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NEPHROPROTECTIVE PROFICIENCY OF PHYMIN-22 AGAINST CISPLATIN-INDUCED NEPHROTOXICITY IN HUMAN EMBRYONIC KIDNEY- 293 (HEK-293) CELLS AND IN-SILICO EXPERIMENTS

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Abstract

The present study was designed to evaluate the nephroprotective effect of PHYMIN-22 in human embryonic kidney (HEK)-293 cells and in-silico experiments. Invitro studies were investigated in HEK-293 cells to assess the cytoproliferative potential of PHYMIN-22 with cisplatin (CP) as a nephrotoxic agent. Further, Molecular docking studies in silico experiment were conducted to find an understanding of the potential interplay between Hsp90 and RIP1kinase proteins and PHYMIN-22. Treatment with PHYMIN-22 alone did not cause any considerable effect on cell survival. However, the cisplatin (20 μM) treatment induces significant ($P < 0.001$) effect on the HEK-293 cell viability. PHYMI-22 treatment significantly ($P < 0.001$) increased the cell viability at concentration of 125 and 250 μg/ mL. The microscopical results depict that cisplatin treatment induce irregular morphology and cell death, PHYMIN-22 treatment revert the irregular morphology into normal. The DNA fragmentation is considered as a hallmark of cytotoxicity, Slight DNA smash/damage has observed in cisplatin (20 μM) treated cells, after the treatment of PHYMIN-22 by different concentration (62.5, 125 and 250 μg/ml) reduced the DNA fragmentation to normal. In molecular docking insilico experiment the docking score values indicates that, the PHYMIN-22 have high docking in negative with HSP90 as -7.6 *kcal/mol.* Compared with RIP1 kinase protein, it has high binding affinity. Therefore, PHYMIN-22 treatment was confirmed to harbor proliferative and anti-apoptotic effects in Hek-293 cells with lower cytotoxicity by its cytoprotective functions.

Keywords : Nephroprotective, PHYMIN-22, Cytotoxicity, Molecular docking, HEK-293.

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Introduction

Nephrotoxicity is most common kidney problems which cause loss of kidney function caused by direct exposure to environmental toxins, drugs and chemicals renowned as nephrotoxic agents (Alsalame et al., 2018). Some, anti-cancer, antibiotics and non-steroidal anti-inflammatory drugs are the most common nephrotoxins affects human (Kovacic and Jacintho, 2012). A potent antineoplastic medication and other diseases utilized in chemotherapy and other chemical based drugs for the management of disease control. Several recent studies reported that the interactions of these drugs to mitochondrial and DNA create nephrotoxicity (Sadeghi et al., 2020). The major common mechanisms of nephrotoxicity are oxidative stress, inflammatory response, and apoptosis (Sohn et al., 2009; Meng et al., 2017). The World Health Organization wished-for that early diagnosis of kidney problems may prevent, reduce or reverse the loss of renal function in acute and chronic kidney malfunction or disease by the use of cheap intervention of natural product based medicines (World Health Organization, 2018).

Currently, various approaches have been evaluated to reduce the some chemicals and drugs induced nephrotoxicity and need to produce the alternative remedy which must be effective, and non-toxic to human health. Scientifically authorized and recognized herbal-based medicines may transform the pharmaceutical approach based on traditional data. According to the Indian system of medicine, a *Physalis minimum* is a significant medicinal plant (Joseph and Ravi, 2022). It has been discovered that this plant possesses anti-inflammatory, diuretic, and laxative qualities. Additionally, the plant's potential for nephroprotective action has not been fully explored (Tienda-Vázquez et al., 2022). The plant's primary chemical components, alkaloids, phenolics, and soon, suggest that it may have potent nephroprotective properties (Pradeepkumar and Muthukrishnan, 2022). Additionally, it has been revealed that the bioactive compounds in this plant have anti-diabetic, analgesic, anti-cancerous, anti-pyretic, and anti-inflammatory activities (Tran et al., 2020; Prasathkumar et al., 2021). Accordingly, the present studwork was designed to evaluate nephroprotective and medicinal potential of *Physalis minimum* compound PHYMIN-22 against HEK-293 cells and insilico method.

Materials and methods

PHYMIN-22

We previously isolated the bioactive compound from *Physalis minima* fruits using column chromatography, purified using thin layer chromatography and the structure prediction was done with ^{1}H-NMR and ^{13}C-NMR spectroscopy and named the bioactive compound as PHYMIN-22 (Pradeepkumar and Muthukrishnan, 2022).

In vitro cell survival assay

To detect cell survival and proliferation after exposed to various concentration of cisplatin and PHYMIN-22. Approximately $1x10^5$ cells/well of HEK-293 cells were introduced in RPMI culture medium (100 µl) in 96-well plate then incubated overnight with humidified air by following the method of Ahmad et al., with minor modifications (Ahmad et al., 2017). The PHYMIN-22 stock solution (v/v) was prepared using phosphate buffer saline (PBS) and diluted in culture medium to the preferred concentrations (PHY 25, 50, 100, 200, 300 and 400 µg/ mL ; control, drug control PHY 250 µg/ml, CP 20 µM, PHY 62.5, 125 and 250 µg/ml), then added to the wells. After incubation period (24 h), 10 µl of MTT (5 mg/ml in PBS) reagent was added and then incubated until purple formazan crystals developed at 37 °C. Then the crystals were dissolved in 100 µl of dimethyl sulfoxide

DMSO and read at 540 nm using ELISA reader. Cell without treatment was considered as negative control and cells without bacterial infection and treatment was considered (untreated) as control. The plot of percentage of cell survival versus PHYMIN-22 concentrations was used to analyze the lethal concentration of the cells. The Hek-293 cellular morphological modifications were observed under microscope.

DNA damage/fragmentation analysis by agarose gel method

Cells were cultured in a 96 well plates ($1x10^5$ cells/well of HEK-293 cells) and allowed for 24 h adherence. Cells were exposed with various concentrations (control, drug control PHY 250 µg/ml, CP 20 µM, PHY 62.5, 125 and 250 µg/ml) for 48 h. DNA was extracted from cells using an readymade DNA extraction kit, and evaluation of damaged or fragmented DNA was separated by agarose gel electrophoresis method using a agarose gel (1.5 %) in tris-borate-EDTA TBE buffer at constant 100 mA for 120 min. (Pandurangan et al., 2016).

Dual staining (AO/EB) of HEK-293 cells

The morphological changes as well as viable and dead cells in the HEK-293 cells, which exposed to different dosages (control, drug control PHY 250 µg/ml, CP 20 µM, PHY 62.5, 125 and 250 µg/ml) were assessed through AO/EB fluorescence staining by following the modified methodology of Fan et al., (Fan et al., 2017). Briefly, Hek-293 cells (1×105 cells per well) were seeded in a 6-well plate and incubated for 24 hrs. After incubation cells were under the treated with various concentrations of PHYMIN-22 with positive control and incubated for 24 h. Then the cells were rinsed twice in PBS and stained by 1 mL cold PBS containing 40 µL mixture of 1 mg mL–1 acridine orange (AO) and 1 mg mL–1 ethidium bromide (EB) and then kept for 10 min under dark condition. After staining cells morphology were observed using a fluorescence microscope.

Molecular docking study

Chem Draw software was used to drawn the structures of ligand. The three-dimensional crystal structure of hsp90 (ID:2K5B) (Sreeramulu et al., 2009) and RIP1 kinase (ID:4NEU) (Harris et al., 2013) were retrieved from protein data bank. Docking study was carried out by Auto dock vina version 1.1.2 (Trott and Olson, 2010). The PyMol software was used to analyze the intermolecular interactions between the ligand and protein (The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC).

Results and Discussion

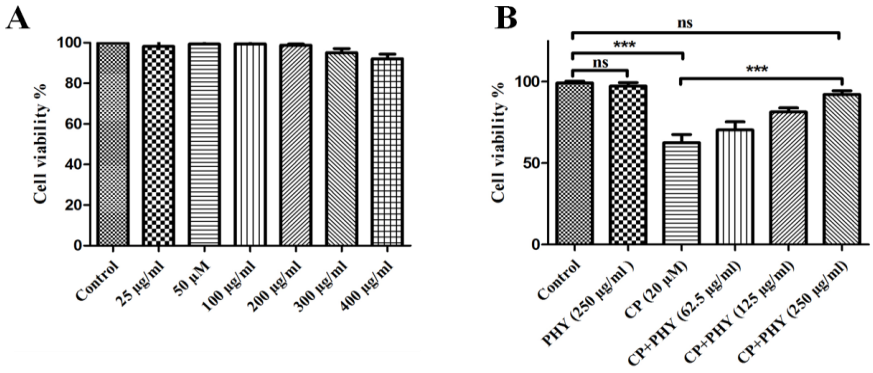

Fig. 1. Cytoprotective effect of PHYMIN-22 in cisplatin provoked toxicity in HEK-293 cells.

[A] HEK-293 cells were treated with PHYMIN-22 (25–400 µg/mL) alone for 24 h, and subjected to MTT experiment to detect its cytotoxicity. [B] HEK-293 cells were treated with CP (20 µM) and PHYMIN-22 (62.5–250 µg/mL) for 24 h and evaluated for cell survival by MTT assay. CP- cisplatin, PHY- PHYMIN-22. The values are expressed as Mean ± SD. For statistical analysis used One-way ANOVA followed by Tukey's Multiple Comparison Test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Effect of PHYMIN-22 in cisplatin induced cytotoxicity in HEK-293 cells

Cell viability was evaluated to study the cytoprotective assessment of PHYMIN-22 alone and against cisplatin induced toxicity in HEK-293 cells. HEK-293 cells were treated with different concentrations of PHYMIN-22 (25, 50, 100, 200, 300 and 400 µg/ mL) alone and cisplatin (20 µM) plus PHYMIN-22 (62.5, 125 and 250 µg/ mL) for 24 h. Treatment with PHYMIN-22 alone did not induce any considerable effect on cell viability (Fig. 1A). However, the cisplatin treatment significantly ($P < 0.001$) affected the cell viability at concentration of 20 µM. PHYMI-22 treatment significantly ($P < 0.001$) increased the cell viability at concentration of 125 and 250 µg/ mL (Fig. 1B). The micrograpical results depict that cells of untreated control group and drug control (PHYMIN-22 250 µg/ mL) were healthy, with normal morphology. However, cisplatin treated cells showed irregular morphology, floated cells in culture medium, and reduction of cell density with number of dead cells. PHYMIN-22 treated modified the irregular morphology into normal (Fig. 2).

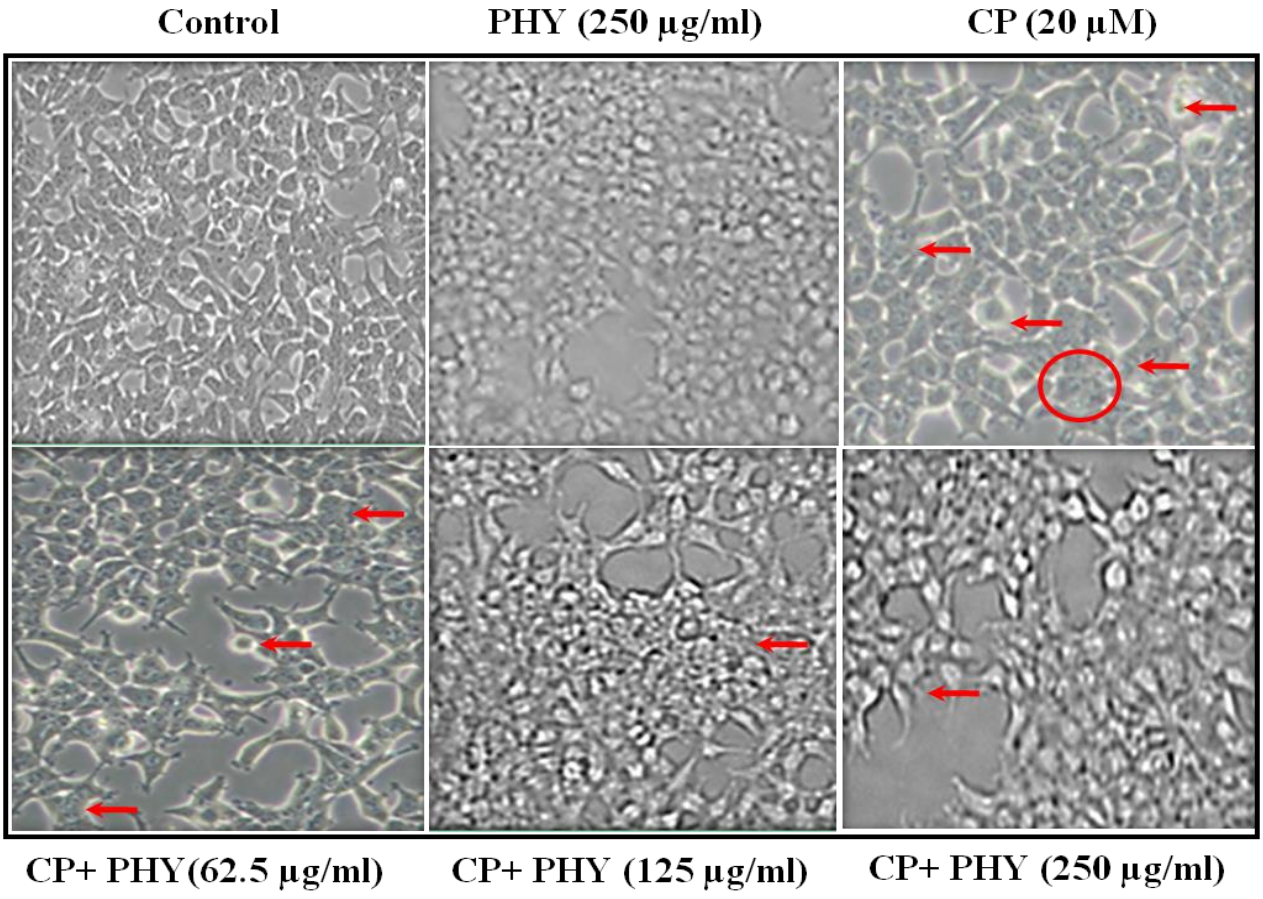

Fig. 2. Effect of PHYMIN-22 against cisplatin induced cellular morphological modifications in HEK 293 cells.

Microscopic images of HEK-293 cells, morphological modifications after treatment with CP (20 μM) and PHYMIN-22 (62.5–250 μg/mL) for 24 h. CP-cisplatin, PHY- PHYMIN-22.

Dual staining (AO/EB) of HEK-293 cells

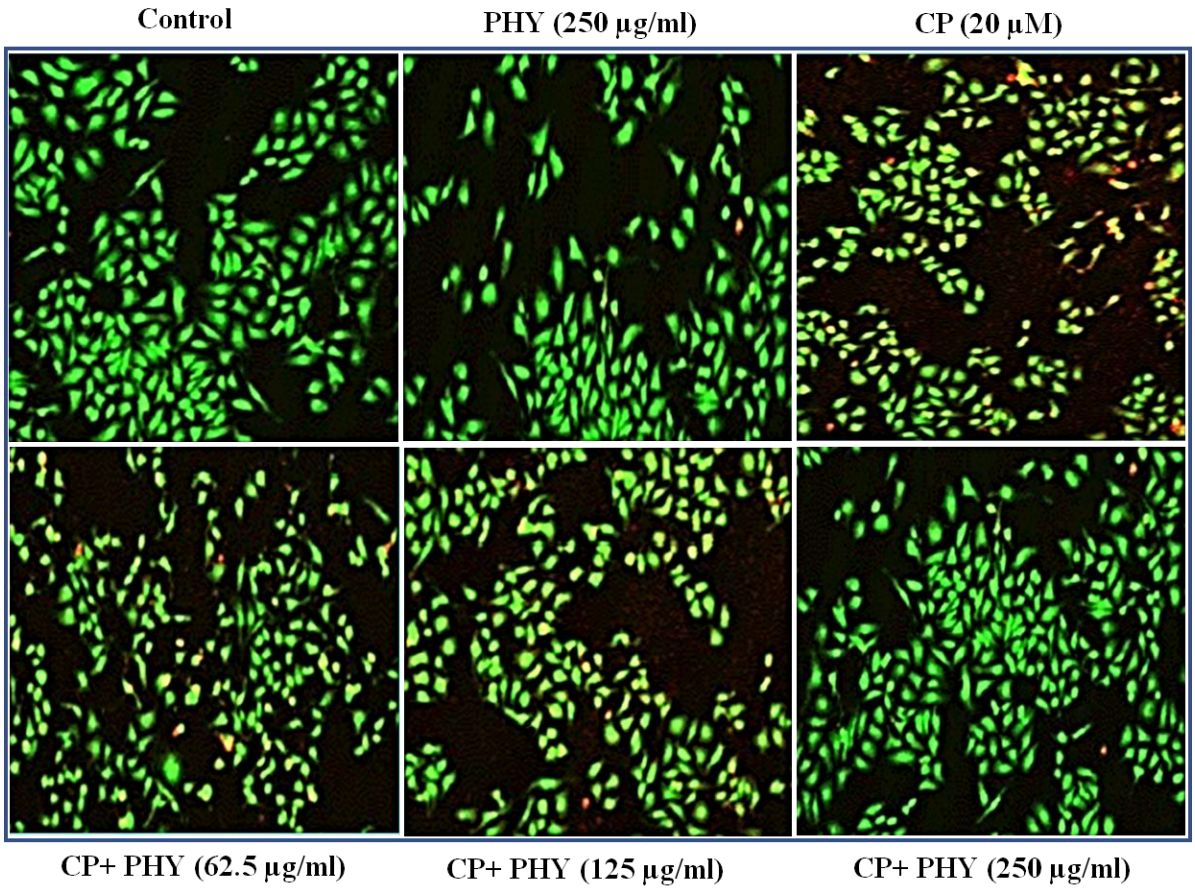

Fig. 3. Fluorescence microscopic observation of PHYMIN-22 with different concentrations on cisplatin treated HEK-293 cells. The green color indicates the live cells, orange color indicates apoptotic cells and red color necrotic cells respectively. CP- cisplatin, PHY- PHYMIN-22 .

Effects of PHYMIN-22 on Hek-293 Cell Morphology and Apoptosis

Dual staining results showed that the control cells appear green absence of orange and red stains, which denotes the healthy and live cells. However, exposures to cisplatin induce damage and death of kidney cells hence the cells showed orange and red nuclei in cell population. The number of orange-red stained nuclei was reduced by the treatment of PHYMIN-22 to increasing concentration (Fig. 3).

DNA Fragmentation

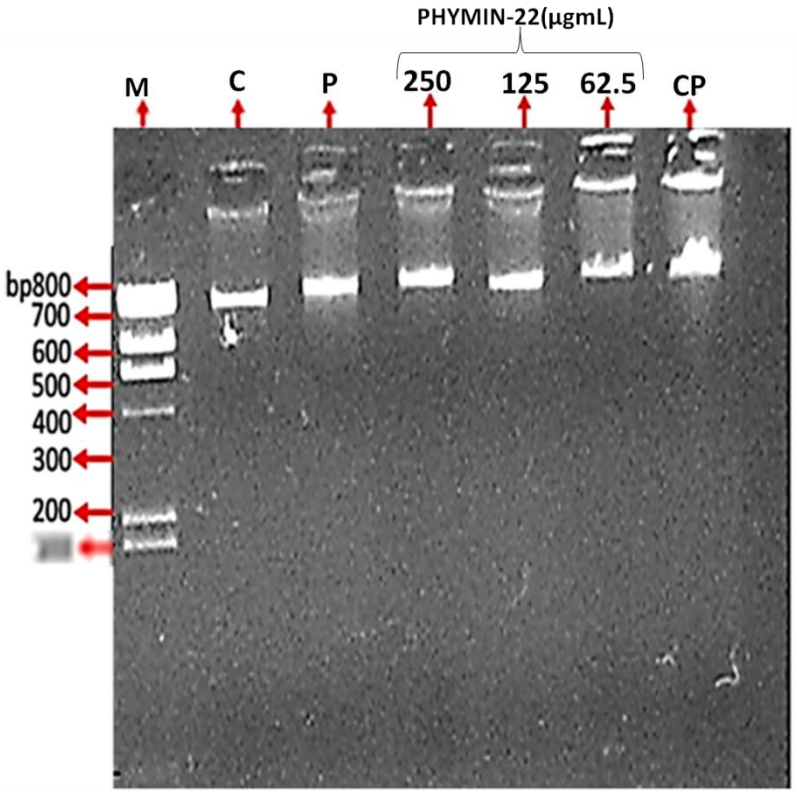

Figure 4. Effect of PHYMIN-22 on cisplatin induced DNA fragmentation in HEK-293 cells on agarose gel electrophoresis. M-Marker, C-Control, P-PHYMIN-22 (250 µg/ml), CP-Cisplatin

Slight DNA smash/damage has observed on cisplatin (20 µM) treated cells, after the treatment of PHYMIN-22 by different concentration (62.5, 125 and 250 µg/ml) reduced the DNA fragmentation to normal for 48 h. There was no considerable DNA fragmentation has noticed on drug control group (PHYMIN-22 (250 µg/ml)) compared to the normal control HEK-293 cells. The DNA was extracted from Hek-293 cells treated by cisplatin and PHYMIN-22 as described above and separated by agarose gel electrophoresis method and photographed by gel docking instrument. The DNA fragmentation is précised as a characteristic of cytotoxicity, and endonuclease cleaves DNA into many fragments, which appear as a DNA ladder. Multiple oligomers and DNA smear did not occur in the control cells, however a damaged DNA bands were appeared only in cisplatin treatment (Fig. 4).

Molecular docking results

The docked complexes of PHYMIN-22 with HSP90 and RIP1 proteins were obtained from the molecular docking studies. Based on the docking score values and intermolecular interactions, each complex were chosen from the different conformers. The docking score values of PHYMIN-22 with HSP90 and RIP1 were calculated as -7.6 and -6.6 *kcal/mol* respectively.

Table.1 Docking score values PHYMIN-22 with proteins.

Target Protein	**Dock Score *(kcal/mol)***
HSP90	-7.6
RIP1	-6.6

Intermolecular interaction analysis

PHYMIN-22 -HSP90

In PHYMIN-22-HSP90 complex, PHYMIN-22 forms the hydrogen bonding interactions with the Asn51, Asp54, His210, Glu47, Gly132, Gln133, Ser50 and Ser53 with the distances 2.2, 2.5, 2.8, 2.1, 2.3, 3.4, 2.6 and 2.7 Å respectively. There is no other type of interactions are found by the molecular docking analysis as found in the PHYMIN-22-HSP90 complex.

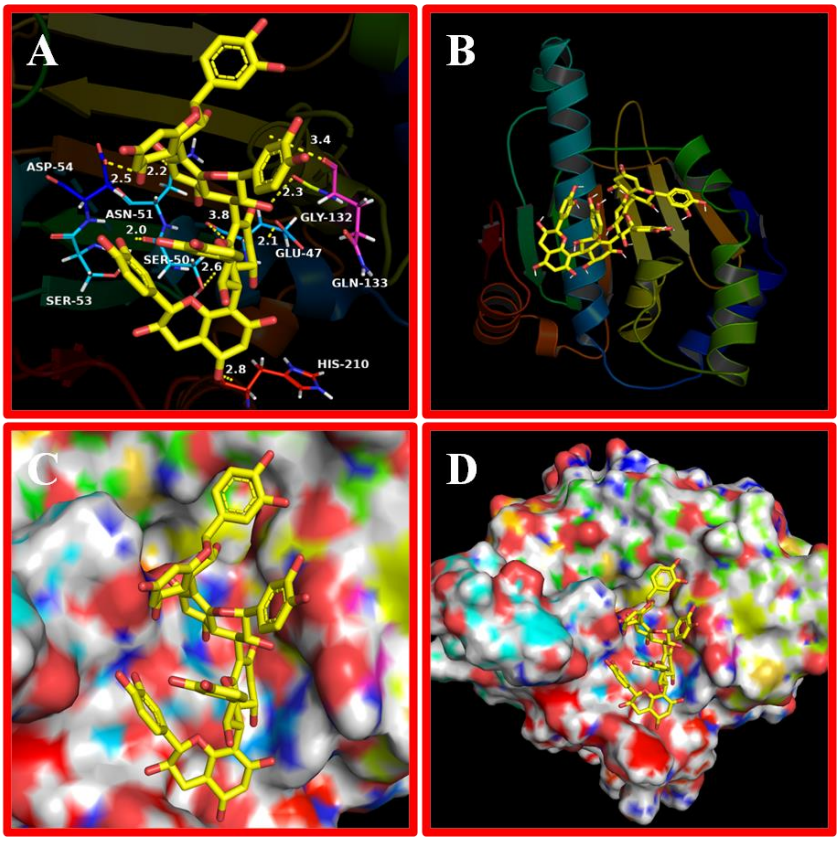

Fig. Intermolecular interaction betweenPHYMIN-22 andHSP90 kinase obtained from the molecular docking(A) interactions with active site residues, (B) Cartoon view, (C) Surface view and (D) Cavity view.

Table 3 Intermolecular interactions distance between PHYMIN-22 with active site residues of HSP90.

PHYMIN-22 ⋯ HSP90	**Distance (Å)**
	Docking
Hydrogen bonding interactions	
OD1⋯Asn51	2.2
OD1⋯Asp54	2.5
O⋯His210	2.8
OE1⋯Glu47	2.1
O⋯Gly132	2.3
O⋯Gln133	3.4
OG⋯Ser50	2.6
HG⋯Ser53	2.7

PHYMIN-22 -RIP1

In PHYMIN-22 -RIP1 complex, PHYMIN-22 forms the hydrogen bonding interactions with the RIP1 kinase active site residues Asn99, Asp24, Glu226, Glu142, Met101, His101 and Glu22 with the distances 3.1, 2.3, 2.9, 2.4, 3.1, 2.9 and 3.7 Å respectively. Hydrophobic interactions have been formed with the residues His102 and Ala21 with the distances 2.8 and 4.4 Å respectively.

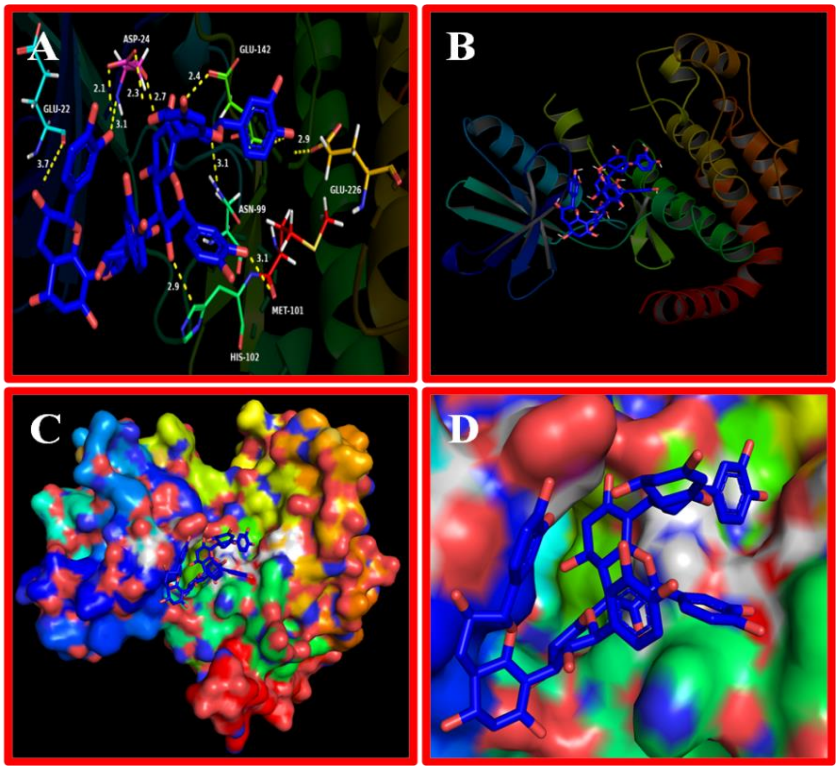

Fig. 6 Intermolecular interaction between PHYMIN-22 and RIP1 kinase obtained from the molecular docking(A) interactions with active site residues, (B) Cartoon view, (C) Surface view and (D) Cavity view.

Table 4 Intermolecular interactions distance between PHYMIN-22 with active site residues of RIP1 kinase.

PHYMIN-22 ⋯RIP1 kinase	**Distance (Å)**
	Docking
Hydrogen bonding interactions	
ND2⋯Asn99	3.1
OD2⋯Asp24	2.3
OE2⋯Glu226	2.9
OE1⋯Glu142	2.4
O⋯Met101	3.1
CD2⋯His101	2.9
O⋯Glu22	3.7
Hydrophobic interactions	
Lig⋯His102 (Alkyl⋯Pi-Orbitals)	2.8
Lig⋯ Ala21 (Alkyl⋯Alkyl)	4.4

Overall molecular docking results have been analyzed to understand the binding affinity of the PHYMIN-22 molecule with the active site region of HSP90 and RIP1 kinase. The docking score values indicates that, the PHYMIN-22 have high docking in negative with HSP90 as -7.6 *kcal/mol.* Compared with RIP1 kinase protein, it has high binding affinity. From the intermolecular interaction analysis, PHYMIN-22 forms a greater number of interactions with the active site residues of HSP90 while the other protein have less, which reflects the binding score values. Among these results, the high binding affinity of the PHYMIN-22 molecule while its bound in the active site of HSP90 has been confirmed.

Discussion

Cisplatin is a platinum based chemotherapy and used for the various cancer treatment option (Singh et al, 2018). However, usage of cisplatin has been limited by severe dose-dependent toxicity or side effects (Trendowski et al., 2019). Its effects is generally related with nephrotoxicity, provoked by formation of stress, inflammation and apoptosis through formation of reactive oxygen species and etc (Eslamifar et al., 2021). Accordingly, the approaches to target different pathophysiological processes include repression of oxidative stress, inflammation, and kidney cytoprotection to avert cisplatin-induced toxicity (Domitrović et al., 2014).

Currently the researchers are come back to plant based medicine because chemodrugs are more toxic to normal cells which kill the tumor cells and also the normal cells. Present study was performed to study the protective efficacy of PHYMIN-22 against cisplatin induced toxicity in HEK-293 cells. Notably, the study revealed that PHYMIN-22 treatment did not affect any cytotoxic on normal HEK-293 cells. However, cells treated with cisplatin showed increased apoptosis or necroptosis mediated programmed cell death along with modified cellular morphology. Co-treatment of cells with cisplatin and PHYMIN-22 with increased concentration (62.5, 125 and 250 μg/mL) showed significant increase in cell growth as

compared with the cisplatin alone treated toxic group highlighting the cytoprotective property of PHYMIN-22. Recent studies used ascorbic acid and α-ketoanalogue for its nephroprotective capacity by variation of oxidative, inflammatory, and apoptotic stress (Abdel-Daim et al., 2019; Chewcharat et al., 2020).

Our docking score values indicate that the PHYMIN-22 has high docking in negative with HSP90 as -7.6 kcal/mol. Compared with RIP1 kinase protein, it has a high binding affinity in silico study. In vitro study, the PHYMIN-22 treatment confers protection against cisplatin-induced human epithelial kidney cell damage that may be caused by inhibition of apoptosis or necroptosis a programmed cell death. Necroptosis is also a programmed cell death caused by plasma membrane damage that enables cytoplasm and cellular components to permeate, it leads to a progression of inflammation (Gao et al., 2016). Necroptosis is a process that mainly relies on a RIPK1protein and is altered by RIPK3 and its substrate, mixed lineage kinase domain-like protein (MLKL) (Dannappel et al., 2014; Mulay et al., 2019). The Hsp90 complex, in healthy cells, normally functions with its wide range of client proteins. Approximately 175 Hsp90 client proteins are identified they involve in biological functions, include cell cycle process, apoptosis, signal transduction in cell proliferation, and tumorogenesis (Gao et al., 2016). Cdc37 is an Hsp90 cochaperone and its a kinase-specific and disruption of connection between these two proteins straightforwardly suppressing Cdc37 that may leads to make some lockage between Hsp90 kinase and its clients, which improve it a promising target for drug development (Müller and Klempnauer, 2021). In a recent study, the authors concluded that C-316-1 a drug that protects the renal damage induced by cisplatin/ischemia–reperfusion in vitro by restrained programmed cell death (Liu et al., 2022). Our invitro cytoprotective effect of PHYMIN-22 on cisplatin induced HEK-293 cells may also result from inhibition of HSP90 and its client RIP1Kinase.

Conclusion

Our findings confirmed that PHYMIN-22 applied its nephroprotective effect against cisplatin-induced toxicity in vitro models established in HEK-293 cells. We propose that PHYMIN-22 can be considered as a safe nephroprotective agent during cisplatin based chemotherapy treatment. These observed positive actions can be recognized to sustained cellular integrity, opposing nephrotoxicity and motivating proliferation in Hek-293 cells. A molecular docking study proved that the molecular interaction with PHYMIN-22 may improve the nephroprotective efficacy. **Acknowledgements**

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References

Alsalame, H.A.A.A., Al-Aameli, M.H., Al-Taee, R.A.M., Al-Bazii, W.G.M., 2018. Protective role of alchoholic extract of fennel seed in nephrotoxicity induced by cisplatin in male rabbits. Biochem. Cell. Arch. 18 (1–6).

World Health Organization, 2018. Bulletin of world health organisation 96, 369-440.

Kovacic, P., Jacintho, J., 2012. Mechanisms of carcinogenesis focus on oxidative stress and electron transfer. Curr. Med. Chem. 8, 773–796. https://doi.org/ 10.2174/0929867013373084.

Sadeghi, H., Mansourian, M., Panahi kokhdan, E., Salehpour, Z., Sadati, I., Abbaszadeh-Goudarzi, K., Asfaram, A., Doustimotlagh, A.H., 2020. Antioxidant and protective effect of Stachys pilifera Benth against

nephrotoxicity induced by cisplatin in rats. J. Food Biochem. 44. https://doi.org/10.1111/jfbc.13190.

Sohn, S.H., Lee, H., Nam, J. young, Kim, S.H., Jung, H.J., Kim, Y., Shin, M., Hong, M., Bae, H., 2009. Screening of herbal medicines for the recovery of cisplatininduced nephrotoxicity. Environ. Toxicol. Pharmacol. 28,206-212. https://doi. org/10.1016/j.etap.2009.04.005.

Meng, H., Fu, G., Shen, J., Shen, K., Xu, Z., Wang, Y., Jin, B., Pan, H., 2017. Ameliorative effect of daidzein on cisplatin-induced nephrotoxicity in mice via modulation of inflammation, oxidative stress, and cell death. Oxid. Med. Cell. Longev. 2017, 1– 10. https://doi.org/10.1155/2017/3140680.

Joseph L, Ravi C. Physalis minima L (2022) Fruit-A Promising Approach To Alzheimer's Disease. Pharmacological Research-Modern Chinese Medicine 2:100038. https://doi.org/10.1016/j.prmcm.2021.100038

Tienda-Vázquez MA, Morreeuw ZP, Sosa-Hernández JE, Cardador-Martínez A, Sabath E, Melchor-Martínez EM, Iqbal HMN, Parra-Saldívar R (2022) Nephroprotective Plants: A Review on the Use in Pre-Renal and Post-Renal Diseases. Plants (Basel) 11:818. https://doi.org/10.3390/plants11060818

Pradeepkumar S, Muthukrishnan S (2022) Structural Prediction of Bioactive Compound Elucidated Through 1H and 13C NMR Based Metabolomics from Physalis Minima. J Adv Mater Sci Eng; 2:1-8.

Tran N, Pham B, Le L (2020) Bioactive Compounds in Anti-Diabetic Plants: From Herbal Medicine to Modern Drug Discovery. Biology (Basel) 9:252. https://doi.org/10.3390/biology9090252

Prasathkumar M, Anisha S, Dhrisya C, Becky R, Sadhasivam S (2021) Therapeutic and pharmacological efficacy of selective Indian medicinal plants–a review. Phytomed Plus 1:100029. https://doi.org/10.1016/j.phyplu.2021.100029

Ahmad, M., Akhtar, J., Hussain, A., Arshad, M., Mishra, A., 2017. Development of a new rutin nanoemulsion and its application on prostate carcinoma PC3 cell line. EXCLI journal 16, 810.

Fan, G., Liang, X., He, Y., Ren, H., Zhao, J., Liang, T., Wei, J., Wang, T., Zhang, F., 2017. Brucine sensitizes HepG2 human liver cancer cells to 5-fluorouracil via Fas/FasL apoptotic pathway. INTERNATIONAL JOURNAL OF PHARMACOLOGY 13, 323-331.

Pandurangan, M., Enkhtaivan, G., Young, J.A., Hoon, H.J., Lee, H., Lee, S., Kim, D.H., 2016. In vitro therapeutic potential of Tio2 nanoparticles against human cervical carcinoma cells. Biological trace element research 171, 293-300.

Trott O., Olson A.J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry. 2010;31:455-61.

Wang Z.-m., Ho J.X., Ruble J.R., Rose J., Rüker F., Ellenburg M., et al. Structural studies of several clinically important oncology drugs in complex with human serum albumin. Biochimica et Biophysica Acta (BBA)-General Subjects. 2013;1830:5356-74.

Sreeramulu S., Jonker H.R., Langer T., Richter C., Lancaster C.R.D., Schwalbe H. The human Cdc37· Hsp90 complex studied by

heteronuclear NMR spectroscopy. Journal of biological chemistry. 2009;284:3885-96.

Harris P.A., Bandyopadhyay D., Berger S.B., Campobasso N., Capriotti C.A., Cox J.A., et al. Discovery of small molecule RIP1 kinase inhibitors for the treatment of pathologies associated with necroptosis. ACS medicinal chemistry letters. 2013;4:1238-43.

Singh, M.P., Chauhan, A.K. and Kang, S.C., 2018. Morin hydrate ameliorates cisplatin-induced ER stress, inflammation and autophagy in HEK-293 cells and mice kidney via PARP-1 regulation. *International immunopharmacology*, *56*, pp.156-167.

Trendowski, M.R., El Charif, O., Dinh Jr, P.C., Travis, L.B. and Dolan, M.E., 2019. Genetic and modifiable risk factors contributing to cisplatin-induced toxicities. *Clinical Cancer Research*, *25*(4), pp.1147-1155.

Eslamifar, Z., Moridnia, A., Sabbagh, S., Ghaffaripour, R., Jafaripour, L. and Behzadifard, M., 2021. Ameliorative effects of gallic acid on cisplatin-induced nephrotoxicity in rat variations of biochemistry, histopathology, and gene expression. *BioMed Research International*, *2021*.

Gao, L., Wu, W.F., Dong, L., Ren, G.L., Li, H.D., Yang, Q., Li, X.F., Xu, T., Li, Z., Wu, B.M. and Ma, T.T., 2016. Protocatechuic aldehyde attenuates cisplatin-induced acute kidney injury by suppressing nox-mediated oxidative stress and renal inflammation. *Frontiers in Pharmacology*, *7*, p.479.

Müller, J.P. and Klempnauer, K.H., 2021. The CDC37-HSP90 chaperone complex co-translationally degrades the nascent kinase-dead mutant of HIPK2. *FEBS letters*, *595*(11), pp.1559-1568.

Liu, X.Q., Liu, M.M., Jiang, L., Gao, L., Zhang, Y., Huang, Y.B., Wang, X., Zhu, W., Zeng, H.X., Meng, X.M. and Wu, Y.G., 2022. A novel small molecule Hsp90 inhibitor, C-316-1, attenuates acute kidney injury by suppressing RIPK1-mediated inflammation and necroptosis. *International Immunopharmacology*, *108*, p.108849.

Gao, L., Wu, W.F., Dong, L., Ren, G.L., Li, H.D., Yang, Q., Li, X.F., Xu, T., Li, Z., Wu, B.M. and Ma, T.T., 2016. Protocatechuic aldehyde attenuates cisplatin-induced acute kidney injury by suppressing nox-mediated oxidative stress and renal inflammation. *Frontiers in Pharmacology*, *7*, p.479.

S.R. Mulay, M.M. Honarpisheh, O. Foresto-Neto, C. Shi, J. Desai, Z.B. Zhao, J. A. Marschner, B. Popper, E.M. Buhl, P. Boor, A. Linkermann, H. Liapis, R. Bilyy, M. Herrmann, P. Romagnani, I. Belevich, E. Jokitalo, J.U. Becker, H.J. Anders, Mitochondria Permeability Transition versus Necroptosis in Oxalate-Induced AKI, J. Am. Soc. Nephrol. 30 (10) (2019) 1857–1869.

M. Dannappel, K. Vlantis, S. Kumari, A. Polykratis, C. Kim, L. Wachsmuth, C. Eftychi, J. Lin, T. Corona, N. Hermance, M. Zelic, P. Kirsch, M. Basic, A. Bleich, M. Kelliher, M. Pasparakis, RIPK1 maintains epithelial homeostasis by inhibiting apoptosis and necroptosis, Nature 513 (7516) (2014) 90–94

R. Domitrović, I. Potočnjak, Ž. Crnčević-Orlić, M. Škoda, Nephroprotective activities of rosmarinic acid against cisplatin-induced kidney injury in

mice, Food Chem. Toxicol. 66 (2014) 321–328.

Abdel-Daim MM, Abushouk AI, Donia T, Alarifi S, Alkahtani S, Aleya L, *et al.* The nephroprotective effects of allicin and ascorbic acid against cisplatin-induced toxicity in rats. Environ Sci Pollut Res Int 2019; 26: 13502-13509.

Chewcharat A, Takkavatakarn K, Wongrattanagorn S, Panrong K, Kittiskulnam P, Eiam-Ong S, *et al.* The effects of restricted protein diet supplemented with ketoanalogue on renal function, blood pressure, nutritional status, and chronic kidney diseasemineral and bone disorder in chronic kidney disease patients: A systematic review and meta-analysis. J Ren Nutr 2020; 30: 189-199