



Studying the green tea-based radioprotective potential for mice tissue application

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Received 17/7/2026, Received in revised form 28/8/2026, Accepted 5/9/2026, Published 15/10/2026

Ionizing radiation (gamma ray) generates numerous reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). These free radicals interact with living cells, causing significant damage and leading to cell death. The aim of this research is to prepare green tea nanoparticles for studying the structural and optical properties of its resulting then use these nanoparticles to protect living rat tissue. The size, shape, and distribution of green tea nanoparticles are characterized by UV-visible absorption spectra, atomic force microscopy (AFM), X-ray diffraction (XRD), and scanning electron microscopy (SEM). Then six groups of mice, part of mice groups are injecting with green tea nanoparticles to study the protected and treated living cells from radiation-induced diseases. By analyzing the histopathological of skin tissue, we conclude that the anti-cancer activity increases with increasing concentrations of green tea nanoparticles and thus, the synthesized nanoparticles are more efficient in biomedical applications in cancer treatment due to their high anti-cancer activity.

Keywords: GPTN; Living tissue; Anticancer; Gamma rays.

1. INTRODUCTION

Gamma rays, which is one type of ionizing radiation, produces a large number of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot OH$) and superoxide ($O_2^{\cdot -}$). These reactive molecules (free radicals) interact with living cells, causing extensive damage that can ultimately lead to cell death [1].

Green tea contains many secondary metabolites, which have therapeutic and preventive properties against a large number of functional diseases, in addition to a large group of minerals and vitamins. Scientific research has found that green tea contributes to the process of lowering blood sugar through its effect on insulin levels in the body, and its effect on the percentage of fats in the blood. It has been noted that green tea works to reduce the level of triglycerides associated with heart and arterial diseases, through the inhibitory role played by catechins available in green tea against the effectiveness of the enzyme (Squalene epoxidase). This enzyme works to convert (Squalene) into cholesterol [2]. In addition, green tea is found to increase the effectiveness of the antioxidant enzyme Superoxide dismutase by 90.8%. Green tea is also found to have a significant inhibitory role for the harmful compound Malon di aldehyde, which is one of the products of lipid peroxidation resulting from oxidative stress in body tissues, as the inhibition rate for this compound is about 25.4%. Studies have also shown that green tea reduces the accumulation of fat in the liver and thus reduces its weight. Recent studies have shown that an individual's consumption of ten cups or more of green tea per day may lead to an increase in the level of high-density lipoprotein cholesterol and a decrease in the level of harmful low-density lipoprotein cholesterol, in addition to reducing the concentration of peroxides in the liver [2]. Research has shown that the increase in green tea consumption has increased the formation of the Endothelial Cells as a medium to expand the blood vessels in the body, as it is found that having 500 ml of tea per day leads to the expansion of the artery diameter by about 40% compared to the control groups, i.e. from the normal level by 6.3%. Then, this reduces high arterial pressure. As well as, it contributes to the blood reaching the body tissues in abundance. [3]. The potential mechanism of action of catechins and vitamin C in green tea is their ability to increase the bioavailability of nitric oxide in the body. Additionally, they may activate the production of NOS enzymes that contribute to the enhancement of nitric oxide formation [4]. Catechins in green tea also reduce the inflammatory response by controlling the formation of nitric oxide. EGCG has been found to inhibit the activity of the enzyme that produces nitric acid synthase, which reduces the inflammatory processes associated with nitric oxide. Green tea also contains about 20 amino acids, the three most important of which are:

- 1- The amine: Found only in green tea and plays a major role in enhancing the effectiveness of anti-cancer drugs.
- 2- Hydroxyanisole: Works to combat atherosclerosis.
- 3- Aminobutyric acid: Contributes to lowering high blood pressure [5].

The aim of this research is to prepare green tea nanoparticles for studying the structural and optical properties of its resulting, then use these nanoparticles to protect living rat tissue

1.1. Nanoparticle manufacturing

There are two methods to manufacture nanoparticles: In the first method, known as the “top-down” method, solids are gradually broken down into nanomaterials. In the second method, the bottom-up method, atoms or molecules are assembled to form a nanomaterial. The bottom-up method relies on

chemical and biological processes to manufacture nanoparticles. However, conventional physical and chemical methods for manufacturing nanoparticles produce toxic by-products, creating environmental hazards [6,7].

In addition, these particles cannot be used in medicine due to health-related issues. Conventional methods can be used to produce nanoparticles in large quantities with specific sizes and shapes in a shorter period; however, these techniques are complex, expensive and inefficient [8]. In recent years, there has been a growing interest in obtaining environmentally friendly nanoparticles that do not produce toxic waste during the manufacturing process [9]. This can only be achieved through benign synthesis procedures of biological nature using biotechnological tools that are safe and environmentally sound for manufacturing nanomaterials as an alternative to conventional physical and chemical methods [10].

This has led to the emergence of the concept of green technology or green nanotechnology. In general, green nanobiotechnology refers to the synthesis of nanoparticles or nanomaterials using biological methods, such as those based on microorganisms, plants, viruses, or their by-products such as proteins and lipids, with the help of various biotechnological tools.

Nanoparticles produced by green technology are much better than those manufactured by traditional physical and chemical methods in several aspects. For example, green technologies eliminate the need for expensive chemicals, consume less energy, and produce environmentally friendly products [11].

Accordingly, green nano biotechnology is a promising alternative route for the synthesis of stable and biocompatible nanoparticles [12]. However, plants remain the best choice for the production of metal nanoparticles and metal oxides. This is due to the rapid reaction rate with low energy consumption, the presence of many biomolecules, cost-effectiveness, good stability, as well as the absence of hazardous chemicals and the ease and safety of operational procedures [13].

2. EXPERIMENTAL WORK

In order to prepare green tea nanoparticles (GTNP), samples of green tea, the leaves are taken, which are medicinal plants, are purchased from the local market in Baghdad. After that, the leaves are first dried, then 10 grams of green tea are taken and ground using an electric grinder into a fine powder.

The powder is sieved using a 58 μ l sieve, then 200 ml of ionic water is added to the solution and placed in a water bath containing boiling water inside a clean glass beaker. Ten grams of the plant powder is added to the solution, then heated at 50 °C for 2 hours.

After this period, the solution is filtered using filter paper, then filtered using a Bir filter, preferably using a vacuum to improve filtration. It is preferable to use water from a good ionic source. The solution is kept at a temperature of 6 °C throughout the study period.

3. RESULTS AND DISCUSSION

To study the ability of using (GTNP) as anticancer in living tissue, GTNP are examined in deionized water. Samples of water are exposed to 2mSv from gamma ray to induce free radicals, and these quantities of free radical are evaluated using DPPH and UV-vis spectroscopy. Figure shows tubes containing DPPH and deionized water exposed to radiation with a different concentration of GTPN.

The first purple tube contains DPPH and deionized water exposed to gamma ray only, while the other tubes contain DPPH and deionized water exposed to gamma rays with concentrations of GTNP added after irradiation.

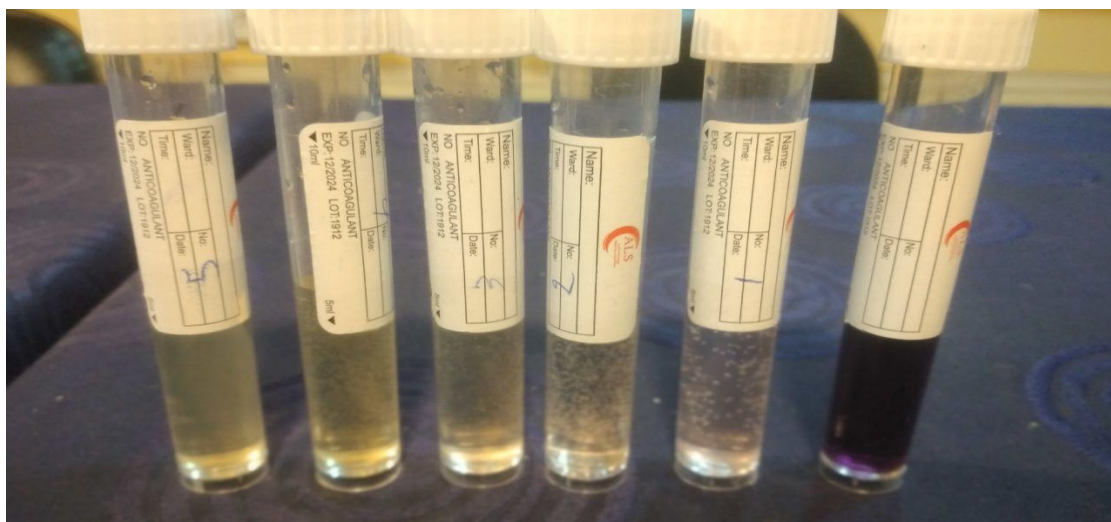


Figure 1 Tubes containing DPPH and deionized water exposed to radiation with a different concentration of GTPN.

Then absorbance spectra of exposed water contained DPPH and GTNP are evaluated using UV-Vis spectroscopy. Figure 2 shows the absorbance spectra for exposed water (added the GTNP) to 2 Sv gamma ray. From these spectra the peak absorbance is evaluated, and the efficiency of removing free radicals in water are calculated as shown in Table 2.

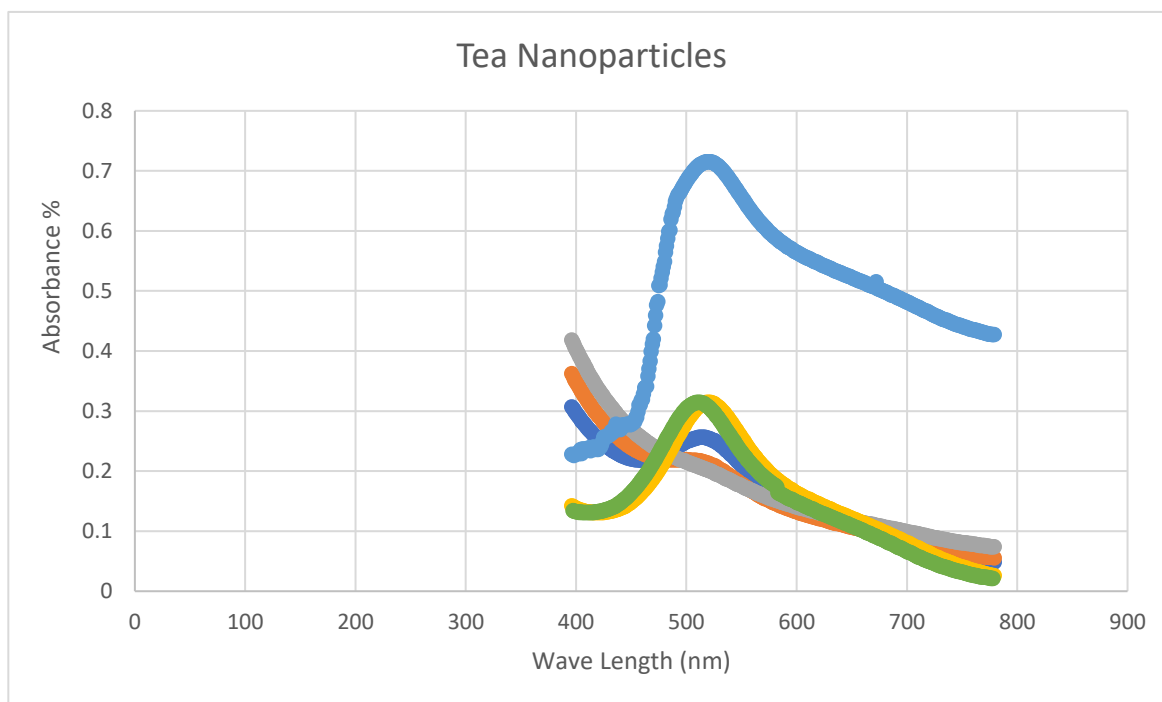


Figure 2 Spectra of UV-vis test results for GTNP and DPPH absorption for all deionized water samples at 524 nm with different concentrations of GTNP (0.0, 0.2, 0.4, 0.6, 0.8, 1mg/ml).

Table 2 Concentration of GTNP mg/ml, peak absorbance, (the quantity of free radicals) and the efficiency of removing free radicals %.

Concentration of GTNP mg/ml	Peak absorbance (Quantity of free radicals)	Efficiency of removing free radicals %
0.0	0.73	0.00
0.2	0.34	51.4
0.4	0.33	52.9
0.6	0.28	60.0
0.8	0.22	68.6
1.0	0.20	71.4

The efficiency of removing free radicals is increased when the concentration of GTNP increases till reach the efficiency 71.4 %, this value is very excellent to protect the deionized water from exposure to r x-ray and gamma ray.

3.1. Field emission scanning electron microscopy results

Figure 3 presents a magnified FSEM image for GTNP. It is noted from the figure that the particles are nanomaterials of different shapes and sizes, with dimensions ranging between (31.57- 77.48 nanometers), and they are gathered in groups due to the property of the material [14].

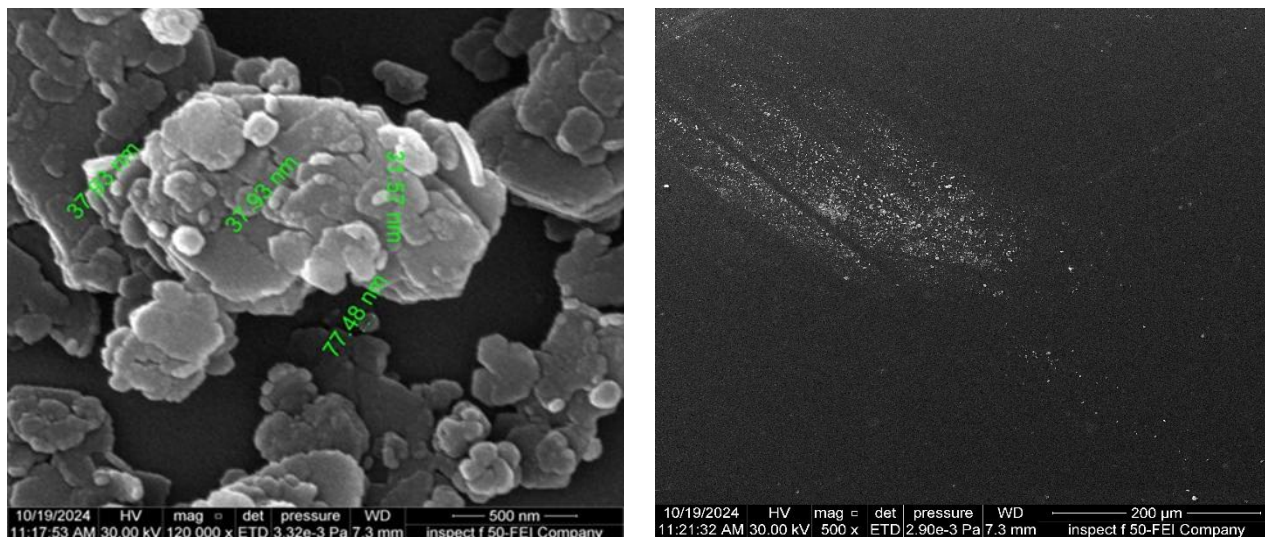


Figure 3 FESEM images of examination results of green tea nanoparticles.

Then the GTNP are examined using X-ray diffraction to evaluate the properties of its. Figure 4 shows images of the XRD test results for GTNP. It is clear that these particles do not have any crystallization, but rather are amorphous in nature [15].

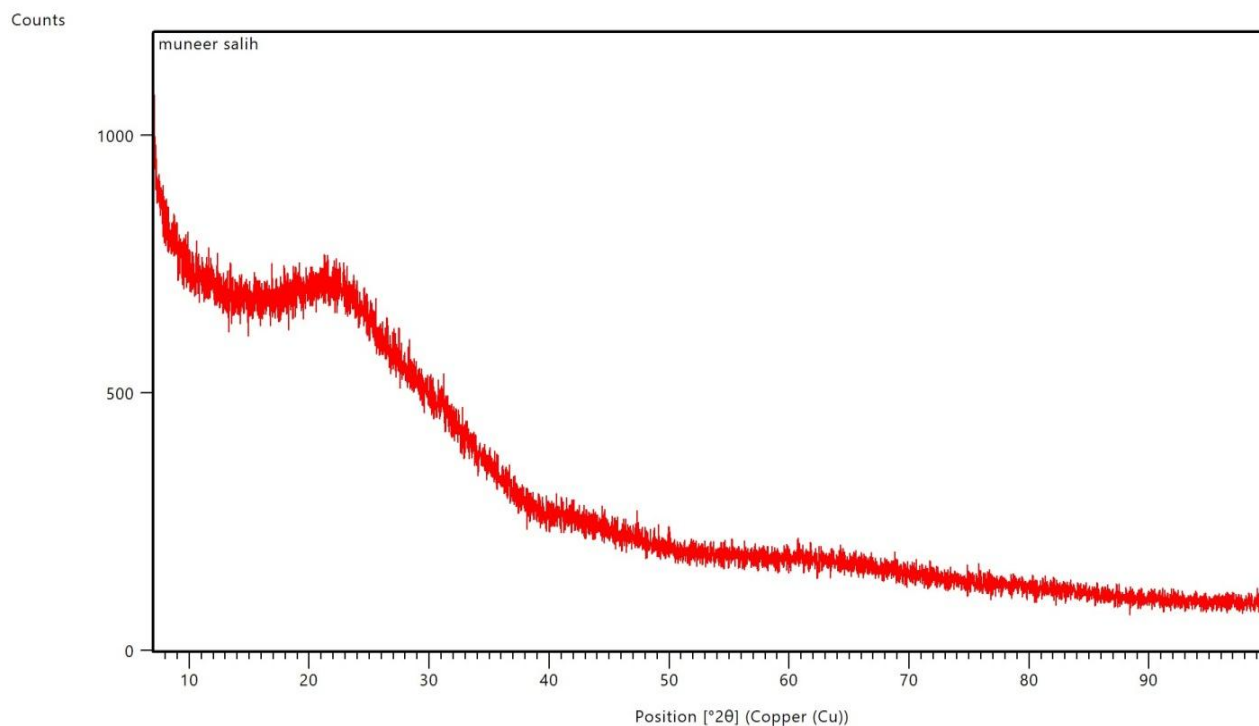


Figure 4 XRD pattern of GTNP.

The results of using GTNP in vivo to protect mice skin exposed to gamma rays from cancer, the mice are given a dose of 1 mg per milliliter GTNP as following:

Four groups of mice are taken, healthy group without any irradiation, as a control sample [14], group of mice are irradiated with 2 Sv of gamma ray, the third group are the mice given the GTNP before exposed to gamma ray, and group 4 the irradiated mice that taken 1mg/ml of GTNP after irradiated to gamma ray (treatment).

Figure 5 shows the normal tissue of control mice, Figure 6 shows the irradiated mice by 2Sv gamma rays, Figure 7 shows the irradiated mice that taken 1mg/ml of GTNP before irradiated to gamma ray and Figure 8 shows the irradiated mice that taken 1mg/ml of GTNP after irradiated to gamma ray (treatment) [16-20].

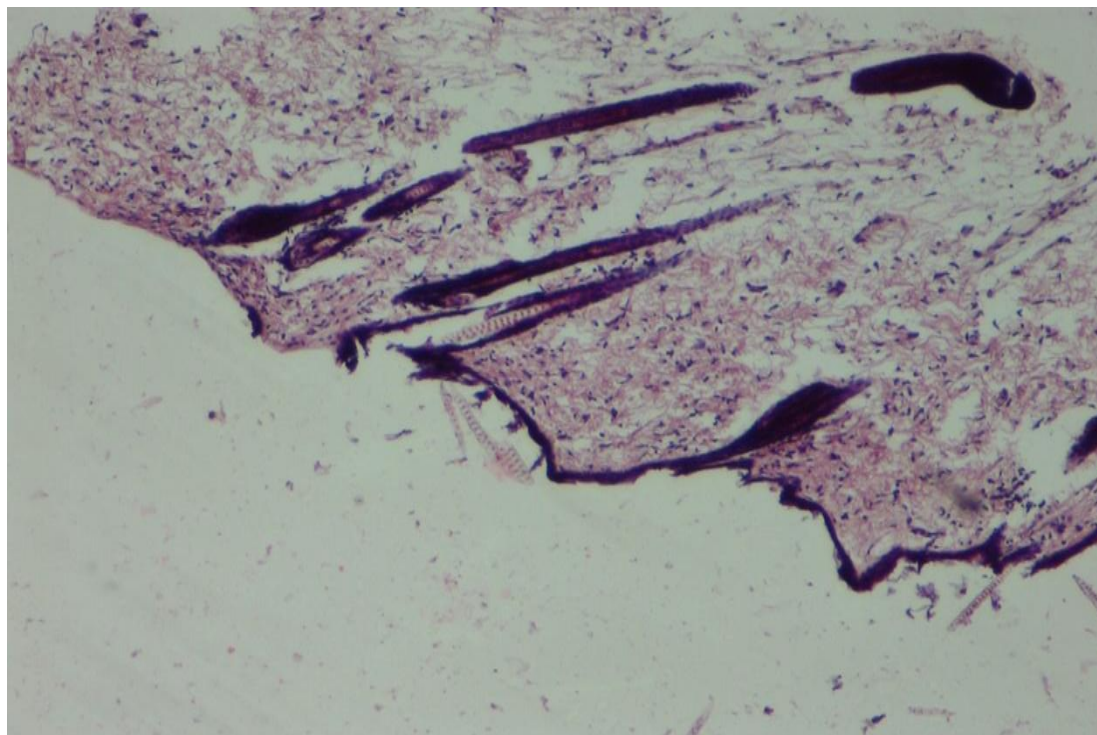


Figure 5 Section of skin (control group) showing normal epidermis, hair follicles, and normal dermal fibroblast tissue. Hematoxylin and eosin stain. 100x.

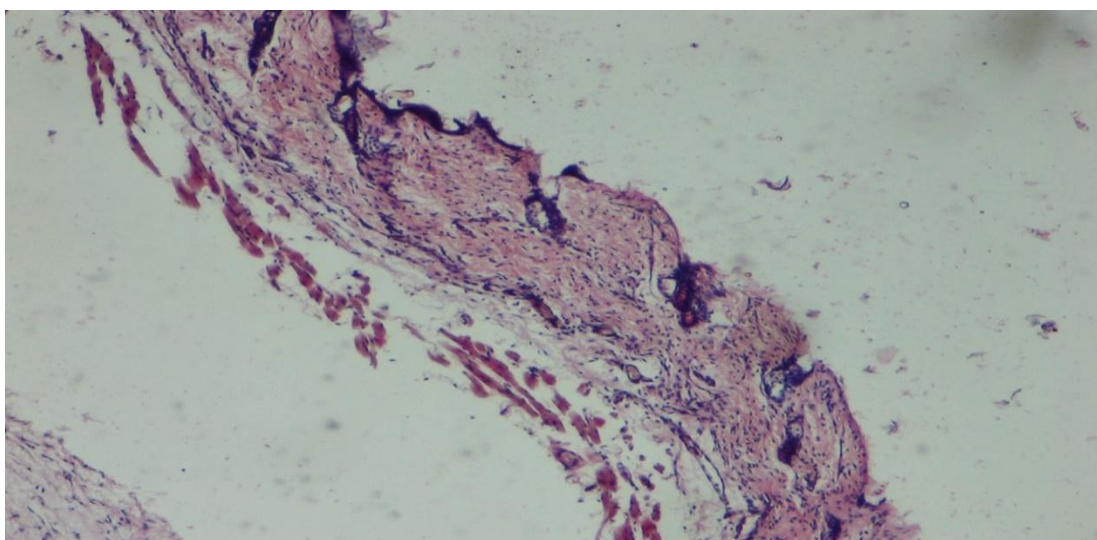


Figure 6 Section of skin (irradiated). Hematoxylin and isopropylene stain 100x.

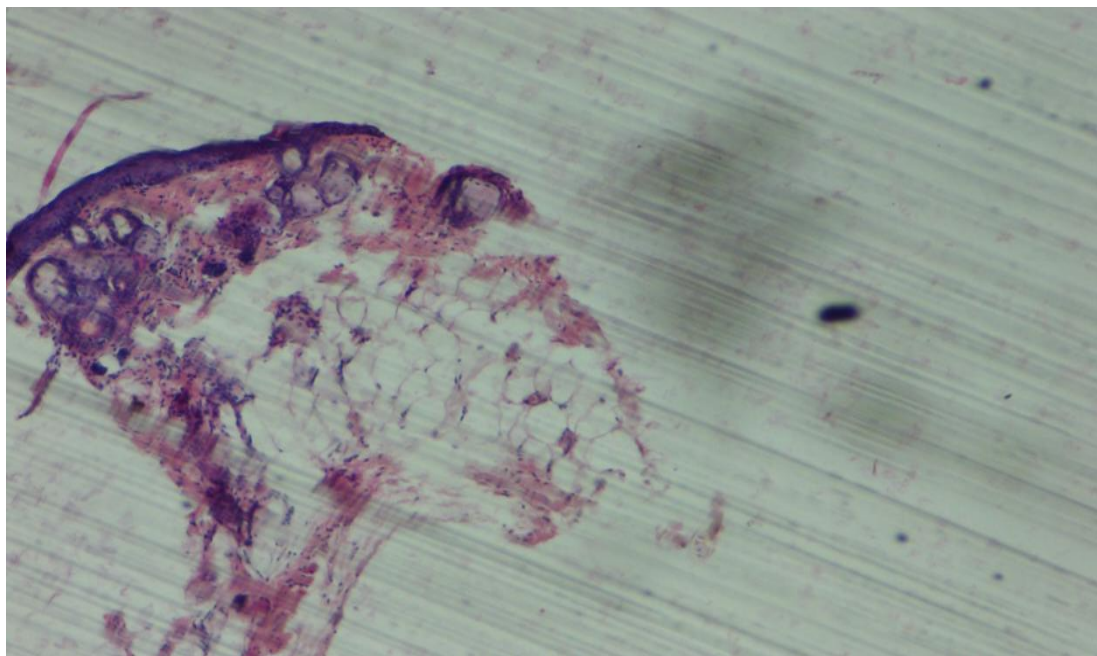


Figure 7 Section of skin protected with green tea nanoparticles. Hematoxylin and isopropylene stain 100x.

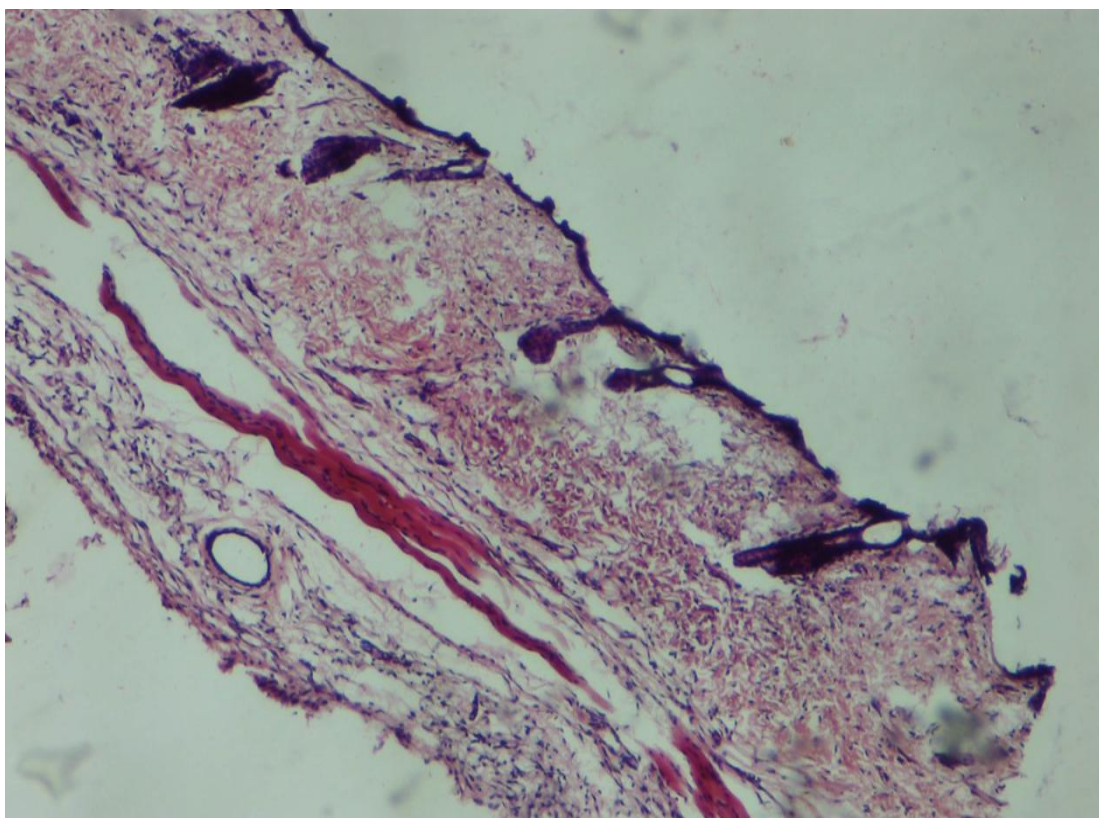


Figure 8 Section of skin (prevention) treated with GTNP after irradiated mice to 2Sv of gamma ray dose. Hematoxylin and isopropylene stain 100x.

Group 2 (irradiated): Animals are exposed to ionizing radiation (gamma rays) from cesium-137 at an equivalent dose of 2 Sieverts. Histopathological findings of the skin showed severe thickening of the epidermis associated with acute and chronic inflammation. The skin showed liquefied necrosis in the dermis, as well as infiltration of inflammatory cells, especially neutrophils, around hair follicles and sebaceous glands, with moderate proliferation of fibrous connective tissue. The results showed the presence of histopathological changes in various tissues (skin), such as the accumulation of lymphoid immune cells and inflammatory cells. The whole body of the animals is exposed to gamma radiation at a dose of 2 Sieverts. (Mice dissected after 4 days) by day 4, acute effects are prominent: inflammation, cell death, and early signs of tissue remodeling. This time frame aligns with the latent period of acute radiation syndrome, where initial damage starts to become evident histologically [21].

Group 3 (Protection): The purpose of the current group is to study the effect of pre-irradiation treatment. The prevention groups consisted of pre-irradiation groups. The animals are treated before exposure with 1mg/ml (0.5 ml daily) two times. After that, they are exposed to radiation. The results showed that dosing the animals with tea clearly reduced the histological effects resulting from the radiation [22, 23].

Group 4 (treatment): The purpose of this group is to study the effect of treatment the irradiated mice by 1 mg/ml of GTNP. The prevention groups consisted of irradiated mice group. The animals are treated after exposure with 1mg/ml (0.5 ml daily) two times. The results showed that dosing the animals with tea clearly reduced the histological effects resulting from the radiation [24].

4. CONCLUSIONS

In this study, green tea nanoparticles were prepared, and then the necessary examinations were carried out. The scanning electron microscope results showed that the particles varied in shape and size, with dimensions ranging from (31.57 - 77.48 nm). The tea nanoparticles were also examined using (XRD) and (UV) spectroscopy, and the results showed that the particles were within the nanoscale range. These results showed that injecting mice with nanoparticles protected and treated living cells from radiation-induced diseases, as shown by histopathological examination of skin tissue. We conclude from this work that the anti-cancer activity of GTNP were injected to mice before irradiated them was better than the injected mice after irradiated. And thus, we can conclude that the synthesized nanoparticles were more efficient in biomedical applications in cancer treatment due to their high anti-cancer activity.

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