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Allicin functionalized locust bean gum nanoparticles for improved therapeutic efficacy: An *in silico*, *in vitro* and *in vivo* approach

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ABSTRACT

The field of nanotechnology has overgrown over the past few years and has even ventured into the field of medicine. The aim of the present study is to develop a novel allicin functionalized locust bean gum nanoparticle using the nanoprecipitation technique. The synthesized nanoparticles were characterized by dynamic light scattering, scanning electron microscopy and transmission electron microscopy. The characterization study revealed the nanoscale structure (~100 nm) of the prepared particles. *In silico* toxicology analysis were carried out to assess the drug-like properties and virtual toxicity of allicin. Toxicity of the prepared nanoparticles were carried out in RAW 264.7 cell lines *in vitro* and *in vivo* studies were carried out in Sprague-Dawley rats. In *in vitro* study, LBGAN showed a maximum toxicity of 10.51% in MTT assay, no reactive oxygen species generation on DCFDA staining and LBGAN was effective to protect the cells from apoptosis. In *in vivo* toxicity studies LBGAN showed no significant change in the activities of the marker enzymes like LDH, CK-MB, ALP, ACP, AST and ALT. Thus, the functionalization of nanoparticles with allicin has the benefit of providing protection and stability to the allicin, in addition to increasing its pharmacological activity.

1. Introduction

Nanotechnology is widely used in many fields of science and medicine. Applying nanoparticles gained immense popularity in the last decade, due to their prospective to improve the therapeutic effects of the encapsulated drugs by protecting drugs from enzymatic degradation, providing their controlled release and prolonged blood circulation; changing their pharmacokinetics, decreasing their toxicity and limiting their nonspecific uptake [1]. Polymeric nanoparticles have been offered as promising tools to meet the challenge of delivering biopharmaceuticals successfully. Natural polymers are widely used in drug delivery systems, as they simply fulfill the conditions of biocompatibility, biodegradability, non toxicity and overall cost of production. Moreover, these have some exceptional merits over synthetic ones, due to improved capacity for cell adhesion and mechanical properties similar to natural tissues [2].

Locust bean gum (LBG) is a neutral polysaccharide (galactomannan) being extracted from the seeds of the carob tree (*Ceratonia siliqua*) having a chemical structure composed of both galactose and mannose units. Due to particular features of LBG mainly related with its gelling capacity and synergies with other polysaccharides, a growing interest is being observed regarding its biopharmaceutical use [3]. There is not much information regarding the use of LBG as drug delivery agent, and this is the first report on the use of LBG nanoparticles in biomedical application.

From the time immemorial, garlic is extremely useful in day to day life. The medicinal activities of garlic are attributed to the thiosulfonate compounds, of which allicin is about 75%,and it is responsible for the typical, pungent odour of garlic, which is generated when the enzyme alliinase reacts with its substrate alliin [4,5]. Allicin has a variety of biological effects including antimicrobial, hypolipidemic, antithrombotic, anticancer, and antioxidant activities [6,7]. However, under acidic pH (*e.g.*, internal human body) allinase is irreversibly deactivated thus allicin is not produced in the body from the consumption of garlic. Allicin is also unstable when utilized, degrading rapidly with heat and more slowly upon standing at room temperature.

In the present study, LBG was used to prepare nanoparticles as it is a natural food additive and has the property for reducing the cholesterol level, improves blood sugar level, improves digestion and is effective against colon cancer. Here, allicin was used as a therapeutic agent. Taking all these into account this is the first report on exploring the beneficial role of allicin functionalized locust bean

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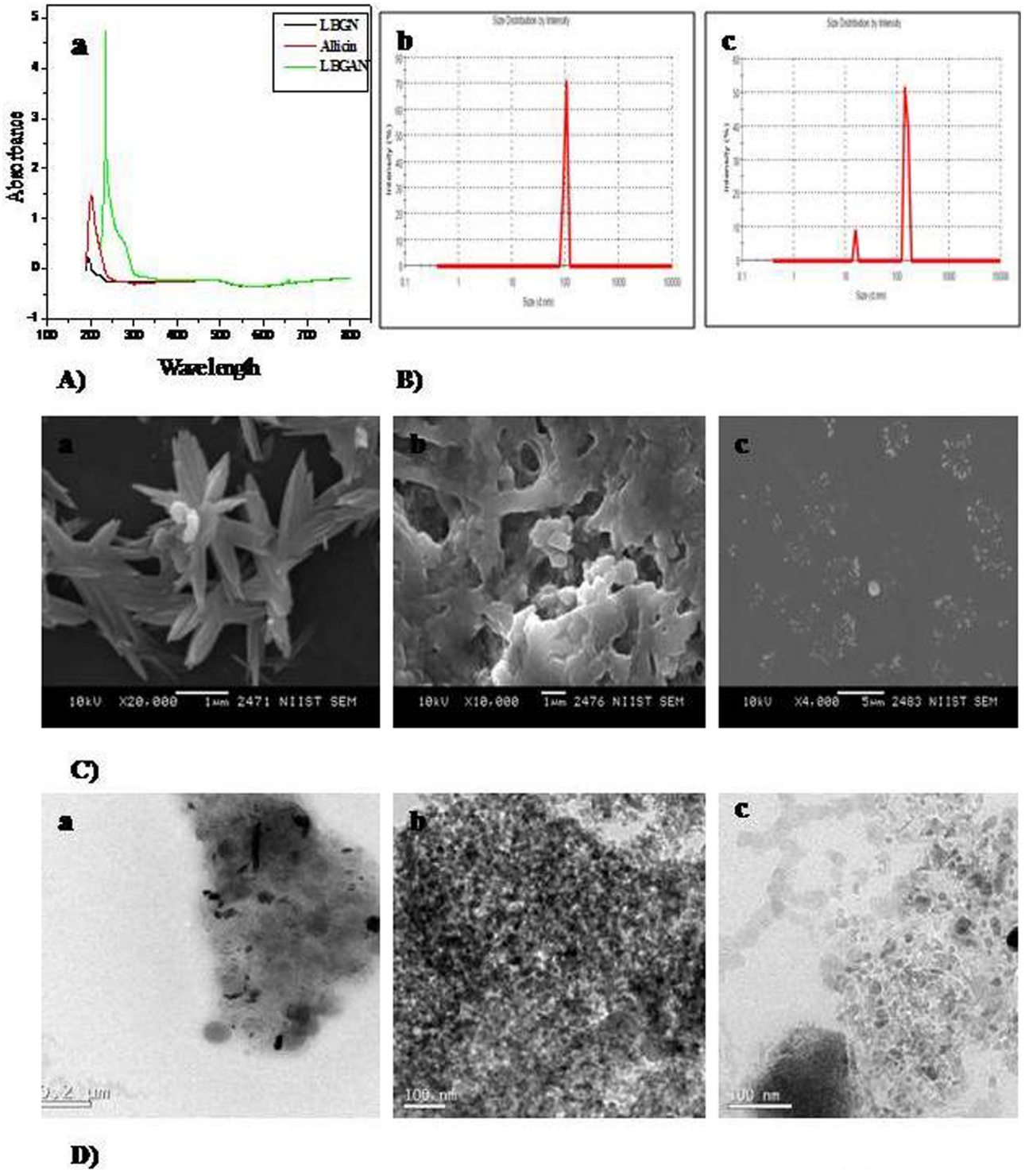

Fig. 1. A (a) UV absorption analysis of allicin, LBG nanoparticles and LBGAN. 1.B (a) particle size distribution of LBG nanoparticles (b) particle size distribution of LBGAN. 1.C (a) SEM images of allicin (b) LBG nanoparticles (c) LBGAN, 1.D **(**a) TEM images of allicin (b) LBG nanoparticles (c) LBGAN.

gum nanoparticles (LBGAN) on *in silico*, *in vitro* and *in vivo* experiments to check whether the effect of allicin is enhanced when it is incorporated to a bioactive polymer LBG nanoparticle and assessed the non toxic nature of LBGAN.

2. Materials and methods

Locust bean gum powder, α-galactosidase enzyme from coffee beans, allicin from garlic extract, triton X-100, isopropanol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), tripolyphosphate, dimethyl sulfoxide (DMSO), 2′,7′dichlorodihydrofluorescein diacetate (DCFH-DA), acridine orange (AO), ethidium bromide (EtBr), 2,3 diaminonaphthalene, JC-1 (5,5′,6,6′-tetrachloro- 1,1′,3,3′ tetraethylbenzimidazolyl carbocyanine iodide), 4′,6-diamidino-2-phenylindole (DAPI), were purchased from Sigma Chemicals, USA. Synthesis and purification of allicin were performed as previously described [8] with slight modification, yielding allicin preparations with a purity of >98%.