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Hesperetin conjugated PEGylated gold nanoparticles exploring the potential role in anti-inflammation and anti-proliferation during diethylnitrosamine-induced hepatocarcinogenesis in rats

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ABSTRACT

Liver cancer is the fifth most common cancer and one of the leading causes of death in the world, and second most common cause of death in men. Natural products emerge as the most enduring approaches in the development of anticancer targeting drug. Hesperetin (HP), one of the abundant flavonoids found naturally in citrus fruits, has received considerable attention in anti-cancer promotion and progression. The present study was conducted to decipher the role of 0.5 ml hesperetin conjugated gold nanoparticles (Au-mPEG$_{(5000)}$-S-HP NPs) during diethylnitrosamine (DEN)-induced hepatocarcinogenesis in male Wistar albino rats and shows the better antioxidant that possesses anti-inflammatory, anti-proliferation and anticarcinogenic properties and may modulate signaling pathways. The confirmation of polymer functionalized gold nanoparticles and drug loaded polymer gold nanoparticles were characterized by HR-TEM with EDAX, and DLS with Zeta potential techniques. The drug encapsulation efficiency and release properties were carried out in PBS at pH 7.4 for Au- mPEG$_{(5000)}$-S-HP and compared with the control pure hesperetin (HP). Here, we review the role of mast cell counts, tumor necrosis factor alpha (TNF-α), transcription factor nuclear factor-κB (NF-κB), levels of glycoconjugates, proliferating cell nuclear antigen (PCNA) and argyrophilic nucleolar organizing regions, are the master regulator of inflammation and proliferation, in the development of hepatocellular injury, liver fibrosis and HCC. DEN-administered animals showed increased mast cell counts, tumor necrosis factor alpha, transcription factor nuclear factor-κB, glycoconjugates, proliferating cell nuclear

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antigen, and argyrophilic nucleolar organizing regions. Whereas Au-mPEG$_{(5000)}$-S-HP NPs supplementation considerably suppressed all the above abnormalities. These results suggest that the Au-mPEG$_{(5000)}$-S-HP NPs exhibited the better potential anticancer activity by inhibiting cell inflammation and proliferation in DEN-induced hepatocellular carcinogenesis.

1. Introduction

Hepatocellular carcinoma (HCC) is the most frequent form of primary liver cancer and it is the fifth most common cancer in the world and third most common cause of cancer mortality [1]. The incidence of HCC is rising across the globe, especially in the United States, with 71% increase over the last 25 y [2]. The incidence of liver cancer has been increasing in recent years in India and the major risk factors of HCC may be age, gender, hepatitis B and C, alcohol consumption, hormone exposure, haemochromatosis, vinyl chloride, arsenic poisoning, aflatoxin B1, obesity, diabetes, renal transplant patients, tobacco smoking and parasitic infections such as clonorchiasis and schistosomiasis which results in liver damage such as cirrhosis [3]. The main denominator in HCC of different etiology is the induction of oxidative stress by inflammatory cells, resulting in chronic hepatic injury and cell death, followed by oncogenic transformation of surviving hepatocytes and compensatory proliferation that leads to oncogenesis [4,5]. Diethylnitrosamine (DEN) or N-nitrosodiethylamine (NDEA) is a powerful environmental carcinogen that has been used as an initiating agent for hepatocarcinogenic activities. N-nitroso compounds are considered to be a tragic health hazards to man, and these compounds were present in tobacco products, cheddar cheese, cured and fried meals, occupational settings, cosmetics, agricultural chemicals and pharmaceutical agents [6]. The pre-treatment and the post-treatment of cancer is still a big challenge in medicine and the chemoprevention serves as an attractive and alternative to prevent cancer [7]. The term cancer chemoprevention is the use of several natural, synthetic, and biological agents to reverse, inhibit, or delay carcinogenic progression to invasive cancer which has been identified as a novel approach against several types of cancers [8]. Considerable efforts were taken to search for naturally occurring compounds that can curtail several stages of carcinogenesis. Plant derived substances have recently gained importance, owing to their versatile applications such as quenching reactive oxygen species and protect critical cellular components like DNA, proteins, and lipid from oxidative damage [9]. It may also interfere with intracellular signaling pathways which as regulate cell proliferation, initiation of apoptosis, and response to oxidative damage [10]. Epidemiology and animal studies have suggested that a high intake of flavonoids may be linked to a reduced risk of cancer.

Hesperetin (5, 7, 3'-trihydroxy-4'methoxy flavanone), a Chinese traditional medicine, is a bioflavonoid occurring abundant in citrus fruits which occurs as hesperidin (its glycoside form) in nature and it has received considerable attention in cancer prevention [11]. It exhibits various pharmacological activities, such as anti-inflammatory, anti-hypertensive and anti-atherogenic effects [12–14]. Despite the challenging application of hesperetin (HP) in cancer therapy, but the clinical use of HP was restricted because of the poor water solubility. Therefore, many researchers are now focusing on improving its bioavailability through several approaches including innovative drug delivery systems [15–17]. In this condition we need an effective drug delivery system with the help of various biomaterials such as biodegradable nanoparticles (NPs). To enhance the efficacy and the solubility of the cancer therapeutic agent, the use of nanoparticle-based drug formulation is an important aspect of nanomedicine [18]. In particular, gold NPs possess biological activities like antioxidant, anti-inflammatory, anti-angiogenesis and anticancer properties and therefore it has been used for the delivery of drugs, proteins, peptides and oligonucleotides etc. [19]. The uses of biocompatible functionalized polymers emerged as an attractive candidate for the delivery of various therapeutic agents and also playing a dual role as reducer and stabilizer [20]. The major advantage of using a polymer as a stabilizing agent not only the enhancement of their long-term stability, adjustment of the solubility and amphiphilicity, but also their functionalization with polymers to achieve higher and tunable surface-density of shell/brush morphology and to tailor its properties, beyond promoting their compatibility and processibility of the nanoparticles by preventing particle agglomeration [21,22]. Hence, it is clear that the binding ability of the AuNPs to the cell membrane and the functionalization of the m-PEG-thiol polymer on the AuNPs make it to serve as a good drug carrier and solubility.

In our previous study we have introduced a new method for effective drug delivery system to improve the drug efficacy, solubility and bioavailability with the help of nanomaterials by synthesizing gold (Au) NPs stabilized and reduced with polymer O-[2-(3-mercaptopropionylamino)ethyl]-O'-methyl polyethylene glycol (mPEG$_{(5000)}$-SH). Further, it is capped with anticancer drug-HP for effective chemotherapy drug to treat DEN induced HCC in male Wistar albino rats. The effect of better anti-inflammatory effect and anti-proliferative effect of hesperetin conjugated gold nanoparticles (Au-mPEG$_{(5000)}$-S-HP NPs) is not yet documented. Hence the present study was aimed to elucidate the better protective role of Au-mPEG$_{(5000)}$-S-HP NPs on the expressions of cell inflammation and cell proliferation during diethylnitrosamine-induced hepatocarcinogenesis in male Wistar albino rats.