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Research article

The effect of silver nitrate on the alkaline phosphatase activity, TNF and IL-10 gene expression; Experimental and computational studies

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ABSTRACT

The present study examines the effects of silver nitrate on the activity of the enzyme alkaline phosphatase (ALP) and its regulatory influence on inflammatory cytokines, specifically interleukin-10 (IL-10) and tumor necrosis factor-alpha (TNF-α). These parameters are critical for elucidating the biochemical and immunological responses associated with metal exposure. The research employed enzyme-linked immunosorbent assay (ELISA) techniques to quantify IL-10 and TNF-α levels using serum assay kits. Additionally, gel filtration chromatography with Sephacryl S300 demonstrated that serum from silver nitrate-treated groups exhibited a significantly elevated concentration of high molecular weight alkaline phosphatase compared to control groups. In the TNF-α ELISA assay, no significant differences were observed between the control and treatment groups 15 days post-injection of silver nitrate. However, a statistically significant alteration was detected in both groups 45 days post-injection ($P < 0.005$). Molecular docking analysis further revealed that the optimal binding pose for ALP, based on the docking score, was −5.28 kcal/mol, with a re-ranking score of −7.43 kcal/mol. Collectively, the findings indicate that silver nitrate exerts a significant impact on ALP activity, leading to a sustained increase in TNF-α levels over time, while IL-10 levels remain unaffected.

1. Introduction

Technological advancements have instigated substantial changes in the environment and the Earth's crust, leading to the contamination of water and food supplies with various elements. This contamination negatively impacts the functioning of multiple bodily systems, resulting in a range of complications [1]. The role of these elements in influencing the natural metabolism of tissues has attracted significant attention from researchers. Numerous studies underscore the essential functions of elements such as iron, nickel, zinc, and chromium in biochemical reactions in both humans and animals [2,3].

Silver ions have long been recognized for their antibacterial properties. The literature extensively documents the utilization of silver ions, particularly those derived from soluble silver nitrate ($AgNO_3$) and silver sulfadiazine, in the fight against both gram-positive and gram-negative bacteria [4]. Historically, silver has been used for various purposes, including the storage of drinking water in silver

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vessels due to its antibacterial properties [5]. In recent years, novel silver metal complexes have gained significant attention for their potent anticancer activity, fewer side effects, and lower potential for resistance development compared to cisplatin, carboplatin, and oxaliplatin [6,7].

Silver nitrate can cause severe chemical burns and is highly cytotoxic. A study by *Sodhi* et al. documented cases where silver nitrate was involved in accidental chemical injuries, leading to skin and ocular damage. The study emphasized the need for proper handling and application in clinical settings to prevent adverse reactions [8]. A review by *Rajan* et al. highlighted the environmental hazards associated with silver nanoparticles and silver compounds, including silver nitrate. The authors noted that exposure to silver can lead to bioaccumulation, leading to toxic effects in aquatic organisms and potential toxicity in humans through ingestion or dermal contact. The systemic toxicity includes effects on various organs, including the kidneys and liver, raising concerns about both environmental safety and human health [9]. *Janzadeh* et al. investigated the neurotoxic effects of silver compounds and reported that silver nitrate exposure could induce neurotoxicity in mammalian models. The study provided evidence that chronic exposure to silver could lead to cognitive deficits and neuronal damage, highlighting the potential risks associated with therapeutic or occupational use of silver salts [10].

Alkaline phosphatase (ALP) is predominantly expressed in mineralized tissue cells and is integral to the process of hard tissue formation. Regular reviews of the current literature on this enzyme are essential for advancing our understanding of its role in mineralization [11,12]. ALP contributes to increased local concentrations of inorganic phosphate, thereby facilitating mineral deposition, while simultaneously decreasing extracellular pyrophosphate concentrations, which serve to inhibit mineral development. Mineralization is characterized by the synthesis of hydroxyapatite crystals within matrix vesicles, which are derived from the outer membrane of osteoblasts and hypertrophic chondrocytes [13]. These hydroxyapatite crystals infiltrate the extracellular matrix and become interspersed between collagen fibers. Among the various isoforms of ALP, the tissue-nonspecific isozyme (TNAP) is notably expressed in bone, liver, and kidney tissues, playing a critical role in the calcification of bone [14,15].

Tumor necrosis factor-alpha (TNF-α) was initially identified as a tumor necrosis factor but has since been recognized as a key pathological component of autoimmune diseases. TNF-α binds to two distinct receptors, initiating signal transduction processes that lead to various cellular responses, including cell survival, differentiation, and proliferation [16]. However, inappropriate or excessive activation of TNF signaling is associated with chronic inflammation and can lead to the development of pathological conditions such as autoimmune diseases [17]. The understanding of TNF-α signaling mechanisms has expanded, facilitating the development of effective therapeutic tools, including TNF-α inhibitors [16,18]. Clinically approved TNF-α inhibitors have demonstrated remarkable efficacy in treating various autoimmune diseases, and new inhibitors are under clinical evaluation. The IL-10 family of cytokines can be broadly categorized into three subgroups based on their functions. The first group contains IL-10, which targets both innate and adaptive immune responses, exerting immunosuppressive functions to reduce tissue damage caused by excessive and uncontrolled inflammatory responses [19]. IL-10 also maintains intestinal bacterial homeostasis, especially during the resolution of infection and inflammation. The IL-10 family cytokines are potent immune mediators with versatile functions, and efforts have been made to regulate these cytokines as a therapeutic strategy for diseases such as autoimmune disorders and cancer [20,21]. However, no clinically approved drugs targeting the IL-10 family exist due to the complex and sometimes contradictory biological effects. For example, IL-10 exhibits both immunosuppressive and immunostimulatory effects, while IL-22, which promotes tissue repair in the intestine, can cause acanthosis in dermatitis [22,23].

Despite studies on the effects of metal exposure on the concentration of ALP and its isoenzymes, research specifically examining the impact of silver on ALP is lacking. This study investigates the effect of intraperitoneal injection of silver nitrate on the activity of total serum ALP, interaction enzyme-ligand by molecular docking, and its high molecular weight (HMW) and low molecular weight (LMW) isoenzymes, as well as serum changes in IL-10 and TNF-α over short-term (15 days) and long-term (45 days) periods.

2. Materials and methods

2.1. Animals and treatments

The study adhered to ethical standards for the treatment of laboratory animals. All procedures were approved by the relevant institutional animal care and use committee (ethical certificate: IR. IAU.NAJAFABAD.REC.1400.196). The laboratory animals utilized in this study were male Wistar rats (*Rattus norvegicus*). A total of 48 rats, with an average weight ranging between 200 ± 7 and 250 ± 7 gr, were randomly assigned to eight experimental groups. Throughout the study, the animals were housed under standard laboratory conditions, which included access to a healthy diet and clean water, a controlled 12-h light/dark cycle, and a temperature-regulated environment. All procedures involving the animals were conducted in strict compliance with ethical guidelines for the care and use of laboratory animals. To minimize the risk of potential infections, oral ampicillin was administered via drinking water at a concentration of 250 mg/L until the initiation of the injection phase. These measures ensured the health and well-being of the animals throughout the experimental period.

2.2. Experimental design: treatment protocols

2.2.1. Short-term period (15 Days)

Control Group: Rats in the control group will receive daily intraperitoneal injections of 0.5 mL of sterile normal saline for a duration of two weeks.

Silver Nitrate Group: Rats in the treatment group will receive daily intraperitoneal injections of 0.5 mL of silver nitrate solution at a

concentration of 8 mg per kilogram of body weight for two weeks.

2.2.2. Long-term period (45 Days)

Control Group: Rats in the control group will receive daily intraperitoneal injections of 0.5 mL of sterile normal saline for a duration of 45 days.

Silver Nitrate Group: Rats in the treatment group will receive daily intraperitoneal injections of 0.5 mL of silver nitrate solution at a concentration of 4 mg per kilogram of body weight for 45 days.

2.3. RNA isolation and cDNA synthesis

2.3.1. RNA isolation

Total RNA was extracted from the collected tissue samples using a commercially available RNA isolation kit (e.g., TRIzol reagent or equivalent), following the manufacturer's protocol [24]. Briefly, tissue samples were homogenized in the lysis buffer provided in the kit to ensure complete disruption of cells and release of RNA. The homogenate was then subjected to phase separation by adding chloroform, followed by centrifugation at 12,000×*g* for 15 min at 4 °C. The aqueous phase containing RNA was carefully transferred to a new tube, and RNA was precipitated by adding isopropanol. After centrifugation, the RNA pellet was washed with 75 % ethanol, air-dried, and dissolved in nuclease-free water. The purity and concentration of the isolated RNA were assessed using a spectrophotometer (e.g., NanoDrop) by measuring absorbance at 260/280 nm and 260/230 nm ratios. RNA integrity was further confirmed by agarose gel electrophoresis.

2.3.2. cDNA synthesis

First-strand complementary DNA (cDNA) was synthesized from the isolated RNA using a reverse transcription kit (e.g., RevertAid First Strand cDNA Synthesis Kit or equivalent). For each reaction, 1 μg of total RNA was mixed with oligo (dT) primers or random hexamers, depending on the experimental requirements, and reverse transcriptase enzyme in a reaction buffer provided by the kit. The reaction mixture was incubated at 42 °C for 60 min to allow reverse transcription, followed by enzyme inactivation at 70 °C for 5 min. The synthesized cDNA was stored at −20 °C until further use in downstream applications such as quantitative PCR (qPCR) or other gene expression analyses. The amplicon sizes and GenBank accession numbers for the target and reference primers are provided in Table 1.

2.4. qRT-PCR

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) will be conducted to assess changes in TNF-α gene expression, with beta-actin serving as the housekeeping gene. Primers will be designed using Primer 3 software (Fig. S1).

2.4.1. Obtaining of the blood samples and enzyme analysis

Blood will be drawn from the hearts of the rats, and serum will be separated from the clot by centrifugation. The serum level of TNF will be measured using Karmania Pars Gene kits, and protein levels will be determined according to *Lowry's* method [25]. Total serum ALP levels will be measured using a modified method by *Bessy* and colleagues [26] and a Pars Azmon kit. These measurements will be taken using a spectrophotometer at 405 nm. To separate high and low molecular weight isoenzymes of ALP, gel filtration chromatography on Sephacryl S-300 will be used. Samples (0.5 mL) will be diluted with an equal volume of Tris buffer (50 mM, pH 7.4) and then applied to a column (50 × 9 cm) containing Sephacryl S-300. Fractions of 2 mL will be collected by adding Tris buffer (50 mM, pH 7.4) at a flow rate of 10 mL/h. The ALP activity and protein concentration of each collected fraction will be measured according to the method of Bessy et al. and compared with the control group. Chromatography tests will be conducted at laboratory temperature.

2.5. Preparing 3D structures of ALP and silver nitrate

Initially, the amino acid sequences of the ALP enzyme (PDB ID: 1ANI) were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/). Subsequently, silver nitrate, with the molecular formula AgNO3 (CID_24,470), was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [27,28] (Fig. 1A–C).

Table 1
Gene reference, accession number, sequence and amplicon size of primers used to analyze gene expression by qPCR.

Gene	Primers (5′-3′)	Amplicon size	Gene reference	Accession
FactBrat	5′- GCCTTCCTTCCTGGGTATGG -3′	136bp	*ActB*	NM_031144.3
RactBrat	5′- GCCTGGGTACATGGTGGTGC-3′			
FTNf	5′-TACTGAACTTCGGGGTGATCG-3′	124bp	*TNFα*	NM_001278601.1
RTNf	5′-CCACTCCAGCTGCTCCTCC-3′			
FTGFB	5′-AGACATTCGGGAAGCAGTGC-3′	*148bp*	TGFb	AY550025
RTGFB	5′-CAGCAGCCGGTTACCAAGG-3′			

2.6. Molecular docking

The ligand molecular docking process to the ALP enzyme (PDB ID: 1ANI) was evaluated using a flexible docking approach with AutoDock Vina [29,30]. The side chains of both the enzyme and the ligand demonstrated sufficient flexibility, facilitating interactions with the enzyme through AutoDock Vina. AutoDock (version 1.5.6) was employed to define the permissible ligand torsions, add polar hydrogen atoms to the enzyme, and specify the search space coordinates [31]. These parameters were input into AutoDock Tools, and a grid box with dimensions (XYZ) (−35.34, −49.13, −62.71 Å) was configured after selecting the active site residues. AutoDock [32] was used to determine the lowest binding energy of the ALP-ligand complex, which was considered the initial 3D structure for further analysis in Discovery Studio.

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism (Version 9). Spearman correlation analysis was employed to assess relationships between variables, while t-tests were utilized for variance analysis (ANOVA) and post hoc tests. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

In the liver TNF-α qRT-PCR analysis, no significant changes were observed at both the 15-day and 45-day time points (Fig. 2A and B). Similarly, the serum TNF-α ELISA assay revealed no significant differences between the control and treatment groups 15 days post-silver nitrate injection. However, at the 45-day mark, a significant increase in serum TNF-α expression was noted in both groups ($P < 0.005$) (Fig. 3A and B).

Regarding the chemical assay of ALP enzyme activity, a statistically significant increase in ALP activity was recorded at the 15-day interval when compared to the control group ($P < 0.005$). This increase became markedly significant at the 45-day interval, suggesting a substantial rise in serum ALP activity and levels ($P < 0.001$) (Fig. 4A and B).

To further investigate the effects of silver nitrate on high and low molecular weight ALP activity in serum, gel filtration chromatography was employed. Serum samples from both silver-treated and untreated control rats were analyzed. Results presented in Fig. 5A and B indicate that high molecular weight (HMW) ALP activity was exclusively detectable in the serum of treated rats. A significant increase (7-fold–9-fold) in HMW-ALP was observed at both 15 and 45 days following silver treatment ($P < 0.05$) compared to control specimens, while no significant changes were recorded in low molecular weight ALP activity. Additionally, Spearman's correlation test indicated a significant relationship between serum TNF-α expression and ALP activity at both 15 and 45 days post-injection.

The molecular docking method was employed to investigate the interactions between silver nitrate and ALP at specific binding sites. This approach is vital for understanding the enzyme-ligand interactions that can influence the functionality and therapeutic potential of the compounds in question. Docking studies were performed using AutoDock software, which enabled the evaluation of binding affinities and the identification of specific interaction sites between silver nitrate and the ALP molecule. The docking analysis employed a grid resolution of 0.43 Å as the scoring function, allowing for high precision in modeling the spatial arrangements of the ligand-receptor complex. The protocol encapsulated essential characteristics of ligand-protein interactions, including internal electrostatic interactions and hydrogen bonding. Fig. 6A–D presents detailed representations of the interaction interface, showcasing the specific binding locations and types of interactions occurring at the molecular level.

The Docking Simulation Engine (Dock SE) algorithm was utilized to investigate the binding interactions of selected ligands with ALP. A total of 100 docking runs were conducted for each ligand to ensure statistical robustness and capture a comprehensive range of possible binding conformations. Following the docking procedure, energy minimization and optimization of hydrogen bonds were performed to refine the ligand conformations within the active site of ALP. An energy threshold was established at 200.00 kcal/mol, facilitating the exclusion of similar poses to focus on the most relevant docking outcomes.

The docking results were systematically evaluated utilizing both AutoDock and reranking scores. The reranking score serves as an estimate of the interaction quality, providing insight into the strength and specificity of the ligand-ALP binding interactions. Among the various poses evaluated, the optimal pose for the ALP enzyme was identified based on its docking score. This pose demonstrated a binding energy of −5.28 kcal/mol and a reranking score of −7.43 kcal/mol, indicating a favorable interaction with the enzyme

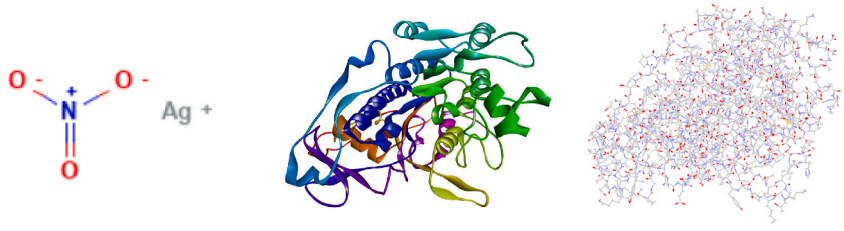

Fig. 1. (A) 2D structure of silver nitrate. (B) and (C): Structure of ALP enzyme.

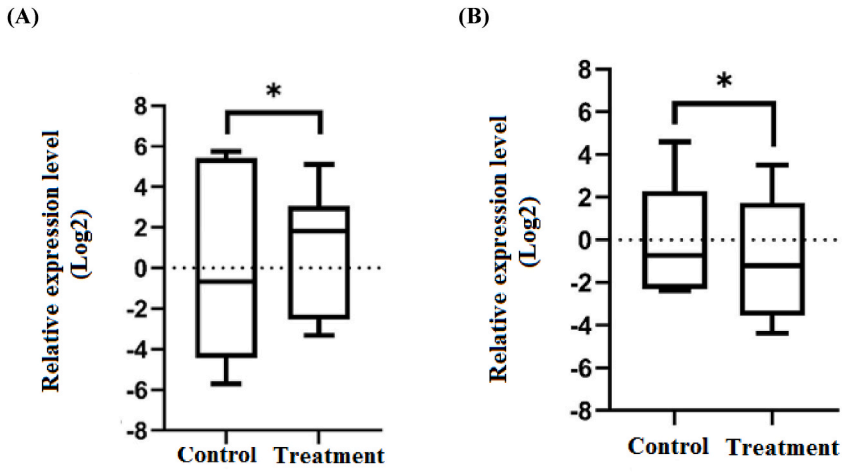

Fig. 2. TNF-α qRT-PCR in (A) 15 days and (B) 45 days by injection of Silver nitrate. * Compared to the starch group, it is significantly different ($P < 0.05$).

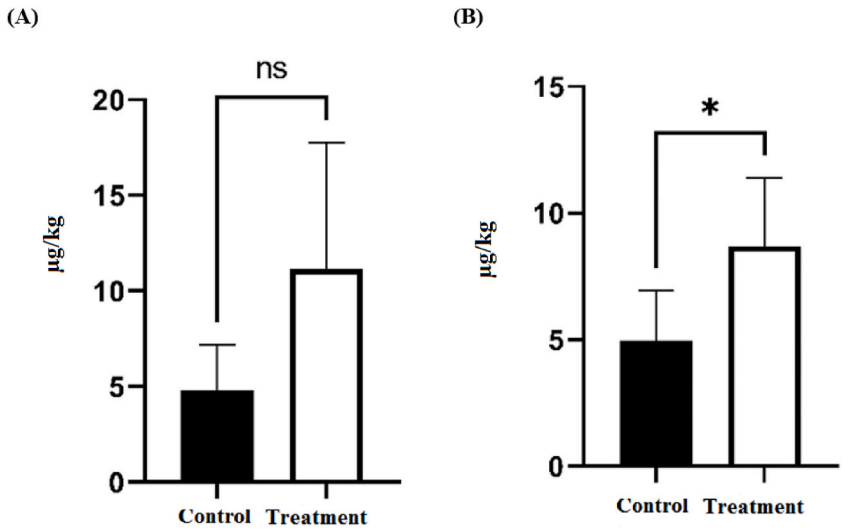

Fig. 3. Serum TNF-α assay in (A) 15 days and (B) 45 days by injection of Silver nitrate. * Compared to the starch group, it is significantly different ($P < 0.05$); NS indicates not significant.

(Table 2).

4. Discussion

This study aimed to elucidate the effects of silver nitrate exposure on serum ALP levels and its implications for liver function. The findings demonstrated a significant elevation in total serum ALP activity in rats treated with silver nitrate at both 15-day and 45-day intervals compared to the control group. Notably, the gel filtration chromatography results identified high molecular weight ALP (HMW-ALP) as the primary contributor to the observed increases in serum ALP activity [33]. This suggests that exposure to silver nitrate may be linked to liver dysfunction, potentially through the stimulation or release of HMW-ALP, particularly from biliary cells or through enhanced biosynthesis of this isoenzyme following treatment [34,35]. Although the study did not directly assess serum IL-10 levels, the findings underscore the necessity for further investigation into the precise molecular mechanisms by which silver nitrate influences liver function and immune responses. Understanding these mechanisms is vital in developing strategies to mitigate the long-term impacts of silver nitrate exposure on living organisms [36]. The measurement of ALP isoenzyme activities is traditionally utilized for diagnosing and monitoring diseases associated with these specific isoenzymes. In clinical settings, biliary ALP (HMW-ALP) has been identified in the serum of patients suffering from biliary obstruction and metastatic liver cancer. Previous studies have posited that HMW-ALP can serve as a reliable tumor marker for both liver and colorectal cancers. Importantly, the development of liver disease

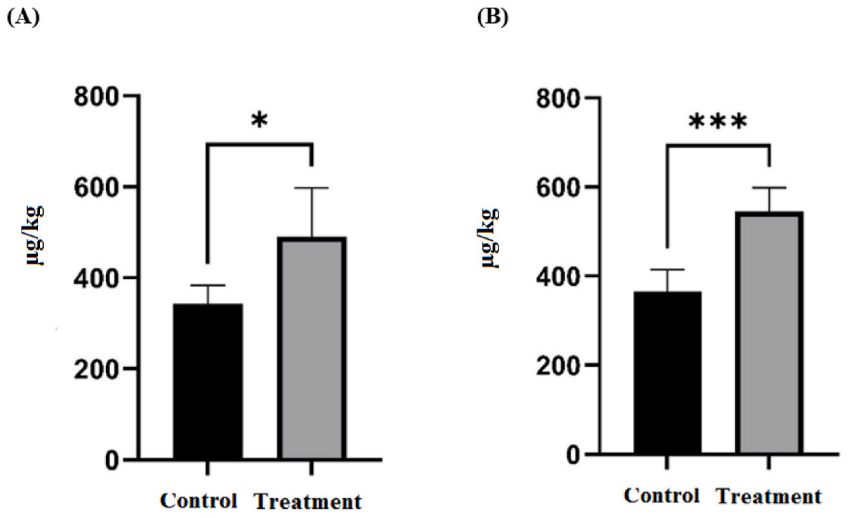

Fig. 4. Serum ALP activity assay in (A) 15 days and (B) 45 days by injection of Silver nitrate. * Compared to the starch group, it is significantly different ($P < 0.05$).

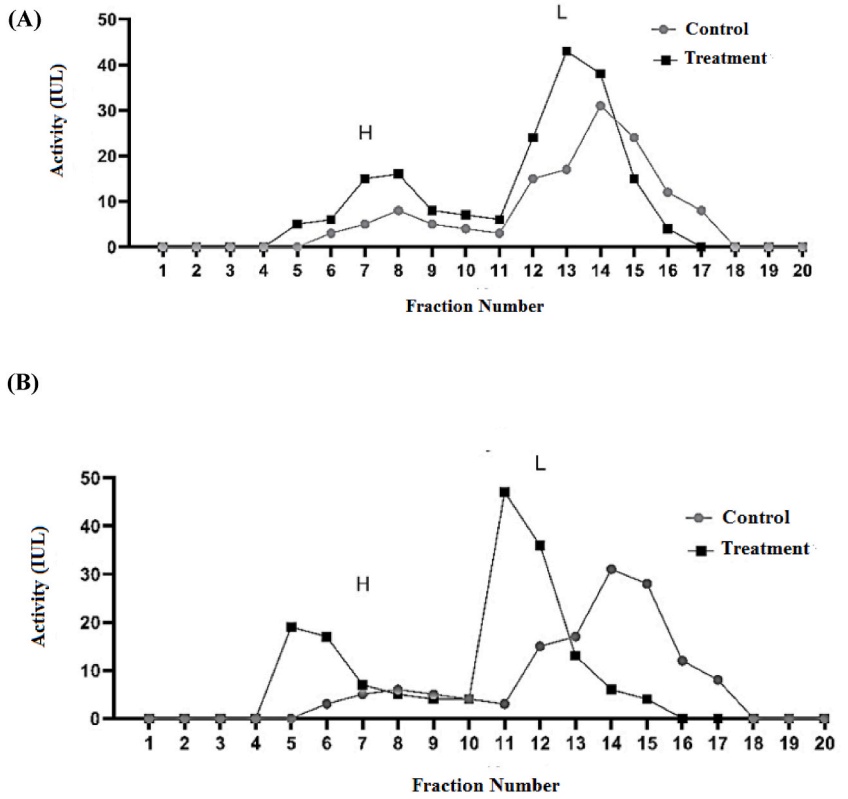

Fig. 5. Elution profiles of high and low molecular weight ALP in serum of control and silver nitrate treated groups in (A) 15 day and (B) 45 day.

and the manifestation of this ALP isoenzyme in serum may correlate with long-term changes in TNF-α levels, positioning ALP as a valuable biomarker for detecting copper poisoning over extended periods [37,38]. Interestingly, the data presented here show a significant ($P < 0.05$) increase in total serum ALP activity with silver nitrate treatment both at 15 and 45 days, as illustrated in Figs. 3–5. Moreover, it was noted that approximately half of the cases demonstrated elevated silver nitrate levels following one month of treatment. Predictive factors for systemic silver absorption, as established through multivariate analysis, included variables such as wound area, anemia, and malnutrition, highlighting the complex interplay of these factors in silver toxicity [39]. Our research

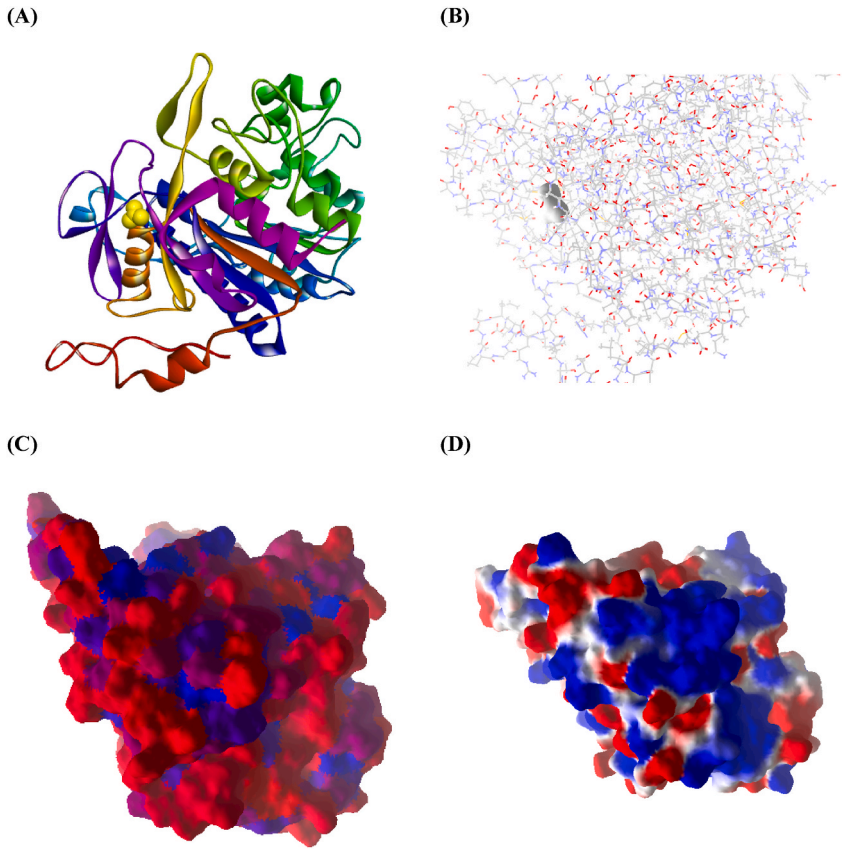

Fig. 6. (A) 3D and (B) overall of Interaction ALP-silver nitrate complex. (C) The hydrophobicity and (D) electrostatic regions.

Table 2
Docking score of top five poses of silver nitrate to ALP.

No.	Ligand	Docking score	Re-docking score
1	$AgNO_3$	−5.28	−7.43
2	$AgNO_3$	−5.14	−6.52
3	$AgNO_3$	−5.11	−6.43
4	$AgNO_3$	−3.75	−4.59
5	$AgNO_3$	−3.51	−4.46

reinforces the notion of long-term and excessive toxic effects associated with silver nitrate exposure, pointing towards potential chronic sequelae, such as kidney dysfunction, particularly in scenarios involving early-life exposure. Furthermore, exposure during gestation has been shown to result in persistent inflammatory responses, alterations in immune defense mechanisms against foreign antigens, and metabolic disturbances that may predispose individuals to pancreatic and renal damage later in life [40]. This investigation concentrated on the changes in inflammatory factors and the overarching immune system, with the limitation of not exploring other organ systems. The significant increase in ALP activity, alongside the identification of HMW-ALP as a key contributor, suggests a critical need for additional studies focused on the underlying molecular mechanisms by which silver induces HMW-ALP production [41]. Future research will also need to expand upon the effects of silver poisoning, explicitly concerning the implications for the immune system and tissue histopathology, to enhance our understanding of the long-term consequences of silver exposure on biological systems.

Understanding the binding location of silver nitrate on the ALP molecule is crucial for elucidating the mechanism of action and the resulting functional implications of this interaction. The insights gained from molecular docking analyses can inform the design of novel therapeutic agents targeting ALP, enhancing their efficacy and specificity. The flexibility of side-chain residues, a key feature of the AutoDock software, plays a significant role in accurately simulating ligand-protein interactions. This flexibility allows for a more realistic representation of conformational changes within the receptor upon ligand binding, which is critical for exploring potential dynamic behaviors of biomolecules in drug design [42–46]. The identification of specific residues interacting with silver nitrate offers a foundation for further studies aimed at understanding the pharmacodynamics of silver compounds. By delineating the nature of these

interactions, researchers can gain insights into how alterations in ALP activity may influence physiological processes, especially in the context of silver nitrate's therapeutic applications.

Moreover, the information derived from this molecular docking study underscores the importance of computational approaches in drug discovery. The ability to predict binding affinities and identify critical interactions can streamline the development of new drug candidates, potentially accelerating their progression from laboratory research to clinical application. In conclusion, this molecular docking analysis not only sheds light on the interactions between silver nitrate and ALP but also highlights the critical role of computational techniques in advancing our understanding of enzyme modulation and therapeutic potential. Further experimental validation will be necessary to confirm these interactions and elucidate their biological significance.

The application of the Dock SE algorithm in this study provided an effective methodology for elucidating the binding interactions between ligands and alkaline phosphatase. The execution of 100 docking runs allowed for a thorough exploration of the conformational space, thereby enhancing the reliability of the results obtained. The subsequent energy minimization and optimization of hydrogen bonds are critical steps that help to stabilize the ligand-enzyme complex and provide a more accurate representation of the interactions at play. The established energy threshold of 200.00 kcal/mol was instrumental in filtering docking poses, ensuring that only the most relevant conformations were considered for further analysis. By disregarding similar poses, the robustness of the selected ligand-conformation pairs was increased, facilitating a focused evaluation of the most promising binding interactions.

The analysis of docking scores and reranking scores reveals valuable information regarding the binding affinity and interaction quality between silver nitrate and ALP. The identified optimal pose, characterized by a binding energy of −5.28 kcal/mol, suggests a moderately strong interaction, while the reranking score of −7.43 kcal/mol indicates an even higher quality of interaction. These values are indicative of the potential efficacy of silver nitrate as a therapeutic agent targeting ALP. Overall, these results underscore the utility of molecular docking simulations in predicting ligand binding and evaluating the potential biological implications of such interactions. Future studies should aim to validate these findings through experimental approaches, such as site-directed mutagenesis and kinetic assays, to confirm the functional relevance of the identified binding interactions. Furthermore, understanding the structural basis of these interactions can aid in the rational design of novel inhibitors or activators of ALP, enhancing therapeutic strategies targeting this important enzyme.

5. Future directions

Based on the provided information regarding alkaline phosphatase, silver nitrate, IL-10, gene expression, and TNF-α, several potential future lines of research can be identified: Investigate the molecular mechanisms by which silver nitrate modulates alkaline phosphatase activity and its subsequent effects on gene expression, particularly focusing on inflammatory markers like TNF-α. Explore the role of interleukin-10 (IL-10) as a regulatory cytokine in the context of silver nitrate exposure, assessing its potential protective effects against inflammation induced by TNF-α. Conduct long-term studies assessing the chronic effects of silver nitrate administration on physiological and biochemical parameters, including changes in alkaline phosphatase levels and inflammatory cytokine profiles. Examine the dose-response effects of silver nitrate on alkaline phosphatase activity and associated inflammatory markers to determine optimal dosing for therapeutic applications. Investigate the effects of different silver compounds (e.g., silver sulfadiazine, silver nanoparticles) on alkaline phosphatase activity and their interaction with pro-inflammatory cytokines. Explore the clinical implications of regulating alkaline phosphatase and cytokine levels in conditions such as osteoarthritis, chronic inflammation, or wound healing, where silver nitrate could be applied therapeutically. Study the genetic regulation of alkaline phosphatase and TNF-α in various cell types in response to silver nitrate to clarify the underlying pathways of gene expression modulation. Investigate how silver nitrate and associated treatments may affect gut microbiota composition and its interaction with the immune system, particularly focusing on cytokine production and overall inflammatory response. By pursuing these lines of research, scientists can deepen their understanding of the effects of silver nitrate on biochemical pathways and their potential therapeutic applications.

6. Conclusions

This investigation examined the impact of silver nitrate on serum ALP levels and its potential implications for liver function. The findings indicated that silver nitrate administration led to a significant increase in ALP activity, which was predominantly associated with HMW-ALP. This suggests that exposure to silver nitrate may have detrimental effects on liver health, potentially through the stimulation or release of HMW-ALP from bile duct cells. Furthermore, the study highlights the necessity for additional research to elucidate the molecular mechanisms underlying silver-induced liver toxicity and its effects on overall immune function. Molecular docking analyses confirmed the interaction between silver nitrate and ALP, providing a foundation for understanding these relationships. Comprehensive studies addressing silver poisoning, particularly its immunological effects and histological impacts, are essential for a complete understanding of the long-term consequences of silver exposure on living organisms.

CRediT authorship contribution statement

Nader jadid: Software, Investigation, Formal analysis. **Ali Asghar Moshtaghie:** Writing – original draft, Supervision. **Hashem Nayeri:** Data curation. **Masoud Foladgar:** Formal analysis, Data curation. **Maryam Ostadsharif:** Data curation.

Data availability

Not applicable.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e42132.

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