

## **IN VITRO ANTICANCER ACTIVITY OF 5' FLUOROURACIL COATED CHITOSAN NANOPARTICLE**

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### **ABSTRACT**

**Objective:** 5' Fluorouracil (5'FU) loaded chitosan nanoparticle (5FCN) was synthesized and tested for its anticancer activity against Hep G2, the liver cancer cell line.

**Methods:** The morphological characteristic of the 5FCN was visualized in Scanning Electron Microscope (SEM).

**Results:** The 5FCN revealed an IC<sub>50</sub> value of 49.50 µl/ml. The study revealed that the 5FCN exhibited excellent anticancer activity against Hep G2 cell line which can be further studied for its application in cancer treatment.

**Conclusion:** Further studies would be focused onto report the drug release efficiency under different physiological conditions and evaluate them in animal model.

**Keywords:** 5'Fluorouracil, Chitosan nanoparticle, IC<sub>50</sub> values, SEM, Anticancer activity

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### **INTRODUCTION**

Cancer is a group of diseases which cause an abnormal and uncontrolled cell division along with malignant behavior such as invasion and metastasis. A tumor malignant is a neoplasm characterized by a failure in the regulation of tissue growth. The abnormal and uncontrolled proliferation of tissues is caused by the mutations in genes. Genes include oncogenes, the promoter of cell growth and reproduction, and tumor suppressor gene, the inhibitor of cell division and survival. Changes in multiple genes required to create normal cells into an abnormal cancer cell [1]. The risk factors cancers include behavioral factors like consumption of alcohol, tobacco usage, high fat containing food as well as viral infections, environmental factors and immune system perturbations [2]. It holds highest mortality rate in the world compared to the other diseases mainly due to non-availability of diagnosis at an earlier stage and proper treatment at an advanced stage. Hepatocellular carcinoma (HCC), one of the important cancer type, is considered as the third most common cause of cancer-related death worldwide, in which, it accounts for over half a million deaths per year. The chemo resistance, particularly to adriamycin (ADM), cisplatin and doxorubicin [3] by which it displays high morbidity and mortality rates.

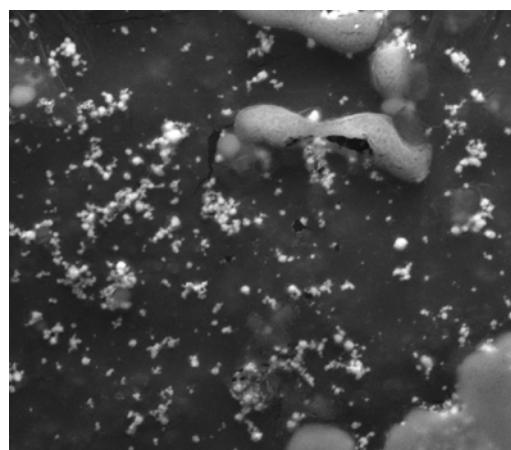
Current cancer therapies, radiation and chemotherapy, have adverse side effects in the cancer individual that lead to the death of the majority of the patients. It was identified the non-specificity of drugs to the target tissues was the main cause of side effects. Numerous works have been streamlined to deliver the chemotherapeutic drug precisely, but the search still existed despite vast drug delivery vehicle. Polymeric nanoparticles may represent the most effective nanocarriers for prolonged drug delivery. The early *in vitro* and *in vivo* development of polymeric nanoparticles loaded with drugs in the 1980s using poly alkyl cyanoacrylate-based nanoparticles releasing doxorubicin [4] led to multiple reports using polymer-based materials for drug delivery. Langer and Folkman [5] identified the first controlled release of macromolecules using polymers, which allowed the development of anti-angiogenic drug delivery systems for cancer therapy and opened new areas for the delivery of macromolecules. In 1994, Langer *et al.* described nanoparticles composed of poly (lactic acid)/poly (lactic-co-glycolic acid) (PLA/PLGA) and PEG block copolymer as "long-circulating nanoparticles" due to their stealth properties [6], leading to an increased interest in polymeric nanoparticles for therapeutic

applications. Two types of polymers can be used in nano delivery which is natural and synthetic. The PNPs are obtained from synthetic polymers, such as poly-ε-caprolactone, polyacrylamide and polyacrylate, polylactic acid or natural polymers, e. g., albumin, DNA, chitosan, gelatin, pectin. Hence in the present investigation, chitosan nanoparticles were used for the delivery of 5' Fluorouracil (5-FU) for the efficient anticancer activity against Hep G2 cell line.

### **MATERIALS AND METHODS**

#### **Preparation of 5 fluorouracil loaded chitosan nanoparticles**

5FU loaded chitosan nanoparticles were prepared by using ionic gelation method. Chitosan was dissolved in glacial acetic acid 1% [v/v], 5 mg of 5 Fluorouracil was added to the above solution and under constant magnetic stirring followed by addition of aqueous TPP solution in a dropwise manner, then the solution was kept on constant stirring for 30 min. The nanoparticle suspension was centrifuged at 13,000 rpm and 4 °C for 30 min using a centrifuge to remove excessive amounts of TPP and unencapsulated 5 Fluorouracil. The pellets were dispersed in deionized water. The structure of the 5FCN was observed in SEM (fig. 1).



**Fig. 1:**

## Cytotoxic assay

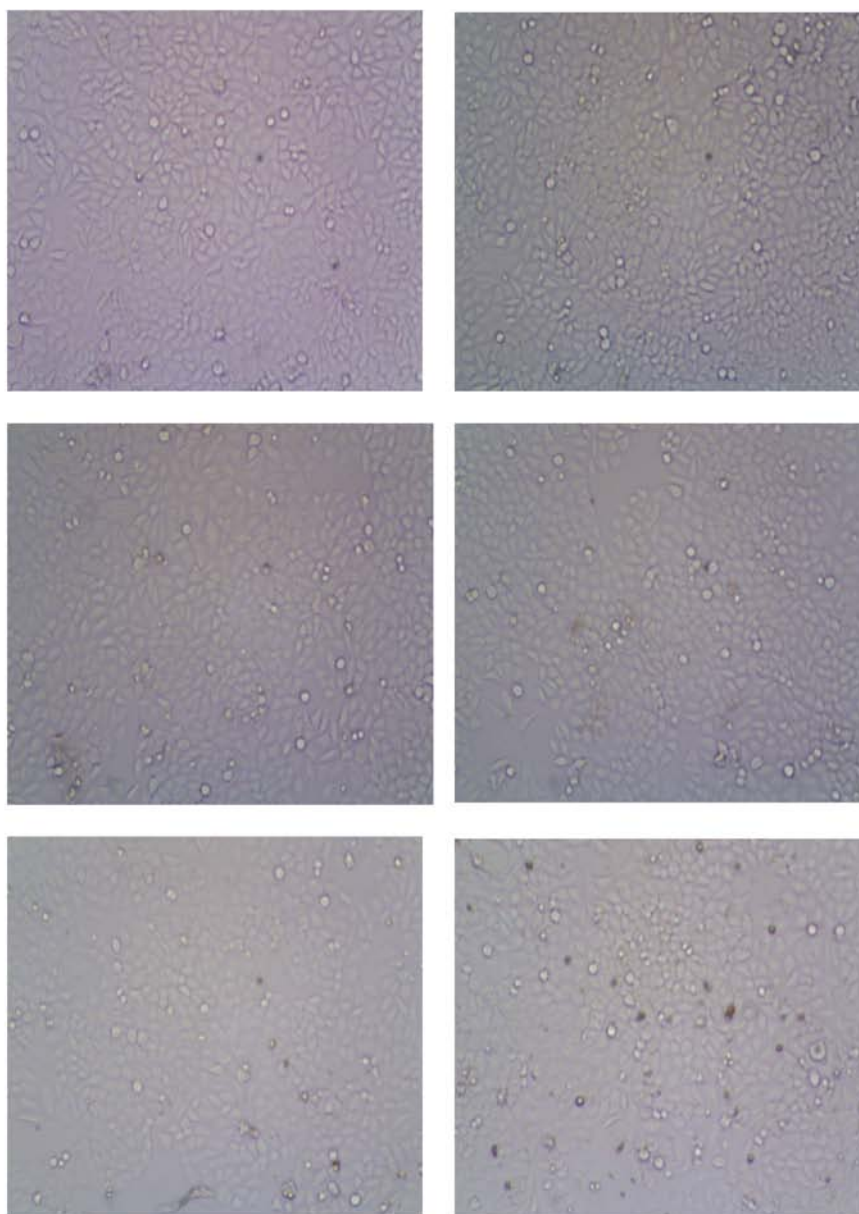
### Cell culture

Human Hep G2, the Liver cancer cell lines used in this study were procured from National Centre for Cell Science, Pune. All cells were grown in Minimal essential medium supplemented with 4.5 g/l glucose, 2 mmol L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37 °C in 5% CO<sub>2</sub> incubator. MTT assay was performed by using the assay developed by Mosmann [7].

### RESULTS AND DISCUSSION

5'Fluorouracil loaded chitosan nanoparticle (5FCN) was synthesized in the present study and was examined for its anticancer activity against Hep G2 (Liver cancer cell line). The structure of the chitosan

nanoparticles was observed from the SEM analysis and is depicted in fig. 1. The chitosan nanoparticles loaded with 5' FU exhibited excellent anticancer against Hep G2 cell line. The IC<sub>50</sub> value of (41.52µg/ml) (fig. 2) the 5FCN was low compared to the chitosan oligosaccharide loaded with ATP (154.8µg/ml) against Hep G2 cell lines [8]. Elkholi *et al.* [3] used trimethyl chitosan nanoparticles against Hep G2 cell lines and its IC<sub>50</sub> value was fivefold increased in concentration compared to the IC<sub>50</sub> of the present study. Anitha *et al.* [9-10] have tested 5FCN and 5FU-N,O, carboxymethyl chitosan nanoparticles against colon cancer cell line (HT-29) and found more than 80 and 75% dead cells respectively. Besides that, they also reported the hemocompatibility of chitosan nanoparticles for the delivery of the drug. Similarly, other nanoparticles such as hydroxyapatite and titanium dioxide showed IC<sub>50</sub> value of 49.02µg/ml and 60µg/ml respectively against.



**Fig. 2: Cytotoxic activity of chitosan nanoparticles added with 5 Fluro uracil against Hep G2, the Liver cancer cell line. a: Control; b-f: Hep G2 Liver cancer cell line treated with (3.125, 6.25, 12.5,25 and 50µg) different concentrations of chitosan nanoparticle**

Hep G2 cell line. Benito-Miguel *et al.* [11] also reported the similar result against HeLa cells. A similar result was also observed by Nivethaa *et al.* [12] who reported excellent anticancer activity of 5FU encapsulated Chitosan/silver/multiwalled carbon nanotube against

MCF-7 cell line and found an IC<sub>50</sub> value of 50µg/ml. Rajan *et al.* [13] supported the role of chitosan as a potential carrier of enzymes which in turn support the present study in which 5FU delivery by chitosan was evidenced. David *et al.* [14] reported 45% reduction in

viability of pancreatic cancer cell line treated with 5FCN. Chen and Gong [15] reported the reduction in growth (62.05%) of H22 hepatoma solid tumor by 5FU-poly-ethylene glycol monomethyl ether (mPEG) nanoparticles. Rejinold *et al.* [16] have also supported the chitosan-g-poly(N-vinyl caprolactam) as a potential carrier for the delivery of 5FU, which exhibited cytotoxic activity against MCF-7, KB and PC3, the breast, oral and prostate cancer cell line respectively. Martino *et al.* [17], for instances, used an alternate polysaccharide based nano complex for the 5FU delivery. Bwatanglang *et al.* [18] noted the similar impact by 5FU loaded folic acid-chitosan nanoparticles-Mn-Zns conjugates against breast cancer cell line. Hence it was observed from the present investigation that, the cytotoxic activity of 5-FU loaded chitosan nanoparticles provided a platform for treating cancer with biopolymer nanomaterials. Chitosan is safe to use as a carrier for the drug 5-FU, which was observed from the present investigation. This could contribute to the discovery of a new method for the treatment of liver cancer that may overcome the difficulties in the currently available procedures or therapies. Further studies would be focused onto report the drug release efficiency under different physiological conditions and evaluate them in an animal model.

#### CONFLICT OF INTERESTS

Declare none

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