Formulation and Evaluation of Ligand-Nanocarriers Targeted Delivery for Enhanced Cardiovascular Therapy

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SUMMARY

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Summary

Cardiovascular diseases (CVDs) impose significant health and economic burdens globally. Cardiovascular diseases are anticipated to emerge as a significant contributor to global mortality in the upcoming years. MI is a severe manifestation of IHD which impacts cardiac function impairment [1]. Naringin is a significant and functional flavanone glycoside found in grapefruit and kindred citrus species. It has been utilized in diverse pharmacological procedures, encompassing anti-inflammatory, anti-angiogenic, anti-viral, anti-fibrotic, and anti-neoplastic activities. Previous investigations have revealed that naringin serves efficient in animal models of cardiovascular disease. However, its limited solubility and physicochemical properties have hindered its clinical effectiveness. The formulation of NR-LGNPs was accomplished by employing an emulsion solvent evaporation method. The utilization of this approach has been observed in various studies to generate consistent nano-compositions of diverse therapeutic agents. The stable formation of NR-LGNPs is achieved through the process of organic solvent emulsion evaporation. The NR-LGNPs were optimized utilizing the 2^3 factorial design of the experiment subsequently an optimized formulation as per the suggested values was prepared and evaluated for dependent variables. There was not a noticeable variation between the suggested and observed values of variables indicated formulation optimization. The SEM and TEM study demonstrated the uniform distribution of nanoparticles along with a spherical shape and smooth surface. The particle size as indicated in the SEM/TEM study was in correlation with the size data obtained through the dynamic light scattering study. The FTIR study revealed the presence of all the characteristic peaks of NR in the prepared NR-LGNPs suggesting drug excipient compatibility. The results of the ISO-induced MI study indicated the potential benefits of using NR-LGNPs for cardiac injury. The ISO-induced myocardial infarction model is a widely recognized method for evaluating novel cardioprotective agents. The findings from in vivo studies suggest that the administration of NR-LGNPs may exhibit significant cardioprotective effects. Furthermore, it has the potential to result in a significant decrease in inflammatory markers, infarct size, lipid peroxidation, as well as cardiac markers, while concurrently elevating the concentrations of antioxidant enzymes [2]. The primary impact of myocardial infarction (MI) is the significant size of the infarct area in the heart, which is assessed through the utilization of the TTC staining technique. The size of the infarction region in rats induced with ISO is greater due to elevated myocardial damage [3]. Administration of NR-LGNPs to rats before inducing myocardial infarction through isoproterenol can significantly modulate oxidative stress, thereby protecting the heart against

subsequent damage. As previously indicated, various pathophysiological and biochemical events, such as oxidative stress, has been identified as the primary causative factor of myocardial infarction [4]. Therefore, the current study was intended to evaluate the antioxidant effect. In addition, myocardiocytes exhibit a high susceptibility to free radical-induced damage owing to their heightened requirement for polyunsaturated fatty acids and increased energy (ATP) demands. In rats injected with ISO, a decrease in the activity of multiple cardiovascular antioxidants (CAT and SOD) was observed. This was accompanied by an exponential increase in MDA levels due to the heightened production of free radicals and subsequent lipid peroxidation in myocardiocytes. In contrast, the administration of NR-LGNPs to rats for 21 days resulted in a significant improvement in their antioxidant status and a reduction in MDA output. This was achieved through the enhancement of myocardiocyte integrity and the suppression of lipid peroxidation [5].

The evaluation of cardiac diagnostic biomarkers associated with myocardial infarction-induced cardiotoxicity is an essential protocol. The present study observed a noteworthy elevation in the levels of various cardiac marker proteins, including LDH and CK-MB, in animals that were subjected to ISO-induced intoxication. Cardiomyocytes exhibited elevated levels of lipid peroxidation, resulting in the leakage of cardiovascular proteins into the extracellular fluid plasma. Rats that received NR-LGNPs exhibited reduced levels of marker enzymes such as LDH and CK-MB, which can be attributed to the protective membrane or anti-lipid peroxidation properties of the nanoparticles [6]. Numerous researches have suggested that oxidative stress is crucial in the initiation of myocardial infarction [7]. The hypothesis posited that NR-LGNPs possess antioxidant properties that enable them to regulate cellular activity [8, 9]. Histopathological evaluation is an essential aspect to confirm the biochemical and molecular alterations mentioned above during MI induced by ISO. The myocardial infarction induced by ISO exhibited disorientation of myofibril (degeneration) along with significant infiltration of neutrophils and multiple necrotic alterations. The administration of NR-LGNPs in rats resulted in a reduction of neutrophil infiltration and necrotic modifications in the cardiac segment, accompanied by an improvement in myofibrillar structure. This effect can be attributed to the potent antioxidant property of NR-LGNPs.

The successful synthesis of NR-LGNPs was achieved through a modified phase separation technique. The prepared NR-LGNPs were optimized using a 2^3-factorial design of

experiment considering polymer amount and sonication amplitude as independent variables while particle size, zeta potential, and drug release as dependent variables. The findings of the SEM/TEM assessment validated the presence of nanoparticles that exhibit a fine, smooth, and spherical morphology. The results of the in vitro drug-release experiments confirmed the nanoparticles' sustained-release characteristics over an extended duration. The intravenous administration of nanoparticles that were prepared resulted in the improvement of the effects of Isoproterenol-induced myocardial infarction in experimental rats. Furthermore, the *in vivo* investigations conducted on the rat model with ISO-induced myocardial infarction revealed a considerably greater extent of infarcted region in comparison to the control cohorts. This was evidenced by improvements in the majority of the estimated parameters. Additionally, the histological images depicting the myocardial infarction evinced the nanoparticles' cardioprotective efficacy. The present study demonstrated that the administration of NR-LGNPs exhibited a more pronounced reduction in the extent of cardiovascular infarction, levels of oxidative stress markers, and cardiovascular markers in comparison to NR treatment. The findings indicate that the polymeric nanoparticles that were developed possess remarkable cardioprotective properties in rats induced with ISO. Therefore, it can be inferred that the nanoparticles have potential therapeutic applications in the treatment of cardiovascular diseases. Enhancing the scale-up capacity of this particular formulation has the potential to facilitate future commercialization. Furthermore, it may serve as a valuable tool for potential cardioprotective interventions in individuals with myocardial infarction. Nevertheless, additional clinical investigations should be pursued to authenticate our findings.

Cardiotoxicity (CT) is a severe condition that negatively impacts heart function. β-sitosterol (BS) is a group of phytosterols and known for various pharmacological benefits, such as managing diabetes, cardiac protection, and neuroprotection. This study aims to develop niosomes (NS) containing BS, utilizing cholesterol as the lipid and Tween 80 as the stabilizer. The research focuses on designing and evaluating both conventional BS-NS and hyaluronic acid (HA) modified NS (BS-HA-NS) to enhance the specificity and efficacy of BS within cardiac tissue.

To evaluate cytotoxicity on H9c2 heart cells, the MTT assay was used. The cellular uptake of BS-NS and BS-HA-NS was confirmed by confocal microscopy on H9c2 cardiac cells. Administering BS-NS and BS-HA-NS intravenously at a dose of 10 mg/kg showed the ability

to significantly decrease the levels of cardiac troponin-I (cTn-I), creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and lipid peroxidation (MDA). Tissue histopathology indicated a substantial potential for repairing cardiac tissue after treatment with BS-NS and BS-HA-NS and strong cardioprotection against ISO induced myocardial tissue damages. Thus, enhancing BS's therapeutic effectiveness through niosome surface modification holds promise for mitigating cardiac damage resulting from CT.

This study demonstrates the successful development and optimization of NS containing BS with a surface modification of hyaluronic acid. The formulated nanoparticles exhibited favourable characteristics, including a well-defined spherical shape, optimal size including higher entrapment potential, and remarkable drug loading percentage. The *in vitro* and *in vivo* assessments revealed the pronounced cardioprotective potential of both conventional BS-NS and surface modified BS-HA-NS formulations. Administering these NS intravenously resulted in a noteworthy reduction in cardiac biomarkers and lipid peroxidation levels, indicative of their ability to mitigate the adverse effects of cardiotoxicity. Histopathological analysis further supported the therapeutic efficacy of BS-NS and BS-HA-NS in promoting cardiac tissue repair. Particularly, the HA-modified NS exhibited enhanced effectiveness in reducing the size of cardiac infarction, highlighting their robust cardioprotective capabilities against myocardial damage induced by ISO. Overall, this research underscores the promise of surface-modified NS as a strategy to enhance the therapeutic efficacy of BS, offering potential avenues for the treatment of cardiotoxicity and related cardiac conditions.