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"Anti-Inflammatory Effects and EDX Spectra Analysis of Zinc Oxide Nanoparticles Synthesized Using Phoenix dactylifera Extracts: An In Vitro Study "

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***Abstract* :**

Aim: The goal of this project was to synthesize and characterize zinc oxide (ZnO) nanoparticles using an aqueous extract of Phoenix dactylifera and to evaluate their anti-inflammatory properties. **Methods:**Using an aqueous extract of Phoenix dactylifera as the reducing agent, ZnO nanoparticles were produced. Utilizing Energy-Dispersive X-ray (EDX) spectroscopy, characterization was carried out. The anti-inflammatory properties were evaluated through the albumin denaturation assay, where varying concentrations (10µL, 20µL, 30µL, 40µL, and 50µL) of the nanoparticles were tested. **Results:** EDX analysis confirmed the presence of ZnO nanoparticles, showing 8.5% zinc and 35.2% oxygen by weight. Additional signals for silver, sulfur, and carbon were attributed to biomolecules from the extract. The albumin denaturation assay revealed that the anti-inflammatory activity of the ZnO nanoparticles increased with concentration, achieving maximum effectiveness at 50µL. **Discussion**: EDX analysis verified that ZnO nanoparticles had formed successfully. The nanoparticles synthesized using Phoenix dactylifera extract demonstrated significant anti-inflammatory properties, consistent with previous findings on ZnO nanoparticles' ability to inhibit mast cell proliferation and block pro-inflammatory cytokines. The peak effectiveness at 50µL suggests a concentration-dependent response. **Conclusion**: ZnO nanoparticles synthesized using Phoenix dactylifera exhibit notable anti-inflammatory properties, making them promising candidates for therapeutic applications with minimal side effects. Further in vivo studies and comprehensive nanoparticle characterization are recommended to explore their full potential in biomedicine.

***Keywords* :** Anti-inflammatory, Characterization, Phoenix dactylifera, Synthesis, Zinc oxide nanoparticles.

Background:

For thousands of years, dates have been a staple meal throughout the Middle East. Date palms, or Phoenix dactylifera, are among the first plants to be cultivated, serving as a source of sustenance for over 6,000 years. With more than 200 varieties, dates are widely available worldwide [1]. An essential aspect of disease prevention is the regulation of transcription factors, which play a key role in controlling gene expression. Transcription factor inhibitors are crucial for preventing the activation of these factors; however, many available inhibitors are

associated with negative side effects and high costs. Plant-derived substances like flavonoids and phenolics have been demonstrated in earlier research to have strong anti-inflammatory qualities [2].

Nanotechnology has recently become one of the most significant and exciting frontier fields in physics, chemistry, engineering, and biology [3]. It holds great potential for fostering numerous innovations that are expected to shape the future of technology across a wide range of applications [4]. The unique properties of nanomaterials, such as their high surface area to volume ratio, are enhanced as the particle size decreases, and their dispersion and shape become more refined [5]. These improved physical characteristics also contribute to the increased reactivity of the nanoparticles [6].

In biomedicine, zinc oxide is recognized as a promising candidate among various metal oxides, especially for its applications in biosensing, cell imaging, anti-cancer, anti-bacterial, anti-diabetic, and anti-inflammatory treatments [7,8]. Several studies have demonstrated that zinc oxide nanoparticles can induce a range of adverse effects, such as oxidative stress, cytotoxicity, and genotoxicity, which vary based on factors like concentration, dosage, route of administration, and duration of exposure [9,10]. Despite these concerns, zinc oxide nanoparticles have also been reported to exhibit anti-inflammatory properties [11]. These effects are achieved by inhibiting the proliferation of mast cells, suppressing LPS-induced COX-2 production, and blocking pro-inflammatory cytokines [12].

Inflammation is the body's immune response to injury or infection. It involves white blood cells and various inflammatory molecules in the bloodstream, which work together to protect the body from harmful invaders such as bacteria and viruses. [13]. A localized inflammatory response following injury is vital for the healing process [14]. Green synthesis of nanoparticles using plant components has gained recognition, with phytochemical metal nanoparticles being utilized in various applications [15].

The rationale for this study stems from the lack of previous research investigating the properties of Zinc Oxide nanoparticles enhanced with Mabroom dates. Therefore, the study aimed to evaluate these nanoparticles by characterizing them using Energy-Dispersive X-ray (EDX) spectra and assessing their anti-inflammatory activity when synthesized with Phoenix dactylifera.

Materials and Methods:

EDX Spectra Characterization:

Utilizing an energy-dispersive X-ray spectrometer (EDX), the elemental composition of the generated particles was investigated. This method was used to evaluate the composition of the needle-like structures that formed at higher oxidizing temperatures as well as the nanostructures that formed at lower oxidizing temperatures.

Albumin Denaturation Assay of ZnO:

A 0.05 mL solution was mixed with 0.45 mL of bovine serum albumin (1% aqueous solution) and 10µL, 20µL, 30µL, 40µL, and 50µL of dates and zinc oxide nanoparticles. The pH of the mixture was then lowered to 6.3 by a little addition of 1N hydrochloric acid.

The samples were incubated for 20 minutes at room temperature and then heated in a water bath for 30 minutes at 55°C.

Egg Albumin Denaturation Assay:

0.2 mL of egg albumin that had been isolated from a hen's egg was combined with a 5 mL solution of recently made phosphate-buffered saline with a pH of 6.3. Syzygium caryophyllatum was then synthesized in various quantities (10µL, 20µL, 30µL, 40µL, and 50µL) individually. A positive control was employed, which was diclofenac sodium. After 15 minutes of heating at 37°C in a water bath, the combinations were allowed to cool to room temperature before their absorbance at 660 nm was measured.

Results :

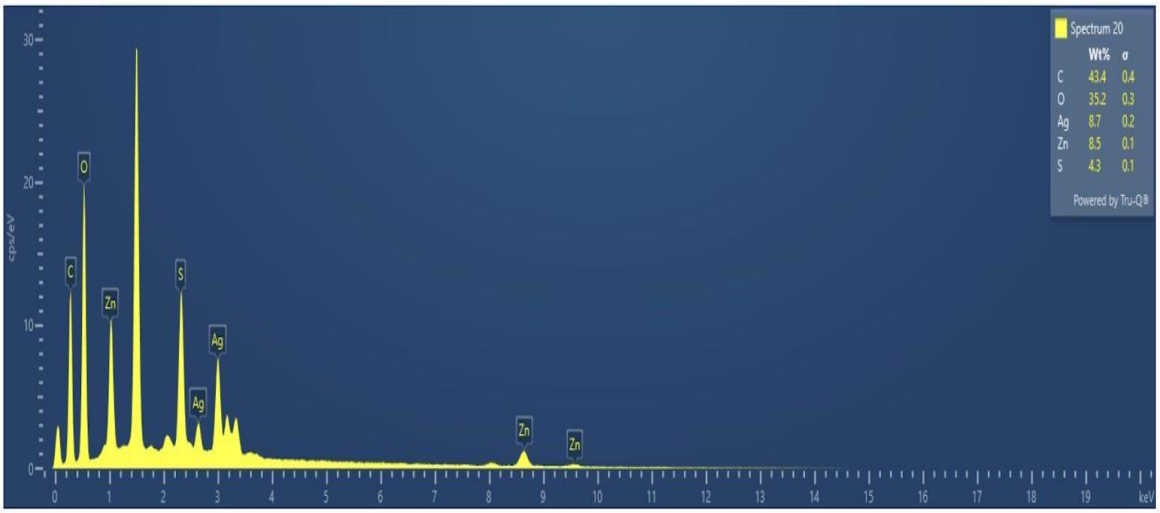

Figure 1 shows the results of the EDX analysis, which confirms the presence of ZnO nanoparticles, indicated by small peaks corresponding to zinc (8.5%) and oxygen. Additional signals for silver (Ag), sulfur (S), and carbon (C) are likely due to the presence of biomolecules from the Phoenix dactylifera extract that adhered to the nanoparticle surfaces.

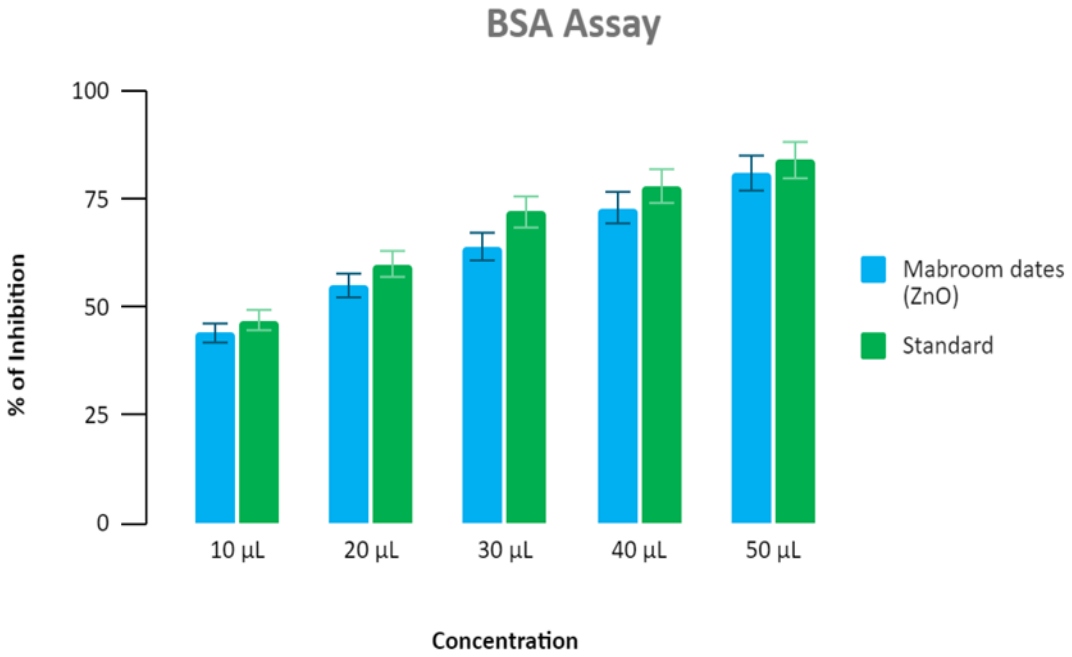

Figure 2 demonstrates the anti-inflammatory activity measured by the inhibition of albumin denaturation, following the method outlined by Leelaprakash and Mohan Das (2010). The data indicate. hat the inhibition percentage reaches its peak at a concentration of 50µL of the extract hat the inhibition

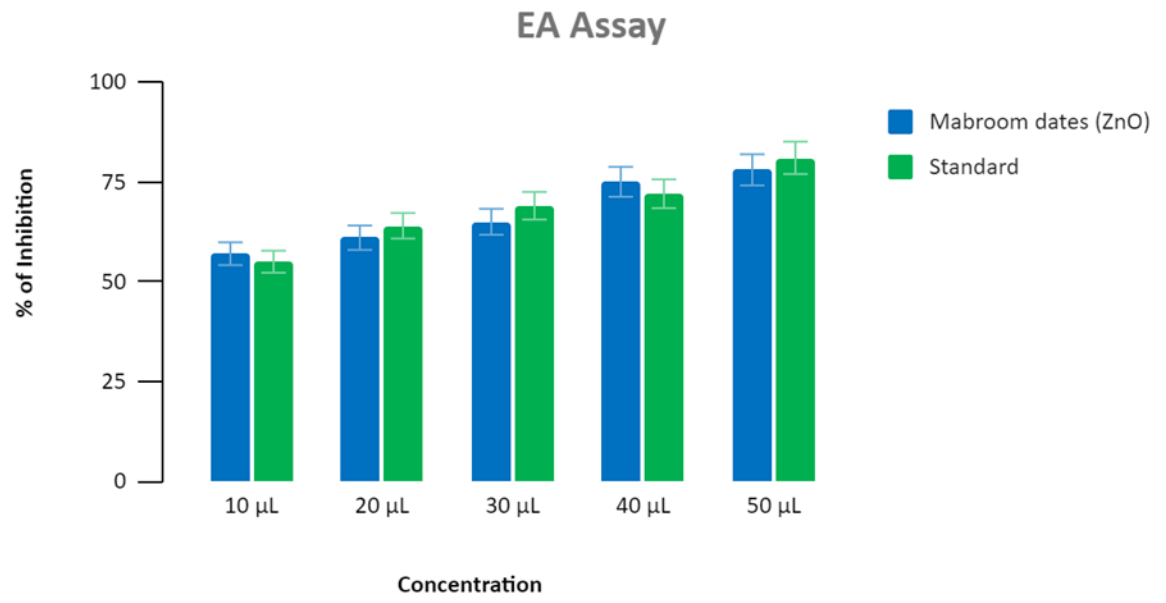

Figure 3 employs the technique outlined by Leelaprakash and Mohan Das (2010) to demonstrate the anti-inflammatory efficacy as determined by the suppression of egg albumin denaturation. The analysis reveals that the extract exhibits higher inhibition percentages at concentrations of 10µL and 40µL compared to the standard.

Discussion:

Inflammation is the body's natural response to various microorganisms, irritants, damaged cells, and harmful stimuli. Zinc oxide nanoparticles (ZnO-NPs) help reduce inflammation by inhibiting the production of inflammatory cytokines. Current medications for various inflammatory diseases often fall short in effectively treating the condition, slowing its progression, or alleviating symptoms[17]. In this study, the distinct peaks observed for zinc and oxygen atoms indicate the successful formation of zinc oxide nanoparticles, consistent with the findings of Rajeshkumar S et al., 2018 [18].

In the assessment of anti-inflammatory effects using the albumin denaturation inhibition assay, ZnO nanoparticles synthesized with grape seed extract showed the highest absorbance at a 50µL concentration, reaching 84.6% [19]. The anti-inflammatory properties of ZnO nanoparticles are well-documented, including their ability to inhibit mast cell proliferation, block pro-inflammatory cytokines, and reduce COX-2 expression induced by LPS. Previous research indicates that ZnO nanoparticles, like those synthesized with P. odoratissimum ALE, exhibit concentration-dependent anti-inflammatory effects, with greater protection observed at higher concentrations [20].

In our research, we investigated zinc oxide nanoparticles produced with dates and assessed their anti-inflammatory effectiveness using an albumin denaturation inhibition assay. The analysis revealed that the extract showed the highest absorbance, reaching 78%, at a concentration of 50µL. Additionally, the EDX spectra provided insights into the elemental composition of the ZnO nanoparticles, confirming the formation of zinc oxide nanocrystals.

Limitations:

The study primarily focused on the anti-inflammatory properties of zinc oxide nanoparticles synthesized with Phoenix dactylifera extract, without extensive investigation of other biological effects such as cytotoxicity or genotoxicity. It was conducted in vitro, lacking in vivo validation. The characterization of nanoparticles was limited, requiring further analysis using techniques like TEM or SEM for detailed size and morphology

assessment. Additionally, the natural variability in Phoenix dactylifera extracts could affect result consistency, and the study did not address the long-term stability and safety of the nanoparticles.

Future scope:

Future research should include in vivo studies to validate the anti-inflammatory efficacy and overall safety of zinc oxide nanoparticles synthesized with Phoenix dactylifera extract. Further characterization using TEM, SEM, and XRD can provide detailed insights into the nanoparticles' properties. Exploring other therapeutic applications, such as antimicrobial and anticancer effects, could expand their utility. Standardizing the extraction process and conducting long-term stability studies will enhance result consistency and safety. Finally, assessing the scalability and environmental impact of the synthesis process is essential for potential commercial applications.

Conclusion:

The zinc oxide nanoparticles synthesized using extracts from Phoenix dactylifera demonstrate notable anti-inflammatory properties. These nanoparticles effectively reduce inflammation while producing minimal side effects, making them a promising option for therapeutic applications.

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