# In Vivo Genotoxicity Assessment of Gold Nanoparticles of Different Doses by Comet Assays

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## Abstract

Gold nanoparticles were synthesis by green method using the soaked orange peels (*citrus Sinensis*) as reducing and stabilizing agent. The gold nanoparticles were diagnosed using the approved method, including the transmission electron microscope and the visible UV spectroscopy. Thus, the results of the diagnosis showed the formation of spherical particles with a size of 36 nanometers. Their biological effects on the bone marrow cells of albino mice were studied.

Male albino mice were used in this study and randomly divided into seven groups. The first group was the control group injected with the physiological solution (Normal Saline) and the other six groups were injected with different doses of the solution of the gold nanoparticles (1, 2, 4, 6, 8 and 10) Mg/kg. The effect of gold nanoparticles on the DNA damage by bone marrow cells was studied using comet assay according to the following criteria:

(DNA in Head%, Comet Length, Tail Length, DNA in Tail%, Olive Moment), the results showed a significant decrease ($P \leq 0.01$) in the rate of DNA in Head%, while it showed a significant increase in Comet Length, Tail Length, DNA in Tail%, Olive Moment compared to the control group and all Doses studied. The effect of gold nanoparticles was directly proportional to the increase in the studied doses. The dose was 10 mg/kg, which was the highest toxicity, followed by the dose of 8 mg/kg, then the rest of the doses was descending.

***Keywords:*** *Gold nanoparticles, Comet assay, Orange peel, toxicity.*

## Introduction

Nano molecules have been designed and widely used in various technologies. Nanoparticles are generally defined as molecules sized range between 1-100 nanometers. It is believed to be within this size. They gain unique characteristics that differ from their properties when they are the largest size. (1) The evaluation of the cellular toxicity for gold nanoparticles is very important because it has a potential impact on DNA damage. This can lead to cancer and mutations in the long-term.

(2)The comet assay or single cell gel electrophoresis (SCGE) assay assesses the DNAdamage resulting from the breakage of one or two double strand. Besides, this shows increasing DNA migration in the electric field. The method has become one of the standard method for assessing the DNAdamage of cells (3). It is preferable to use gold nanoparticles in the medical field because these molecules have unique properties that distinguish them from other minerals, including high biological compatibility with cells and living tissues (4), the affluence of attachment to biomolecules such as polyethene sugars that have wide medical applications, groups of carboxyl, amine, DNA, RNA, antibodies Peptides and others (5) and chemical stability (6) have the ability to form geometries (7).

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Gold nanoparticles may cause stimulation for the immune system due to their size close to the size of proteins and even the sizes of viruses [8]. There is little information about the inflammatory properties of gold nanoparticles and their role as antibodies. Some studies have shown that nanoparticles of gold nanoparticles can accumulate in the reticuloendothelial system of the liver and spleen, causing thus poisoning them. [9]

## Materials and Method

- **Synthesis of gold nanoparticles:** Gold nanoparticles synthesis in an environmentally friendly green method by soaking the orange peels as a reducing agent and stabilizer[10]. The gold nanoparticles produced using the visible UV spectroscopy[11] and the transmission electron microscope[12] were described.
- **Determination of Lethal Dose LD50:** The median lethal dose was determined in male albino mice using the Dixon method [13]
- **The effect of gold nanoparticles on the DNA damage by bone marrow cells:** Depending on Allen's method[14], 6 different doses of the solution of gold nanoparticles (1, 2, 4, 6, 8, and 10) were prepared mg/kg in a volume of 0.3 ml and injected under the peritoneum of the mice of the studied groups by 3 mice per group. As well as, the control group was injected with a volume of 0.3 ml of normal Saline. After 18 hours, the groups studied were injected with a solution of cholchicine (prepared at a concentration of 1 mg/ml) with a volume of 0.3 ml. After 2 hours, the mice were drugged with chlorofum and then dissected to obtain bone marrow from the femur. To determine the effect of gold nanoparticles synthesized according to the present study on the level of the genetic material of the cell, tests were conducted of the effect of gold nanoparticle on the DNA damage of bone marrow cells using Comet assay via the following criteria (DNA in Head%, Comet Length, Tail Length, DNA in Tail%, Olive Moment. The results were extracted based on the comet score program. In addition, this test was performed according to the method followed by[15].

## Results and Discussions

**Characterization of produced gold nanoparticles:** The first way to describe the biosynthesis of gold nanoparticles was to measure ultraviolet light spectrum. After 12 hours of manufacture, the color of the solution changed from yellow to ligth pink, then absorbance was measured. Also, a peak appeared at wavelength (533 nm) and absorbance (0.710).

The results showed that color change plays an important role in detecting the formation of nanoparticles. This is confirmed by the appearance of the peak in conjunction with absorbance after 12 hours. The peak gave a spectral evidence for the formation of surface plasmon resonance of the golden nanoparticles [16]. This is consistent with [17]who concluded that the color change of the solution from blue to pink light rosy is evidence of the reduction of gold salts and the formation of gold nanoparticles. This reduction and color change can be attributed to the presence of phytochemicals reduced in the soak plant. This agreed with[18] who resulted that phyto-chemicalshave high ability to rapidly reduce mineral solutions to nanoparticles. Citrus sinensiscontains proteins and glucose molecules in addition to vitamin C, which are reducing agents for the manufacture of gold nanoparticles [19].

Figure (1) shows a picture of the electron microscope forproduced gold nanoparticles using soaked orange peels *(Citrus Sinensis*). The results showed that spherical nanoparticles of 36 nm with no clusters or conglomerations of these particles. Due to the repulsive nature of the reducing agent that enclosed the surface of the gold nanoparticles, they stabilize as well as biomolecules helped to form spherical molecules[20].

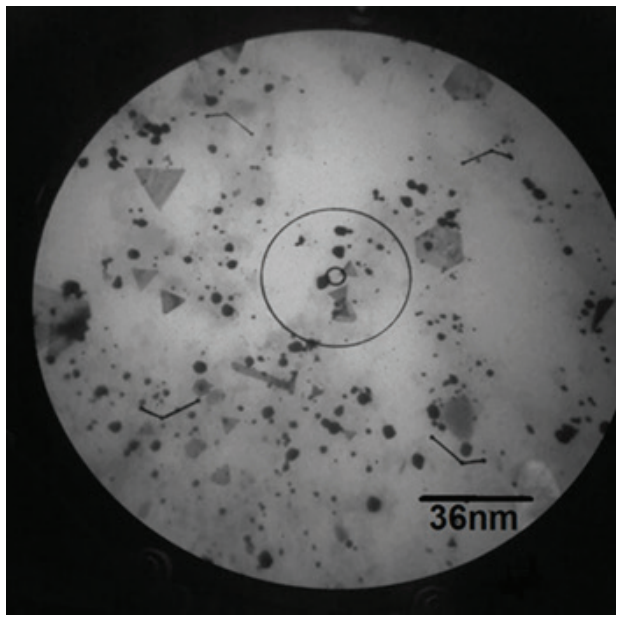


**Figure 1. Transmission electron microscopic (TEM) images of Au NPs Synthesized using soaked orange peels.**

The genotoxic effect in bone marrow of albino mice:

- **DNA in Head%:** The toxic effect of gold particles was directly proportional to the increase in dose in the percentage of DNA head.(Figure 2).

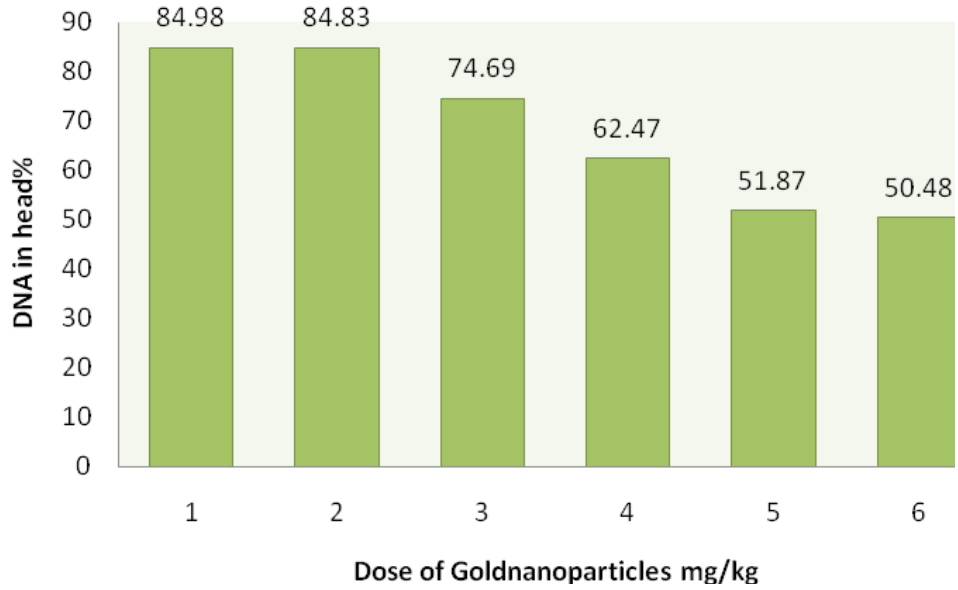


**Figure 2. The effect of Goldnanoparticles on DNA in head% in albino mice bone marrow cells.**

Doses 10 and 8 mg/kg showed the highest toxicity effect in the genetic material of bone marrow cells. The ratio of DNA in head (50.48 ±3.04 and 51.87± 2.89)%,respectively, compared to the control group that reached (84.98±2.020)%. Significant differences (P≤0.01) were found among the different doses, except for doses 1 and 2 mg/kg.While the percentage of head DNA ratio was (2.09 ± 84.55 and 2.31 ± 84.83)%respectively.

- **Comet Length (px):** The results (Figure 3) showed a significant increase in comet length, and significantly (P≤0.01) was directly proportional to the increase in the dose used. Obviously, the dose of 10 mg/kg showed the longest comet, reaching (95.44 ± 3.92) px compared to the control group (39.82 ±1.69)px.

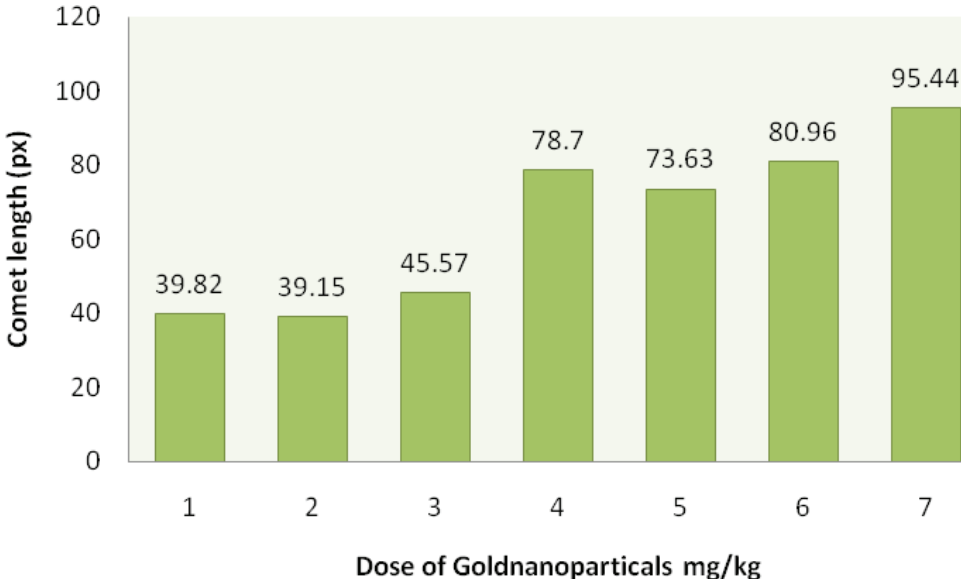


**Figure 3. The effect of Goldnanoparticles on Comet Length (px) in albino mice bone marrow cells.**

Then the effect decreased by diminuting the dose (4,6,8) mg/kg at a rate of (78.70 ±1.04)and (73.63±1.84) and (80.96 ± 3.59) px, respectively. A significant difference did not indicate between them (P> 0.01), while it increased significantly for the two doses (1,2) mg/kg and the control group.

For the two doses (1,2) mg/kg, they did not show a significant difference between them, nor did they show a significant difference when compared to the control group at the rate of (39.15±1.09) and (45.57 ± 2.01) px, respectively.

- **Tail Length:** The results of the Tail Length (figure 4) showed a significant height (P≤0.01) when using high doses 6, 8 and 10 mg/kg. The tail length reached (13.32 ±1.62), (13.78±1.33), (22.20±1.95) px, respectively, compared to a control group of (4.36±1.47)px. The doses 1, 2 and 4 mg/kg did not have a significant effect (P> 0.01) compared to the control group. When comparing the concentrations between them, the results showed that the length of the tail increased with an increase in the doses used.

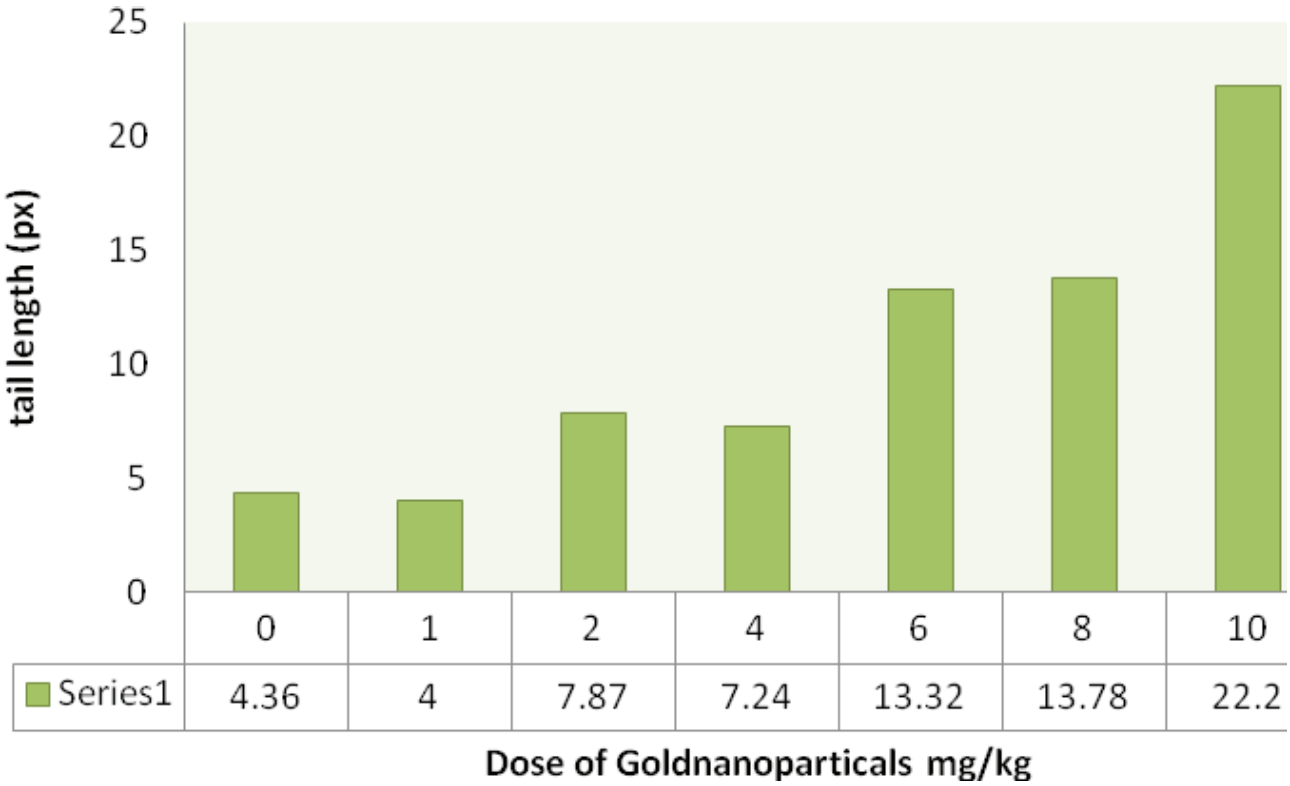


**Figure 4. The effect of Goldnanoparticles on Tail Length (px) in albino mice bone marrow cells.**

- **DNA in Tail %:** Gold nanoparticles caused a toxic effect on bone marrow cells, depending on the DNA in the tail at the (P≤0.01) level compared to the control group. The DNA break increased with a higher concentration (Figure 5). The doses 10 and 8 mg/kg showed the highest DNA percentage in the tail reached (49.52 ±3.04) and (48.12±2.89)%, respectively, compared to the control group (15.17±2.31)%. For doses 1 and 2 mg/kg, they did not cause toxic effects in the genetic material of marrow cells. There were no significant differences in the proportion of tail DNA, which reached (15.67±2.21),% (15.02 ±2.02), respectively, compared to the control group.

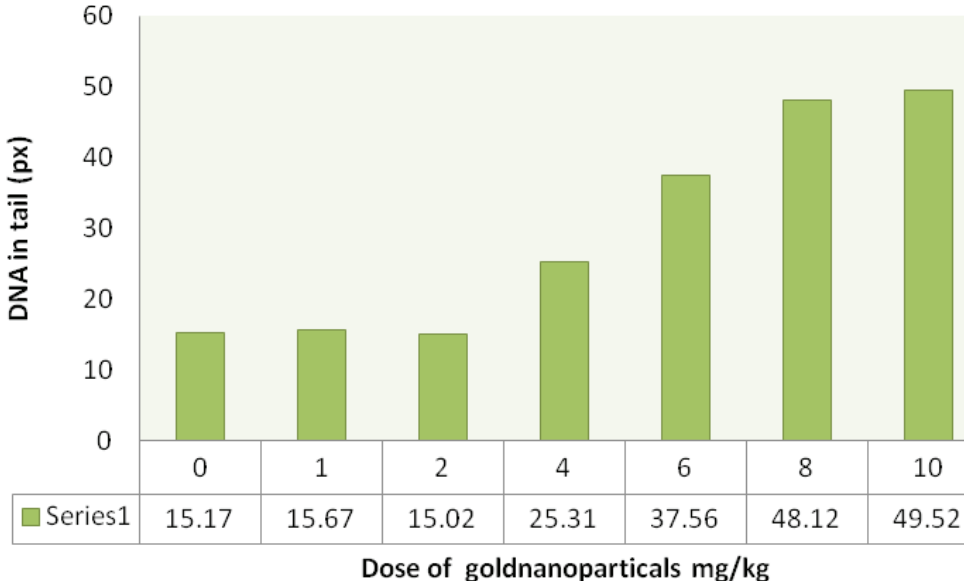


**Figure 5. The effect of Goldnanoparticles on DNA in Tail %in albino mice bone marrow cells.**

- **Olive Moment:** The results in Figure (6) showed a significant increase (P≤0.01) for all studied doses compared to the control treatment. The increase in the value of Olive Moment increases with increasing dose value. The dose 10 mg/kgshowed the highest toxic effect on the genetic material of marrow cells, as the value of Olive Moment (11.19±0.91) compared to the control group which reached (4.05±0.52). According to this study, low doses 1, 2 and 4 mg/kg, however, did not show a significant effect compared Control group.

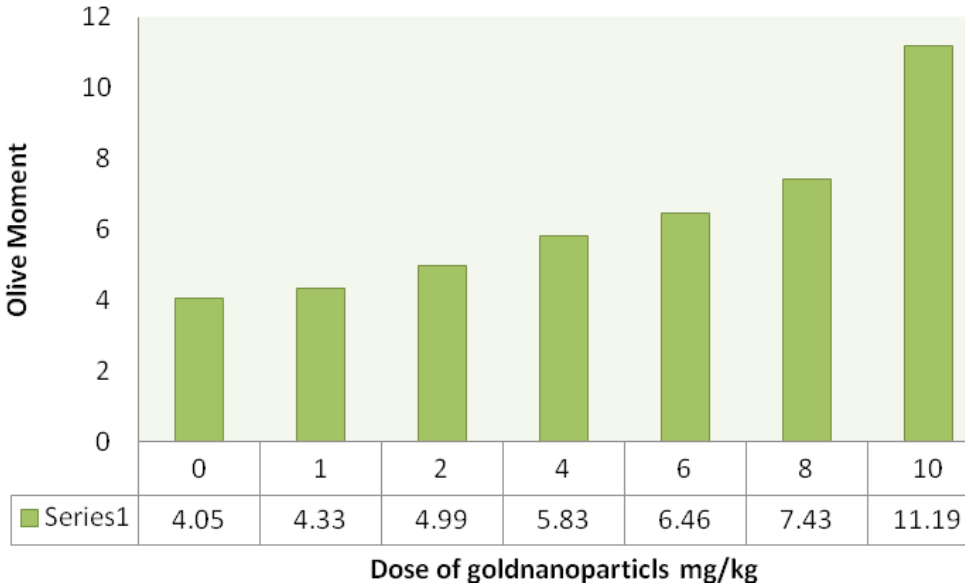


**Figure 6. The effect of Goldnanoparticles on Olive Moment in albino mice bone marrow cells.**

The results of the present study agreed with Olive [15] who studied a modified method to reduce the toxicity of gold nanoparticles for DNA damage by encapsulating these nanoparticles with glucose molecules. This indicates the toxic effect of nanoparticles on the DNA.

According to the results obtained, it was found that gold nanoparticles synthesizedby the environmentally friendly biological method using soaked orange peel had a toxic effect on the DNA. It could be directly proportional to the high dose used.

The toxic effect of synthesized gold nanoparticles may induce mutations [21] and DNA strand damage [22] by stimulating the production of free oxygen radicals, causing destruction of cell membranes, proteins, and DNA [23] and stimulating DNA damage signals [24]. Toxicity may also be caused by the direct association of these nanoparticles with the DNA strand due to their small size, where are able to cross cellular barriers or by stimulating oxidation systems, repairing proteins of the DNA strand and causing damage to the DNA strand[25] in addition to its ability to stimulate apoptosis of the cells [26]. It can be the size of the nanoparticles used in the study (36 nanometers) has a great role in the toxicity of these molecules. The sizes of 30-50 nanometers consider the most toxic ones compared to other sizes because of their high ability to cross cell membranes [27]. The accumulation within the lysosome stimulates the mechanism of autophagosome,causing cell death [28]. Besides, the spherical shape of these molecules is five times more toxic than other forms [29].

**Conclusion:**

The soakorange peelscontains reducing agents and stabilizer for making spherical nanoparticles in an environmentally friendly green method . The results of the visible UV spectroscopy and the Transmission electron microscopy for characterization of synthesized nanoparticles are the reductive ability of the soakorange peels The gold nanoparticlessynthesized have a toxic effect on the DNA of bone marrow of albino mice. Thus, the toxic effect depended on the doses used in this study. It showed a direct increase with the rise in the studied doses. Its toxicity was evaluated according to the comet assay test.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** Non

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