

The Impact of *Nasturtium officinale*-MgO Nanoparticle on the Immunity of Hypomagnesemic rats

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ABSTRACT

This study explores the green synthesis of magnesium oxide nanoparticles (MgONPs) via *Nasturtium officinale* (watercress) extract and assesses their physicochemical properties and therapeutic effects against furosemide-induced hypomagnesemia in rats. X-ray diffraction (XRD), energy-dispersive spectroscopy (EDS), scanning electron microscopy (SEM), atomic force microscopy (AFM), UV-Vis spectroscopy, and Fourier-transform infrared spectroscopy (FTIR) were used to confirm the biosynthetic process. In this experiment, 40 adult female rats were divided into five groups: control, furosemide-treated (T1), MgONPs-treated (T2), watercress extract-treated (T3), and magnesium sulfate-treated (T4). The antioxidant enzyme activity (catalase and superoxide dismutase), and serum magnesium levels were examined. MgONPs increased granulocyte proliferation, boosted antioxidant enzyme activity, and successfully restored serum magnesium levels. Validated MgONPs' protective function in maintaining immune function. These findings imply that watercress extract and biosynthesized MgONPs have great promise for treating hypomagnesemia and related immunological dysfunctions.

Keywords: Magnesium oxide nanoparticles, *Nasturtium officinale*, hypomagnesemia, immune modulation, antioxidant defense.

1. INTRODUCTION

Magnesium is an important mineral involved in many physiological functions, such as immune response, oxidative stress management, and enzyme activation. Magnesium (Mg) is an important factor for enzymes involved in carbohydrate metabolism (Hamdan, 2011). Dietary Mg²⁺ deficiency is associated with ischemic heart disease, congestive heart failure, sudden cardiac death, arrhythmias, vascular complications of diabetes mellitus, (Obaid, and Khaleel, 2019)., given magnesium with KCl we observed moderate increase in nuclear and cytoplasmic staining. Hypomagnesemia is an electrolyte imbalance induced when there is a low amount of serum magnesium (less than 1.46 mg/dL) in the blood, (Obaid, and Khalil, 2021). Chronic diuretic use frequently causes hypomagnesemia, which can result in oxidative stress, inflammatory diseases, and immune disruptions. Supplementing with magnesium is a common treatment; however, conventional magnesium salts exhibit limited bioavailability. Nanotechnology offers a possible alternative through the use of MgONPs, which gives better bioavailability and stability. Nanobiotechnology is a rapidly developing topic that incorporates elements from many academic disciplines, including biology, chemistry, physics, and medicine (Panda et al, 2021). typically ranging of NPs from 1 to 100 nanometers in size, possess a large surface area to volume ratio, high stability, their small size allows for easy penetration into cells and tissues, MgO nanoparticles can be produced by physical, chemical, or biological processes. Physical and chemical synthesis is not the ideal choice due to its high cost, energy requirements, toxicity to the environment, and unsuitability for biological applications. (Daharwal and Kujur, 2021). Biosynthesis techniques, however, may also be beneficial in terms of reduced costs and environmental friendliness. The advantages of capping and stabilizing can also be obtained by using nontoxic extracts or microbiological cultures (Ahmed, 2022).

Medical plants take a vital part in our life since they're broadly used for the prevention and treatment of different diseases (Mashi and Dheyab, 2018). In order to produce green nanoparticles, more plant materials have recently been added to the process of using phytochemicals like leaf extracts, fruit, bark, fruit peels, roots, and callus as bio-reductants. (Abuzeid, 2023). Rocket plant (*Nasturtium officinale*) has gotten more value as a vegetable, and spice around the world (Mashi, 2017), further it is considered to be an important chemoprotective plant. In this study, we attempted to manufacture MgONPs utilizing *Nasturtium officinale* extract and evaluate their potential in alleviating furosemide-induced hypomagnesemia and immunological dysfunction. The electrolyte such as Mg²⁺ disturbances could be mediated by the tumor pathology (Abdulrahman, et al, 2021).

2. MATERIALS AND METHODS

Synthesis and Characterization of MgONPs MgONPs were biosynthesized using an aqueous extract of *Nasturtium officinale* as a reducing and capping agent. The formation of MgONPs was monitored through colorimetric changes and confirmed using UV-Vis spectroscopy, FTIR, XRD, SEM, EDS, and AFM.

Experimental Design

Forty female Wistar rats (8 weeks old) were randomly assigned into five groups (n=8 per group):

Control group: The control group were treated with normal saline orally.

T1 group (Hypomagnesemia) : animals received furosemide at dose of 40mg/kg BW . Administration by injection (**Yelitza Berné et al.,2005**)

T2 group: Animals in this group received furosemide at dose of 40mg/kg BW plus watercress MgONPs at does 12.5µg/kg BW.

T3 group: animals received furosemide at dose of 40mg/kg BW plus watercress aqueous extract at dose 500mg/kg BW (**Aires, et al, 2013**)

T4 group: rats in this group administered furosemide at dose of 40mg/kg BW With Mg sulphate at dose 0.1mg /kg BW.(**constable et al.,2016**).

Biochemical Analyses

1-Serum magnesium levels were measured using atomic absorption spectrometry.

2-Antioxidant Markers

Antioxidant enzyme activities (catalase and superoxide dismutase) were analyzed spectrophotometrically.

3. RESULTS AND DISCUSSION

Characterization of MgONPs

A peak at 380 nm was found in the UV-Vis spectra, confirming the production of MgONP. The presence of carboxyl and hydroxyl functional groups, as shown by FTIR spectra, stabilized the nanoparticles. MgONPs' cubic crystalline structure was validated by XRD analysis, although SEM and AFM images revealed a homogeneous spherical morphology with little aggregation.

Monitoring color shifts of Biosynthesis MgONPs

No changes in table color, which is a first sign of nanoparticle formation, were seen during the biosynthesis of magnesium oxide nanoparticles (MgONPs) using the aqueous extract of watercress (*Nasturtium officinale* L.) (Pal et al., 2019). The magnesium nitrate solution had no color at first (Figure-A). The solution changed color as the Watercress extract was added gradually; after 20 minutes, it took on a yellowish hue (Figure-B). After approximately one hour, the mixture's appearance evolved to a cloudy consistency (Figure -C). By the 48-hour mark, the solution had attained a stable dark yellow color, which persisted until the synthesis process was completed. Following these observations, the reaction mixture was centrifuged and washed five times to purify the MgONPs, as illustrated in (Figure -D).

The successive color variations observed during the biosynthesis process are crucial markers of the nanoparticle creation and growth mechanism. The initial change from a colorless solution to a yellowish hue suggests the onset of the bioreduction process, where bioactive compounds present in the Watercress extract begin reducing magnesium ions to form MgONPs(Kumar et al., 2021). After an hour, the subsequent change to a hazy look most likely represents the nucleation and early growth of nanoparticles, where a growing number of nuclei contribute to light scattering and alter the optical characteristics of the solution.

After 48 hours, the final stable dark yellow color is seen, signifying that MgONP production is complete and the particles have achieved a mature, uniform condition. This color stabilization, which may be the result of localized surface plasmon resonance (LSPR) effects, is compatible with the production of a sufficient number of nanoparticles exhibiting distinctive optical properties. The subsequent centrifugation and washing procedures are crucial for eliminating unreacted precursors and any remaining biomolecules, guaranteeing the stability and purity of the produced MgONPs (Khan et al., 2021).

These findings are consistent with related research on the manufacture of biogenic nanoparticles, which shows that the nucleation and growth stages of nanoparticles are correlated with slow color changes. In addition to aiding in the reduction of magnesium ions, the extract from *Nasturtium officinale* offers natural capping agents that stabilize the nanoparticles, reducing aggregation and improving the material's overall quality.

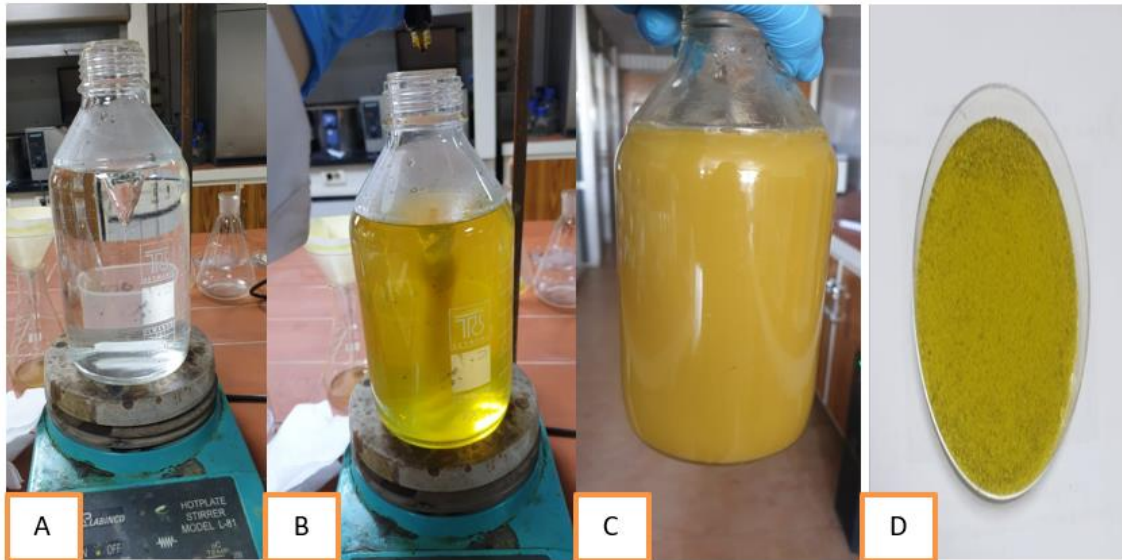


Figure -1: Changes in color during the synthesis of MgO nanoparticles include: A-MgNO₃ alone; B- color shift from white to yellowish when watercress extract and NaOH are added drop by drop; C- color shift after two hours of reaction time; and D-The MgONPs after two days.

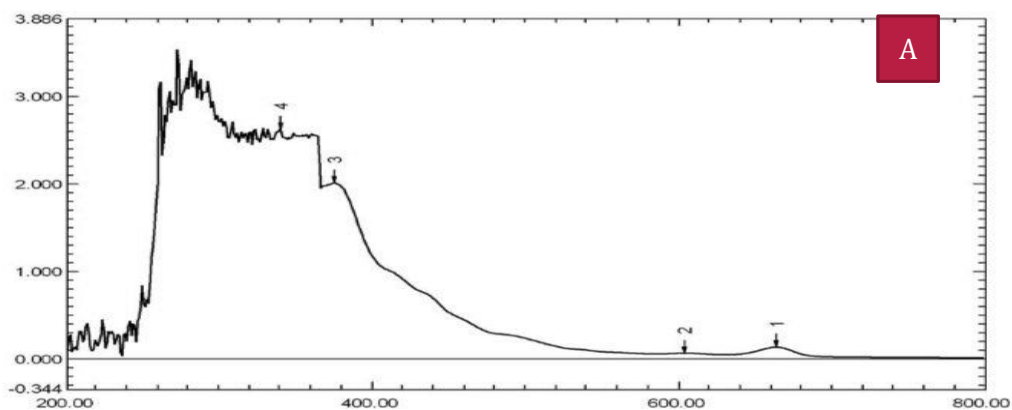
Ultraviolet-Visible(UV-VIS)Spectroscopy for MgONPs

The UV–visible spectroscopy

The UV–visible spectroscopy of the biogenically synthesized magnesium oxide nanoparticles (MgONPs) revealed a prominent absorption band centered at approximately 380 nm. During the synthesis process, a distinct brown color change was observed, correlating with the emergence of this peak.

The creation of MgONPs is indicated by the absorption peak at 380 nm, which is caused by the localized surface plasmon resonance (LSPR) phenomenon. When stimulated by incident light, the collective oscillation of electrons on the nanoparticle surface causes this resonance. As surface plasmon activation increases light scattering, the color shift from light to brown further supports the production of nanoparticles.

at the wavelength of resonance. These optical characteristics, which have already been documented in the literature (Philip & Kumar 2022), validate the efficiency of employing watercress (*Nasturtium officinale*) aqueous leaf extract as a capping and reducing agent in this green synthesis method.



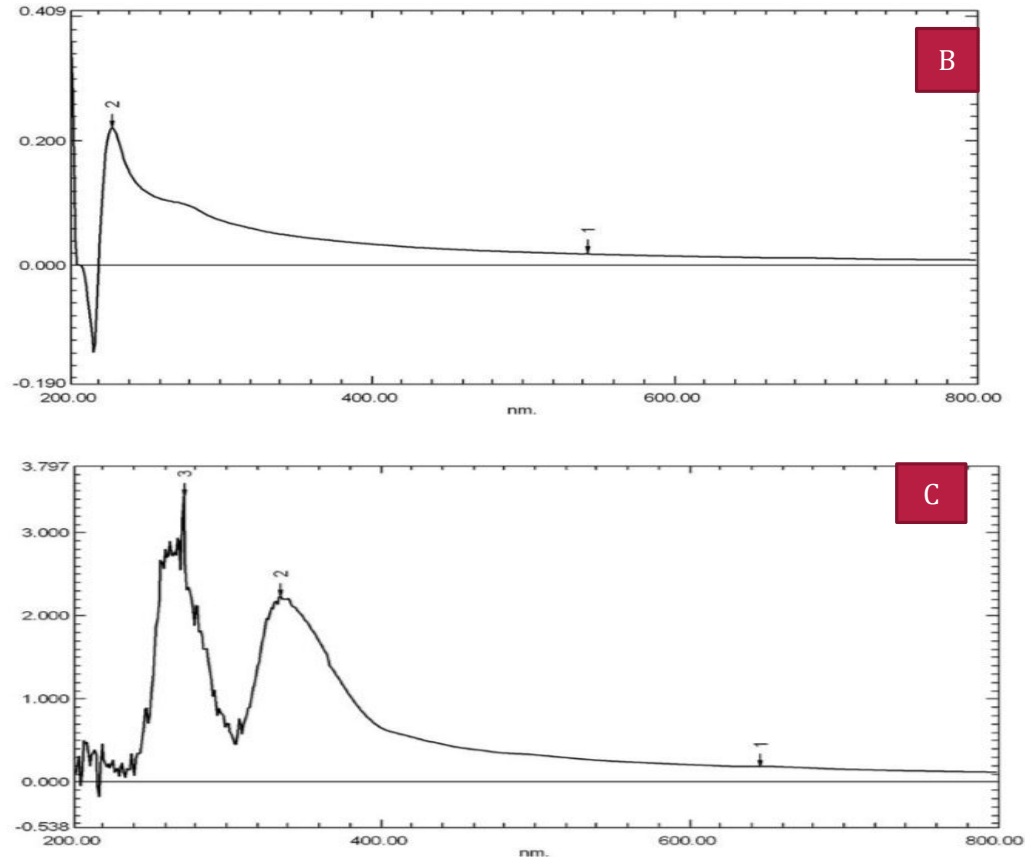
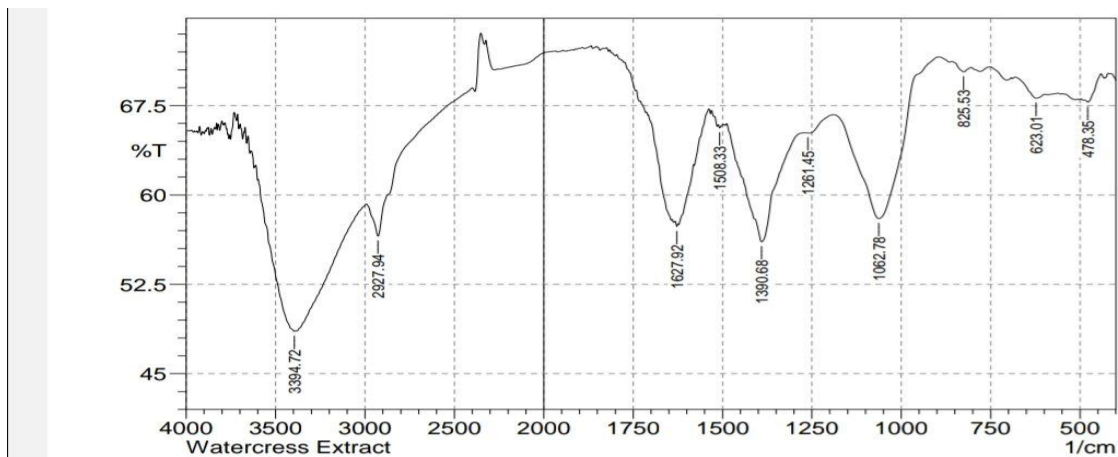


Figure -2 (A): UV-Vis spectroscopy absorbance of watercress extract, (B) UV-Vis spectroscopy absorbance of magnesium nitrate, (C) UV-Vis spectroscopy absorbance of MgONPs.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis for MgONPs

The FTIR spectra of the synthesized MgONPs exhibited several significant absorption bands. Key features included a broad band around 3400 cm^{-1} (indicative of O–H stretching vibrations), a band near 1650 cm^{-1} corresponding to C=O stretching, and additional peaks related to C–O and N–H vibrations.

The bioactive compounds from the watercress extract are confirmed to be involved in the synthesis process by the FTIR analysis. The wide O–H stretching band indicates the presence of hydroxyl groups, most likely from phenolic or alcoholic chemicals, which can serve as stabilizers and reducing agents. Proteins and other biomolecules are adsorbed on the surface of the nanoparticles, acting as capping agents, as shown by the C=O and N–H vibrations (Sidhu et al., 2022). According to related investigations, this dual effect not only helps reduce magnesium ions but also stops excessive agglomeration.



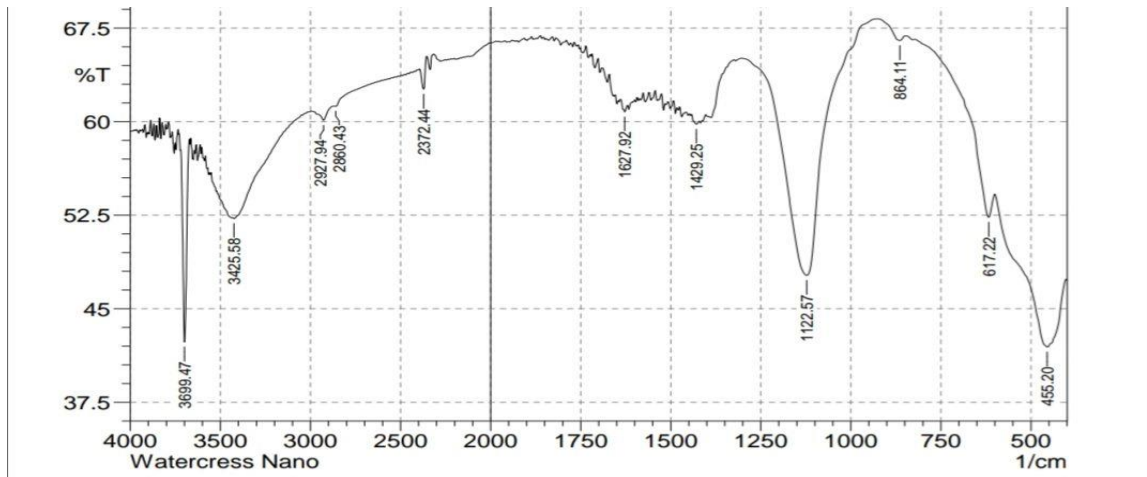
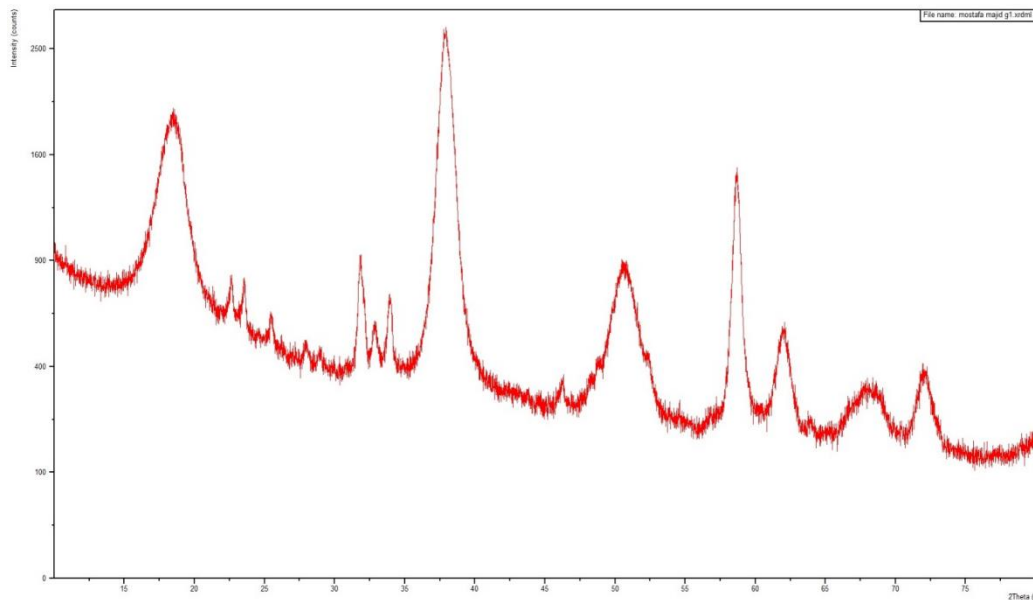


Figure -3 A: FT-IR spectrum of Watery extract of Watercress, B: FT-IR spectrum of functional groups of watercress extract attached to MgONPs.

X-ray Diffraction (XRD) Analysis for MgONPs

XRD analysis of the MgONPs produced distinct and sharp diffraction peaks corresponding to well-defined crystal planes. The pattern is in agreement with the standard cubic phase of magnesium oxide, and the calculated crystallite size (using the Scherrer equation) falls within the nanometer range.

The crystalline character of the MgONPs is clearly shown by the XRD data. The distinct peaks show strong crystallinity and imply that well-ordered nanoparticles are produced by the biogenic synthesis pathway. The diffraction pattern confirms that the target phase was successfully synthesized because it matches standard reference data for MgO (Netzer and Noguera, 2021). Additionally, a somewhat uniform particle size distribution is implied by the low peak widths, which is important for several applications including electronics and catalysis. This controlled crystallinity is in line with other research that synthesizes nanoparticles using plant extracts (Al-Kurdy et al., 2020).



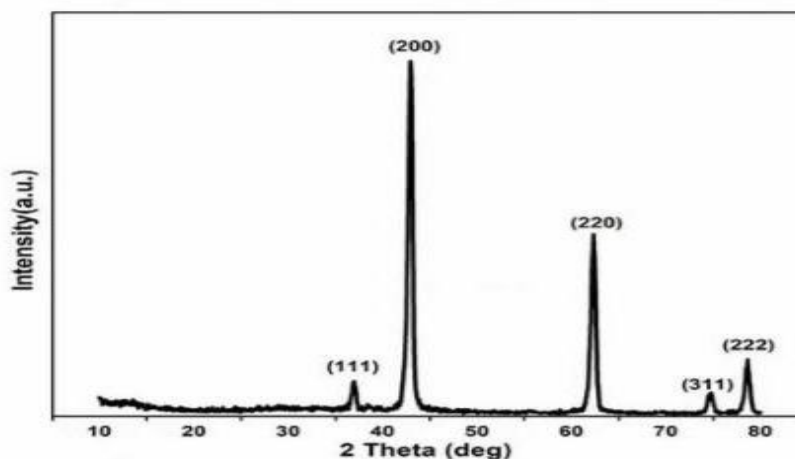


Figure -4 :X-ray diffraction pattern for MgO nanoparticales

Energy Dispersive Spectroscopy (EDS) Analysis for MgONPs

4. RESULTS

EDS analysis confirmed the elemental composition of the synthesized nanoparticles. The spectra predominantly displayed peaks corresponding to magnesium and oxygen, with relative atomic percentages in line with the stoichiometry of MgO. No significant impurities were detected.

The EDS results verify the purity of the MgONPs, as only the expected elements (Mg and O) were present. This high purity is critical for applications that require uncontaminated materials, such as in catalysis or biomedical applications. The absence of extraneous peaks indicates that the use of *Nasturtium officinale* extract did not introduce any unwanted elements into the synthesis process. These findings are consistent with similar studies that emphasize the advantage of biogenic synthesis methods in producing high-purity metal oxide nanoparticles (Kumar et al., 2021)

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
N	17.9	0.3	14.9	0.2
O	68.8	0.4	65.1	0.4
Na	1.3	0.0	1.7	0.1
Mg	8.8	0.1	12.6	0.1
Si	2.1	0.0	3.5	0.0
S	0.5	0.0	1.0	0.0
Ca	0.5	0.0	1.1	0.0

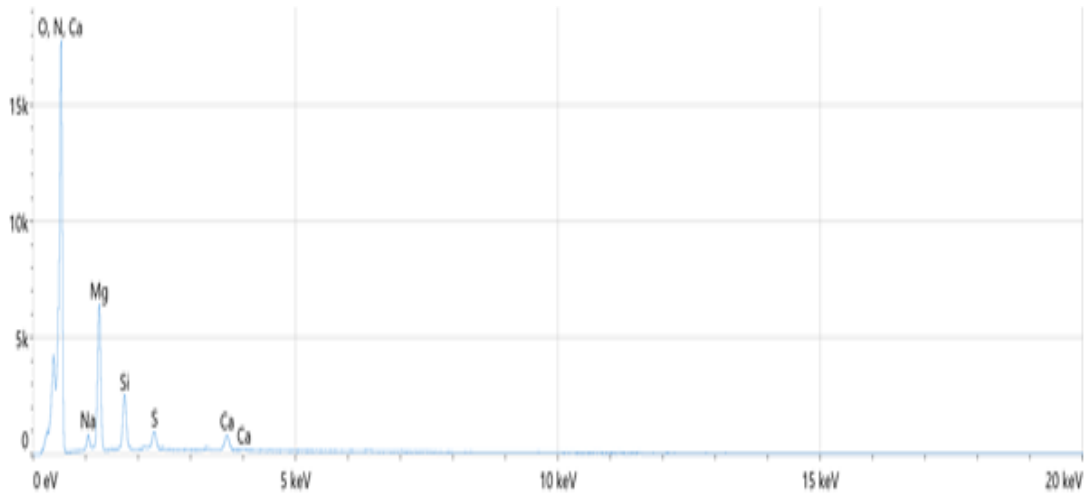
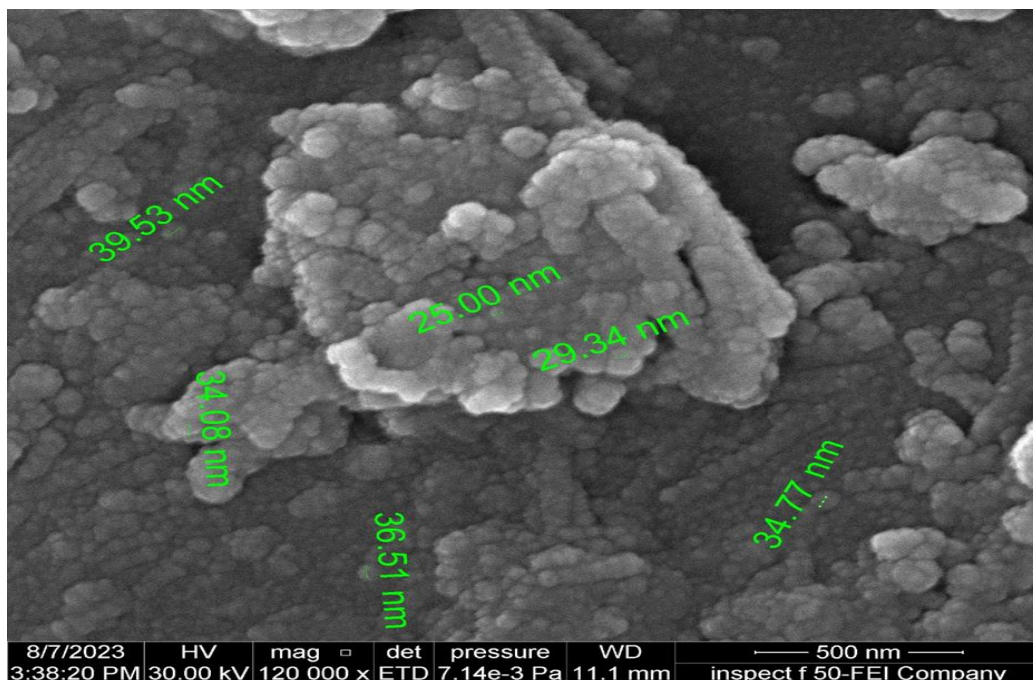


Figure -5: Energy Dispersive Spectroscopy(EDS) results for MgO nanoparticles

Scanning Electron Microscopy (SEM) Analysis for MgONPs

SEM imaging revealed that the MgONPs possess a predominantly spherical morphology with a relatively uniform size distribution. Some regions showed minor agglomeration, which is common in nanoparticle samples prepared via biological routes.

The morphological properties of the produced MgONPs are well demonstrated by the SEM pictures. The homogeneous distribution and spherical shape that were discovered indicate that the biomolecules in the extract of *Nasturtium officinale* effectively encapsulated the nanoparticles, preventing widespread aggregation. The high surface energy that nanoparticles naturally possess during the drying process is usually the cause of the slight agglomeration that was observed (Shrestha et al., 2020). Overall, the morphological characteristics found are consistent with other studies on the synthesis of biogenic nanoparticles (Essien et al., 2020), demonstrating the synthesis method's effectiveness and reproducibility.



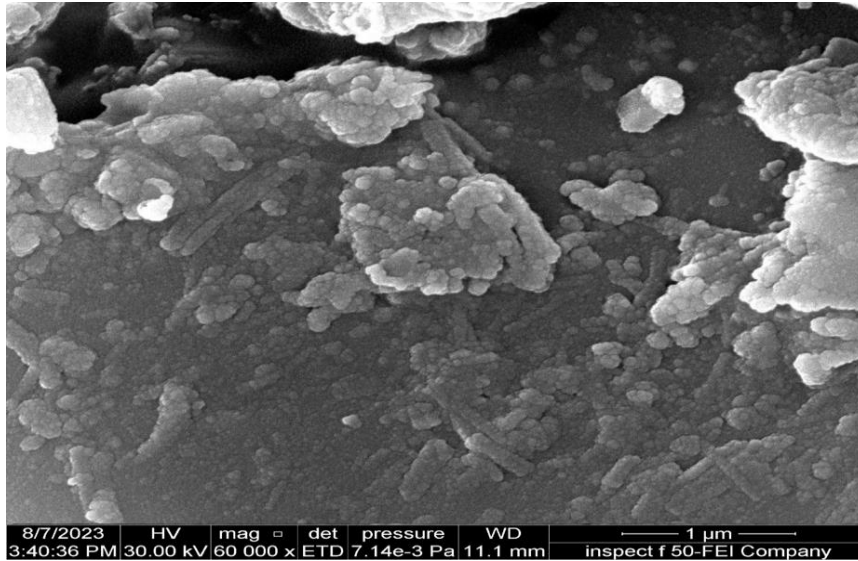
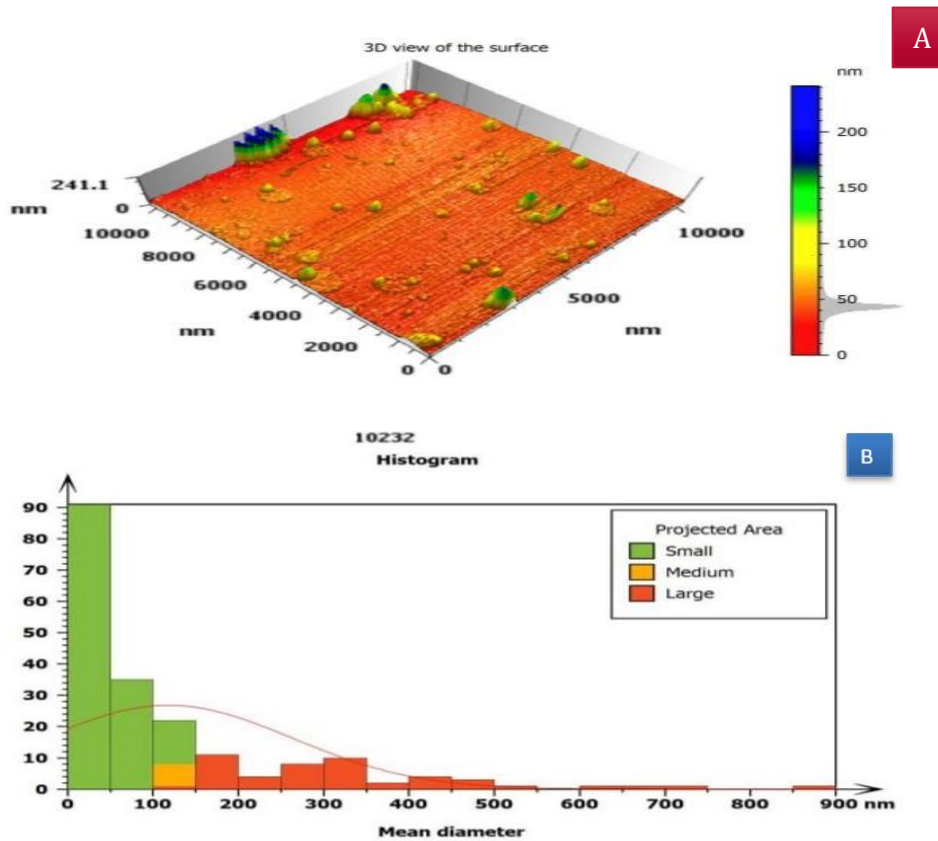


Figure -6: SEM image showed the size and characters of synthesized MgONPs (A) 500nm(25.00-39.53nm) and (B) 1 μm

Atomic Force Microscopy (AFM) Analysis for MgONPs

AFM analysis provided three-dimensional topographical maps of the MgONPs, revealing consistent particle heights and clear surface features. The height measurements corroborated the size estimations obtained from SEM analysis.

Particle height and surface roughness may be precisely measured thanks to AFM's complementing three-dimensional view of MgONP surface morphology. The uniformity of particle size and the effectiveness of the green synthesis approach in controlling nanoparticle dimensions are validated by the consistency between AFM and SEM data. Understanding how surface characteristics may affect the physical and chemical characteristics of the nanoparticles in possible applications requires the use of comprehensive topographical data (Joudeh and Linke, 2022).



Particle #150	Small	4277	60.24	430.0
Particle #157	Small	2415	41.90	456.3
Particle #158	Large	26769	168.1	520.7
Global statistics				
Mean	*****	32416	117.8	488.6
Min	*****	452.9	14.87	446.4
Max	*****	604351	855.3	765.7

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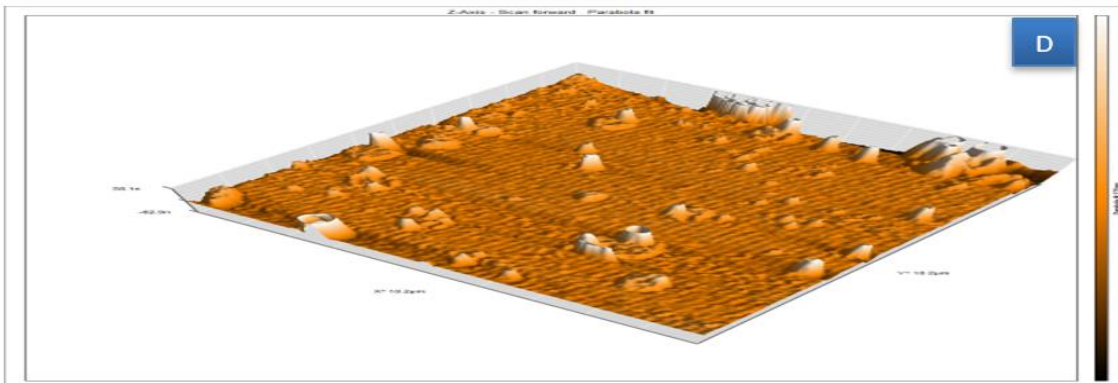


Figure -7: Atomic force microscopy (AFM) for (MgONPs) (A)Three dimensional image, (B) and (C) size distribution histogram of MgO Nanoparticles analyzed by AFM. (d) Two-dimensional.

Effects on Serum Magnesium

When compared to the furosemide group, MgONPs significantly raised serum magnesium levels ($P < 0.05$). Leukocyte equilibrium was restored by the MgONPs and watercress groups, which increased granulocyte numbers and decreased the lymphocyte rise seen in the furosemide group.

Table 1- Showed the Effect of Watercress *Nasturtium officinale L.* extract, MgONPs, furosemide and Mg sulfate on Mg(mg/dL) concentration in adult female rats

Groups	Mg in serum(mg/dL)
Control(C)	1.55±0.22 b
T1	0.33±0.16 c
T2	3.01±0.22 a
T3	2.92±0.23 a
T4	2.03±0.07 a
LSD	0.77

Means having with the different letters in same column differed significantly, * ($P \leq 0.05$). C: control group, T1 Group:

animals received furosemide at dose of 40mg/kg BW . Administration by injection, T2 Group: Animals in this group received furosemide at dose of 40mg/kg BW plus watercress MgONPs at does 12.5µg/kg BW, T3 Group: animals received furosemide at dose of 40mg/kg BW plus watercress aqueous extract at dose 500mg/kg BW, T4 group rats in this group administered furosemide at dose of 40mg/kg BW With Mg sulphate at dose 0.1mg /kg BW.

5. ANTIOXIDANT MARKERS

Detection of Serum Catalase (CAT) Activity

Significant variations in CAT activity between the groups are shown by the data ($P = 0.0001$). The MgONPS group had the highest CAT activity (201.78 ± 18.82 mg/dl), which was far higher than that of any other group. CAT levels were considerably lower in the Furosemide group (104.54 ± 7.42 mg/dl) than in the control group (143.23 ± 7.32 mg/dl). The CAT activity levels in the Control, Watercress (141.38 ± 8.41 mg/dl), and Mg Sulfate (153.29 ± 13.15 mg/dl) groups were intermediate.

Catalase (CAT) is a key antioxidant enzyme that catalyzes the degradation of hydrogen peroxide into water and oxygen, thereby protecting cells from oxidative damage. This conclusion fits with earlier research suggesting that magnesium oxide nanoparticles (MgONPs) can boost antioxidant enzyme activity by lowering oxidative stress and enhancing free radical scavenging (Gatou, et al., 2024). The nanomaterial's capacity to alter redox signaling pathways and boost endogenous antioxidant defenses may be the cause of the increased CAT activity in this group.

By producing reactive oxygen species (ROS) and reducing the activity of antioxidant enzymes, the loop diuretic furosemide has been shown to cause oxidative stress (Sabra et al., 2022). The observed reduction in CAT activity raises the possibility that furosemide has a pro-oxidative effect, which could lead to oxidative tissue damage. Watercress, known for its polyphenol content, may exert a protective effects on oxidative stress (Panahi Kokhdan et al., 2021). However, because Mg Sulfate has little direct contact with redox pathways, it is less likely to dramatically change CAT activity.

Detection of Serum Superoxide Dismutase (SOD) Activity

The study's findings show that the experimental groups' SOD activity varied significantly ($P = 0.0001$), indicating varying antioxidant responses. The MgONPS group had the greatest SOD activity (131.62 ± 4.72 mg/dl), showing a substantial increase above all other groups. In comparison to the Control group (105.20 ± 4.35 mg/dl) and other experimental groups, the Furosemide group had the lowest SOD levels (49.51 ± 4.17 mg/dl). SOD levels were similar in the Control, Watercress (117.45 ± 4.74 mg/dl), and Mg Sulfate (114.36 ± 4.40 mg/dl) groups.

Another essential antioxidant enzyme that acts as the first line of defense against oxidative stress is superoxide dismutase (SOD), which transforms superoxide radicals into hydrogen peroxide. According to previous research, MgONPs can increase SOD activity by increasing enzymatic antioxidant responses and regulating cellular redox equilibrium (Sabra et al., 2022). This group's elevated SOD levels could be a sign of better cellular defenses against oxidative damage.

lowering the amounts of antioxidant enzymes and compromising mitochondrial function, furosemide makes oxidative stress worse (Sharifi-Rad et al., 2020). This decrease in SOD activity draws attention to the possible oxidative stress that furosemide causes, which could be a factor in its well-known detrimental effects on vascular and renal function. As mentioned earlier, watercress includes phytochemicals that may help promote antioxidant defenses, and supplementing with magnesium sulfate has been associated with moderate increases in enzymatic antioxidant activity (Marín et al., 2025). The results underscore the differential impact of the tested compounds on oxidative stress biomarkers, particularly CAT and SOD activity. MgONPS demonstrated the most significant enhancement in antioxidant enzyme activities, suggesting its potential as a protective agent against oxidative damage. In contrast, Furosemide markedly reduced both CAT and SOD levels, supporting its well-established pro-oxidative properties. The intermediate antioxidant enzyme activity of watercress and magnesium sulfate suggest possible minor protective benefits without significant enzymatic change.

Table -2 Showed the Effect of Watercress *Nasturtium officinale L.* extract, MgONPs, furosemide and Mg sulfate on CAT and SOD in adult female rats.

Group	Means \pm SE	
	CAT (mg/dl)	SOD (mg/dl)
Control	143.23 \pm 7.32 b	105.20 \pm 4.35 b
T1	104.54 \pm 7.42 c	49.51 \pm 4.17 c
T2	201.78 \pm 18.82 a	131.62 \pm 4.72 a
T3	141.38 \pm 8.41 b	117.45 \pm 4.74 b

T4	153.29 ±13.15 b	114.36 ±4.40 b
L.S.D.	35.07 **	13.234 **
P-value	0.0001	0.0001

Means having with the different letters in same column differed significantly, * (P<0.05). C: control group, T1 Group: animals received furosemide at dose of 40mg/kg BW . Administration by injection, T2 Group: Animals in this group received furosemide at dose of 40mg/kg BW plus watercress MgONPs at does 12.5µg/kg BW, T3 Group: animals received furosemide at dose of 40mg/kg BW plus watercress aqueous extract at dose 500mg/kg BW, T4 group rats in this group administered furosemide at dose of 40mg/kg BW With Mg sulphate at dose 0.1mg /kg BW.

6. CONCLUSION

This study highlights the promising therapeutic potential of *Nasturtium officinale* extract and biosynthesized MgONPs in mitigating immune dysfunction associated with hypomagnesemia. The MgONPs demonstrated significant improvements in antioxidant defense, immunological balance, and serum magnesium restoration, showing superior efficacy compared to traditional magnesium treatments. These findings suggest that MgONPs could serve as an effective alternative treatment for immune-related conditions and magnesium deficiency.

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Conflict of Interest Statement The authors declare no conflicts of interest.

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