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Nano-encapsulation of capsaicin on lipid vesicle and evaluation of their hepatocellular protective effect

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ABSTRACT

The intention of the study was to evaluate the effectiveness of nanocapsulated food constituent capsaicin in protection of liver oxidative stress. We had prepared phospholipid vesicle (nanoliposome) by formation of thin lipid film followed by hydration when the mean vesicle diameter was found to be 277.7 nm. Protection from sodium fluoride (NaF) induced oxidative stress by capsaicin loaded nanoliposomal formulation were tested in rats where a single dose of capsaicin in free and nanoliposome forms were administered after two hour of exposure to NaF. Membrane in hepatic cells were damaged by NaF and it was evaluated by estimating reactive oxygen species (ROS), lipid peroxidation, and catalase activity when it was observed that free capsaicin produced mild protection whereas liposomal capsaicin exerted a significant result. This can be suggested that liposome encapsulating capsaicin acts as a promising therapeutic agent in reducing liver oxidative stress produced by different stress factors.

1. Introduction

Carcinoma at hepatocellular region is ranked as the fifth commonest cause of malignancy and third cause of death worldwide [1]. ROS is created in all mammalian cells, partially as a result of normal cellular metabolism, and partially owing to activation of membrane-bound enzyme systems in response to exogenous stimuli. ROS is a combined term that includes not only oxygen radicals (hydroxyl and superoxide) but also some non-radical derivatives of molecular oxygen such as hydrogen peroxide. ROS produced from environmental factors or cellular mytochondrial dysfunction, generates oxidative stress which is associated with carcinoma of liver. Accumulated ROS was produce oxidative stress due to decrease of its removal from the system [2]. Antioxidants like glutathione, catalase, and superoxide dismutase etc, protect our body from oxidative stress [3]. Carcinogenesis results from excess production of ROS with damage to nucleic acids, lipids, or proteins, when breaking of DNA strands and formation of abnormal DNA linkages were noted [4–6]. The human body is continually under oxidative stress arising from exogenous origins and endogenous origins. When such oxidative stress exceeds the capability of the oxidation-reduction system of the body, gene mutations may result leading to carcinogenesis. Cellular targets affected by oxidative stress consist of phospholipids, proteins, DNA, and carbohydrates on the cell membrane. Oxidized and injured DNA has the potential to persuade genetic mutation. Oxidized lipids react with metals to produce active substances which have the potential to induce mutation. Research is being carried on worldwide to find the relation between oxidative stress and carcinogenesis and also to find newer potential chemopreventive agents and their delivery approach.

Numerous natural products are potential antioxidants that defend against reactive nitrogen species or reactive oxygen species induced damage and ameliorate oxidative stress. Antioxidants exert their action by numerous mechanisms, like by suppressing the creation of active species by reducing hydroperoxides and hydrogen peroxide, by sequestering metal ions, termination of chain reaction by scavenging active free radicals. Quercetin present in huge amounts in fruits, vegetable, tea and olive oil, has been used to serve as a stress protectant for its capability to interact with free radicals involved in oxidative damage in liver [7].

Due to their potential to decrease oxidative stress, natural dietary agents or their derivatives are used now-a-days extensively to prevent cancers [8], which are almost half of the agents used to treat hepatocellular malignancy [9,10]. Natural products from plant sources which contain active ingredients have been used in therapy from ancient times and also in use at present at different areas of the world [11–13].

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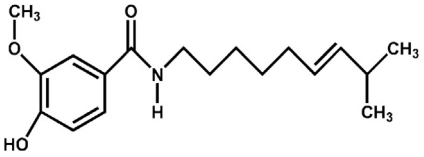

Fig. 1. Chemical structure of CAP.

Red pepper ingredient capsaicin (Fig. 1), pungent in taste, has been used as food additives and drugs since long time [14,15]. Recently, it was observed that capsaicin contains chemopreventive properties against some types of carcinoma [16]. Capsaicin represses the growth of cancer cells through induction of apoptosis [16–19]. Though the anticancer potential of capsaicin is proven, its use in the treatment is fraught with difficulties. As capsaicin has many dose-dependent side effects on oral use e.g, increased salivation, gastric secretion, intense burning sensation, and gastro-intestinal disorders, it is very difficult to use it in cancer treatment. The poor aqueous solubility (10.3 mg/L at 25 °C) of capsaicin is another barrier to use it in high dose. After oral administration of capsaicin (0.4 mg per kg body wt) in male adults, the blood concentration was seen to be 2.47 ng/mL (equivalent to 8.1 nM) [20], so it is not a very effective approach to use simple capsaicin as a therapeutic agent. Recently, nano-liposomes are tremendously used to target the drug to specific site of the body [21]. In order to improve therapy for hepato-cellular oxidative stress, nano-liposome form of capsaicin which deliver the drug in the liver is an ideal approach to protect liver damage when the local concentration of the drug can be increased, adverse drug reactions reduced and maximal therapeutic efficacy can be achieved. Dicetylphosphate is used to increase targeted drug delivery in liver as it is negetivly charged whereas liver is positively charged [22]. It was observed that so far there is no published data on the protective effects of capsaicin in nano-liposomal form against hepatocellular oxidative stress. To address this lack of information, the objective of this study was to investigate the protective effect of nanoliposomal capsaicin against oxidative stress caused by fluoride treatment in rats.

2. Materials and methods

2.1. Materials

Capsaicin (CAP) was purchased from Naturite Agro Products Ltd., Hyderabad, India. L-α-phosphatidylcholine (LP) and Cholesterol (CHL) were purchased from HiMedia Laboratories Pvt. Ltd, Mumbai, India. Dicetyl phosphate (DCP) was purchased from Sigma Chemicals, USA. Choroform was obtained from SD Fine Chem, Mumbai, India. All other ingredients used in the study were of analytical grade.

2.2. Preparation of liposome

Required quantity of CAP, LP, CHL, and DCP (Table 1) was taken and dissolved into chloroform in round bottom flasks (250 mL). The organic solvent was evaporated at 55–60 °C in a rotary evaporator (PMTC-3040, Superfit, India) with rotation speed of 120 rpm and thin film was formed. For complete removal of traces of organic solvent, the flask was kept overnight in a vacuum desicator. The dried film was hydrated at 60 °C in a water bath connected with a rotary evaporator at 120 rpm till compete dispersion of lipid film. The hydration was performed above the phase transition temperature of the phospholipids (55 °C) i.e. at 60 °C [23]. This is in order to make sure that all the phospholipids are dissolved in the suspension medium homogenously and have sufficient flexibility to align themselves in the structure of lipid vesicles.

The dispersion was sonicated at the same temperature in a bath type sonicator (UD200SH-6L, Takashi, Japan) for 1 h in a bath sonicator for reducing the vesicle size. Then the dispersion was kept for one hour at room temperature for vesicle formation and was stored at 4 °C for further use.

2.3. Drug excipients interaction study

2.3.1. Fourier transform infrared spectroscopy

CAP, physical mixture of CAP and excipients, and liposomal formulation of CAP and excipients were mixed with KBr and subsequently pellets were prepared. The pellets were scanned over a wave number range of 4000–400 cm^{-1} using Fourier transform infrared spectroscopy (FTIR) (Bruker, Alpha T, Germany) [24].

2.3.2. Differential scanning calorimetry

Differential scanning calorimetric (DSC) thermograms of CAP, CHL, LP, physical mixture, and complexes of these ingredients were obtained in an inert atmosphere over a temperature range of 30–300 °C in a DSC (Pyris Diamond TG/DTA, PerkinElmer, Singapore) [25].

2.3.3. X-ray diffraction

CAP, physical mixture of CAP and excipients, and liposomal formulation of CAP and excipients were scanned from 5° to 60° diffraction angle (2θ) using X-ray diffractometer (XRD) (Ultima-III, Regaku, Japan) [26].

2.4. Particle size and zeta potential

Size distribution and zeta potential was measured by Zetasizer nano ZS (Malvern, UK).

2.5. Drug entrapment efficiency

The liposomal suspensions (10 mL) containing trapped CAP were alienated from un-entrapped CAP by cooling centrifugation (VCDC4281, Remi Electrotechnik, India) at 4 °C for 1 h at 15,000 rpm. The supernatant was alienated and the precipitate was added with fresh phosphate buffer solution pH 7.4 and centrifuged another 30 min under same conditions. The process was repeated three times. Un-entrapped drug was measured spectrophotometrically (UV-1800, Shimadzu, Japan) at wavelength of 280 nm. The entrapment efficiency percent (%EE) was calculated using the following equation [27]:

$$\%EE = \left[\frac{C_d - C_f}{C_d}\right] \times 100 \quad (1)$$

where C_d is the concentration of total CAP added and C_f is concentration of free CAP detected.

2.6. In vitro antioxidant properties

CAP and CAP loaded liposome was dissolved in methanol for radical scavenging determination. Various concentrations (5, 10, 15 and 20 μg/mL) of CAP and liposome containing CAP (1 mL) were mixed with 1 mL of methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH) (approx. 60 μM). The mixture was left for 30 min with vigorous shaking at room temperature. The radical scavenging activity was measured sphectrophotometrically at 520 nm.

$$\text{Radicalscavengingactivity}\,(\%) = \frac{(A - B)}{A} \times 100 \quad (2)$$