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Iota Carrageenan, A Sustainable Polymer Transformed Into A Nanogel Formulation, By Using Environment Friendly Method For Antitumor Action

Akanksha Bhatt[1], Surbhi Panwar[2], Priyank Purohit[3]*

Abstract

In the pharmaceutical field, the utilization of polymers, both in their natural form and through chemical modifications, holds immense importance. In this context, a novel and eco-friendly method for the synthesis of nano carrageenan-based polymers has been developed. The efficacy of carrageenan in this domain is attributed to its crosslinking and biological properties, which are largely influenced by its sulfate ion content. This study aims to formulate nanogels via a greener approach, eliminating the need for synthetic crosslinking agents, with the goal of specifically targeting tumor cells. The synthesis process involves heating carrageenan in water, followed by the gradual addition of ethanol dropwise, with continuous stirring for up to 3 hours. Later, initial in silico studies indicated strong selectivity towards tumor cells, which was further validated through in vitro cell line experiments. In vitro studies demonstrated the significant selectivity of the carrageenan nanogel towards cancer cells, suggesting its potential for targeted cancer therapy. This novel, green synthesis method not only offers an eco-friendly alternative to conventional practices but also highlights the promising potential of carrageenan nanogels in cancer-targeting applications.

Keywords: Carrageenan, antitumour agent, crosslinking agent, greener method, cell line studies

INTRODCTION

The increased use of synthetic polymers, as well as the improper disposal of medical and pharmaceutical waste, has raised environmental concerns. The environmentally friendly method is a concern for all chemists, especially for heterocyclic synthesis; in addition, polymer functionalization/grafting is also a challenge [1-2]. An environment-free method will have a long-term positive impact on the environment by reducing the negative effect of the process. This approach aims to make the use of chemicals, materials, and energy more sustainable than in previous generations. Greener chemistry promotes environmentally friendly chemical techniques and products, resulting in more cost-effective and innovative solutions while reducing pollution. Additionally, by modifying and grafting polymers, their properties can be enhanced [3]. Greener chemistry is the application of principles (figure 1) that promote the use of natural products while eliminating hazardous chemicals from the design and manufacturing of final products. A key principle is the use of solvent-free chemical processes, as well as benign chemicals and solvents that are both environmentally friendly and cost-effective in the long run [4].

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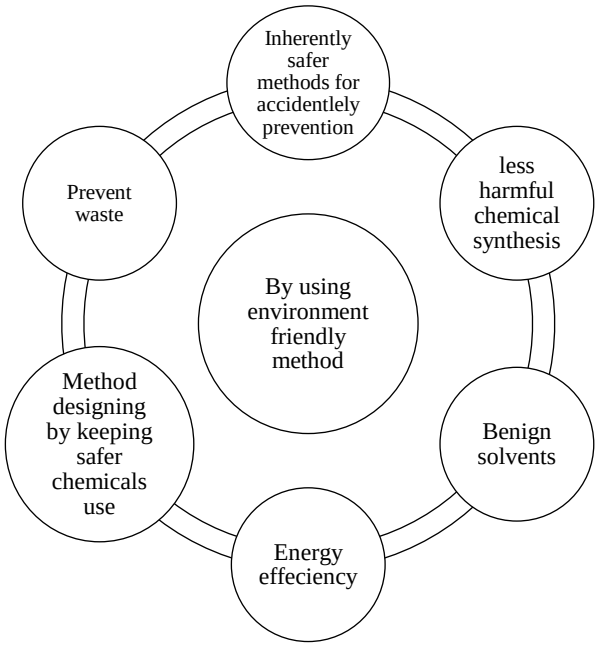

Figure 1. Fundamentals for a sustainable method for drug delivery.

The use of nanotechnology is a cutting-edge field that offers many advantages in the form of nanocrystals[5-6]. Due to their nanoscale size, nanoparticles offer several significant advantages in biomedical applications. One of the key benefits is their ability to facilitate targeted drug delivery, ensuring that therapeutic agents are delivered directly to diseased tissues or cells, minimizing the impact on healthy tissues. This targeted approach enhances the efficacy of the treatment while reducing side effects and toxicity, making it particularly useful in cancer therapies. Additionally, nanoparticles can significantly increase bioavailability, allowing drugs that are poorly soluble or unstable in biological environments to be effectively absorbed and utilized by the body. The small size of nanoparticles also enables them to cross biological barriers, such as the blood–brain barrier or cellular membranes, which are often difficult to penetrate with conventional drugs. However, conventional nanoparticle preparation methods have had a negative impact on the environment because of the use of various chemicals, heat, and solvents. To address these concerns, greener nanoparticle development methods are being investigated to achieve their intended goals (Figure 2) more sustainably. [7-8].

According to research, the size of any formulation influences the release behaviour of the polymer, as does the polymer's functionalization with groups, and changes with the linked ions, as our research group demonstrated the importance of altering the carrageenan hydrogel, which is a widely used polymer for the drug and food industries [9-11]. Carrageenan, an anionic water-soluble gum derived from red seaweed, was used in this study because of its high water affinity, making it ideal for hydrogel formation. Carrageenan's properties, including moisture management, gas barrier effects, biodegradability, and antimicrobial activity, help promote sustainable development through decreasing environmental impact and encouraging environmentally friendly practices [12].

Carrageenan (CGN) is a heterogeneous mixture of sulfated polygalactans with 15–40% ester-sulfate content, which makes it an anionic polysaccharide [13]. It can be prepared from various other species, such as *Gigartina, Hypnea, and Furcellaria.* The main source is *Chondrus crispus.* Three types of carrageenan have been identified that differ in their total sulfate content, distribution and molecular configuration. Kappa (κ) carrageenan forms a linear carbohydrate chain of α-1,3--linked units of carrabiose with alternating sequences of D-galactose 4-sulfate or 3,6-anhydro-D-galactose units. Iota (ι) carrageenan has the same structure as kappa carrageenan with an additional half-sulfate ester group, whereas lambda (λ) carrageenan has varying compositions with equal proportions of 1,3 linked and 1,4

linked D-galactose units. The carrageenan in aqueous solution shows some similar properties, such as a thermoreversible conformational arrangement at high temperature. Both forms also display gelling properties, which are dependent on the concentration and nature of the cations and the presence of 3-6 anhydro-bridges. The gel passes through a random coil to form a helix through a heating and cooling process; moreover, the gel formation depends on the attached cation and its interaction with the sulfate ions [14-15].

Carrageenan (CGN), a sulfated polysaccharide derived from red seaweed, has shown potential as an antimetastatic agent because of its ability to interfere with these processes. The CGN hinders cancer metastasis by disrupting the interaction between cancer cells and ECM proteins. By binding to integrins or modifying the ECM structure, CGN can reduce the adhesive capabilities of cancer cells, thereby impeding their ability to migrate and invade new tissues. Furthermore, CGN may influence downstream signalling pathways that are critical for cancer cell movementfor example, by blocking integrin-ECM interactions, CGN can suppress the activation of the PI3K/Akt, MAPK/ERK, and FAK/Src pathways, which are crucial for promoting cellular motility, survival, and invasion. As a result, this disruption could reduce cancer cell migration, limit their invasiveness, and ultimately prevent the formation of metastases. Given this mechanism, CGN holds promise as a potential therapeutic agent for preventing cancer metastasis. By targeting the cancer cell-ECM interactions that are essential for tumor spread, CGN could be developed as an adjunct therapy to conventional cancer treatments, potentially reducing the risk of tumor recurrence and improving patient outcomes [16-18].

The other mechanisms through which carrageenan has anticancer activity include the induction of apoptosis via intrinsic and extrinsic pathways and cell cycle arrest. In addition to binding to TLR-4, CGN activates nuclear factor-kappa B (NF-κB), a transcription factor that is central to regulating immune and inflammatory responses. NF-κB is normally found in an inactive state in the cytoplasm and is bound to the inhibitory protein IκB. However, the activation of TLR-4 leads to the phosphorylation and degradation of IκB, which releases NF-κB and allows it to translocate into the nucleus. Once inside the nucleus, NF-κB promotes the transcription of a wide range of proinflammatory cytokines, chemokines, and other immune-related genes [19-20]. Carrageenan has also been shown to be selective for various attached ions because of the different dissociation energies; among the various attached ions, the barium-linked ions were more selective than the parent carrageenan [21]. Compounds with different functional groups and ions have been utilized, but for the first time, the gel used as an API (an active pharmaceutical ingredient) with anticancer activity is represented by a validated protocol as well as an explanation. The carrageenan and its nano conversion through green protocol has been utilized here in for the evealution of prostate cancer activity through cell assay *in-vitro* test. Carrageenan and its nanoformulation, synthesized using an environmentally friendly green protocol, have been explored in this study for their potential in prostate cancer targeting. The nanoformulation was evaluated for its anticancer activity through *in-vitro* cell assays, aiming to assess its efficacy, selectivity, and biocompatibility in prostate cancer cells. This approach highlights the utility of carrageenan-based nanomaterials in cancer research, emphasizing sustainable methods in their development.

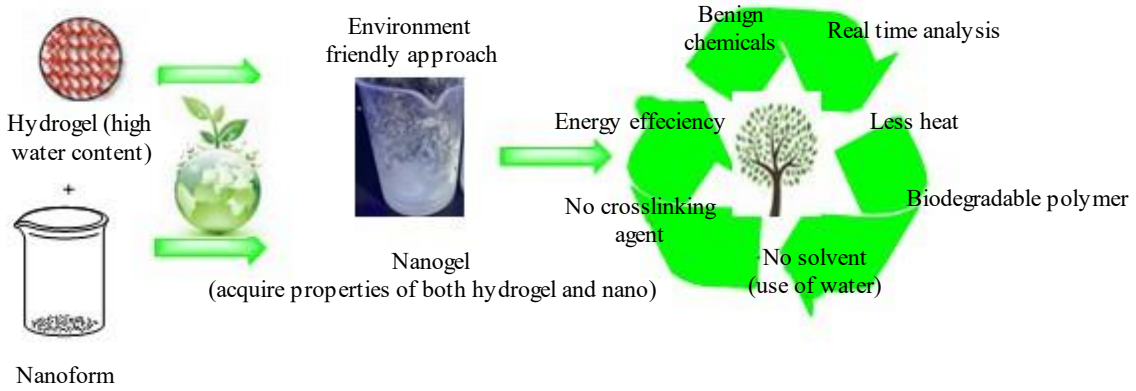

Figure 2. Overview of the benefits of using green chemistry.

MATERIAL AND METHODS

Iota carragennan was purchased from Marine Hydrocolloids (cochins) along with standard operating procedures. The solvents of regent grade were purchased from Merck Aldrich, and the glassware and instruments were validated before use.

Preparation of Simple Gels and Nanogels

Water was used as the solvent, and heat was used to keep the solution in its original form by adding carrageenan to create a uniform gel. For the nanogel, a desolvation agent, ethanol, was added dropwise to reduce the gel size while continuously stirring until a homogeneous mixture was achieved, which took approximately 3 hours. The desolvation agent was omitted from the simple gel formulation as it is depicted in the below Figure 3. The carrageenan favours a lower energy state that promotes gel formation with a helical conformation. Heat converts it from this lower energy state to a higher energy state, keeping it in solution (an unfavourable condition). Conformation is important in gels because it controls interactions and three-dimensional structures within the gel. The validated protocol for the cytotoxicity of PC3 (Prostate Cancer) was subsequently developed. LLC-MK2 (normal cells) was used to calculate the cell viability (%) = average number of cells/average number of cells in control wells. The IC_{50} values were subsequently calculated via Prism 10 [21].

Conductivity

A Hanna Instruments EC 215 conductivity meter was used to determine the conductivity of both the sample and the nanogel, and a conductivity meter calibrated with distilled water solution was used.

Antitumour action

In silico studies

Computational analysis was performed via the DeMap portal of the CCLE cancer cell line encyclopedia, which also supports the expression of the TLR4 synthase gene in the PC3 cell line, which is known for prostate cancer.

RESULTS AND DISCUSSION

The simple gel and nanogel were formed via the desolvation method, where conformational changes are crucial for the state transitions. Heat was applied to maintain the water temperature and ensure that the system remained in solution form, leading to the formation of a homogenous gel. However, this heating step poses formulation challenges, as it increases the system's energy state, promoting network formation within the gel. Ethanol was used for desolvating the nanogel, which involves a transition from a helical to a coiled conformation (Figure 4).

Without heat, carrageenan tends to form a gel with water prematurely, preventing the formation of a true solution and thus hindering the formation of a homogenous gel, unlike when hot water is used. (Figure 5)

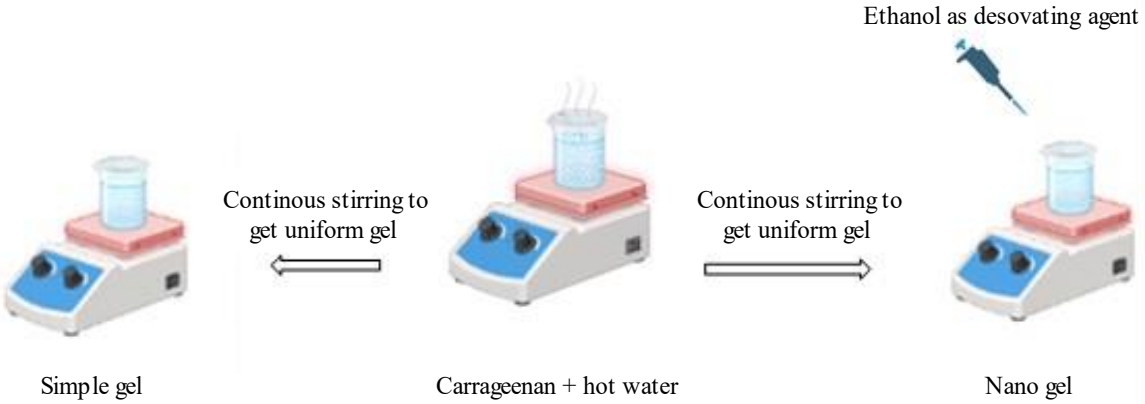

Figure 3. Overview of simple carrageenan and nanogel formulations.

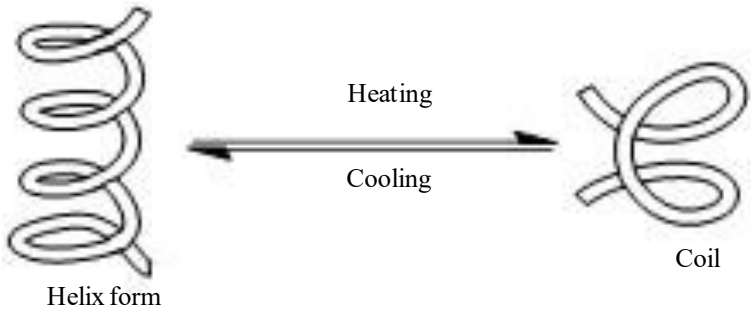

Figure 4. Helix to Coil through heating and coil to helix to cool.

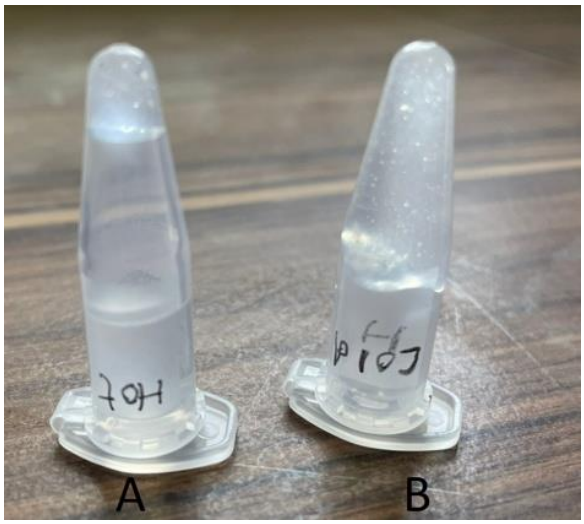

Figure 5. Carrageenan in hot and cold water.

By using SEM, the gel was examined to obtain images of particle size with verified lines given with scales in each image. The particle size was further studied and measured through line and circle areas via ImageJ software. The mean final data were taken in this case, and particles come under the nanorange compared with its powder form. The particle means were determined to be approximately 160 nm, indicating that the gel network is in the nano gel (Table 1). The conductivities of both gels were measured, and the results revealed that the nanogel had greater conductivity (0.64 million) than did the simple gel (0.01). Owing to the higher surface-to-volume ratio, more interactions with ions in solution increase the overall conductivity, and the smaller size facilitates ionic movement within the gel network and allows ions to move within.

Table 1. Particle size distribution inside the nano gel.

	Label	Mean	StdDev	Min	Max	Angle	Circ.	Length
1		222.039	11.457	200.037	250.227	-79.592	0.258	138.375
2		211.59	14.233	184	239.406	145.125	0.318	111.724
3		244.752	6.173	222	253.449	132.879	0.222	159.193
4		196.602	13.123	174.206	221.942	141.009	0.482	75.044
5		193.265	16.907	141	222.5	97.125	0.263	134.358
6		185.46	18.649	163.461	246	-163.61	0.36	98.435
7		175.88	18.417	128	204	90	0.545	66.66
8	Mean	204.227	14.137	173.243	233.932	51.848	0.35	111.97
9	SD	23.621	4.435	32.645	18.223	122.76	0.122	34.225
10	Min	175.88	6.173	128	204	-163.61	0.222	66.66
11	Max	244.752	18.649	222	253.449	145.125	0.545	159.193

In silico studies were performed using depmap to determine the selectivity of carrageenan towards the PC-3 cell line. Prostate cancer is a major contributor to global morbidity and mortality rates. (Figure 6) The prostate gland consists of various cell types, including luminal epithelial cells, which play a key role in its physiology. These luminal cells highly express androgen receptors and differentiation markers such as cytokeratin 8 and prostate-specific antigen. The other cells are basal cells expressing lower levels of androgen receptors (ARs) and neuroendocrine cells. [22-23] Additionally, prostate glands have double-layered, glandular, pseudostratified cells. The top layer, called the luminal layer, consists of receptors that respond to estrogen and androgen, whereas the bottom layer, known as the basal or myoepithelial layer, consists of cells that express a protein called p63. This protein is encoded by the TP63 gene and has an important role in controlling various pathways needed for the growth and development of ectoderm tissues.

In the literature, carrageenan has been reported to have good antitumour activity and was found to be a potent anticancer agent (Table 2), which was proven via *in silico* studies via computerized techniques such as the DepMap portal and the cancer cell line encyclopaedia shown in Fig. 6, showing the dependency of TLR4 on PC3.

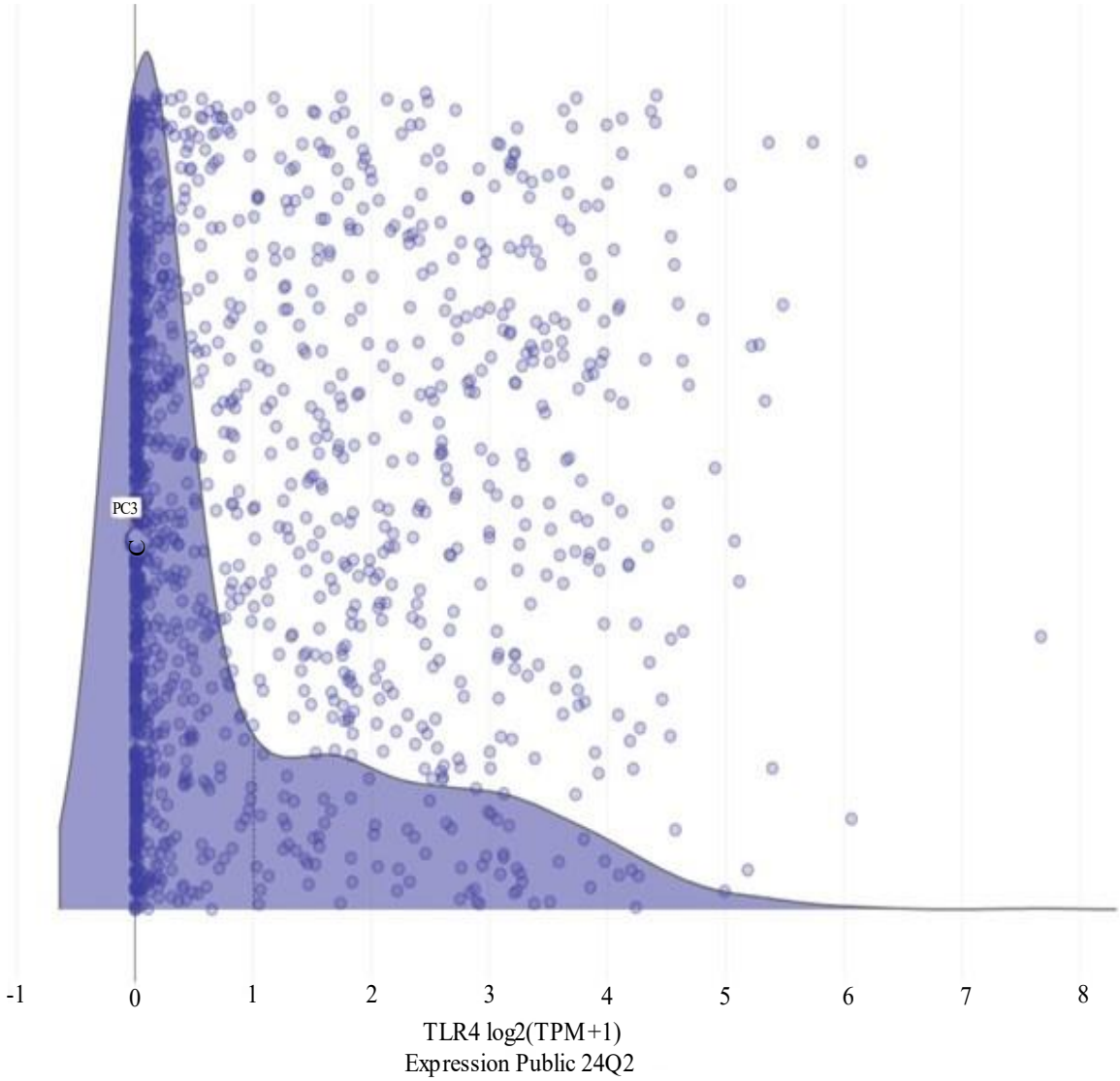

Figure 6. The selectivity of PC3 with respect to TLR4 gene expression (carrageenan) was found to be greater initially than that of the other strains via DepMap software under CCLE.

Table 2. Percent inhibition of the nanogel PC3 and LLC-MK2 cell lines.

PC3 Cell Line						
Conc.	**Run1**	**Run2**	**Run3**	**Mean**	**% Viability**	**% Inhibition**
0	1.13	1.13	1.13	1.13	100	0
6.25	1.021	1.021	1.021	1.021	90.37	9.63
12.5	0.82	0.82	0.82	0.82	72.39	27.61
25	0.55	0.55	0.55	0.55	48.65	51.35
50	0.323	0.323	0.323	0.323	28.59	71.41
100	0.092	0.092	0.092	0.092	8.2	91.8
LLC-MK2 (normal cell)						
ref	**Run1**	**Run2**	**Run3**	**mean**	**% viability**	**% inhibition**
0	0.764	0.766	0.765	0.765	100	0
6.25	0.679	0.679	0.682	0.68	88.97	11.03
12.5	0.639	0.637	0.641	0.639	83.53	16.47
25	0.615	0.615	0.615	0.615	80.44	19.56
50	0.569	0.566	0.566	0.567	74.15	25.85
100	0.548	0.544	0.546	0.546	71.38	28.62

The in vitro biological activity of both simple and nanogel formulations was assessed via the IC_{50} value via the GraphPad Prism online tool, specifically for the PC3 prostate cancer cell line and the LLC-MK2 healthy cell line (Table 1). [24-25] The results demonstrated that carrageenan has significant greater antitumour activity towards PC3 cancer cells than does VEL. These findings indicate the markedly increased potency of the nanogel in targeting and inhibiting cancer cell proliferation. The enhanced activity of the nanogel can be attributed to its increased surface area and more effective cellular uptake, which allows a higher concentration of the therapeutic agent to act directly on the cancer cells. In contrast, the selectivity of the nanogel towards LLC-MK2 healthy cells was approximately 28 times greater (Figure 7) than that of the simple gel.

These findings suggest that the nanogel remains highly effective against cancer cells. The substantial difference in activity indicates that the nanogel formulation is less likely to interfere with healthy cells, thereby enhancing its potential as a safer therapeutic option. [24-25]. Overall, these findings highlight the superior efficacy and selectivity of the nanogel formulation over the simple gel. The nanogel not only demonstrates a more potent antitumour effect but also shows a greater degree of selectivity, reducing the potential for collateral damage to healthy tissues. This makes the nanogel a promising candidate for targeted cancer therapy with minimal side effects; moreover, the regulatory agencies, will align the current protocol because of the absence of toxic crosslinking agents and solvent, moreover the use of a biorelevant polymer withr minimizing patient risk and environmental impact [26-27].

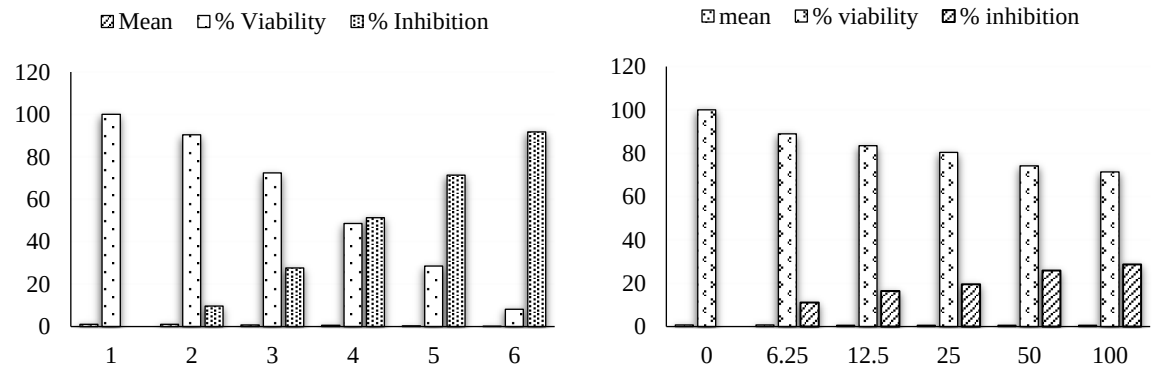

Figure 7. % inhibition and % viability of the gel in PC3 (left) and LLC-MK2 (normal) cells.

CONCLUSION

Cancer is a critical global issue and continues to have a profound impact on the ongoing health challenges faced worldwide. It is a heterogeneous disease influenced by a wide number of components; thus, its diverse intricate pathology makes its prevention, diagnosis, treatment and survival challenging. Globally, prostate cancer remains a key challenge for healthcare systems, highlighting the need for improved diagnostic tools, more effective therapies, and greater awareness for early detection and prevention strategies to reduce its impact on men's health. Nanogels prepared from carrageenan offer several benefits over simple gels in terms of conductivity and fluorescence activity because of their nanosized particles. In terms of cancer action, nanogel formulations outperform simple carrageenan gels. Nanogels perform better because of their smaller size and more effective interaction with cancer cells, leading to better drug delivery and therapeutic outcomes. This highlights the potential of nanogels as a more advanced and efficient approach to targeted cancer treatment than traditional carrageenan gel formulations. The future of nanogels in prostate cancer therapy is very promising. They are capable of delivering therapeutic agents in a targeted and controlled manner, can improve imaging and diagnostics, and combination therapies make them important tools in the fight against prostate cancer.

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Conflict of Interest

None

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