Longev*. 2021;2021:3687700. Doi: 10.1155/2021/3687700. PMID: 34707776; PMCID: PMC8545549.
- [3] Awosika AO, Farrar MC, Jacobs TF. Paclitaxel. 2023 Nov 18. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. PMID: 30725602.
- [4] Flatters SJL, Bennett GJ. Studies of peripheral sensory nerves in paclitaxel-induced painful peripheral neuropathy: Evidence for mitochondrial dysfunction. *Pain*. 2006;122(3):245-57. Doi: 10.1016/j.pain.2006.01.037. Epub 2006 Mar 13. PMID: 16530964; PMCID: PMC1805481.
- [5] Kumazawa A, Katoh H, Nonaka D, Watanabe T, Saotome M, Urushida T, et al. Microtubule disorganization affects the mitochondrial permeability transition pore in cardiac myocytes. *Circ J*. 2014;78(5):1206-15. Doi: 10.1253/circj.cj-13-1298. Epub 2014 Mar 10. PMID: 24614510.
- [6] Kidd JF, Pilkington MF, Schell MJ, Fogarty KE, Skepper JN, Taylor CW, et al. Paclitaxel affects cytosolic calcium signals by opening the mitochondrial permeability transition pore. *J Biol Chem*. 2002;277(8):6504-10. Doi: 10.1074/jbc.M106802200. Epub 2001 Nov 27. PMID: 11724773.
- [7] Staff NP, Fehrenbacher JC, Caillaud M, Damaj MI, Segal RA, Rieger S. Pathogenesis of paclitaxel-induced peripheral neuropathy: A current review of in vitro and in vivo findings using rodent and human model systems. *Exp Neurol*. 2020;324:113121. Doi: 10.1016/j.expneurol.2019.113121. Epub 2019 Nov 21. PMID: 31758983; PMCID: PMC6993945.
- [8] Duggett NA, Griffiths LA, McKenna OE, de Santis V, Yongsanguanchai N, Mokori EB, et al. Oxidative stress in the development, maintenance and resolution of paclitaxel-induced painful neuropathy. *Neuroscience*. 2016;333:13-26. Doi: 10.1016/j.neuroscience.2016.06.050. Epub 2016 Jul 5. PMID: 27393249; PMCID: PMC4996646.
- [9] Klein I, Lehmann HC. Pathomechanisms of paclitaxel-induced peripheral neuropathy. *Toxics*. 2021;9(10):229. Doi: 10.3390/toxics9100229. PMID: 34678925; PMCID: PMC8540213.
- [10] Desforges AD, Hebert CM, Spence AL, Reid B, Dhaibar HA, Cruz-Topete D, et al. Treatment and diagnosis of chemotherapy-induced peripheral neuropathy: An update. *Biomed Pharmacother*. 2022;147:112671. Doi: 10.1016/j.biopha.2022.112671. Epub 2022 Jan 29. PMID: 35104697; PMCID: PMC11118018.
- [11] Zhang S. Chemotherapy-induced peripheral neuropathy and rehabilitation: A review. *Semin Oncol*. 2021;48(3):193-207.
- [12] Avallone A, Bimonte S, Cardone C, Cascella M, Cuomo A. Pathophysiology and therapeutic perspectives for chemotherapy-induced peripheral neuropathy. *Anticancer Res*. 2022;42(10):4667-78. Doi: 10.21873/anticancer.15971. PMID: 36191965.
- [13] da Costa R, Passos GF, Quintão NLM, Fernandes ES, Maia JRLCB, Campos MM, et al. Taxane-induced neurotoxicity: Pathophysiology and therapeutic perspectives. *Br J Pharmacol*. 2020;177(14):3127-46. Doi: 10.1111/bph.15086. Epub 2020 Jun 3. PMID: 32352155; PMCID: PMC7312267.
- [14] Zhou L, Yang H, Wang J, Liu Y, Xu Y, Xu H, et al. The Therapeutic potential of antioxidants in chemotherapy-induced peripheral neuropathy: Evidence from preclinical and clinical studies. *Neurotherapeutics*. 2023;20(2):339-58. Doi: 10.1007/s13311-023-01346-8. Epub 2023 Feb 3. PMID: 36735180; PMCID: PMC10121987.
- [15] Kottschade LA, Sloan JA, Mazurczak MA, Johnson DB, Murphy BP, Rowland KM, et al. The use of vitamin E for the prevention of chemotherapy-induced peripheral neuropathy: Results of a randomized phase III clinical trial. *Support Care Cancer*. 2011;19(11):1769-77. Doi: 10.1007/s00520-010-1018-3. Epub 2010 Oct 9. PMID: 20936417; PMCID: PMC3329941.
- [16] Leal AD, Qin R, Atherton PJ, Haluska P, Behrens RJ, Tiber CH, et al. Alliance for Clinical Trials in Oncology. North Central Cancer Treatment Group/Alliance trial N08CA-the use of glutathione for prevention of paclitaxel/carboplatin-induced peripheral neuropathy: A phase 3 randomized, double-blind, placebo-controlled study. *Cancer*. 2014;120(12):1890-97. Doi: 10.1002/cncr.28654. Epub 2014 Mar 11. PMID: 24619793; PMCID: PMC4047184.
- [17] Masoud M, Youness E, El-Laithy N, Mohamed N, Omara E, Sayed EM, et al. Amifostine silica nanoparticles characterization and effect on neuronal damage in cisplatin-treated rats. *Egypt J Chem*. 2023;0(0):0-0.
- [18] Schloss JM, Colosimo M, Airey C, Masci PP, Linnane AW, Vitetta L. Nutraceuticals and chemotherapy induced peripheral neuropathy (CIPN): A systematic review. *Clin Nutr*. 2013 Dec;32(6):888-93. Doi: 10.1016/j.clnu.2013.04.007. Epub 2013 Apr 13. Erratum in: *Clin Nutr*. 2015;34(1):167. PMID: 23647723.
- [19] Maleki SJ, Crespo JF, Cabanillas B. Anti-inflammatory effects of flavonoids. *Food Chem*. 2019;299:125124. Doi: 10.1016/j.foodchem.2019.125124. Epub 2019 Jul 3. PMID: 31288163.
- [20] Chagas MDSS, Behrens MD, Moragas-Tellis CJ, Penedo GXM, Silva AR, Gonçalves-de-Albuquerque CF. Flavonols and flavones as potential anti-inflammatory, antioxidant, and antibacterial compounds. *Oxid Med Cell Longev*. 2022;2022:9966750. Doi: 10.1155/2022/9966750. PMID: 36111166; PMCID: PMC9470311.
- [21] Chirumbolo S. The role of quercetin, flavonols and flavones in modulating inflammatory cell function. *Inflamm Allergy Drug Targets*. 2010;9(4):263-85. Doi: 10.2174/187152810793358741. PMID: 20887269.
- [22] Shoaib M, Ali Shah SW, Ali N, Shah I, Umar MN, Shafiqullah, et al. In vitro enzyme inhibition potentials and antioxidant activity of synthetic flavone derivatives. *J Chem*. 2015;2015:01-07.
- [23] Sulaiman CT, Jyothi CK, Unnithan JK, Prabhukumar KM, Balachandran I. Phytochemical comparison and evaluation of anti-inflammatory and anti-diabetic activity of three source plants of Jivanti—an important Ayurvedic drug. *Future J Pharm Sci*. 2021;7:52. Doi:10.1186/s43094-021-00201-X.
- [24] Ramasamy K, Shanmugasundaram J, Manoharan R, Subramanian V, Kathirvelu P, Vijayaraghavan R. Anti-neuropathic effect of 7,3'-dihydroxyflavone in paclitaxel induced peripheral neuropathy in mice involving GABAA, KATP channel and adenosine receptors. *Neurochem Int*. 2022;159:105388. Doi: 10.1016/j.neuint.2022.105388. Epub 2022 Jul 6. PMID: 35809719.
- [25] Li Y, Tatsui CE, Rhines LD, North RY, Harrison DS, Cassidy RM, et al. Dorsal root ganglion neurons become hyperexcitable and increase expression of voltage-gated T-type calcium channels (Cav3.2) in paclitaxel-induced peripheral neuropathy. *Pain*. 2017;158(3):417-29. Doi: 10.1097/j.pain.0000000000000774. PMID: 27902567; PMCID: PMC5303135.
- [26] Ko MH, Hu ME, Hsieh YL, Lan CT, Tseng TJ. Peptidergic intraepidermal nerve fibers in the skin contribute to the neuropathic pain in paclitaxel-induced peripheral neuropathy. *Neuropeptides*. 2014;48(3):109-17. Doi: 10.1016/j.npep.2014.02.001. Epub 2014 Mar 3. PMID: 24630273.
- [27] Chen Y, Lu J, Pan H, Zhang Y, Wu H, Xu K, et al. Association between genetic variation of CACNA1H and childhood absence epilepsy. *Ann Neurol*. 2003;54(2):239-43. Doi: 10.1002/ana.10607. PMID: 12891677.
- [28] Viggiano M, D'Andrea T, Cameli C, Posar A, Visconti P, Scaduto MC, et al. Contribution of CACNA1H variants in autism spectrum disorder susceptibility. *Front Psychiatry*. 2022;13:858238. Doi: 10.3389/fpsy.2022.858238. PMID: 35350424; PMCID: PMC8957782.
- [29] Stringer RN, Jurkovicova-Tarabova B, Huang S, Haji-Ghassemi O, Idoux R, Liaschenko A, et al. A rare CACNA1H variant associated with amyotrophic lateral sclerosis causes complete loss of Cav3.2 T-type channel activity. *Mol Brain*. 2020;13(1):33. Doi: 10.1186/s13041-020-00577-6. PMID: 32143681; PMCID: PMC7060640.
- [30] Mezzanotte JN, Grimm M, Shinde NV, Nolan T, Worthen-Chaudhari L, Williams NO et al. Updates in the Treatment of Chemotherapy-Induced Peripheral Neuropathy. *Curr Treat Options Oncol*. 2022;23(1):29-42. Doi: 10.1007/s11864-021-00926-0. Epub 2022 Feb 15. PMID: 35167004; PMCID: PMC9642075.

- [31] Vijayarajan K, Ramasamy K, Kadal KK. Neuroprotective effect of 7,3'-dihydroxyflavone against paclitaxel-induced neurotoxicity in SH-SY5Y neuroblastoma cell line: An in vitro model. *Asian J Pharm Clin Res.* 2025;18(3):130-35.
- [32] Pandey P, Kumar A, Pushpam D, Khurana S, Malik PS, Gogia A, et al. Randomized double-blind, placebo-controlled study of oral gabapentin for prevention of neuropathy in patients receiving paclitaxel. *Trials.* 2023;24(1):79. Doi: 10.1186/s13063-023-07126-1. PMID: 36732774; PMCID: PMC9896696.
- [33] Behery SA El Sayed, Toam MM, Hegazy MW, Abdelhady El, Balata R. Gabapentin efficacy in preventing peripheral neuropathy following taxane administration in breast cancer. *Zagazig Univ Med J.* 2025;31(5):2097-108. Doi: 10.21608/zumj.2025.370027.3889.
- [34] Zhi YR, Cao F, Su XJ, Gao SW, Zheng HN, Jiang JY, et al. The T-Type calcium channel cav3.2 in somatostatin interneurons in spinal dorsal horn participates in mechanosensation and mechanical allodynia in mice. *Front Cell Neurosci.* 2022;16:875726. Doi: 10.3389/fncel.2022.875726. PMID: 35465611; PMCID: PMC9024096.
- [35] Chemin J, Monteil A, Perez-Reyes E, Bourinet E, Nargeot J, Lory P. Specific contribution of human T-type calcium channel isoforms (α_1G), α_1H and α_1I) to neuronal excitability. *J Physiol.* 2002;540(Pt 1):03-14. Doi: 10.1113/jphysiol.2001.013269. PMID: 11927664; PMCID: PMC2290209.
- [36] Alam KA, Svalastoga P, Martinez A, Glennon JC, Haavik J. Potassium channels in behavioral brain disorders. Molecular mechanisms and therapeutic potential: A narrative review. *Neurosci Biobehav Rev.* 2023;152:105301. Doi: 10.1016/j.neubiorev.2023.105301. Epub 2023 Jul 4. PMID: 37414376.
- [37] Cai S, Gomez K, Moutal A, Khanna R. Targeting T-type/CaV3.2 channels for chronic pain. *Transl Res.* 2021;234:20-30. Doi: 10.1016/j.trsl.2021.01.002. Epub 2021 Jan 7. PMID: 33422652; PMCID: PMC8217081.
- [38] Eckle VS, Shcheglovitov A, Vitko I, Dey D, Yap CC, Winckler B, et al. Mechanisms by which a CACNA1H mutation in epilepsy patients increases seizure susceptibility. *J Physiol.* 2014;592(4):795-809. Doi: 10.1113/jphysiol.2013.264176. Epub 2013 Nov 25. PMID: 24277868; PMCID: PMC3934715.
- [39] Zhang Y, Chen D, Ennis AC, Polli JR, Xiao P, Zhang B, et al. Chemical dispersant potentiates crude oil impacts on growth, reproduction, and gene expression in *Caenorhabditis elegans*. *Arch Toxicol.* 2013;87(2):371-82. Doi: 10.1007/s00204-012-0936-x. Epub 2012 Sep 19. PMID: 22990136.
- [40] Choi S, Yu E, Hwang S, Park SY, Kim C, Ahn D. Targeting Cav3.2 T-type calcium channels for the treatment of neuropathic pain. *Transl Res.* 2021;230:1-16. Doi: 10.1016/j.trsl.2021.01.002.
- [41] Abd-Elsayed A, Jackson M, Gu SL, Fiala K, Gu J. Neuropathic pain and Kv7 voltage-gated potassium channels: The potential role of Kv7 activators in the treatment of neuropathic pain. *Mol Pain.* 2019;15:1744806919864256. Doi: 10.1177/1744806919864256. PMID: 31342847; PMCID: PMC6659175.
- [42] Siddiqui M, Abdellatif B, Zhai K, Liskova A, Kubatka P, Büßelberg D. Flavonoids alleviate peripheral neuropathy induced by anticancer drugs. *Cancers (Basel).* 2021;13(7):1576. Doi: 10.3390/cancers13071576. PMID: 33805565; PMCID: PMC8036789.
- [43] Suraweera LT, Rupasinghe HPV, Delleire G, Xu Z. Regulation of Nrf2/ARE pathway by dietary flavonoids: A friend or foe for cancer management? *Antioxidants (Basel).* 2020;9(10):973. Doi: 10.3390/antiox9100973. PMID: 33050575; PMCID: PMC7600646.
- [44] Ramasamy K, Shanmugasundaram J, Subramanian V, Manoharan R, Kathirvelu P, Vijayaraghavan R. 3', 4'-dihydroxyflavone ameliorates paclitaxel model of peripheral neuropathy in mice by modulating KATP channel, adenosine (A3) and GABAA ($\alpha 2$ subunit) receptors. *Bioinformation.* 2023;19(6):754-63. Doi: 10.6026/97320630019754. PMID: 37885774; PMCID: PMC10598353.
- [45] Flatters SJ, Bennett GJ. Studies of peripheral sensory nerves in paclitaxel-induced painful peripheral neuropathy: Evidence for mitochondrial dysfunction. *Toxicol.* 2012;291(1-3):77-85. Doi: 10.1016/j.tox.2011.10.019.
- [46] Choi JI, Lee BH. Targeting calcium signaling for the treatment of paclitaxel-induced peripheral neuropathy. *Cell Calcium.* 2023;112:102762. Doi: 10.1016/j.ceca.2023.102762.
- [47] Siau C, Bennett GJ. Dysregulation of cellular calcium homeostasis in chemotherapy-evoked painful peripheral neuropathy. *Anesth Analg.* 2006;102(5):1485-90. Doi: 10.1213/01.ane.0000204318.35194.ed. PMID: 16632831; PMCID: PMC1805480.
- [48] Son DB, Choi W, Kim M, Go EJ, Jeong D, Park CK, et al. Decursin alleviates mechanical allodynia in a paclitaxel-induced neuropathic pain mouse model. *Cells.* 2021;10(3):547. Doi: 10.3390/cells10030547. PMID: 33806325; PMCID: PMC8001788.

PARTICULARS OF CONTRIBUTORS:

1. Postgraduate Student, Department of Pharmacology, Sri Ramachandra Medical College and Research Institute, Chennai, Tamil Nadu, India.
2. Professor and Head, Department of Pharmacology, Sri Ramachandra Medical College and Research Institute, Chennai, Tamil Nadu, India.
3. Associate Professor, Department of Pharmacology, Sri Ramachandra Medical College and Research Institute, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Kavitha Ramasamy,
Professor and Head, Department of Pharmacology, Sri Ramachandra Medical College and Research Institute, Chennai, Tamil Nadu, India.
E-mail: r.kavitha@sriramachandra.edu.in

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: May 17, 2025
- Manual Googling: Jun 18, 2025
- iThenticate Software: Jul 05, 2025 (7%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: May 09, 2025

Date of Peer Review: Jun 02, 2025

Date of Acceptance: Jul 08, 2025

Date of Publishing: Aug 01, 2025