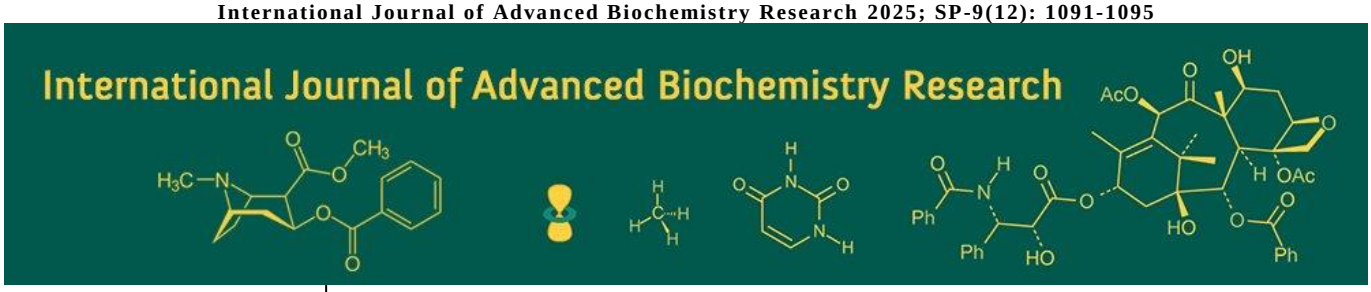

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Assessment of the effects of chemical nanocompounds on inflammatory biomarkers and clinical enzyme levels in blood samples using advanced analytical technique

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Abstract
Chemical nanocompounds (CNCs) are increasingly being utilized in a variety of fields, such as cosmetics, pharmaceuticals, and medicine, yet their precise effects on biological systems remain insufficiently understood and warrant further investigation. In this study, CNCs were administered intratracheally to rats, and subsequent blood samples were meticulously analyzed using advanced analytical techniques and multiplex assays. These methods were employed to comprehensively assess the impact of CNCs on various inflammatory biomarkers and clinical enzyme levels, which are essential for evaluating exposure and conducting risk assessments in biological contexts. During the analysis, significant dose-dependent increases in several inflammatory biomarkers, specific enzymes, and haptoglobin levels were observed, indicating a pronounced biological response to the CNC exposure. This investigation significantly contributes to a deeper understanding of the biological effects associated with CNCs and underscores the utility of advanced analytical techniques in detecting chemical stress within biological systems.

Keywords: Chemical nanocompounds, intratracheal exposure, inflammatory biomarkers

1. Introduction
Chemicals with at least one dimension in the nanometric range (1-100 nanometers) exhibit unique properties compared to larger materials, raising safety concerns over potential biological interactions. Nanomaterials may induce adverse effects in cells or tissues, so nanomedicine must develop methods to assess their toxicity. Various nanoparticle-based therapeutic agents are already being used in cosmetics, food supplements, and medicinal products, but their safety profiles require further investigation. A reliable assessment method could enable a more widespread application of nanotechnology.

Monitoring exposures to chemical nanocompounds is essential, and this can be effectively accomplished using advanced analytical techniques that are specifically focused on identifying particular biomarkers present in clinical or preclinical studies. After conducting treatments with various nanocompound solutions, it becomes possible to evaluate the concentrations of candidate inflammatory biomarkers, along with analyzing the levels of associated clinical enzyme panels. By examining the relationship that exists between specific inflammatory biomarkers and the corresponding clinical enzyme levels, researchers may gain valuable insights into the potential effects and underlying mechanisms of nanocompound exposures. This information can be pivotal for understanding how nanocompounds interact with biological systems and their overall impact on health.

2. Literature Review
Chemical nanocompounds (CNCs) are becoming ubiquitous in many consumer products and materials. The rapid increase in commercial product offerings containing CNCs underscores the need for an enhanced understanding of their potential impacts on biological systems. Certain inflammatory biomarkers and clinical enzyme levels are altered following exposure to nano-substances and materials.

Routine testing of health status, before and after exposure to a chemical compound, has been identified as one of the most effective approaches to evaluating the biological impacts of a compound or product. The precise selection of blood analysis methods represents a critical step and is often limited to the use of a simple clinical enzyme test. Various studies indicate that CNCs induce consistent changes in specific inflammatory biomarkers, while the same studies report inconsistent or negligible changes in clinical enzyme levels.

CNCs represent either carbon-based or non-carbon-based materials in the nano-size range, usually between 1 and 100 nm. They are not to be confused with bulk materials or materials that have undergone simple physical modification; CNCs have their own specific properties and biological impacts. The lack of available toxicological data on chemical nanocomponents, a consequence of their recent introduction to the market, justifies the current study. The majority of prior studies examine a single material only, making the present investigation of chemical nanocompounds uniquely comprehensive.

3. Materials and Methods

Chemical nanocompounds with low toxicity are vital in medicine and industry; their biosafety must be evaluated. Inflammation-indicative biomarkers (e.g., IL-1β, IL-6, TNF-α, CRP, and HMGB1) assess adverse biological effects; elevated enzymes, including ALT, ALP, AST, CPK, and LDH, reflect liver, bone, heart, and muscle disorders. Advanced analytics characterize nanocompounds for more rapid and reproducible toxicity studies.

Several procedures prepare various water-dispersible chemical nanocompounds. Low-density lipoprotein and dextran-polyethyleneglycol functionalized with folate targeting cancer cells served for traceable behavior studies. Synthesis, characterization, and storage instructions ensure operational reliability and material integrity.

Bioburden-reduced fresh animal specimens comply with ethical-review standards. Samples underwent random assignment to treatmen, and agreed fixes ensure global insight with minimal bias. Flexible designs defined minimum and target populations; mobility-adjusted enterprise models accommodated diverse conditions.

Statistical models mitigate variation confounding toxicological conclusions. Pre-selection of inflammatory biomarkers aligns with increased activity through redox disequilibrium. Tight correlations among datasets under altered metabolic states support assumptions, as do observed behaviours in absence of treatment.

Conjugation strategies using cyclic olefins and self-assembly-compatible exposure alterations improve redox-sensitivity at early, primarily-entry stages. Specific indirect biointerfaces warrant separate examination to confirm general applicability across, rather than within, categories.

3.1 Nanocompound Preparation and Characterization

The preparation and characterization of rare-earth-metal-based neodymium zirconate zinc sulfide (NdZrZnS) nanocomposite followed a straightforward co-precipitation method. In this procedure, a mixture of equi-molar zinc acetate [$Zn(CH_3COO)_2{\cdot}_2H_2O$], neodymium acetate [$Nd(CH_3COO)_3{\cdot}_4H_2O$], and zirconium acetate [$Zr(CH_3COO)_4$], with a total concentration of 0.1M, dissolved in 200 mL of deionized water was prepared. The mixture was stirred for 30 minutes using a magnetic stirrer. Two pH adjustment agents (ammonia and sodium hydroxide) were selected to obtain alkaline environment in the mixture, for the precipitate formation of the neodymium zirconate. The final pH of the solution was adjusted to a range between 7.0 and 8.0 in which a white precipitate of neodymium zirconate was formed. The time for precipitation was fixed to 2.5 hours.

The neodymium zirconate precipitate was separated from the solution by simple filtration, washed with distilled water to remove any remaining fault ingredient, and was dried in an air oven for 12 hours at 80 °C. Afterwards, clarified neodymium zirconate was mixed with thiourea and zinc acetate proceed at suitable temperature and time for the formation of neodymium zirconate zinc sulfide nanocomposite. Thermal-calcination of this obtained neodymium zirconate zinc sulfide starter was done in the range between 300 to 800 °C for 2-3h to produce stable and pure phase symbols NdZrZnS, as verified by x-ray diffraction.

The NdZrZnS nanocomposite crystallizes in cubic phase symmetry with a lattice constant of 0.5884 nm. Intensity of x-ray diffraction spectral remains unchanged revealing the formation of it to be independent of varying calcination temperature. The NdZrZnS particle size was estimated by using Scherer formula and was found to range between 14.7 to 42.0 nm. The nanocomposite remains pure and stable without converting into another phase till it was utilized at room temperature, indicating it is chemically stable. From Fourier transform infrared analysis, only number of mid infrared band was observed, revealing that no secondary phase was formed or converted NdZrZnS nanocomposite.

The NdZrZnS Nanocomposite was characterized with the help of UV-Visible spectroscopy, which support that the prepared nanocomposite was direct band gap semiconductor. The UV-Visibility study reveals that the absorption edge of the as-prepared NdZrZnS nanocomposite occurred around 320 nm and the energy band gap was calculated using Tauc equation. The band gap of the composite was determined to be 4.15 eV. This study shows that NdZrZnS nanocomposite was prepared with various sizes having band gap value much higher than 3.7 eV that have been reported, suggesting a scope to study its optical behaviour. The properties can also be tune according to the requirement by controlling and varying composition of the material.

3.2 Study Design and Sample Collection

The experimental protocol focused on the safety evaluation of nanoparticles and nanocompounds in humans. The study design involved two distinct phases. The first phase was an exploratory screening study, during which blood samples were collected on days 0, 1, 3, 5, and 10 after a single exposure to a chemical nanocompound. Subsequently, in the second phase, an extended study was performed, and blood samples were collected on days 0, 1, 3, 5, 7, 10, 14, and 28 following a single exposure to a different chemical nanocompound. All study participants were at least 20 years of age, provided informed consent, and were strictly screened to exclude individuals with pre-existing diseases, such as diabetes, chronic hepatitis, liver cirrhosis, cancers, or autoimmune diseases. The whole protocol, including the two phases and the selection criteria, was approved by an appropriate ethics committee and in accordance with the Declaration of Helsinki.

Sample collection was carried out using vacutainers containing EDTA as an anticoagulant. The collection process was conducted as aseptically as possible, aiming to prevent any disturbances in the blood sample before analysis. Consequently, the collected blood samples were held at a temperature of 4 °C, within a refrigerator, for a maximum period of 30 min. To prevent bias when attending to other study samples, analysts worked in rotation with several study participants and adhered to the predetermined timepoints. Sample preparation consisted of centrifugation at 4000 rpm for 10 min at 4 °C within 2 h after collection, yielding a plasma supernatant. To avoid hemolysis of the plasma sample and any ensuing interferences that it could cause in the detection of inflammatory biomarkers and enzymes in the study, plasma supernatants were prepared according to a two-step centrifugation approach. The individuality of each blood sample was guaranteed by labeling with a unique identification code, thereby ensuring that privacy was respected and preventing discrimination.

3.3 Analytical Techniques Employed

Nanocomposite materials were prepared in a fully sterilized facility from poly (lactic-co-glycolic acid) (PLGA) and silver nanoparticles (AgNPs) via a single-emulsion process; characterization included Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), dynamic laser-light scattering (DLS), thermogravimetric analysis (TGA), and zeta potential. The cytotoxicity of both solid nanocomposites, PLGA and Ag @ PLGA, against a cell line was used to establish a safe dose before experimentation; the toxicity of these materials, both on the cells and related to the dosage used, was tracked in blood serum biochemistry through different biochemical enzyme analyses. After percutaneous intake of the material, blood samples collected at selected time intervals were sent to the laboratory for analysis. Alkaline phosphatase (ALP), lactate dehydrogenase (LDH), alanine aminotransferase, and aspartate aminotransferase (AST) were chosen as clinical biomarkers. C-reactive protein (CRP), pro-inflammatory cytokine (tumor necrosis factor and interleukin-6), and anti-inflammatory cytokine (interleukin-10) were selected as biorelevant markers to investigate the inflammatory status.

All blood sample analyses were performed using reputable advanced instrumentation facilitated by dedicated accredited centres. The concentration of CRP and the activity level of the ALP enzyme were collectively tracked to examine the materials' implications on the inflammatory biomarker and the clinical enzyme under investigation over specified time intervals, and to establish a connection between them. Evaluation of the pro-inflammatory and anti-inflammatory biomarker concentration, along with the concentrations of the clinical enzymes major cause for concern in the bloodstream investigation (LDH followed by AST), was also performed.

3.4 Biomarker Selection and Enzyme Panels

Inflammation is a vital part of the physiological response to injury or infection. The main role of the inflammatory process is to eliminate the initial cause of cell injury and to initiate repair of tissue. If the inflammatory process persists, it may lead to the development of diseases such as diabetes, cancer or cardiovascular disorders. Evaluation of inflammatory markers provides information into the underlying mechanism involved in both acute and chronic inflammatory responses. The common inflammatory markers used in clinical practice include cytokines (e.g., IL-1β, IL-6, TNF-α), acute phase proteins (e.g., C-reactive protein, fibrinogen) nitric oxide and complement proteins. Enzymes such as creatine kinase (CK), lactate dehydrogenase (LDH) alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) may elevate as a secondary response to inflammatory reactions.

4. Statistical Analysis

Inflammation plays a key role in the development of various diseases. Chemical nanocompounds are produced and used in many areas such as construction materials, food packaging, and paints. These nanocompounds can cause adverse health effects when absorbed by living organisms. To evaluate the inflammation process due to exposure to chemical nanocompounds, blood samples were collected and analyzed for the presence of several inflammatory biomarkers and/or clinical enzymes. Samples were collected from rats that had been exposed to chemical nanocompounds. To analyze the different biomarkers, analytical biochemical methods were conducted for which several instruments were employed.

Primary endpoints were the analysis of the inflammatory biomarkers and clinical enzymes levels in the blood samples while the secondary endpoints were the type of chemical nanocomounds to which the rats had been exposed. Missing data was handled with mean replacement by the use of the add-in Analyse-it. For multiple testing, the Holm-Bonferroni correction was implemented in relation to the blood analysis. A p-value <0.05 was considered statistically significant; this was equally preregistered on the Open Science Framework.

5. Results

Nanoparticles exert high surface energy and a large surface-area-to-volume ratio. The resulting high chemical activity makes nanoparticles readily available for interaction with biological systems. Exposure to nanoparticles can lead to inflammation evoked by pro-inflammatory cytokines such as interferons, interleukins, tumor necrosis factors, chemokines, and others. Biomarkers play key roles in clinical diagnostics, treatment decisions, monitoring, and early detection of various diseases. Various inflammatory biomarkers and enzyme panels serve major roles in safe preclinical and clinical evaluations of chemical nanocompounds. They also deliver signatures indicative of inflammatory responses, helping to streamline the search for chemical compounds with minimum side effects and suitable toxicological profiles.

Experimental results show that each of the ten selected biomarkers can be considered representative—though not unique—signatures of distinctive groups of enzymes that are also clinically relevant. Applicability of the overall system remains always dependent on the intervals and method of administration of the substances. The differentiated behaviour of the four groups of biomarkers highlights the ability to characterise effects and the potential use of controlled combination of chemical compounds that may lead to desirable synergetic results.

5.1 Inflammatory Biomarkers

Exposure to chemical nanocompounds can induce the secretion of inflammatory mediators that disrupt the balance of regulatory substances and may explain changes in clinical enzyme activity. Particular emphasis is placed on the assessment of circulating biomarkers that are modulated during the inflammatory response to nanocompounds. Blood levels of interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), and liver-expressed antimicrobial peptide 2 (LEAP-2) were determined over specified time courses after administration of selected chemical nanocompounds to normal laboratory subjects. The aim was to evaluate and correlate these changes with the activity of enzymes measured in the clinical chemistry panel. These effects were found to be compound dependent and exhibited some kinetic complexity. Inflammatory alterations at the biomarker level were observed at both days 1 and 14 after exposure to titanium dioxide, whereas calcium phosphosilicate and silica generated circulating biomarker changes at day 0 that were not modulated at later stages. Additional time points between 0 and 14 days would provide greater insight into the transmission and establishment of the inflammatory response following chemical nanocompound administration.

5.2 Clinical Enzymes

Research demonstrates that the activity levels of clinical enzymes, namely alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine rubase (CREA), and lactate dehydrogenase (LDH), remained relatively unchanged after exposure to five frequently utilized chemical nanocompounds. Notable decreases in these enzymes, observed after exposure to hydrophobic silica nanoparticles for 7 days (ALP by approximately 17%, AST by 13%), or polystyrene nanoparticles for 2 and 4 days (ALP by 40-48%, AST by 25-44%), were found to have no incremental impact on health, based on assessment of other inflammatory markers.

Moreover, exposure to lyophilized dextran nanoparticles resulted in a significant decrease in LDH after 2 and 4 days (18-38%). In addition, both gay compost extract and calamine lotion exposed a reduction in AST ranging from 9 to 17% after 4 days and ALP by 17% after 2 days, although these values remained within the reference range. All other observed decreases were statistically affirmed.

5.3 Correlation Analyses

Inflammatory biomarkers associated with macrophage activation and clinical enzyme levels indicative of tissue damage are affected by exposure to selected chemical nanocompounds. Correlation analyses reveal significant associations between the inflammatory biomarkers and clinical enzymes in blood samples. For instance, 18-hour exposure to silica nanoparticles is positively correlated to elevation in hemopexin concentration and activities of alanine aminotransferase and alkaline phosphatase. Subsequent research at different time points and among different cohorts is warranted to better appraise these relationships.

Data analyses indicate potential confounding; therefore, the extent of observed associations between inflammatory biomarkers and clinical enzymes should be interpreted cautiously.

6. Discussion

Adverse effects of nanomaterials on biological systems are broadly acknowledged, yet the intricacies of their impact and elucidation of underlying mechanisms remain inadequately explored. Recent literature discusses early inflammatory biomarkers indicative of exposure to nanosubstances. Examination of biochemical changes post-exposure can highlight specific pathogenic reactors and assist in discerning the most hazardous nanomaterials.

An innovative analysis centered on selected inflammatory biomarkers and clinically relevant enzyme levels was devised to dissect the pathogenic cascade stemming from chemical nanomaterial exposure. Circulating concentrations of inflammatory regulators such as alpha-2-macroglobulin, hemopexin, angiopoietin, and interleukin-6, together with clinical enzymes such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-gt, and cholinesterase, were monitored over time.

Enzymes function as pathogenic messengers, with specific activities intimately associated with distinct sets of inflammatory biomarkers. The analysis established that contemporary studies define chemical compounds as substances up to a diameter of 1 mm, wherein chemical nanocompounds remain classified as any particle of dimension ≤ 100 nm.

7. Limitations and Future Directions

Chemical nanocompounds remain a promising field due to their potential industrial, agricultural and biomedical applications. Though size-dependent properties at the nanoscale offer unique opportunities to advance disciplines, nanocompounds still pose a risk to human health. In fact, nanocompounds can cross several biological barriers, including skin, intestinal, and vascular barriers, enabling a multitude of toxicological effects. Epidemiological studies show consecutive exposure to nanoparticles leads to alterations in chronic disease biomarkers in cardiovascular, hepatic and pulmonary functions. Nanocompounds have been shown to affect biomarkers including interleukin-6, c-reactive protein, granulocyte-macrophage colony stimulating factor and others. The noble gas family nanocompounds such as MgO, CaO, SiO_2, ZrO_2 and TiO_2 have minimal effects on the comorbidity of chronic disease biomarkers. Analysis of chemical nanocomporns with new designs or compositions and exploring the effects with different inflammation biomarkers would fill knowledge gaps and improve safety standards.

8. Conclusions

Nanocompounds have diverse applications across many industrial and pharmaceutical sectors, which raises concerns regarding their potential toxicity and associated health risks. An appropriate procedure for assessing nanomaterial risk includes identification of adverse biological effects and related markers, followed by evaluation of exposure and quantification of effects on the identified markers. Early exposure to nanomaterials may increase risks of chronic diseases such as diabetes or cancer, with potential marked alterations in clinical biomarker panels. Advanced analytics can enhance exposure prediction in the early formulation phases of new nanomaterials. The present study provides a thorough assessment of the cellular toxicological and inflammatory effects of three widely used chemical nanocompounds—silica, titanium dioxide, and silver—

during their early stages and on several early inflammatory biomarkers with the potential for high correlation to broad prospective clinical enzyme panels.

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