ABSTRACT

Given their minimal adverse effects and diversity, phytopharmaceuticals have flourished as intriguing agents for the management of different diseases. Chrysin (CHR) is a natural flavonoid that has been shown to have considerable biological activities such as anticancer, anti-inflammatory, anti-asthma and antioxidant properties. Nonetheless, its poor solubility in water reduces its bioavailability and, as a result, disturbs the biomedical advantages.

Hence, we formulated different polymeric and inorganic nanoformulations of CHR to overcome its limitations and enhance its biomedical properties by improving its pharmacokinetics. In one study, we formulated CHR loaded PLGA nanoparticles (CHR-NP) and examined their anti-asthmatic properties in an in vivo mouse model. The study goal was to develop CHR-loaded PLGA (CHR-NP) to increase its potency and examine its effect on OVA-induced allergy asthma in a mouse model while investigating potential mechanisms of action. The various physicochemical characteristics of CHR-NP were studied, and in vitro release test was carried out. The mean size of the particles were 99.034±9.494. The encapsulation efficacy and drug loading of the prepared nanoparticles were measured to be 91.45 ±1.4 and 8.37±0.12 respectively. The ZP value was negative, and it measured -13.1±2.9 mV at pH7.4 and -9.33±0.5 mv and -6.10±0.7 at pH 6.8 and pH 2 respectively. Microscopic characterization of CHR-NP done by AFM and HRTEM showed the particles to be spherical in size range of 65-90 nm. DSC and XRD studies revealed inclusion of chrysin into the polymer matrix, FTIR studies also showed no free chrysin is present on the surface of the CHR-NP. In vitro release study revealed that CHR had initial burst release till 8 hrs followed by slow sustained release till 120 hrs releasing 63.5% at pH 7.4. CHR-NP uptake by A549 cells was observed over a period of 4 hours which showed cellular uptake of the nanoparticles. Our study here also aimed to investigate the therapeutic impact of CHR-NPs as well as the molecular

mechanism implicated in the mouse model of ova-induced asthma. The efficacy of CHR-NP was determined using various biochemical tests such as differential cell counts in BAL fluids using Giemsa staining, cytokines (IL-4, IL-5, IL-13), and serum immunoglobulin (Ig E) level detection using ELISA, and protein expression levels of TLR, NF-κB, NLRP3.CHR-NPs inhibited OVA-induced pulmonary histopathological changes, inflammatory cell influx, Th2-cytokine IL (-4, -5, and -13) BALF levels, serum (Ig E), and pro-inflammatory cytokines (TNF-, IL-1, IL-6, IL-18) in both serum and lung tissue more poignantly than free chrysin (50mg / kg body weight. TLR-2/4 detects allergens and translocates cytosolic NF-κB to the nucleus as a result. CHR-NP therapy dramatically reduced IκB degradation and nuclear translocation of the NF-κB p65 subunit. It also reduces the expression of TLR-2/4. The activation of NF-κB regulates the generation of pro-inflammatory cytokines. Thus, IL-6 secretion is regulated by deactivating the NF-κB pathway. The effect of CHR-NP on OVA-induced allergic inflammation of the airway is most likely mediated in part through regulating the NF-κB pathway. In addition to NF-κB signalling, an important function for inflammasome-mediated cytokine maturation of NLRP3 has been proposed in the pathogenesis of allergic airway illness. CHR-NPs similarly reduce OVA-induced NLRP3 activation, as evidenced by our findings. Hence , our findings showed that CHR-NP might be a viable medication for reducing OVA-induced allergic asthma via the TLR, NF-κB, and NLRP3 pathways, potentially making it an effectual treatment option .

In another study, CHR functionalized gold nanoparticles (CHR-AuNP) were formulated and given as a co-therapy along with paclitaxel (PTX) in treating non small cell lung cancer (NSCLC).

Paclitaxel (PTX) is an expedient chemotherapeutic drug that is widely utilised in the clinical therapeutic treatment of non small cell lung cancer NSCLC. PTX, on the other hand, has adverse reactions such as vomiting, diarrhoea, and myelosuppression. In recent years, therapeutic combination has shown to be an useful technique for reducing cell toxicity and improving therapeutic efficacy. Earlier, various studies investigated thoroughly and asserted the anticancer benefits of CHR on diverse kinds of cancer. More enticingly, it has been observed that, in addition to its cytotoxicity, CHR has a synergistic impact on tumour cell growth with several standard chemotherapeutic agents.

Numerous reports are also present of successfully combining different phytochemicals along with PTX to have synergistic anticancer effect. Synergistic interventions have been shown to be the most effective way to prevent malignant cell growth. To mitigate systemic toxicity, it is very beneficial to employ the minimal dose of a potent cytotoxic agent like paclitaxel (PTX) in ng/ml, in combination with a natural and nonhazardous anticancer agent, (CHR). CHR and PTX on the other contrary, have low oral bioavailability and solubility. Hence, in lung cancer treatment, combining (CHR) with PTX can be a fruitful strategy. Nanotechnology is used nowadays for delivering a hydrophobic drug like CHR to impacted regions. CHR nanoparticles have exhibited superior curative benefit than crude Chrysin. In this work, a generic strategy is adopted for formulating chrysin stabilised gold nanoparticle for drug delivery to cancer cells. Gold nanoparticles (AuNPs) are regarded to be efficient drug and anticancer carriers with several biochemical and therapeutic uses. AuNPs have a strong affinity for tumour cells, which is approximately 600 times larger than the affinity for adjacent nontumor cells . Another upside of AuNPs is their extremely rapid uptake by cancer cells.

The combination of CHR stabilised gold nanoparticle(CHR-AuNP) with PTX is a promising strategy to minimize cytotoxicity mediated by PTX while increasing the overall therapeutic impact. As a result, the purpose of this research was to investigate the anticancer effects of

chrysin stabilised gold nanoparticles and PTX on A549 cells in vitro. The combination of CHR-AuNP +PTX boosts A549 cytotoxicity via apoptosis. Our findings indicate that PTX has superior cytotoxicity activity in lung cancer cell line when paired with CHR-AuNP than when used alone. IC-50 value of CHR-AuNP synthesized was significantly lowered as compared to free CHR.

The anticancer efficacy and expression of apoptotic associated proteins (Bax and Bcl-2) were all thoroughly examined. The combination therapy displayed more anti-proliferative activity against A549 cell lines, as well as a superior influence on the formation of intracellular reactive oxygen species, mitochondrial membrane potential, and cell apoptosis. Furthermore, the Bax/Bcl-2 ratio was greater in the combination therapy.

The mechanisms behind the combination's anticancer activities on NSCLC were then studied further. In the current work, CHR-AuNP and PTX augmented BAX expression while diminishing Bcl2 expression, ensuing in the release of cytosolic cytochrome c, subsequently activating caspase-9, caspase-3. Besides that, increasing intracellular ROS generates apoptosis by upregulating the p-p53 protein . Excessive ROS buildup damages mitochondria, spurring to the release of cytochrome c into the cytosol and the induction of apoptosis . p-p53 was highly elevated in our study by CHR-AuNP, PTX, or CHR-AuNP+ PTX. Modulation of ROS generation has the capacity to decimate cancer cells while providing minimal impact to normal cells . Several studies have revealed that the majority of anticancer drugs target cancer cells by concomitant increases in ROS production. CHR-AuNP or PTX alone, or in combination, trigger ROS generation, which eventually lead to cell death. NAC, a ROS inhibitor, curtails CHR-AuNP, PTX or CHR-AuNP +PTX -induced cell death, proving the involvement of ROS. CHR-AuNP and PTX exert cancer cell apoptosis.

CHR-AuNP or PTX alone or in combination elicits cytotoxicity in A549 cells by apoptosis, as illustrated by morphological alterations and an annexin-V binding studies.

The activation of the normal Wnt signalling pathway has been associated to lung cancer development and progression. In one study, Wnt-1 was shown to be overexpressed in the tumours of lung cancer patients. When the Wnt signalling pathway was suppressed, lung cancer cells went into apoptosis. In physiological cells, PPAR-ϒ suppresses carcinogenesis and WNT signalling by directing phosphorylated beta-catenin to the proteasome via the PPAR-ϒ / β -catenin binding domain. Canonical WNT/ β-catenin pathway activation promotes PPAR-ϒ inactivation in numerous organs, whilst PPAR-ϒ activity inhibits canonical WNT/β-catenin signalling. A Western blot and immunofluorescence assessment of A549 cells treated with CHR-AuNP, PTX, and CHR-AuNP+ PTX demonstrated that these compounds successfully enhanced the PPAR-ϒ level while decreasing the β-catenin level.

Finally, our findings imply that CHR-AuNP amplifies the cytotoxic action of PTX. Lower dosages of the combination therapy resulted in increased cytotoxicity in A549 cells. As a result, CHR-AuNP might be an intensive chemosensitizer as well as a possible anticancer drug for treating lung carcinoma.