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EVALUATION OF THE BIOMEDICAL APPLICATIONS OF NiO NANOPARTICLES SYNTHESIZED WITH COW MILK AND AQUEOUS ETHYLENE GLYCOL

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ABSTRACT

NiO-NPs were prepared using cow milk (CM) and 30% ethylene glycol (EG). The production of NiO-NPs was indicated through a color transformation in the solution mixture from yellow to black. To evaluate the effectiveness of NiO-NPs as anti-inflammatory and antidiabetic agents. The *in vitro* antimicrobial activity tests were carried out through diffusion in agar agar against microorganisms at different concentrations of the sample. Our results in terms of IC_{50} indicate that the mean value was 14.95±0.02 and 11.89±0.06 for antidiabetic activity and anti-inflammatory activity, respectively. The study led to the finding that NiO-NPs, due to their toxic nature, have anti-inflammatory and antidiabetic activities.

Keywords: Anti-Inflammatory, Anti-Diabetic, Cow Milk, Nickel Oxide Nanoparticles, Modified Polyol Synthesis.

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INTRODUCTION

The global market for nanomaterial-based products is calculated commercially about $55 billion in 2020 having growth rate of 20 percent annually.[1] In recent years, among various nanomaterials, nickel oxide nanoparticles have been evaluated in the field of catalysis,[2] magnetic materials, and optical materials.[4] In particular, nano-dimensional nickel oxide nanoparticles (NiO-NPs) with a high surface area have shown an extensive range of applications in fuel cells, gas sensors, electrochemical films, nonenzymatic glucose sensors, and charge storage materials such as electrodes, batteries, and photoelectronic devices.[5,6] The nanoparticles of nickel oxides have gained importance in recent years due to their biological and pharmacological activities.[7,8,9] In the future, nanoparticle-pharmaceuticals may create a new field in medicines and pharmaceuticals. NiO-NPs have been prepared using various chemical and physical methods, such as sol-gel, precipitation, solvothermal, and hydrothermal techniques.[10,11] These methods are non-ecofriendly and expensive. Now eco-friendly approach to synthesizing the nanoparticles has been recommended.[12-15] In the polyol method, the polyol can also function as a protective agent to prevent particle aggregation.[16-18] A survey of the literature reveals that silver nanoparticles can be synthesized using milk as one of the precursors.[19-22] The production of silver nanoparticles in all the studies is an environmentally friendly method of synthesis. Nickel belongs to the transition metals and is an essential trace element for mammals. The requirements of nickel for adults should not exceed 20-35 milligrams per day.[23] A report indicates that NiO-NPs demonstrate notable antibacterial activity against various bacterial strains.[24] An additional report explores the antileishmanial activity alongside antibacterial activities.[25] This research aims to expand the preparation of NiO-NPs using a minimum amount of 30% ethylene glycol (EG) in water, along with 10% cow milk (CM), and to evaluate their biomedical applications.

EXPERIMENTAL

Materials and Methods

For this investigation, the precursors EG (Merck), nickel acetate hexahydrate (Merck), and NaOH (Merck) were used as received, without any further purification. The fresh CM was sourced from a local farm in the

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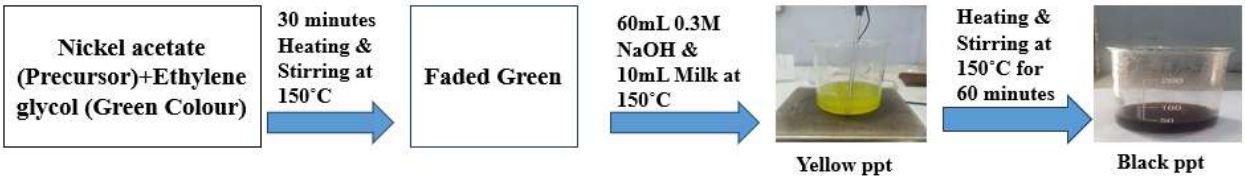
Almora District, Uttarakhand, India. The fresh and standardized solution of known concentration was prepared using double-distilled water.

General Procedure

The procedure used for synthesizing NiO-NPs is depicted in Scheme-1.



Scheme-1: Preparation of NiO-NPs

The volume and quantity of the precursors used in the synthesis were nickel acetate (0.75 g), EG (30 ml), NaOH 0.3M (60 ml), and CM (10 ml). In the polyol medium process of hydrolysis and condensation has been reported in the literature[26] having the following formula, where M is Ni.

$$M(OH)_{1-x}(OAc)_X.\ nH_2O$$

(M= Co-Ni, Zn, Ni, and Co)

The hydrated nickel acetate results in the formation of nanoparticles in the presence of CM.

Detection Method

The instrumental analysis of the sample was carried out in different laboratories, namely the Energy Dispersive X-ray spectrometer in the Sophisticated Analytical Instrument Facility, Chandigarh, Panjab University, India. IR spectrometer Varian 7000, (Thermo Scientific, Evolution-201) UV-visible spectrophotometer, (PAN analytical X Pert Pro) X-Ray Diffractometer, and TGA (STA 6000 Perkin Elmer) MNIT Jaipur, India.

Evaluation of Biomedical Applications

Study of *in Vitro* Anti-Inflammatory Activity

NiO-NPs at various concentrations (5-25 µg/ml) were added to fresh albumin protein (200 µl) for the experimental setup. Next, 2.8 ml of PBS (pH 6.4) was combined with this solution, bringing the final volume to 5 ml. After preparation, the solution was maintained at 37°C for fifteen minutes and warmed to 70°C for five minutes. Upon lowering the temperature of the reaction mixture, the absorbance at 660 nm was measured. Diclofenac sodium was utilized as the standard.[27] Formula to determine the % inhibition of anti-inflammatory activity:

$$\text{Percentage Inhibition } (IC_{50}) = [1- (\text{sample/control})] \times 100$$

Study of *in Vitro* Anti-Diabetic Activity

NiO-NPs (5–25 µg/ml) and the acarbose standard drug were mixed with 100 µl of 2 mM PBS (pH 6.9) and 200 µl of α-amylase solution.[28] The solution was incubated for 20 minutes. Subsequently, 100 µl of a 1% starch solution was mixed with the NiO-NPs. After a 5-minute incubation at 37°C, 500 µl of DNSA reagent was added to the solution, and the solution was subsequently boiled in a water bath for 5 minutes. At 540 nm, the absorbance was measured. Formula to determine the % inhibition of anti-diabetic activity:

$$\text{Percentage Inhibition } (IC_{50}) = [1- (\text{sample/control})] \times 100$$

RESULTS AND DISCUSSION

XRD

The XRD of nickel oxide nanoparticles was obtained, as shown in Fig.-1. The data were measured at 15-250 °C (room temp.) through a continuous scan within an angular range of 2θ, from 0° to 80°. Prominent diffraction peaks at 2θ degree of 30.02°, 34.42°, 35.20°, 37.80°and 44.50° along with low diffraction peaks at 2θ 47.50° ,52.50° and 60.0° in decreasing order of intensity. The diffraction peaks at 44°, 52°, and 76° are characteristic of the crystalline structure of nickel,[29] indicating that the NiO-NPs have an FCC structure. The characteristics peak at 35.50°, 37.80°, and 44.75°, which exhibited the FCC structure of NiO nanoparticles.[30,31] The examination of these peaks also reveals that the average crystallite size is ~17 nm. Scherrer's equation was employed to determine the D of the NiO-NPs in their as-prepared state.

$$D = 0.9 \lambda \div \beta \cos\theta$$

Where 'θ' = Bragg's diffraction angle, 'D' = average crystallite size, 'λ' = wavelength of the incident beam (λ = 1.5406 Å), and 'β' = full width at half maximum. 'D' of the NiO-NPs can be accurately determined via this equation.

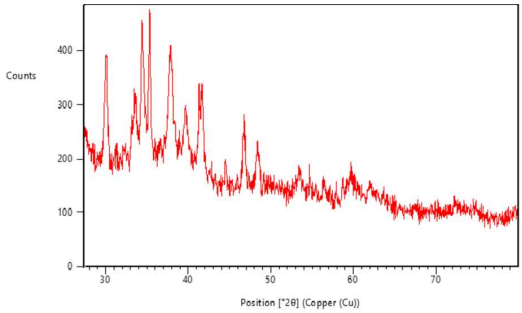


Fig.-1: XRD Spectra of NiO-NPs

IR Spectra

Figure-2 depicts the IR spectra of NiO-NPs in the 4000 cm^{-1} - 400 cm^{-1} range. A broad adsorption band at 3431 cm^{-1} is ascribed to υOH. On the other hand, this band, which appears at 1439 cm^{-1}, can be assigned to OH bending, indicating the existence of a -COOH group in the reactants. Likewise, the signal observed at 1571 cm^{-1} corresponds to the involvement of the nanoparticles, acting as a capping agent, with CM components, as well as molecules adsorbed onto the nanoparticle surface.[32] The peak at 455 cm^{-1} distinctly confirms the presence of NiO.[33] A medium adsorption band at 445 cm^{-1} corresponds to the υNi-O stretch of the octahedral NiO_6 units in the FCC structure of nickel oxide.[34,35,36,37] The very intense peaks noticed in the FTIR spectrum of CM reported in literature[38] at 2935 cm^{-1}, 2869 cm^{-1}, and 1458 cm^{-1} are attributed to bonds belonging to one of the precursor milk. The peaks observed near these vibration modes in our study may be ascribed to the existence of functional groups adsorbed onto the nanoparticle layer.

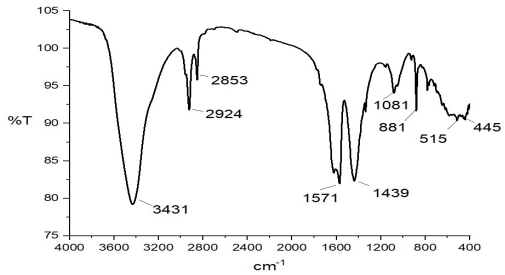
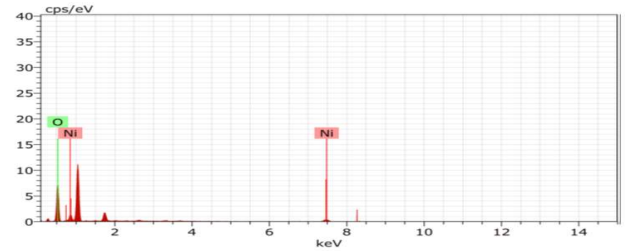


Fig.-2: IR spectra of NiO-NPs

EDX Spectra

The EDX spectra of the prepared NiO-NPs are displayed in Fig.-3, with the EDX analysis of the sample exhibiting signals for Ni and O. The percentage of O and Ni is 87.34% and 12.66%, respectively. Then, using TGA, the sample has been characterized to find out the type of contaminant present on the adsorbent surface.



Spectrum: test 18224

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Nickel	K-series	13.88	34.71	12.66	1.69
Oxygen	K-series	26.10	65.29	87.34	10.24
	Total:	39.98	100.00	100.00	

Fig.-3: EDX of NiO-NPs

TGA Analysis

The normal TG curve of the sample is presented in Fig.-4. As demonstrated in Fig.-4, the TGA curve indicated three weight loss stages. Between 28°C and 150°C, approximately 22 wt% of the crystallization process is attributed to the first stage, which involves the evaporation of water molecules. The second stage, accounting for around 35% weight loss, results from the thermal decomposition of nickel hydroxide to nickel oxide nanoparticles, along with the release of one water molecule, occurring between 150°C and 430°C. In the third stage, which begins at 420°C and ends at 480°C, approximately 50% of the total weight loss is observed. There was no significant decomposition or reactivity above 500°C. Above 400 °C, the TGA curve weight of the precursor doesn't change, in good agreement with the previous report.[39]

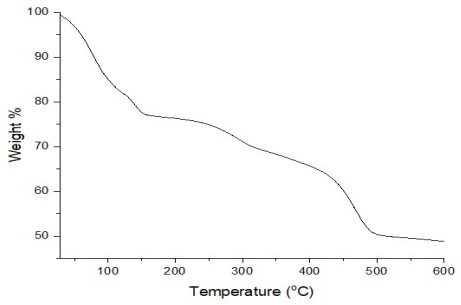


Fig.-4: TGA Spectrum of NiO-NPs

UV–vis Spectrum

The spectrum obtained in the UV spectrum is as shown in Fig.-5. Absorption peak was observed at a wavelength relatively at about 323 nm, assigned to the band gap absorption of NiO-NPs.[40] The UV–Visible absorbance spectrum identifies the band gap (Eg), and an absorption peak at 323 nm of these NPs was derived using the tauc equation, as shown below:

$$\alpha h\nu = (h\nu - Eg)^n A$$

Where n=2 (direct transitions), hυ = photon energy, Eg = band-gap, and α = absorption coefficient. A plot of $(\alpha h\upsilon)^2$ (eV^2. cm^{-2}) vs hν (eV) is presented in the insert of Fig.-5, while the linear part of the curve was extrapolated to the hυ axis, allowing the extraction of Eg. The Eg is determined to be 3.15 eV, a bit higher than the earlier reported value of 3.04 eV, and the absorption peak at 330 nm.[41,42]

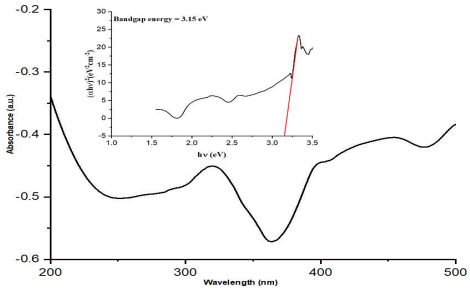


Fig.-5: Ultraviolet and Visible Spectra and Tauc-curve of NiO-NPs

Analysis of *In-vitro* Anti-Inflammatory Activity

Compared to standard (IC_{50} = 7.27±0.04 µg/ml), NiO-NPs exhibited lower activity. The order of inhibition of protein denaturation, along with the corresponding IC_{50} values, was as follows: NiO-NPs (11.89±0.06) < standard (7.27±0.04) (Table-1a).

Table-1a: *In-vitro* Anti-Inflammatory Activity of NiO-NPs

Sample and Standard	Average Anti-Inflammatory Activity Expressed as IC_{50} with Standard Deviation (µg/mL±SD)
NiO-NPs	11.89±0.06
Diclofenac sodium	7.27±0.04

Analysis of *In-vitro* Anti-Diabetic Activity

NiO-NPs demonstrated notable antidiabetic activity, although to a lesser degree than acarbose. The assay results showed that the sample displayed inhibition similar to that of the reference, acarbose. The IC_{50} values were as follows: NiO-NPs (14.95±0.02) < standard (7.17±0.02) (Table-1b).

Table-1b: *In-vitro* Anti-Diabetic Activity of NiO-NPs

Sample and Standard	Average Anti-Diabetic Activity Expressed as IC_{50} with Standard Deviation (µg/mL±SD)
NiO-NPs	14.95±0.02
Acarbose	7.17±0.02

CONCLUSION

This research focuses on the preparation of NiO-NPs using cow milk (CM) in an aqueous alkaline solution of ethylene glycol. The method used in the synthesis of nanoparticles in the study is non-toxic, eco-friendly, and uses of chemicals compared to the physical and chemical methods. This is the first attempt at the preparation of NiO-NPs, which demonstrates the use of CM of biological origin with a green chemistry approach. The preparation of NiO-NPs has been confirmed by analytical techniques such as FTIR, UV, XRD, EDX, and TGA, which reveal an FCC structure with an approximate size of 17 nm. The synthesized nickel oxide nanoparticles show anti-inflammatory and antidiabetic properties. This study could be a helpful contribution and will open new areas to many fields of nanoparticle-based therapeutic applications.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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