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In Vitro antioxidant Behavior and Cytogenetic effects of Zinc Nanoparticles loaded on Banana Peels

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ABSTRACT: The present study investigates the green synthesis of zinc oxide nanoparticles (ZnO-NPs) using aqueous extracts of banana peels and evaluates their antioxidant potential and cytogenetic effects. Zinc nanoparticles were synthesized via an eco-friendly approach and characterized using UV-Vis spectroscopy and atomic force microscopy (AFM) and scanning electron microscopy (SEM), which revealed particle sizes ranging between 23.9 to 27.8 nm and structure varied depending on extract concentration. Antioxidant activity was assessed through DPPH radical scavenging and FRAP assays, showing maximum activity at a concentration of 1.5 M, where DPPH inhibition reached 86.33% and FRAP absorbance peaked at 1.517. In vivo genotoxicity was evaluated using the micronucleus and comet assay on bone marrow cells of Swiss albino mice. ZnO-NPs are the most inducing of DNA damage as compared to negative control. These findings suggest that banana peel-mediated ZnO-NPs exhibit promising antioxidant activity but may elicit cytogenetic effects at elevated doses, highlighting the need for careful biomedical application. The study contributes to sustainable nanomaterial synthesis and offers a low-cost strategy for converting agri-waste into value-added nanproducts.

INTRODUCTION

In recent years, the focus has increased on the waste produced by the vegetable and fruit processing industry, which includes peels, seeds, etc., in order to convert them into components that can be used by extracting their essential components such as vitamins, phenols, flavones, minerals and the like, because of these compounds are antioxidants, used to treat some diseases such as cancer, aging, and antibacterial [1] or used as raw materials for the manufacture of pharmaceuticals, cosmetics or low-cost agricultural fertilizers and animal feed [2, 3]. Bananas are one of the most important food crops whose peels are eliminated, accounting for 30-40% of the full fruit weight [4].

Zinc oxide nanoparticles have excellent properties and are widely used in applications such as pharmaceutical, cosmetic, electronics, optics and photolysis of organic pollutants in water. Zinc oxides are n-type semiconductors at a band gap of 3.37 eV and a high excitonic binding energy of 60 mV [5].

Interest in zinc nanoparticles has emerged due to their eccentric physical and chemical properties such as electronic motion, photosensitivity and stability [6]. Then the use of chemical and physical methods to manufacture nanoparticles of zinc oxide, but these methods have many disadvantages, including high energy consumption, high pressure, temperature, the use of

expensive and unsafe equipment and generate waste harmful to the environment [7], which can cause biological and environmental problems and for the purpose of avoiding such problems, the manufacture of zinc oxide nanoparticles from plant extracts has been resorted to, which is one option, simple, less time-consuming, economical and environmentally friendly, which is banana peel [8].

Banana peel is rich in natural compounds that are biologically active and antioxidant resulting from free radical reactions [9]. Studies have indicated that ripe banana peel contains anthocyanin and cyanidin as well as catecholamines [10, 11] in addition to carotenoids such as beta carotene and xanthophyll, additionally 300-400 µg of luteinizing equivalent per 100 g of banana peels [12].

Recent trends are focused on the isolation and use of natural antioxidants, especially polyphenols, as potential agents for disease prevention and because these compounds are present in most fruit tissues [13], so banana peel has gained attention as a natural source of antioxidants and rich chemical content of active radical species removing compounds [14].

This study aimed to evaluate and compare the phytochemical contents and antioxidant activity of banana peel extract.

MATERIALS AND METHODS

Plant samples collection

Banana (*Musa* sp.) peels were obtained from the local market in Baghdad during July 2024.

Aqueous extraction of plant

The banana peels were ground after drying with a weight of 150 g of the plant and then added to 250 ml of distilled water for 4 hours using the Soxhlet, the heat source was the water bath at 45°C. The banana peel extract solution obtained from the extraction evaporated by rotary where dry of extract was 7.4% by original weight [15].

Biosynthesis of zinc nanoparticles

Zinc nanoparticles (ZnNPs) were synthesized by banana peels aqueous extract as a plant and zinc nitrate (Zn (NO3)2) as zinc source. The mixtures were prepared by mixing 9 mL of (Zn (NO3)2) at varying concentrations (1.0, 1.5, 2.0, and 2.5 mM) with 1 mL of extract. It should be cleared that concentrations of this study (mM) refer to molarity of (Zn (NO3)2) used during nanoparticles synthesis. The Biosynthesis was done in a dark chamber at 30°C overnight without agitation to avoid photoreduction of zinc nitrate. Then the nanoparticles centrifuged at 10000 rpm for 15minutes, three times pellet was washed. Each experiment was performed in triplicate (n = 3) to ensure reproducibility. pH was maintained at 7.0, and visual color change was monitored at regular intervals (every 2 hours) until stabilization. These conditions and selected $(NO_3)_2$ concentrations were adopted from previously reported studies [16].

Detection of Nanoparticles

Visible detection

Nanoparticles were recognized by color changing that depended as an essential way for early detection of nanoparticles green synthetic [17].

Reading by spectrophotometer

Second method for green synthetic nanoparticles detection was evaluating the absorbance at wavelength 433 nm [18].

Scanning electron microscopy (SEM) and Atomic force microscopy (AFM)

(SEM) and (AFM) examination was achieved by NT-MTD scanning prop microscopy. Solution samples of nanoparticles were diluted by DW,

Which positioning on glass slide, the slide was put on the stage after the samples drying and depending to the standard method the analysis was accomplished [19].

DPPH Radical scavenging activity

The antioxidant activity of the banana peel aqueous extract was evaluated by two tests, the free radical scavenging assay (DPPH) and Ferric reducing ability of plasma (FRAP) [20].

Evaluation of the radical scavenging activity

a. Solutions [21].

-DPPH 1,1 Diphenyl-1-2-Picryl Hydrazyl: 4.3 g of DPPH was dissolved in 3.3 ml DMSO-methanol 1:9 size/size where the solution was kept away from light by surrounding the test tubes with thin aluminum foil.

b. Assay procedure: The antioxidant activity of the aqueous extract of banana peels was evaluated on the basis of the radical scavenging activity DPPH and according to the method of [35], as follows: 0.1 ml of banana aqueous extract (0.0625, 0.125, 0.250, 0.500 µg/ml) added to DPPH solution (3.9 ml) in a tube. After it was placed in an incubator with a temperature of 37°C, the absorbance of each solution was determined using a spectrophotometer with a wavelength of 517 nm and three repeaters for each concentration. The radical scavenging ability (DPPH) was evaluated by applying the below:

$$\text{DPPH radical scavenging activity} = \mathbf{1-}\frac{\textit{Absorbance of sample}}{\textit{Absorbance of standard}} \times \mathbf{100}$$

Evaluation of reductive ability

a. Solutions [22].

-0.2 M solution of phosphate buffer (PB):

Buffer solution A: 3.12 g of $NaH_2PO_4.2H_2O$ dissolving in 100 ml of DW.

Buffer solution B: was prepared by mixing 5.36 g of $Na_2HPO_4.7H_2O$ with 100 ml of DW. Finally 62.5 ml of solution A mixed with 37.5 ml of solution B. (pH 6.6).

- Solution of ferric chloride FeCl3 (1%): Prepared by mixing 1 g of FeCl3 in 100 ml of DW.

- Solution of potassium ferrcyanide (1%): 1 g of substance in 100 ml of DW.

- Trichloride acetic acid 10%: 10 g of the acid dissolved in a small amount of distilled water and then complete the volume to 100 ml.

- Trolox is similar to vitamin E dissolved in water: Used as a standard for comparison with the aqueous extract of banana peel. A series of trolox concentrations similar to those of the plant aqueous extract (0.02, 0.04, 0.08, 0.016, 0.032, 0.64 µg/ml) was prepared by dissolving the required weights in distilled water.

b. Assay procedure: The experiment was conducted according to the method of [22] to detect the ability of reductive. One ml of each plant extract concentration (0.02, 0.04, 0.08, 0.016, 0.032, 0.064) µg/ml mixed with 1 ml of phosphate buffer solution 0.2 M (pH 6.6) with 1% potassium ferrcyanide 1.5 ml and placed in incubator at 50°C for 20 min. Then add 1 ml of trichloride acetic acid 10% to the mixture for stoppage of reaction. The mix was placed in the centrifuge for 10 minutes at a speed of 3000 cycles /minute and 2.5 ml of suspension was mixed with 2 ml of DW and 0.5 ml of 1% ferric chloride prepared immediately. The absorption was detected at 700 nm wavelength. This procedure was applied to vitamin E and three repetitions for each concentration.

Animal acclimatization and Experimental design

Animals were Mus musculus Swiss Albino mice from Al-Nahrain University Biotechnology Research Centre. Aged 8-10 weeks, and weighing between 23-27g. Into three groups the mice were divided, six animals for each. Control treated with normal saline. ZnO-NPs-treated group (150 mg/kg). Plant extract group (400 mg/kg)

After that, the mouse was sacrificed and dissected for mice bone marrow for micronucleus and comet assay.

Cytogenetic assay:

Micronucleus assay:

a. Solutions

-Human plasma: Human plasma was supplied by the National Blood Transfusion Center in Baghdad with human plasma AB and then transported to the lab., where the plasma was separated into 5 ml tubes, then were put in a water bath at 56°C for 30 minutes (heat disabling) to be stored at -20°C until used for micronuclei assay [23]. The plasma was sterilized with 0.2 m Millipore Filter before use.

b. Assay procedure: To conduct micronuclei assay Hayashi [24] method was used as shown in the following steps:

- The mice were sacrificed by cutting the spinal cord followed by dissected to obtain the bone marrow. After opening the bone ends, hold the center with forceps in a perpendicular to the edge of the tube, then collect the cells using 2 ml of human plasma by an insulin syringe.

- The test tubes were placed in the centrifuge at 1000 rpm for 10 minutes and the suspension eliminated. Mix the precipitate and make a swab on a glass slide and then leave to dry at temperature of a room.

- For 5 minutes the swab was fixed with methanol absolute reagent and left to dry with the ideal room temperature, then dye with Giemsa for 15 minutes and finally washed with DW. Glass slides examined by the oil lens for 1000 cells to verify the formation of micronuclei. Percentage of micronuclei was calculated by adopting the following equation:

$$\text{Micronucleus index (micronucleus/cell)} = \frac{Number\ of\ Micronuclei}{Total\ Count\ of\ PCE} \times 100$$

Alkaline Comet Assay (Single-cell gel electrophoresis)

Alkaline comet assay measured the DNA damage percentage in mice bone marrow that performed under alkaline conditions according [25]. Agarose coated slides were prepared. Cell suspension was mixed with low melting agarose and speared on slides.in cooling lysis buffer the prepared slides were immersed. After electrophoresis, the slides were stained with ethidium bromide, and examined under fluorescence microscope.

RESULTS AND DISCUSSION

Zinc nanoparticles biosynthesis and detection

The visible detection is the important indicator of the synthesis of ZnONPs in the pre-incubation reaction, the extract has a clear color in distilled water, which transformed to light gray after the addition of Zn^{+2}, which means it was reduced to Zinc nanoparticles as in Figure (1).

This was evaluated by the visible UV spectrometer. Color changes indicated the formation of zinc metal particles because of presence of active components in the extract of banana peel [26], color change is belong to the activation of the Surface plasmon resonance (SPR), the mass oscillation of free electrons in metals that interact with the incident light field leads to the production of surface resonance, which is surface electromagnetic waves and causes the absorption and emission of certain ranges of wavelengths of light, which leads to the emergence of a substance which it gives distinctive colors to human vision [27].

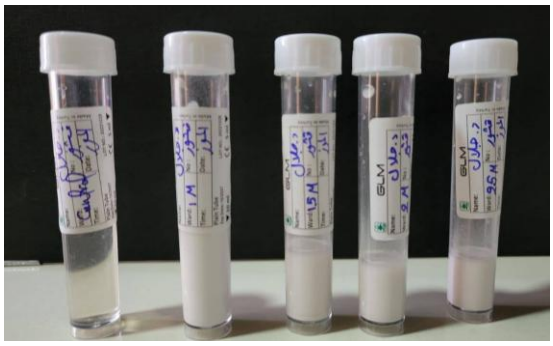


Figure (1): Visual observation of synthesized green nanoparticles

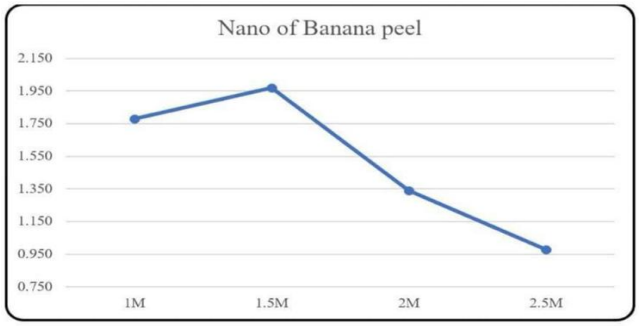


Figure 2: UV visible absorption of nanoparticles

Characterization of zinc nanoparticles

The size and shape of the synthesis zinc nanoparticles were examined by electron microscopy where the particles analysis indicated that the zinc nanoparticles have a mean size based on zinc concentrations (as in the Figure 2).

The results showed that the organic compounds in the plant extract are reason of reducing zinc ions and fixing the resulting nanoparticles, and these components consist basically of cellulose and hemocellulose in addition to pectin compounds [28]. Therefore, functional groups related with such polymers in addition to the protein material may be participated in the reduction of nanoparticles [29]. Studies have shown that reducing compounds are electron-donor compounds that can reduce oxidizing mediated to lipid peroxidation processes, where they can act as primary and secondary antioxidants [30] as in Figure (3).

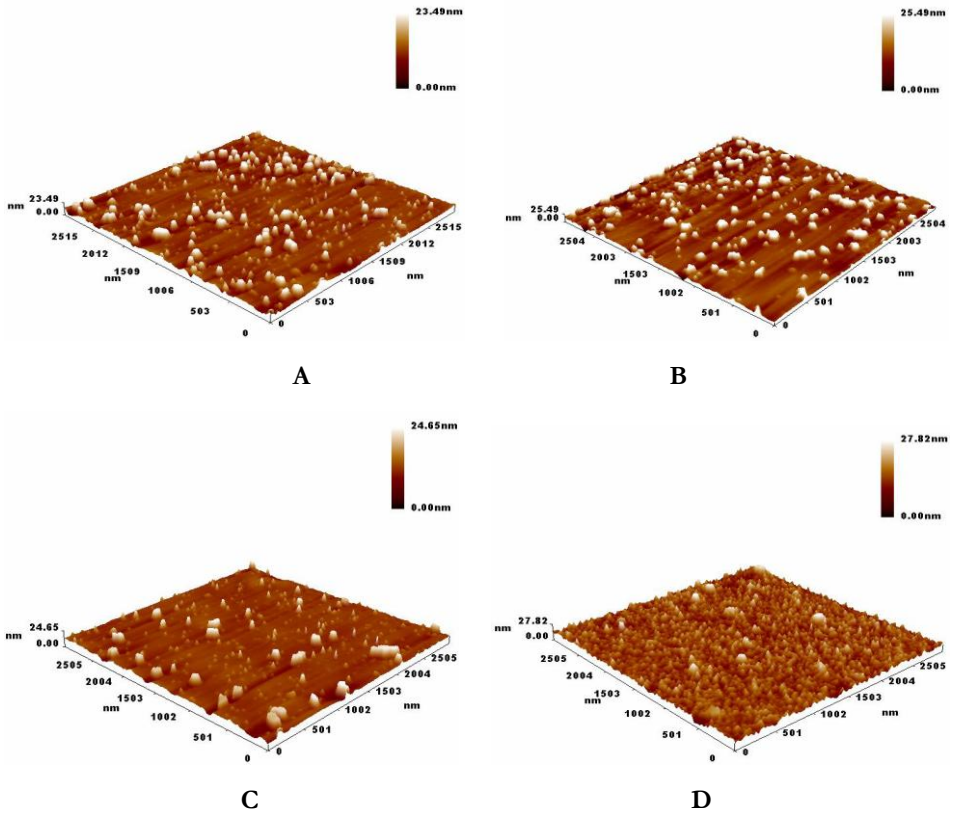
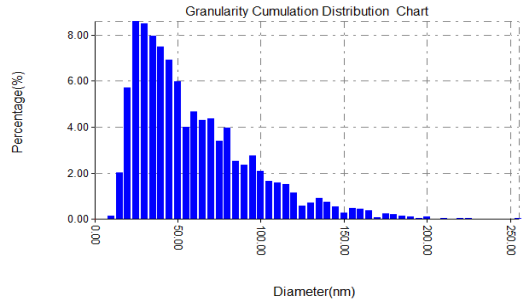
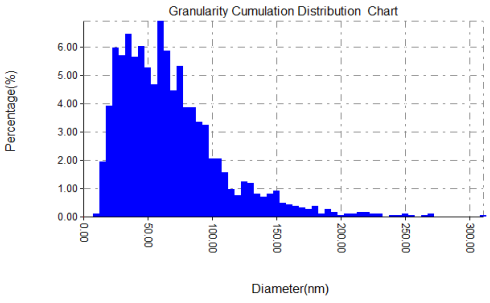


Figure (3): AFM of ZnO nanoparticles of various concentrations:

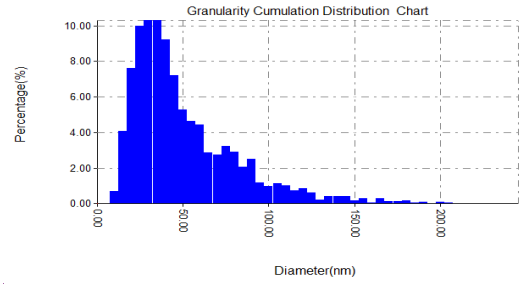
A. 1M of green synthesis NPs with 23.9 nm average size. B. 1.5 M of green synthesis NPs with 25.49 nm average size. C. 2M of green synthesis NPs with 24.65 nm average size. D. 2.5 M of green synthesis NPs with 27.82 nm average size.



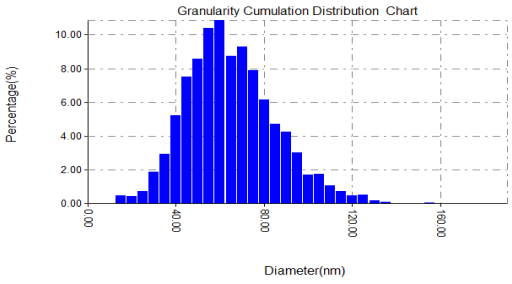
A.Avg. height 57.34 nm and 23.9 nm average size



B.Avg. height 65.53 nm and 25.49 nm average size

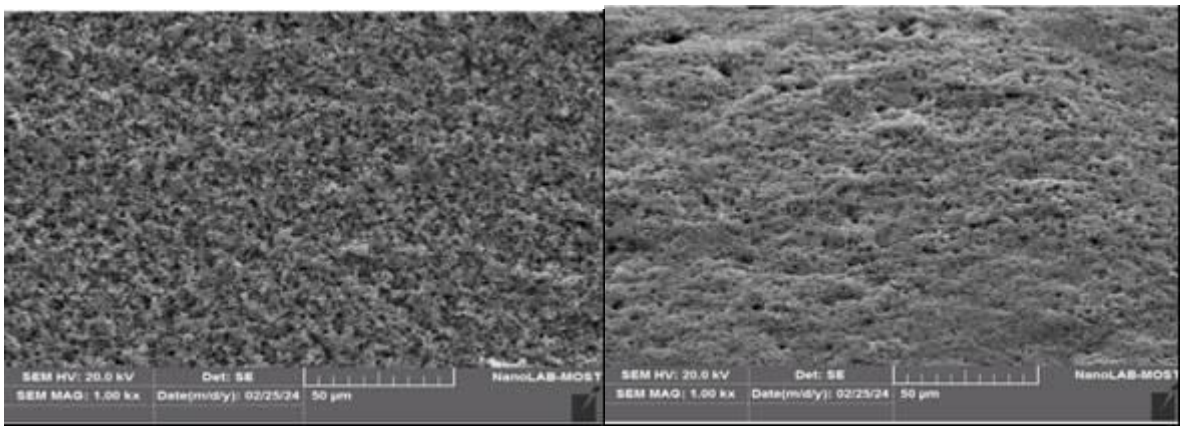


C.Avg. height 48.59 nm and 24.65 nm average size



D.Avg. height 62.83 nm and 27.82 nm average size

The results of SEM showed clear differences in surface morphology and aggregation of green synthesized zinc oxide nanoparticles and it was noted that the peak of zinc oxide nanoparticles was distinct at a concentration of 1.5 M of the extract and as in Figure (4), and the generation of zinc nanoparticles can be attributed to hydrophilic reactions, which leads to the formation of forces between molecules [31] and the difference in biological materials and the concentration of mineral salts has an effect on the synthesis of nanoparticles or the ability of banana peel extract to synthesize ZnONPs can be attributed to the extract's containment of compounds such as polyphenols and flavonoids that act as a reducing agent and catalyst for the formation of zinc nanoparticles [32, 33].



(A) **(B)**

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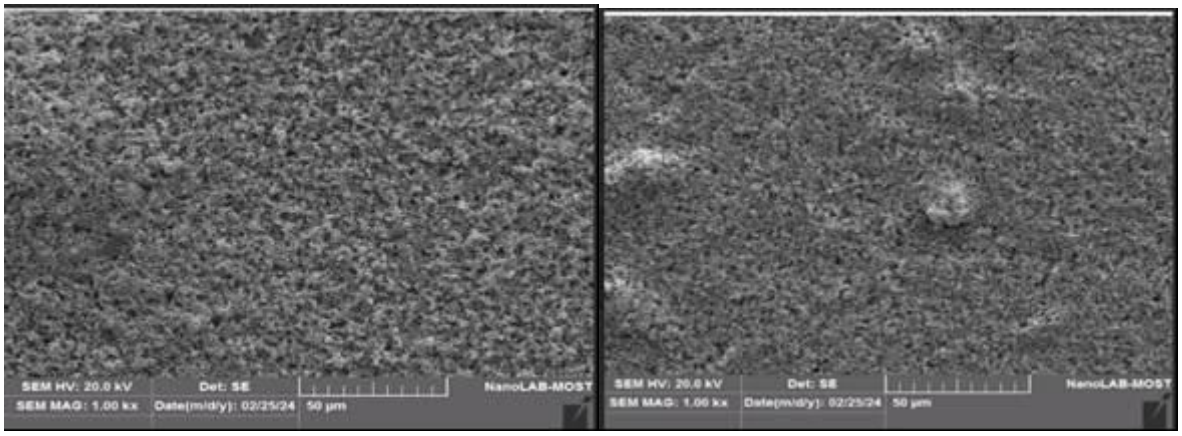


(C) **(D)**

Figure 4: Scanning electron microscopy of green synthesized zinc oxide nanoparticles at concentrations1 M (A), 1.5 M (B), 2 M (C) and 2.5 M (D) with average size 50µm.Image magnification 5.00 kx.

Antioxidant activity DPPH

The free radical scavenging activity of ZnONPs was examined by DPPH method and the results of Table (1) indicated that the scavenging activity of free radical was increased when the concentration of ZnONPs was increased and the concentration of 1.5 M showed an increase of 86.33%, which indicates that this concentration is the best in reaction with free radicals and is the most efficient in producing zinc nanoparticles with antioxidant activity [34]. The synergistic effect between banana peel extract and zinc nanoparticles may be the reason for the increased antioxidant activity at this concentration [35]. This is consistent with [36].

Table 1: Antioxidant activity DPPH of synthesized green of Banana Peel Extract nanoparticles

	DPPH(Mean±SD)				
No.	Conc.	Mean ± SD	No.	Conc.	Mean ± SD
1 M	0.5000	76.67 ± 3.21	2 M	0.5000	80.67 ± 3.79
	0.2500	62.67 ± 2.08		0.2500	73.67 ± 3.06
	0.1250	50.67 ± 2.08		0.1250	66.00 ± 4.36
	0.0625	41.67 ± 2.08		0.0625	53.33 ± 3.51
1.5 M	0.5000	86.33 ± 3.06	2.5 M	0.5000	67.67 ± 5.51
	0.2500	75.00 ± 3.00		0.2500	55.00 ± 2.00
	0.1250	62.67 ± 2.52		0.1250	44.67 ± 3.06
	0.0625	52.33 ± 4.16		0.0625	33.67 ± 3.06

Ferric reducing antioxidant power (FRAP)

The results of Table (2) indicated that the reductive absorption ability of the banana peels extract varies according to the concentrations and it was noted that the concentration of 1.5 M gave the highest value of FRAP, which was 0.6M. This indicates that the concentration1.5 M was the most effective in reducing ferric ion, which was the best in reducing ability, and the reason may be attributed to the extract of banana peels contains phenolic compounds, which have high antioxidant potential, which were very good reducers for metal ions [37]. This is because the banana peels extract contains phenolic

compounds and these compounds contain many hydroxyl groups, including the O-dihydroxy group, which has a very strong free radical removal effect [38].

Table 2: Ferric reducing antioxidant power (FRAP) of synthesized green nanoparticles of Banana Peel Extract

	FRAP(Mean±SD)				
No.	Conc.	Mean ± SD	No.	Conc.	Mean ± SD
1M	0.64	1.281 ± 0.024	2M	0.64	1.118 ± 0.007
	0.32	1.160 ± 0.004		0.32	1.027 ± 0.005
	0.16	0.984 ± 0.011		0.16	0.869 ± 0.018
	0.08	0.444 ± 0.003		0.08	0.417 ± 0.005
1.5M	0.64	1.517 ± 0.057	2.5M	0.64	0.996 ± 0.007
	0.32	1.089 ± 0.007		0.32	0.906 ± 0.005
	0.16	0.909 ± 0.066		0.16	0.629 ± 0.008
	0.08	0.740 ± 0.043		0.08	0.315 ± 0.004

Micronucleus assay

Micronucleus was investigated following the injection with single dose of ZnO-NPs (150 mg/kg), Plant extract treated group (400 mg/kg) for each. The results indicated that ZnO-NPs recorded greater($p \leq 0.05$) percentage of micronuclei namely 12.67±0.50% as compared with negative group 3.76 ± 2.42 % as shown in table 3, figure 5.

Table (3): Micronucleus index (%) of bone marrow in mice treated with ZnO-NP and plant extract

Groups	**MN% (M ±SE)**
Negative control	3.76 ± 2.42C
ZnO-NPs-treated group(150 mg/kg)	12.67± 0.50A
Plant extract treated(400 mg/kg)	2.23 ± 0.58B

Different superscript letters indicate statistically significant differences ($p \leq 0.05$)

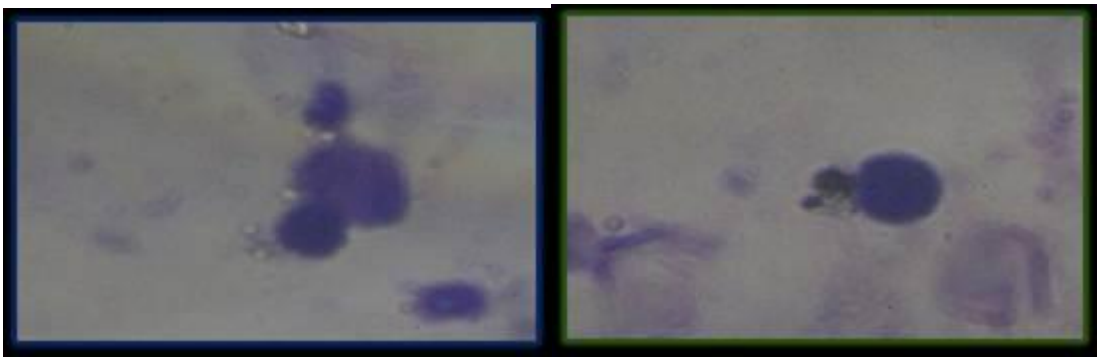


Figure (5): Cytogenetic effects of ZnO-NPs-treated group in mice bone marrow. Magnified view highlighting Micronucleus (400 X)

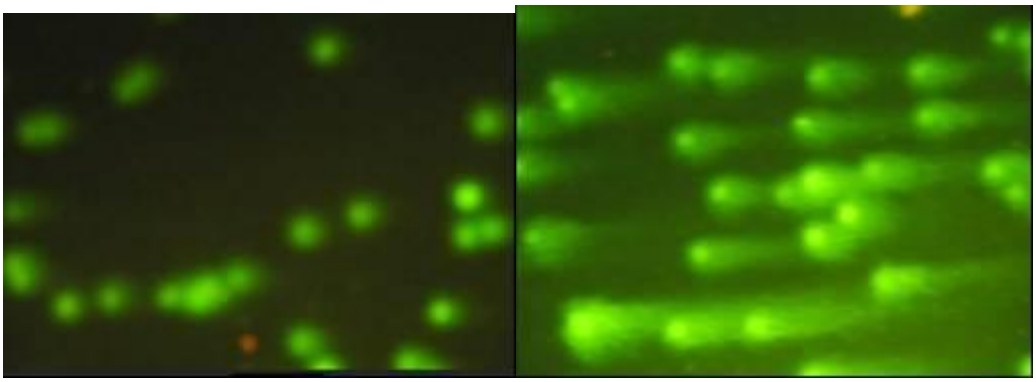
Comet assay

Level of DNA damage was evaluated by alkaline comet assay. The damage was measured by evaluation of distance from DNA of nucleus and tails by system of analysis image. Three parameters used to evaluate the damage: Tail length, Tail moment and % DNA in tail. The results were summarized in (Table 4) and (Figure 6).

Table (4): Comet assay of bone marrow in mice treated with ZnO-NP and plant extract

Groups	Tail length(px)	Tail moment (px)	% DNA in tail
Negative control	1.7 ± 0.06d	0.06±0.0e1	1.23±0.01d
ZnO-NPs-treated group(150 mg/kg)	14.5± 0.57a	25.5±0.20a	17.3±0.06a
Plant extract treated(400 mg/kg)	1.00 ± 0.33e	0.30±0.01d	0.11±0.02e

Different superscript letters indicate statistically significant differences (p≤0.05)



(A) **(B)**

Figure (6): Comet assay in mice bone marrow. Magnified view by florescent microscope (400 X) of control (A), ZnO-NPs (B).

Micronuclei are a small chromatin body outside of the nucleus that occurs during mitosis. Micronuclei are a good indicator of cytotoxicity and chromosomal aberration which is as reason of various diseases including cancer. Micronucleus assay is a predictable procedure of various type of white and red blood cells, which be depended as a useful method to detect the DNA damage that occur in mitosis [39].

Because of extremely the NPs small size, there is a concern that particles interrelate directly with the macromolecules such DNA. In addition the particles induce the oxidative stress by depletion of glutathione, catalase and superoxide dismutase. The Zno NPs have a genotoxic activity in epidermal cells of human because of lipid peroxidation and oxidative stress [40].

The elevation of the concentration of intracellular Zn because of the Zno NPs leading to the over production of the intracellular ROS, plasma membrane leakage, mitochondria dysfunction and death of cells. The dissolved Zn have a major role in the toxic activity of Zno NPs [41].

CONCLUSION

This study showed that banana peel extract can be used as a simple and co-friendly method for synthesizing zinc oxide nanoparticles. The produced nanoparticles exhibited small size and strong antioxidant activity, particularly at a 1.5M concentration. However, the micronucleus assay indicated that higher doses may cause cytogenetic effects and DNA damage. Therefore, banana peel-derived ZnO-NPs are promising for biomedical and pharmaceutical applications, provided they are used carefully and within safe concentration limits to ensure effectiveness without harmful side effects.

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