Research Article

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Green formulation of copper nanoparticles by *Pistacia khinjuk* leaf aqueous extract: Introducing a novel chemotherapeutic drug for the treatment of prostate cancer

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Abstract: Prostate cancer is a cancer prevalent form characterized by serious clinical symptoms. The discovery of a novel supplement or medication for prostate cancer within the realm of chemotherapeutic drugs holds significant value. Our research involved the synthesis of copper nanoparticles (CuNPs) infused with *Pistacia khinjuk* leaf extract to explore their potential anti-prostate cancer, cytotoxic, and antioxidant properties. The green-formulated CuNPs were characterized by TEM, UV-Vis spectrophotometry, FE-SEM, FTIR. The FE-SEM and TEM findings prove the spherical structure with a size of 10–70 nm. The DPPH assay was carried out to examine the effectiveness of antioxidants. CuNPs exhibited the ability to inhibit 50% of DPPH at 215 µg/mL. To assess the anti-prostate cancer properties of CuNPs, an MTT assay was performed on NCI-H660, DU 145, LNCaP clone FGC, and LNCaP clone FGC-Luc2 cells. CuNPs exhibited remarkable efficacy in inhibiting prostate cancer growth, while demonstrating negligible toxicity towards normal cells. The LNCaP clone FGC cell line displayed the most potent anti-prostate cancer effects of CuNPs. IC_{50} of CuNPs was 322, 365, 247, and 273 on NCI-H660, DU 145, LNCaP clone FGC, and LNCaP clone FGC-Luc2 prostate cancer cells. CuNPs containing *Pistacia khinjuk* were found to possess noteworthy anti-prostate carcinoma characteristics and presented excellent antioxidant potentials, as highlighted by this study.

Keywords: copper nanoparticles, cytotoxicity, *Pistacia khinjuk*, prostate cancer

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1 Introduction

Cancer is a special statute characterized by the tissues uncontrolled growth, which arises due to the imbalance between apoptosis and cell division. This imbalance is caused by many intricate factors [1]. The control mechanisms of body are not effectively responded to by carcinoma cells. The proteins of the cell cycle and signaling pathways regulation are altered, causing disruption. As a result, these cells inhibit abnormal needs and behaviors, requiring different treatment methods [2]. This statute is a genetic occurrence compilation and influences linked to one's lifestyle and surroundings [3]. It is a significant contributor to global mortality [4–8]. Despite extensive studies dedicated to understanding cancer, it remains a notable health discussion for the global population. Research in the field of cancer treatment focuses on studying natural origin agents like plant compounds and herbal nanoparticles (NPs) because of the significant drug resistance and side effects associated with chemical drugs commonly used in treatment [9,10]. Hence, it is crucial to endeavor in the exploration of plant compounds and plant NPs [8–10] in order to unveil more effective medications that exhibit reduced resistance and side effects.

Pistacia is from the family *Anacardiaceae* and includes 11 species worldwide. There are many plants of this genus, some of them have therapeutic effects and others have pleasant and edible fruits. *Pistacia Vera* has a large amount of starch and some sucrose in the *Pistacia* kernel. [11] *Pistacia Khinjuk* is a short shrub with a height of 1–3 m, two legs, single comb compound leaves, egg-shaped leaflets, purple-red flowers, compound cluster inflorescences, and small shaft fruits [11–13]. Studies have shown that *Pistacia*

khinjuk resin is used to treat indigestion, toothache, and as an astringent effect in traditional medicine, and *Pistacia khinjuk* fruit is also consumed orally [12]. The aerial part of *Pistacia khinjuk* contains flavonoid glycosides including myrstin-3-glycoside, myrstin-3 retinoside, and myrstin-3 glycoside [13]. Also, myrstin derivatives include 20% of the total polyphenol content of *Pistacia lentiscus* leaves [14,15]. Tohidi reported that the *Pistacia* leaves and gum essential oil have good antioxidant and antifungal activity [16]. In recent times, scientists have directed their attention towards the eco-friendly synthesis of NPs using plants owing to their significant healing properties [17–21].

NPs typically range from 1 to 100 nm [20–23]. The field of nanotechnology is expanding, allowing for precise control over the NPs production and manipulation with diverse distributions, shapes, and sizes. There are numerous hazards for the environment posed by chemical and physical methods, and they can be quite costly. Conversely, using biological methods through plant extracts is both safe and cost-effective [24–27]. Producing NPs through biological means significantly reduces the potential risks to humans [24,25,28]. Copper has historically attracted attention as a disinfectant; however, its effectiveness has been restricted by its low bactericidal characteristics and the emergence of antibacterial substances and antibiotics, leading to limitations in its usage [29–32]. Nevertheless, the advancement in copper nanoparticles (CuNPs) production has caused the flourishing use of copper as potent bactericidal NPs. CuNPs derived from medicinal plants have been effectively utilized in treating various diseases, including different forms of cancer [31–33]. Prior investigations have suggested that using CuNPs leads to the elimination of cancer cells through the elevation of cells' reactive oxygen species levels [24–27,34]. Also, separate research has highlighted the involvement of CuNPs in permeating the membrane of carcinoma cells, resulting in their demise [29–32].

Secondary plant metabolites are organic compounds not directly involved in plant growth and reproduction. Unlike the primary metabolite, these compounds have a limited distribution in the plant kingdom. Alkaloids, terpenoids, and flavonoids are secondary metabolites that have anticancer effects [35]. Current report was conducted to evaluate the cytotoxicity of CuNPs containing *Pistacia khinjuk* on NCI-H660, DU 145, LNCaP clone FGC, and LNCaP clone FGC-Luc2 prostate cancer cells because of the presence of flavonoid glycosides and polyphenol compounds in the *Pistacia khinjuk* leaves and the study lacks the anti-carcinoma efficacies of *Pistacia Khinjuk* green-formulated CuNPs on prostate carcinoma cells.

2 Experimental method

2.1 Materials

The chemicals for the green formulation of CuNPs were acquired from Sigma Aldrich.

2.2 Preparation of NPs

The *Pistacia Khinjuk* leaf was collected and then was subjected to a drying procedure for 15 days at a temperature of 25°C. Before the extraction procedure, the dried materials were converted into smaller fragments with dimensions of 2–4 mm. The extraction was carried out using the percolation (soaking) technique, wherein 0.1 kg plant powder were mixed with 0.8 L of water. After being kept at 25°C for 2 days, the sample was filtered using Whatman filter paper. The extraction process was performed four times, and the entire organic solvent was removed through a rotary evaporator, resulting in the production of crude solid extracts. To ensure the complete elimination of the solvent, a freeze-drying method was then employed.

Following the cooling and filtration of the aqueous extract obtained from *Pistacia Khinjuk* leaf, 40 mL of the extract was combined with 40 mL of $Cu(NO_3)_2{\cdot}3H_2O$ (0.25 M). The resulting mixture was then subjected to reflux for 16 h at 75°C. Consequently, the formation of NPs (Cu@*Pistacia Khinjuk*) occurred, leading to the generation of dark brown precipitates. Following this, the NPs were separated and subjected to four cycles of rinsing with deionized water. The rinsing procedure included periodic centrifugation at a velocity of 11,000 rpm for 8 min. State-of-the-art physicochemical methods like FE-SEM, UV-Vis, TEM, and FT-IR analysis were utilized to analyze the CuNPs synthesized using *Pistacia Khinjuk* extract.

FE-SEM was utilized for the examination of the structure and makeup of CuNPs. The CuNPs size distribution was depicted in a histogram through using imageJ software.

In order to verify the production of CuNPs, a spectrophotometer with a wavelength range of 200–600 nm was utilized. To demonstrate the existence of CuNPs, the synthesized NPs absorption spectrum was generated using the Perkin Emer model 45 UV/Visible-Lambda spectrophotometer from the USA. Multiple samples were extracted from the treated solution at various intervals, and just prior to drying, the sample absorbance was measured within the range of 300–350 nm. The absorption spectrum exhibits fluctuations depending on the morphology of the NPs, which in turn is

associated with the Cu element. This particular range of wavelength is directly linked to these factors.

The identification of the various functional groups accountable for the stabilization and reduction of the formulated CuNPs was accomplished through FT-IR analysis, utilizing the Cary 630 FT-IR model from Tokyo, Japan. The FT-IR analysis was conducted using the KBr technique, where a slice containing 0.3 g of formulated CuNPs mixed with KBr was created under high pressure.

2.3 Anti-prostate carcinoma effects

Synthesized CuNPs' cytotoxic properties on HUVEC (Normal cell), DU 145, NCI-H660, LNCaP clone FGC, and LNCaP clone FGC-Luc2 (prostate cancer cells) were assessed.

In order to assess the NPs' influence on cell morphology, the first stage consisted of placing 100 µL of NPs into 96-well plates. Following this, the cells were exposed to different concentrations of NPs every 72 h. The OLYMPUS microscope was employed to evaluate potential changes in cell morphology after treatment with NPs, in contrast to the untreated cells control group. The growth and viability of cells were assessed using the trypan blue dye exclusion examination and a hemocytometer slide. Afterward, the cells were grown at 10^6 cells per well. Subsequent to this, the initial plate showed no growth after a day, whereas the second plate was exposed to around 1,000 µg/mL of methanol as a control for the solvent. Different doses of NPs were introduced to the rest of the plates, and each dosage was replicated three times. The dishes were subsequently transferred to an incubator adjusted to 95% oxygen, 90% humidity, 37°C, and 5% CO_2 for 24 h. Subsequently, the dishes were analyzed to determine the IC_{50} value. Throughout a span of three days, MTT solution quantities were added and allowed to incubate for 8 h. Afterward, the absorption at 570 nm was quantified using a Biotech ELISA reader produced in Germany [36].

$$\text{Cell viability}(\%) = \frac{\text{Sample A}}{\text{ControlA}}.$$

2.4 Antioxidant effects

To evaluate the antioxidant capacity, the DPPH test was utilized, with the color alteration being quantified using spectrophotometry. A mixture of 1 mL of 1 mM DPPH and 1 mL of NPs at varying concentrations was prepared, thoroughly mixed, and then incubated at 25°C for 0.5 h in the absence of light. Next the measurement of the mixture's absorbance at a wavelength of 517 nm was conducted using blank methanol and DPPH solution. Subsequently, the inhibition of free radicals was determined by applying the following formula [36]:

$$\text{DPPH inhibition}(\%) = 100 \times (\text{A0} - \text{As})/\text{AO}.$$

2.5 Statistical analysis

The normality of the data was first evaluated with Minitab-21. Following this, any data that were not normal was adjusted to achieve normality. Data variance analysis was then carried out using SPSS-22, with visual representations being generated using Excel.

3 Results and discussion

Nanotechnology is currently the focus of significant interest. Extensive research has been carried out in medicine due to the pharmacological characteristics of NPs [27,29,30,34]. Over the past few decades, NPs have been effectively evaluated for their antioxidant properties, demonstrating the capacity to enhance cellular antioxidant activity and decrease oxidative stress with heightened sensitivity. Various types of NPs have been examined for covalent binding or antioxidants encapsulation. Research on this topic is ongoing [26,27,34]. Enhancing the antioxidant capacity of antioxidants can be achieved through encapsulation in nanocarriers or attachment to the nanomaterials surface. It is essential to develop innovative methods for transforming nano-antioxidants and designing modern antioxidant efficacy assays to ensure precise and dependable measurements [29–32]. Hence, an improved comprehension of nanotechnology, nanostructures, novel molecules, and their distinct blend with enhanced antioxidant properties, is poised to revolutionize future therapies utilizing nano-antioxidants [28,37,38].

During the latest examination, an FTIR was followed to evaluate the active and reducing Cu ions groups in 400–4,000 cm^{-1} (Figure 1). The spectrogram illustrates the bioactive potential of *Pistacia khinjuk* leaf in reducing copper ions. The study conducted by Zangeneh et al. [17] reveals that there are distinct peaks observed in the vibration wavelengths of 3,405, 2,912, 1,091, 613, and 566. These peaks correspond to hydroxyl, carbon dioxide, carbonyl, alkene, and alkyl groups, respectively.

During the CuNPs green synthesis, the transition from a light-yellow hue to a rich brown shade in the solution signifies the surface plasmon resonance intensification in CuNPs. Based on the data obtained from UV-Vis, the analysis reported the peak absorption at approximately 338 nm for the NPs that were formulated. The absorption peak

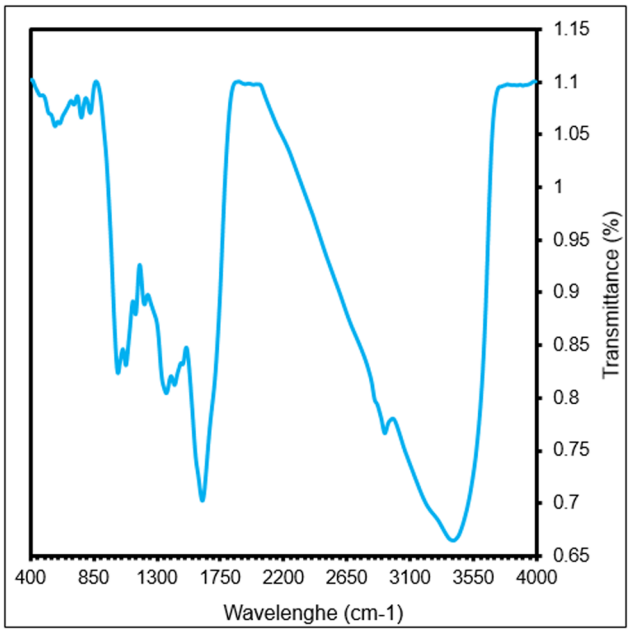

Figure 1: The FT-IR pattern of CuNPs.

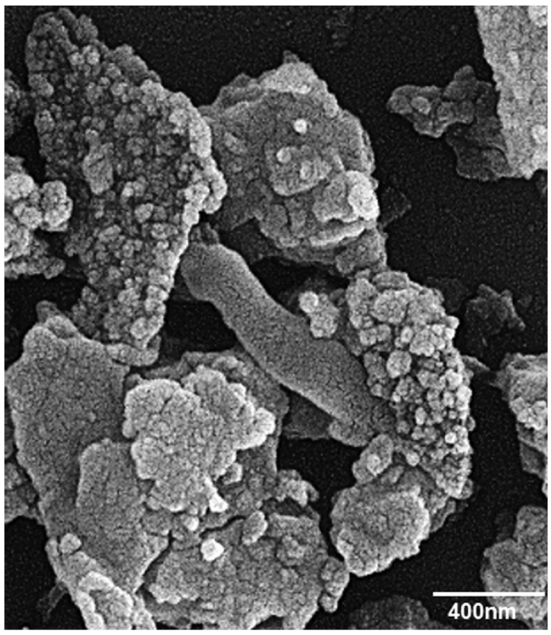

Figure 3: FE-SEM images of CuNPs.

progressively rose until it reached the reaction's stationary phase after 72 h. The bands observed closely resemble those documented in a prior study on CuNPs synthesized using green methods [17] (Figure 2).

The results taken from the TEM and FE-SEM pictures confirmed the CuNPs synthesis. The resulting CuNPs were spherical and ranged from 10–70 nm (Figures 3 and 4). The observed aggregation property aligns with common features of metallic NPs observed in prior report [17].

The field of cancer treatment research is focused on assessing natural origin factors, such as herbal NPs and plant compounds with low harmful effects. With advancements in molecular techniques and bioinformatics, a significant amount of data have been acquired, aiding in the early diagnosis of cancer. Numerous extensive research projects have focused on various herbal NPs, with a particular emphasis on examining their cytotoxic properties and potential anticancer effects [29–31]. A significant number of the compounds utilized in cancer treatment are derived from plants. Plant-derived secondary metabolites exhibit various therapeutic properties, including analgesic, cardiovascular, anticancer, and anti-inflammatory effects [35].

Considering that the investigation of diverse treatment strategies is regarded as a fundamental goal in modern

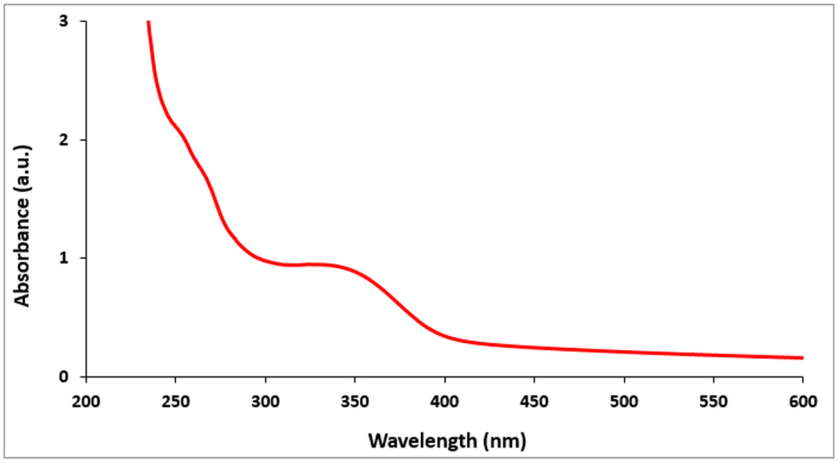

Figure 2: The UV-Visible spectrum of CuNPs.

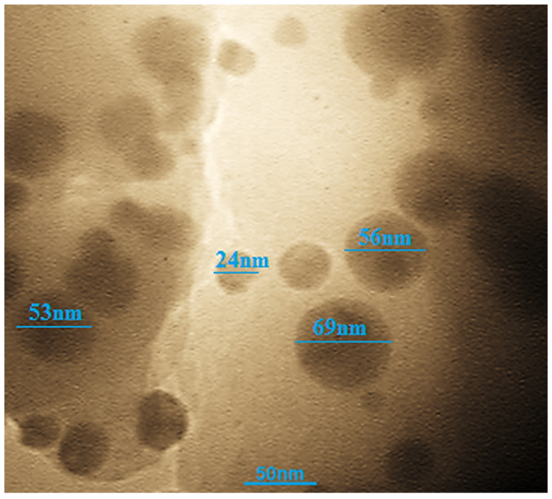

Figure 4: TEM image of CuNPs.

medical research, substantial efforts have been undertaken to reveal effective and appropriate treatment modalities. One notable aspect of these endeavors is the analysis of the cytotoxic characteristics of plant-based compounds in controlled laboratory environments through the MTT assay technique.

The research demonstrated notable antioxidant and anti-prostate cancer properties of CuNPs with *Pistacia khinjuk*.

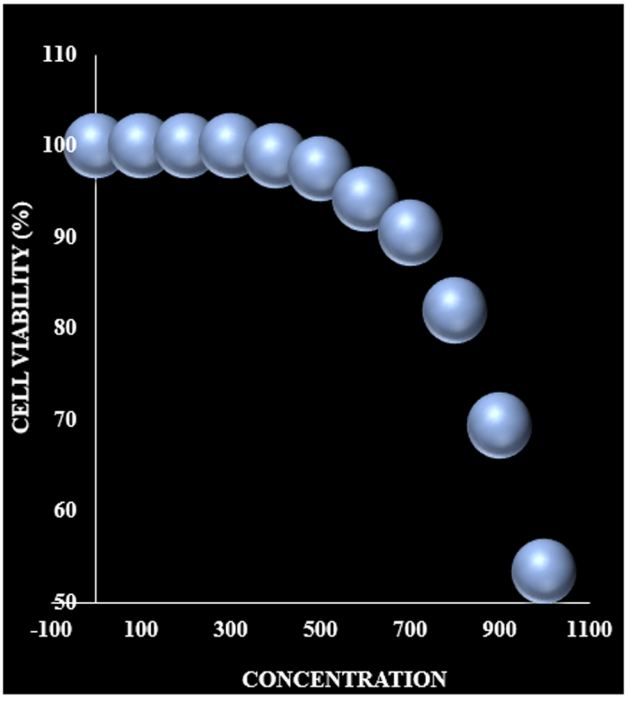

Figure 5: The cytotoxicity efficacy of CuNPs on normal cell (HUVEC).

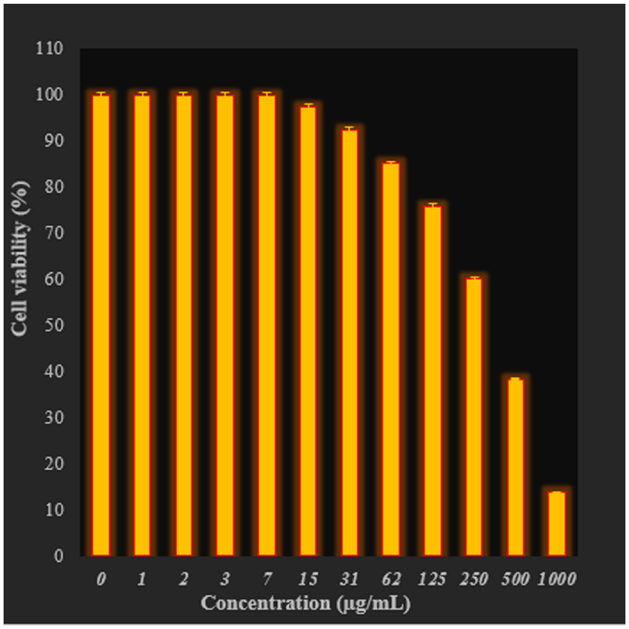

Figure 6: The anti-prostate cancer efficacy of CuNPs on DU 145.

The MTT assay was conducted on DU 145, NCI-H660, LNCaP clone FGC, and LNCaP clone FGC-Luc2 cell lines. The IC_{50} values of CuNPs were found to be 365, 322, 247, and 273 on DU 145, NCI-H660, LNCaP clone FGC, and LNCaP clone FGC-Luc2 prostate cancer cells, respectively. The LNCaP clone FGC cell line exhibited the most promising data regarding anti-prostate cancer properties of CuNPs. The particles demonstrated a strong efficacy against prostate cancer

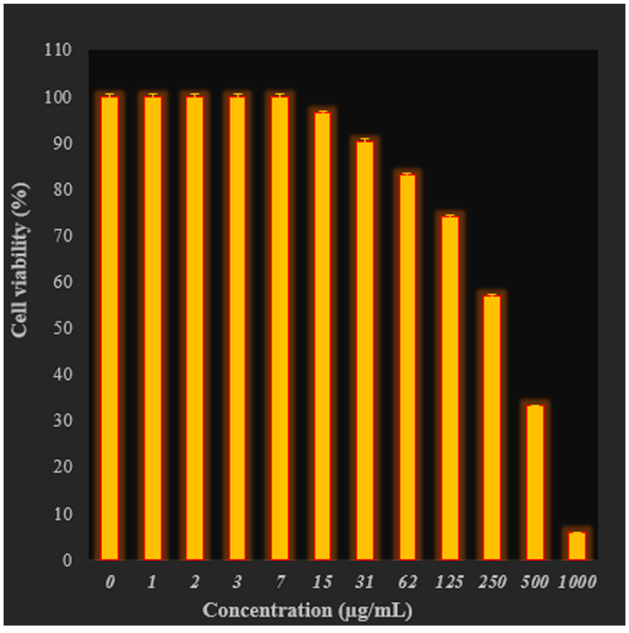

Figure 7: The anti-prostate cancer efficacy of CuNPs on NCI-H660.

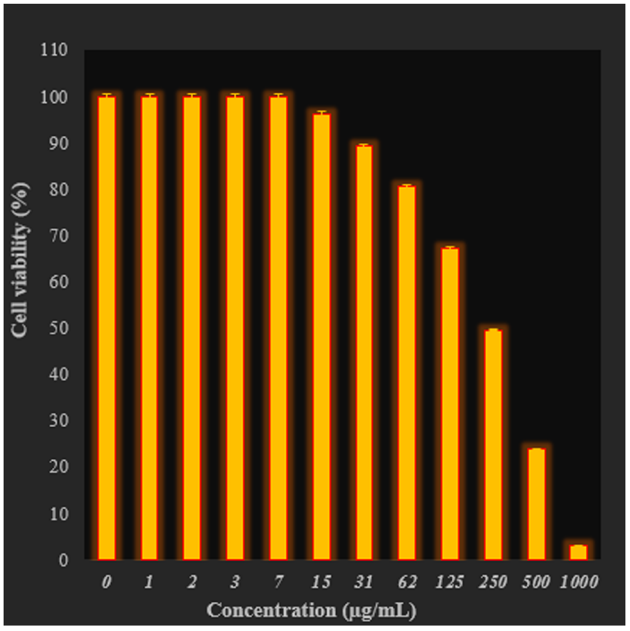

Figure 8: The anti-prostate cancer efficacy of CuNPs on LNCaP clone FGC.

in the mentioned tumor cells, while showing minimal toxicity on HUVEC (Figures 5–9).

The results of the DPPH examination reported an enhancement in prevention percentage as the NPs concentration enhanced. The best antioxidant efficacy was observed at around 100% with a concentration of 1,000 µg/mL. The IC_{50} value was 215 µg/mL (Figure 10).

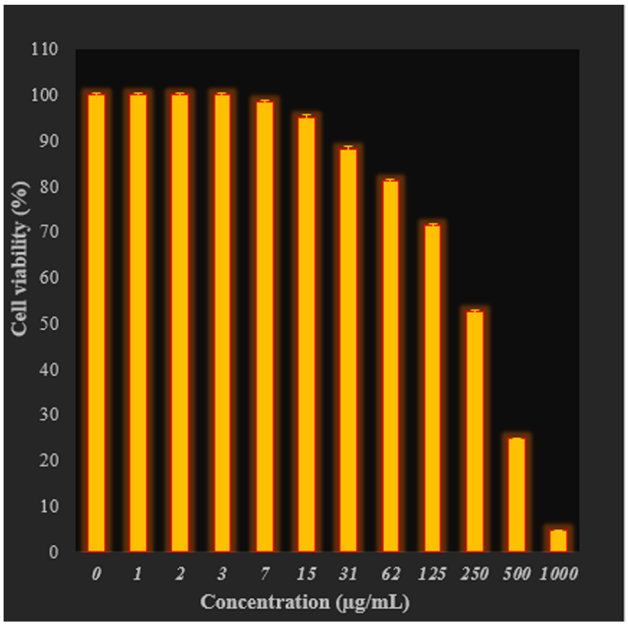

Figure 9: The anti-prostate cancer efficacy of CuNPs on LNCaP clone FGC-Luc2.

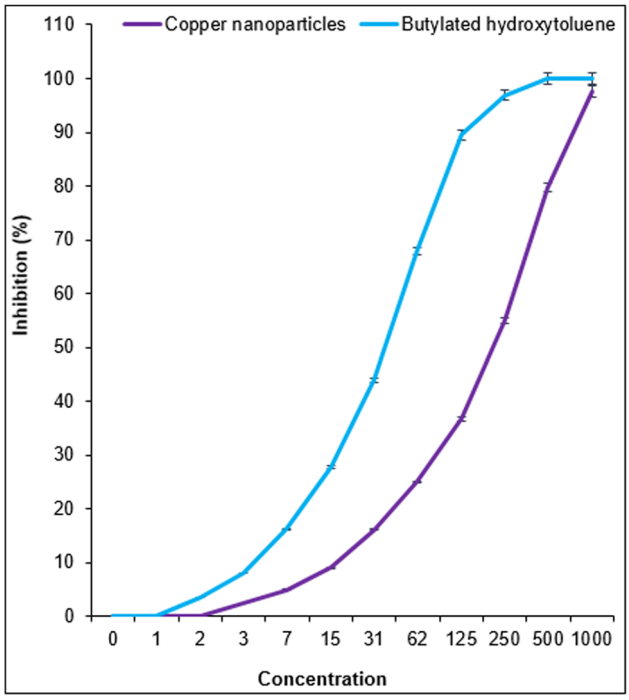

Figure 10: The antioxidant efficacy of CuNPs and butylated hydroxytoluene on DPPH.

Studies have shown that the main components of *Pistacia khinjuk* aerial parts include spathulenol, germacrene B, β-caryophyllene, B-pinene myrcene, and pinene, which have high antifungal and antioxidant effects. [39] The findings taken from the *Pistacia khinjuk* analysis indicated that the percentages of limonene, phellandrene, and α-pinene are 4.08, 15.27, and 52.33, respectively [12]. Also, antimicrobial and antioxidant properties were revealed for the leaves of *Pistacia khinjuk* because of the presence of phenolic compounds [39–41]. By investigating the antitumor activity of *Pistacia* gum, Balan et al. showed that this plant prevents human colorectal tumor cells proliferation *in vitro* by inducing apoptosis [42]. Rezaei et al. reported that *Pistacia* fruit extract inhibits the growth of human carcinoma cells similar to doxorubicin [43]. Almehdar et al. showed that *Pistacia* resin oil has a mild cytotoxic activity on breast cancer and cervical cancer cell lines and thermal melanocytes [44]. Therefore, the results of the recent research are consistent with the confirmation of the role of the results obtained from different types of *Pistacia* and anticancer flavonoids through inhibiting the polymerization of tubulins, preventing the destructive action of free radicals and inducing apoptosis [45]. Comparing the cytotoxicity effects of *Pistacia khinjuk* green-mediated CuNPs on prostate cancer cells showed that these NPs had more toxic effects on LNCaP clone FGC cell than other cells. In all extract concentrations, the growth inhibition percentage of LNCaP clone

FGC cells is higher to some extent. Despite this evidence, it can be expected that the inhibitory activity of CuNPs on cancer cells is maybe because of flavonoid compounds and antioxidant activity in it. Therefore, it seems that the cytotoxic properties of CuNPs can be generally associated with the presence of secondary metabolites, especially flavonoids in the first degree and total phenol present in this plant in the second degree [35].

4 Conclusion

In the current study, we synthesized CuNPs containing *Pistacia khinjuk* leaf aqueous extract for investigating the antioxidant, cytotoxicity, and anti-prostate cancer effects. The DPPH assay was performed to examine the effectiveness of antioxidants. The abovementioned tumor cells exhibited a notable anti-prostate cancer effect when treated with CuNPs, while HUVEC cells did not experience any significant toxicity. CuNPs successfully inhibited 50% of DPPH in the expansion at a concentration of 215 µg/mL. The FE-SEM and TEM data prove the spherical morphology with a size of 10–70 nm. IC_{50} of CuNPs was 365, 322, 247, and 273 on DU 145, NCI-H660, LNCaP clone FGC, and LNCaP clone FGC-Luc2 prostate cancer cells.

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Conflict of interest: There is no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical approval: The experiments were in the *in vitro* condition.

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