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Preparation and Characterization Evaluation The Zno Nanoparticles on Lymphocyte Vitality

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Abstract. Zinc oxide nanoparticles (ZnO-NP) are generally used for a variety of applications in life. Zinc oxide nanoparticles were prepared by biosynthesis route using natural extract of Myrtus communis L as an effective on lymphocyte Vitality. The synthesized nanoparticles were characterized by Ultraviolet visible (UV–Visible), Scanning electron microscopy (SEM), X-Ray diffraction (XRD) spectroscopic techniques. Cytogenetic study on Lymphocyte was carried out by preparing a group of solutions as a Colchicines solution, fixative solution and Giemsa stain, and then blood was collected and cultured with zinc nanoparticles. Cells were examined on wet, cooled, grease-free slides and left to dry at room temperature. The results of this study were i noted that the best absorbance was at wavelength 400 compared to the rest of the wavelengths, It has the lowest nano-size of the prepared particles. a polycrystalline structure with cubic phase. Also, no specific peaks due to any impurities were observed. The SEM examination showed a spherical shape of zno Nanoparticles. Cytogenetic study of the extracted ZnNPs on normal lymphocyte blood cells showed that the effect of zin nanoparticles on lymphocyte . Conclustion, ccording to the results of the current study, it was found that the effect of zinc nanoparticles on white blood cells in increasing the blast index compared with the mitosis index

Highlights:

1. Synthesis and Characterization: ZnO nanoparticles biosynthesized using Myrtus communis L., characterized via UV-Vis, SEM, XRD.
2. Cytogenetic Study: Increased blast index, polycrystalline structure, spherical nanoparticles, minimal impurities observed.
3. ZnO-NPs enhance lymphocyte vitality, showing potential for biomedical applications.

Keywords: ZnNP, Lymphocyte, nanoparticles, zinc oxide, Myrtus communis

Introduction

Engineered nanoparticles (NPs) and nanotechnology attracted a lot of attention from people all across the world recently. Nanoparticles and nanomaterials exhibit unique physical, due to its biological, mechanical, optical, and chemical qualities their significant surface-to-volume ratios [10] there are numerous potential uses for nanoparticles in Medicine, biosensors, cosmetics, food, the car industry, and apparel are

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a few examples of industries and goods [16]. Nanotechnology advancements and the usage of nanomaterials have There are various sorts, sizes, forms, and chemical compositions. resulting in the buildup of NPs in the environment. Consequently, the global attention being paid to the Nano-biosecurity concerns and NPs' effects on living things organisms and the entire environment [15]. Nowadays, nanotechnology, including nanoparticles, has increased widely in different fields [1].

Nanotechnology is the use of materials that are nanoscale in size and is employed in a variety of scientific disciplines, such as biochemistry, materials science, and medicine. For instance, ZnO nanoparticles, which have high electrical conductivity, ultraviolet scattering, chemical sensing, and anti-microbial properties, are used to make a variety of consumer goods, including food packaging, cosmetics, textiles, rubbers, batteries, catalyst for automobile tail gas treatment, and applications in the biomedical field [17]. Importantly, research has revealed that one of the main processes causing the toxicity associated with ZnO NPs is the production of reactive oxygen species (ROS). Cytotoxicity and DNA damage have been shown to be caused by a lack of anti-oxidative activity after a ROS insult9. Additionally, the toxic effects of ZnO NPs have been documented in animal models, such as the invertebrate Drosophila13 and the rodent1 and zebrafish [12].

Myrtle (Myrtus communis) is a well-known medicinal and aromatic plant, belonging to the family of myrtaceae [13], which comprises about 2 genera and more than one specie . It is an evergreen shrub, petiolate oval, lanceolate 3cm long by 1cm wide. They have a bright green color , Its stems are very numerous and ramified. The flowers are white solitary, pedunculate arranged in the leaf axils [8] , Essential oils of leaves and fruits are widely used by Moroccan people for their medicinal treatment. They are used for their digestive, anti-spasmodic , Antiseptic and antimicrobial properties , antioxidant , and Hemostatic activity [5], [6] anti-inflammatory antidiabetic , hypo-cholesterolemiant , scolicidal effect and in the prevention and treatment of Alzheimer's disease [11].

Methods

Plant sample preparing

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Amount of 30g of dry M.. communis leaves were taken and placed in a cylindrical container, a substance made of responsible reagent was placed, a thimble was placed in a designated place in the Soxhlet and 70% methanolic alcohol was added to a volume of 250 ml and extracted for 16 hrs at a temperature of 45Cº ,which is an evaporation temperature, then the extract is filtered with a piece of gauze, then the methanolic extract is concentrated in the incubator for one to two days at a temperature of 37 °C, and the extract is dried. and keeping the primary extract at 4 °C [2].

Preparation of ZnO nanoparticles

Zinc oxide nanoparticles were prepared by dissolving 2.5g of zinc oxide in 20ml of ethanol and the mixture was stirred using a magnetic stirrer for 20 min. at room temperature. 25ml of M.. communis extract at a concentration of 1 mg/ml was added to the zinc oxide mixture and stirred for an hour. The solution was left for the next day, after which a white precipitate appeared at the bottom of the glass flask. The white precipitate was washed with ethanol and distilled water several times. The precipitate was subjected to drying at a temperature of 100°C for 3hrs. Finally, the powder was subjected to a heat treatment for 4hrs. at a temperature of 45 ° C [3].

Cytogenetic study on Lymphocyte of human:

The following solution should be prepared [19]:

a) Colchicines solution was prepared by dissolving 1.0 mg (two tablets)of colchicines in a 10ml D.W.; filtration was then performed stored at 4°C.
b) Hypotonic solution (0.075 M KCl) was done by dissolving 1.1175g of KCl powder in 200ml D. W; the stock solution was stored at 4°C.
c) The fixative employed for cytogenesis studies was a freshly made mixture of absolute methanol and glacial acetic acid in the ratio 3:1 (v/v)
d) Sorenson's buffer was made by dissolving 9.47 g of KH2PO4 into one Liter of D.W., and then the stock solution was stored at the room temperature until use.
e) Giemsa stain in which a stock solution was prepared by dissolving 2g of Giemsa stain powder in a 100 ml of methanol then stirred constantly using a magnetic stiffer at room temperature for two hours. The solution was filtered by a filter paper (Wattman No.1) and stored in a dark tight bottle, on staining 1ml of the stock solution was added to 4 ml of Sorenson's buffer.

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f) Blood collection : Blood was taken from normal adult humans by puncturing using a disposable syringe 5ml of blood was transferred into heparinzed tubes.

Blood culture with ZnNPs:

a) a) Half ml of peripheral blood was added to all test tubes containing 5 ml of culture media (with 20% Fetal calf serum).
b) Phytohaemagglutinin (0.3ml) was added to all test tubes , then the following concentrations (30,60,90µg/ml) of the prepared pure resveratrol solution were added to each tube. (Two replicates for each concentration) and three test tubes of Zinc pure 20 mg\ml positive control.
c) All test tubes were returned to the CO2-incubator for 70hrs., and gently shaken each 12hrs. one tries at least.

Harvesting:

a) After 70 hrs. of incubation, 0.1ml of colchicines solution prepared in section (2-2-6-a) was added to each test tube and then return them back to the incubator to complete 72hrs.
b) Samples were centrifuged for 10min. at 1500 rpm.
c) Supernatant was withdrawn by Pasteur pipette and about 0.5ml of supernatant above the precipitated cells in the test tube was left.
d) Precipitates were mixed very well by the vortex mixer, and then 5-10 ml of warmed 37°C Potassium chloride (0.075M) was added gradually and gently with mixing.
e) Samples were incubated in the shaker water bath for 20 min. at 37°C
f) Samples were centrifuged for 10 minutes at 1500 rpm, and then the supernatant discarded.
g) Few drops of the freshly made fixative (methanol and glacial acetic acid 3:1 v/v) were added drop wise with gently mixing until reaching 5ml; later on centrifugation performed for 10 minutes at 1500 rpm then

fixative decanted off and the process repeated for 2-3 times. At the final change, the cells re-suspended in a 3 ml of freshly made fixative and stored at (–20°C).

Slide preparation

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The procedure was followed according to ICCMGR protocol. The cell suspension was removed from freezer and centrifuged at 1500 rpm for 10 minutes. The supernatant was decanted off and the cells re-suspended in appropriate amount to make thinly cloudy suspension. By using Pasteur pipette, 3-4 drops of cells suspension were dropped evenly from appropriate distance 30 cm onto wet, chilled, grease-free slides and allowed to dry at room temperature.

The slides were stained using freshly made [Giemsa stain stock solution and Sorenson's buffer 1:4 v/v which was applied for two minutes, then rapidly washed with Sorenson's buffer, after that the excess solution was removed from slides by fiber free paper. Microscopic examination under low magnification using 10X objective lens was performed to determine mitotic index (MI %) and blast index (BI %)

MI % analysis: The MI % was determined as a ratio of the mitotic cells
to the cells in interphase in 1000 calculated cells.

M.I. % = (No. of Dividing cells / No. of dividing cells + No. of non- dividing cells) x 100

BI % analysis: The BI% was determined as a ratio of the cells in blast form to the other cells in 1000 calculated cells

Statistical analysis

The SAS (2001) program was used to study the effects of treatments in different trails. The least significant difference (LSD) test was used to signify a comparison between the means.

Result and Discussion

Characterization of nanoparticles

1: Absorbance of zinc nanoparticles

The absorbance was calculated by (UV-Vis) device as the first way to describe the formed zinc oxide nanoparticles. Through the figure 3, it was noted that the best absorbance was at wavelength 400 compared to the rest of the wavelengths, and this indicates that there is a wavelength creep towards lower wavelengths, in other words, it It has the lowest nano-size of the prepared particles.

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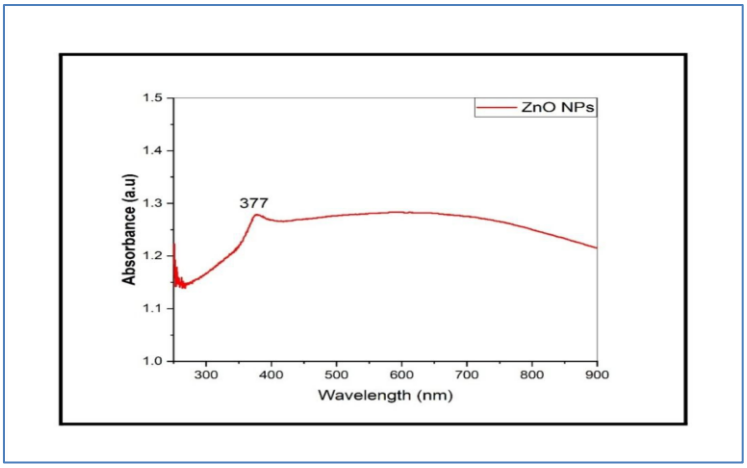
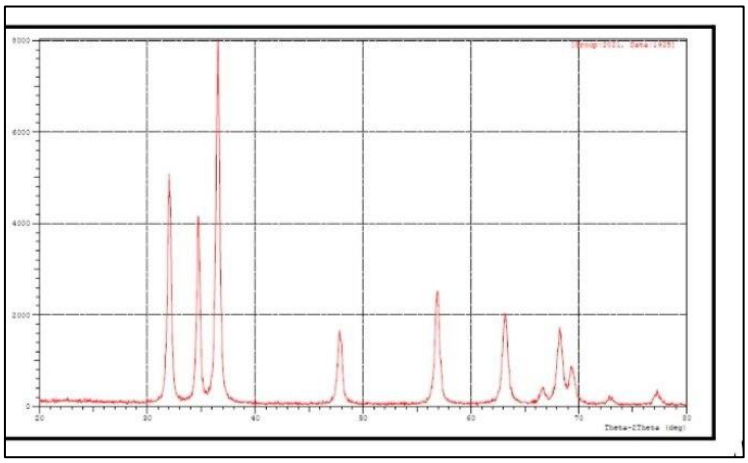


Figure(1)The absorbance of zinc nanoparticles

2: X-ray analysis of zinc nanoparticles

In order to examine the structural properties of the prepared zinc oxide particles, X-ray diffraction was used.

show that her a polycrystalline structure with cubic phase. Also, no specific peaks due to any impurities were observed.



Figure(2) X-ray analysis of zinc nanoparticles

The results of the SEM examination showed that the prepared particles are nanoparticles. The results showed through the image(Figure(5))that the prepared nanoparticles (zinc oxide) have a spherical shape.

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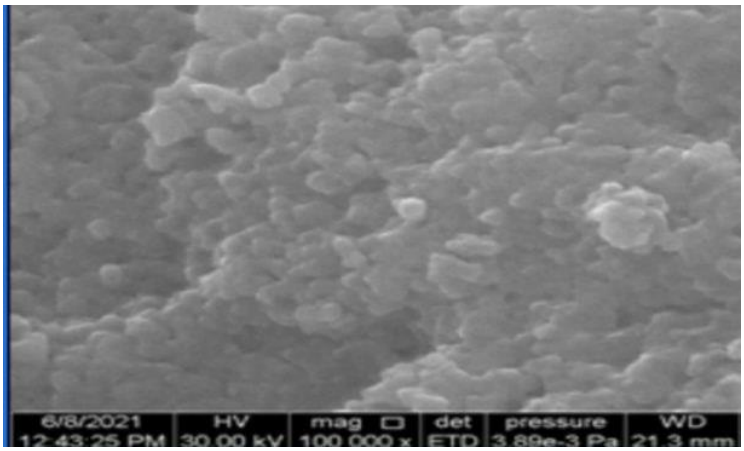

Figure(3): SEM of zinc nanoparticles

Table(1): Cytogenetic study of the extracted ZnNPs on normal lymphocyte blood cell

Concentration	**Mean ± SE**	
µg/ml	**M.I. %**	**B.I. %**
30	**1.17± 0.05 c**	**13.80± 2.00 b**
60	**2.81± 0.50 ab**	**15.35± 1.15 b**
90	**2.10± 0.00 c**	**12.15± 1.55 b**
Zinc 20 µg/ml	**3.59± 0.50 a**	**34.00± 6.00 a**
LSD	**1.0014****	**11.887****
Probability level	**0.0014**	**0.0039**

** (P<0.01).

The means within column difference letter are significant difference.

MI% = mitotic index

BI% = Blast index

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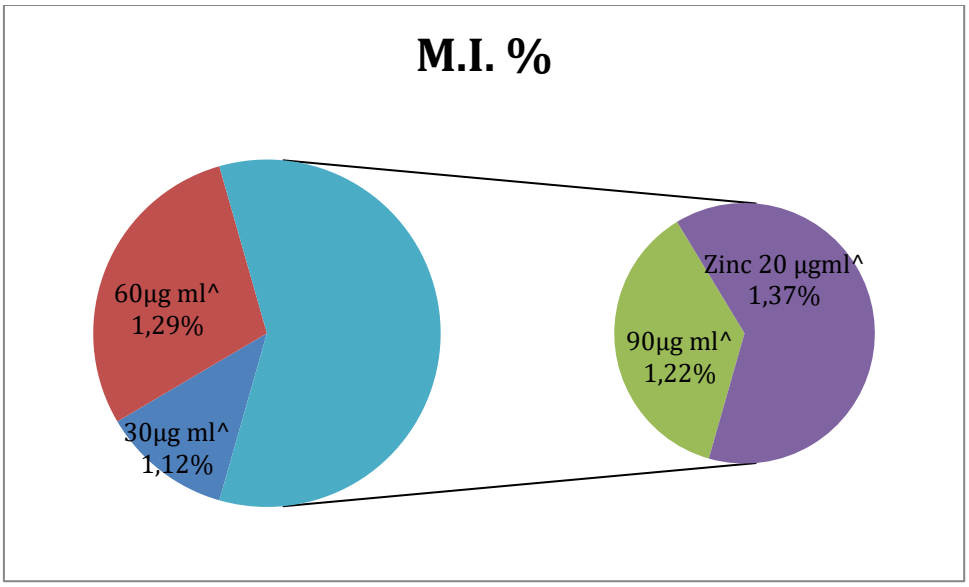

Figure (4): percentage% of concentrations effect on MI

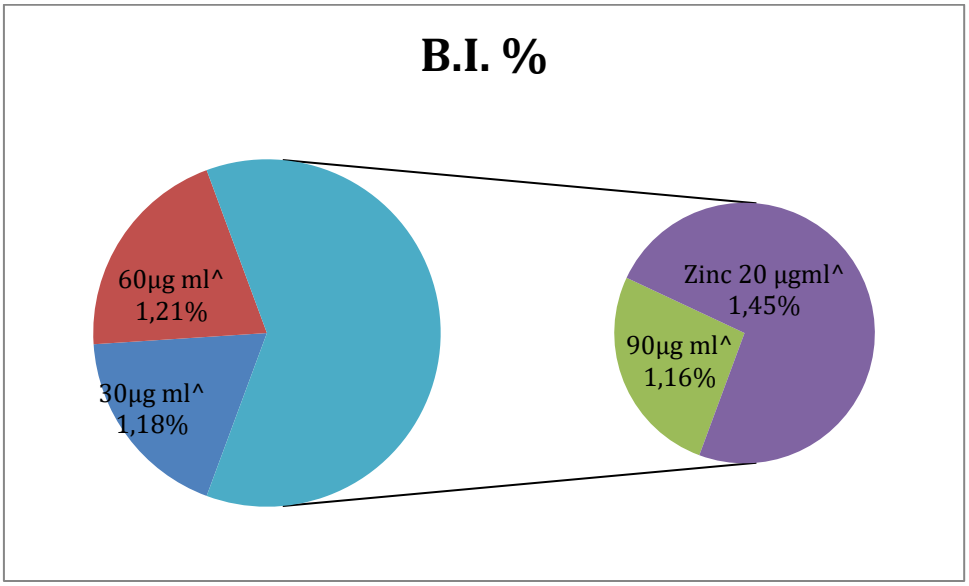

Figure (5) percentage of concentrations effect on BI

Discussion

According to the results of the current study, it was found that the effect of zinc nanoparticles on white blood cells in increasing the blast index compared with the mitosis index. It may be because of that Oxidative stress induced by acute injection of zinc oxide nanoparticles into white blood cells [7]. that this can result from a greater accumulation of High quantities of ZnO NPs in lymphocyte.

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However, following this study, authors it was established that ZnO produced cytotoxicity and oxidative [4] examined primary mouse models of stress. fibroblast cells, and they revealed a notable rise.

but also in the ROS and LDH levels reported a substantial drop in the GSH and SOD level. The level of malondialdehyde (MDA) rose by Nanoparticles of ZnO. They recommended that ZnO nanoparticles produced oxidative stress and cytotoxicity [9]. The capability of ROS to communicate with DNA singleton is caused by biomolecules, especially DNA. or multiple strand breaks, as well as the widely acknowledged Free radicals result from MDA. Several research have indicated corresponding outcomes (Pinho et al.,2020). Our findings also clearly demonstrate that ZnO is clastogenic in human lymphocytes in vitro [7].

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