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Preparation of albumin based nanoparticles for delivery of fisetin and evaluation of its cytotoxic activity

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ABSTRACT

Fisetin is a well known flavonoid that shows several properties such as antioxidant, antiviral and anticancer activities. Its use in the pharmaceutical field is limited due to its poor aqueous solubility which results in poor bioavailability and poor permeability. The aim of our present study is to prepare fisetin loaded human serum albumin nanoparticles to improve its bioavailability. The nanoparticles were prepared by a desolvation method and characterized by spectroscopic and microscopic techniques. The particles were smooth and spherical in nature with an average size of 220 ± 8 nm. The encapsulation efficiency was found to be 84%. The *in vitro* release profile showed a biphasic pattern and the release rate increases with increase in ionic strength of solution. We have also confirmed the antioxidant activity of the prepared nanoparticles by a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Further its anticancer activity was evaluated using MCF-7 breast cancer cell lines. Our findings suggest that fisetin loaded HSA nanoparticles could be used to transfer fisetin to target areas under specific conditions and thus may find use as a delivery vehicle for the flavonoid.

1. Introduction

Nanoparticles (NPs) have drawn significant interest in recent years due to their tremendous applications in various fields especially in the area of drug delivery. Nanoparticles are defined as particles in the size range 10–1000 nm [1]. Various research groups have already shown that nanoparticles not only increase the stability of a drug but can also deliver the drug to its target site, control the release of the drug and reduce its side effects. A number of drugs like aspirin [2], ciprofloxacin [3], rosebengal [4], ganciclovir [5], noscapine [6] have already been encapsulated into nanoparticles to increase their therapeutic efficacy.

Fisetin (3,3′,4′,7-tetrahydroxyflavone), a naturally occurring flavonoid (Fig. 1), found in several vegetables, fruits, nuts and wine [7,8], has been selected as a model compound for our work. It possesses several activities including antioxidant [9], anti-inflammatory [10], and anticancer activities [11–15]. The antiangiogenic activity of fisetin has also been reported by researchers *in vitro* and *in vivo* in mice [16–18]. Fisetin also exhibits anti-allergic, antiviral properties in addition to glucose uptake abilities [19–23]. However, there are some limitations which hinder its effective use in pharmaceutical sciences and this is mainly due to the poor aqueous solubility of fisetin which results in poor bioavailability, instability and permeability. Considering these facts, the purpose of our present work is to prepare fisetin loaded HSA nanoparticles in order to improve the solubility of fisetin. Several polymeric nanoparticles have already been developed for encapsulation of drugs. As nanoparticles enter into the blood stream, the carrier chosen for the preparation of nanoparticles must be biodegradable. In the present study we have chosen human serum albumin (HSA) as a material for nanoparticle synthesis because HSA is biodegradable, biocompatible, soluble in water, easy to prepare and reproducible. Medical studies have proved that HSA based nanoparticles can be easily permitted by the body without any severe side effects which is further supported by the approval of HSA based nanoparticles such as Albunex™ [24] and Abraxane™ [25,26]. Apart from these, a number of clinical attempts have been attempted using albumin bound nanocarrier systems [27].

In this study, the nanoparticles were prepared by a desolvation technique and characterized by transmission electron microscopy (TEM), field emission scanning electron microscopy

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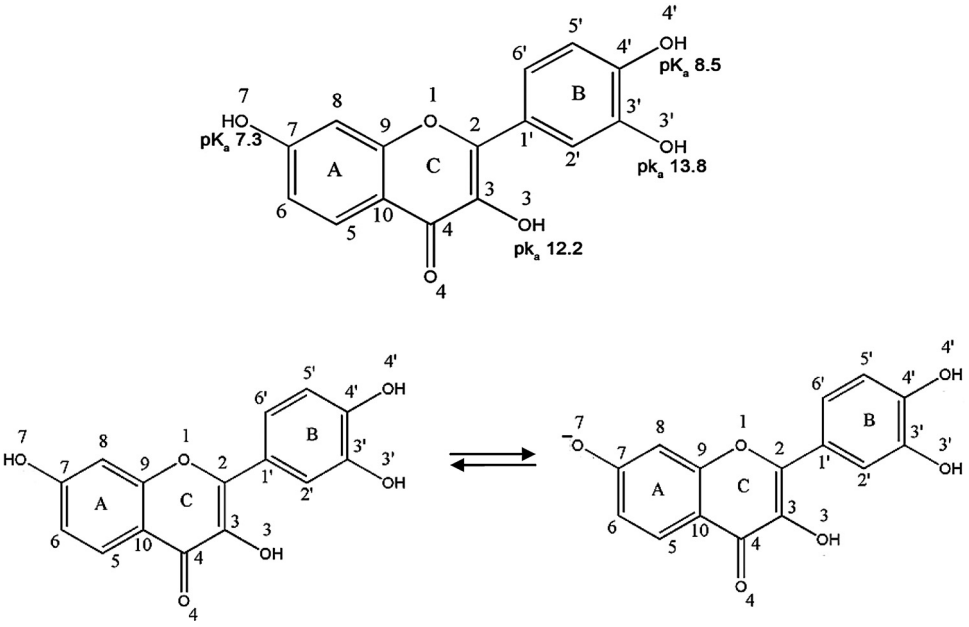

Fig. 1. Structure of fisetin with pK_a values.

(FESEM), atomic force microscopy (AFM), dynamic light scattering (DLS), UV–vis spectrophotometric study, and Fourier transform infrared spectroscopy (FTIR) study. The encapsulation efficiency and *in vitro* release of fisetin from the nanoparticles were also calculated in this study. The antioxidant activity of the nanoparticles was evaluated by the DPPH assay and the toxicity of the nanoparticles checked by a hemolytic assay. A cytotoxicity assay was also carried out to check the efficacy of fisetin and fisetin loaded HSA NPs toward MCF-7 breast cancer cells.

2. Materials and methods

2.1. Materials

Human serum albumin, fisetin and glutaraldehyde were purchased from Sigma Chemical Co. (St. Louis, USA). All other reagents of analytical grade were from SRL, India and used as received. For the cell culture experiments, MCF-7 breast cancer cell line was procured from NCCS, Pune, India. MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), and DMSO (dimethyl sulfoxide) were obtained from Sigma Chemical Co. (St. Louis, USA). DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum was from HIMEDIA (Mumbai, India). Cells were maintained at 37 °C in 5% CO_2 humidified atmosphere.

2.2. Methods

2.2.1. Preparation of fisetin loaded HSA nanoparticles

Fisetin loaded HSA nanoparticles were prepared by a desolvation technique [6,28,29]. 100 mg of HSA was dissolved in 2.5 ml of 10 mM NaCl. 20 mg of fisetin was then added to the solution and incubated for 4 h at room temperature. After the incubation the pH of the solution was adjusted to 8.5 by addition of NaOH. Ethanol was added dropwise at a constant rate of 1 ml/min under continuous stirring until the solution became turbid. The nanoparticles formed were stabilized by crosslinking with 120 μl of 8% glutaraldehyde for 24 h at room temperature. Finally the nanoparticles were purified by centrifugation at 10,000 rpm for 15 min. The pellet was redispersed in 10 mM NaCl. The redispersion process was accomplished in an ultrasonication bath (Oscar Ultrasonic Cleaner, Microclean-101). HSA nanoparticles were also prepared by a similar technique. Fisetin loaded HSA NPs were lyophilized/freeze dried to get the powdered form. For this, the sample is taken in a round bottomed flask and placed in liquid nitrogen until it froze completely. The flask was then connected to a desktop type freeze dryer (EYELA FDU-1200 freeze dryer) equipped with a refrigerator and a vacuum pump keeping the temperature and pressure at −45 °C and <50 Pa respectively.

2.2.2. Characterization of fisetin loaded HSA nanoparticles

2.2.2.1. Spectroscopic characterization.

2.2.2.1.1. UV–vis Spectrophotometer study. HSA, fisetin, HSA nanoparticles and fisetin loaded HSA nanoparticles were characterized by UV–vis spectroscopy (UV-2450, Shimadzu). UV–vis studies were performed at 25 °C in the range 200–600 nm.

2.2.2.1.2. Fourier transform infrared spectroscopy (FTIR) study. FTIR spectra of fisetin and fisetin loaded HSA nanoparticles were obtained on Nexus-870 FTIR spectrometer (Thermo Nicolet Corporation) equipped with a deuterated triglycine sulphate (DTGS) detector, a KBr beam splitter, zinc selenide (ZnSe) attenuated total reflectance (ATR) accessory at room temperature. The spectra were collected using a 256-scan interferogram. The resolution was 4 cm^{-1} and the scanning range 4000–400 cm^{-1}.

2.2.2.2. Transmission electron microscopy (TEM). The size and shape of the nanoparticles were monitored by transmission electron microscopy (TEM). The stock solution of the nanoparticles was diluted 10 fold. A drop of sample was placed on a carbon coated TEM grid that was then air dried and scanned in a TECNAI G^2 20S-TWIN transmission electron microscope operating at an accelerating voltage of 80 kV.

2.2.2.3. Field emission scanning electron microscopy (FESEM). Field emission scanning electron microscopy (FESEM) was used to determine the morphology of the nanoparticles. A drop of sample was mounted on a glass slide which was allowed to dry in air and then scanned in a Carl Zeiss field emission electron microscope operating at 5 kV.