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In silico Investigations of Al_2O_3 and NiO Nanoparticles Interactions with Antioxidant Enzymes of Fresh Water Fish *O.mossambicus*

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Authors' contributions

This work was carried out in collaboration among all authors. The work was carried out and designed by the Author VB and the manuscript was prepared by her. All authors read and approved the final manuscript.

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ABSTRACT

Metal oxide nanoparticles caused hazardous to aquatic organisms, especially fishes. Nanomaterials of Aluminium and nickel oxide are applied in wide range of applications. However the toxicity of these metal oxide nanoparticles on freshwater fish is very scarce. In this study, we

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investigated the toxic impact of these two nanoparticles through an *in silico* approach. The antioxidant enzymes /proteins of SOD, CAT and GST in fish *O.mossambicus* were docked against Al_2O_3 and NiO NPs. These enzymes are responsible for oxidative metabolism of a large number of nanomaterials. Induction of oxidative stress by NPs exposure leads to inhibition of these enzymes. The targeted proteins SOD, CAT and GST were retrieved from Alpha fold database and docked with ligands. Molecular docking was conducted using CB - Dock and AutoDock Vina software tools to find the binding affinity and interaction with amino acids of each protein. The target proteins found to strongly interact with ligands through hydrogen bonding and metal coordination interactions. The lowest binding energy shows strong binding of ligands with proteins. Docking results revealed that the amino acids residues of SOD, CAT and GST enzymes are strongly bind with the Al_2O_3 and NiO NPs in its active site and causes inhibition of the enzymes activities. CAT exhibited lowest binding affinity of (-4.0 Kcal/mol) among all enzymes and significantly inhibited by NiO NPs, followed by GST (-3.4 Kcal/mol), likewise CAT (-1.7 Kcal/mol) and GST (-1.8 Kcal/mol) was moderately inhibited by Al_2O_3 NPs. SOD inhibited insignificantly by both the NPs. In conclusion, NiO NPs effectively inhibit antioxidant enzymes activity than Al_2O_3 NPs.

Keywords: Docking; antioxidant enzymes; Al_2O_3 nanoparticles; NiO nanoparticles; O.mossambicus.

1. INTRODUCTION

Nanotechnology has become revolutionary technology over the past two decades for its extensive use in various disciplines including food, biology, material science, electrical engineering and medicine [1-5]. Ultra-fine particles (less than 100 nm), have demonstrated more toxicity than the larger particles with the same chemical composition [6]. Excessive use of nanoparticles (NPs), in general and particularly metallic nanoparticles such as Aluminium oxide and Nickel oxide has increased their discharge and accumulation in the aquatic biota [7]. Al_2O_3 NPs used in drug delivery, bio sensors, catalysis, clothing and glass formulation, plastics and paints [8] and Nickel oxide NPs drawn a lot of attention by way of broad spectrum applications ranging from catalysts, fuel cells, dye-sensitized photocathodes, electro chromic films, ion storage materials, gas sensors, battery electrodes, photo-electron devices, magnetic materials, thermoelectric materials, and gas sensors [9,10]. Due to over accumulation of NPs, there are possibilities to enter into the aquatic ecosystem. In toxicological study, fishes are the ideal test organisms since they are the direct consumer of the human through food chain and also serve as the indicator of water quality and health status of aquatic ecosystem. Generation of ROS and free radical are the important phenomenon of NPs toxicity which leads to DNA damage, mitochondrial damage and lipid peroxidation. Free oxygen radicals cause deleterious cellular events such as apoptosis during oxidative stress in fish [11]. Fish has developed a complex antioxidant system that protects from ROS and free radicals which are generated by excessive oxidative stress. The antioxidant enzymes of fish *O.mossambicus* include super oxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) is responsible for oxidative metabolisms. By converting superoxide to hydrogen peroxide (H_2O_2), SOD keeps the quantities of superoxide anions in cells within physiological concentrations, further CAT metabolize H_2O_2 to H_2O and O_2, GST is the major enzymes among phase 2 detoxification to protect the cells from nanoparticle toxicity by conjugation with glutathione. Fish can overcome high concentration ROS and free radicals through an action of antioxidant enzymes. In aquatic organisms antioxidant enzymes are applied as biomarkers for metal NPs and organic compounds that cause oxidative stress [12]. The precise mechanism of underlying interaction between nanoparticles and antioxidant enzymes remains unknown [13].

Nowadays bioinformatics tools such as molecular docking and molecular dynamics are used to investigate the toxic potential of metal nanoparticles [14]. Docking offers a rapid and easy way to forecast toxicity by predicting how biological proteins will interact with nanoparticles [15]. Molecular docking studies are play an important role in computer based predictions and estimates the three dimensional structures of protein- ligand complexes and its interactions. We used blind docking web server, named CB dock, which predicts the binding sites of proteins and calculates the centers and sizes with novel curvature based cavity detection approach and perform the docking with the most used docking program called AutoDock Vina [16]. Docking methods predicts the optimal binding energy,

binding mode and time dependent dynamic stability of small molecules against the targets [17].

We have investigated the effect of two metallic NPs (Al_2O_3 and NiO) toxicity and an insight into the specific interaction between the nanoparticles with particular amino acids of target proteins. The binding affinity of ligand with the specific enzymes targets was investigated through docking. Hence this study tailored to evaluate the interactions of Al_2O_3 and NiO NPs with SOD, CAT and GST proteins of fresh water fish *O. mossambicus.*

2. MATERIALS AND METHODS

The following software and online servers were utilized in this current study: UCSF Chimera 1.17.3, the most recent version of the Java Platform binary, and ACD Chemsketch to draw the Aluminium oxide and Nickel Oxide Crystal structures (NPs). The CB-Dock (Cavity-detection guided Blind Docking) server was used for blind molecular docking.

2.1 Protein Data Retrieval

There is no experimental structure available for Superoxide dismutase, Catalase and Glutathione S-transferase. Hence, the Alpha Fold database (https://alphafold.ebi.ac.uk/) was used to obtain the core proteins three-dimensional structures (3D). Alpha Fold database works on the accuracy of protein structure predictions by neural network models and preparing methods in view of the developmental, physical and mathematical requirements of protein structures [18]. The Alpha Fold network straightforwardly predicts the 3D structure of all heavy atoms for a protein by utilizing the amino acid sequence.

2.2 Protein Preparation

The energy minimization was done by utilizing SPDB Viewer software (https://spdbv.unil.ch/disclaim.html#) [19]. The energy minimized Superoxide dismutase, Catalase and Glutathione S-transferase structures were docked with prepared Aluminium oxide and Nickel oxide nanoparticles.

2.3 Ligand Preparation

Molecular docking can assist with approving the Aluminium Oxide and Nickel Oxide nanoparticles in interaction with Superoxide dismutase, Catalase and Glutathione S-transferase from fish *Oreochromis species* (Tilapia). First, we drawn the Aluminium oxide and Nickel Oxide nanoparticles Crystal structures by using the ACD Chemsketch software, and the drawn structure was saved in MDL MOL file format. For docking, the Aluminium oxide and Nickel oxide nanoparticles crystal structure MDL MOL format was converted to the PDB file format. The energy minimization was done by utilizing SPDB Viewer software (https://spdbv.unil.ch/disclaim.html#). The energy minimized Aluminium oxide and Nickel oxide crystal structures were docked with prepared proteins. The binding affinity (minimum binding energy), and interaction with amino acids of each protein was analyzed further.

2.4 Molecular Docking

The server for cavity-detection guided blind docking (CB-Dock) (http://clab.labshare.cn/cb-dock/php/index.php), accessed uses a novel curvature-based cavity detection method to predict a protein's binding sites and calculate their centers and sizes [20]. The server has been carefully optimized to work with AutoDock Vina and has a success rate of over 70% in the developed models. The protein files in .pdb format and the ligand files in .sdf format were used for the analyses, and five possible coupling cavities were found. Based on the lowest Vina value, the one with the lowest binding energy was chosen from these. The Ball and Stick model and Cartoon options were then used to see the ligands and proteins, respectively. The color of the ligand and the proteins was configured by elements for ligands and secondary structure for proteins.

3. RESULTS

The docking of Aluminium oxide and nickel oxide nanoparticles with anti-oxidant enzymes is the subject of this investigation. A total of three antioxidant enzymes were chosen for this study, Superoxide dismutase (SOD - UniProt ID: F5CT17), Catalase (CAT- UniProt ID: A0A669DJW6) and Glutathione S-transferase (GST- UniProt ID: A0A669DBR6) from *Oreochromis species* (Fig. 1).

3.1 Molecular Docking and Protein-Ligand Interaction Analysis

Tables 2,3 & Figs. 2, 3(a,b,c) depicted the CB-DOCK results of molecular docking. Core

proteins (Superoxide dismutase, Catalase and Glutathione S-transferase) as large molecules and the Al_2O_3 and NiO cluster as nanoparticles had been shown different interaction models based on protein-ligand binding affinity prediction using the curvature dependent surface-area model [21]. Binding energy values were obtained (Vina score) inside which the association with the lowest binding energy was chosen (Table 1). In the docking where the minimum binding energy ended up being something very similar, the association in the biggest cavity was selected. The Catalase and Glutathione S- transferase enzymes shown the similar lowest binding energy such as − 1.7 kcal/mol, and − 1.8 kcal/mol respectively, and also with the largest active cavity with Aluminium oxide crystal structure (Table 2). Superoxide dismutase (SOD) presented minimum binding affinity with Aluminium oxide crystal structure, being − 1.1 kcal/mol. Similarly, the Catalase and Glutathione S- transferase enzymes were shown the lowest binding energy with Nickel oxide crystal structure, − 4.0 kcal/mol, and − 3.4 kcal/mol respectively, and also with the largest active cavity (Table 3). Superoxide dismutase (SOD) presented minimum binding energy with Nickel oxide crystal structure (− 2.9 kcal/mol). The secondary structures of the anti-oxidant enzymes (Superoxide dismutase, Catalase, and Glutathione S- transferase) were used to color the crystallized structures, which were (Pink: Alpha Helices; Yellow and white: Beta sheets). Also, the ligand was colored by element (Nitrogen in blue, Oxygen in red, Carbon in grey, Hydrogen in white).

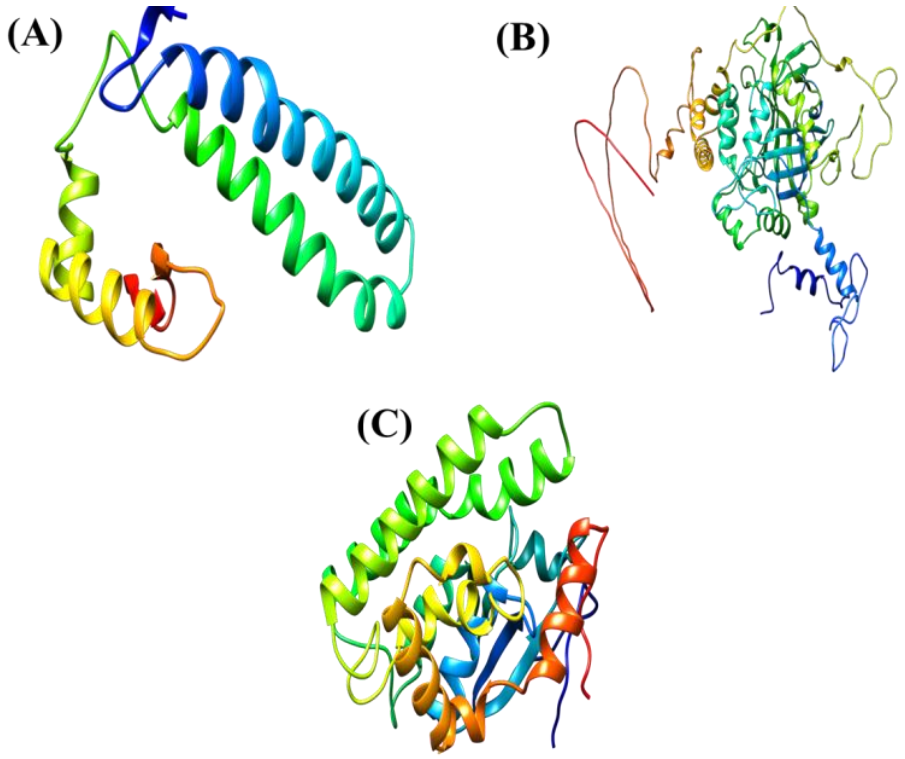


Fig. 1. The cartoon representations of 3D structures of (A) Superoxide dismutase (B) Catalase and C) Glutathione S-transferase

Table 1. Summary of minimum binding energies

S.No.	Protein Names	Minimum Binding Energy (kcal/mol)		Cavity Size	
		Aluminium Oxide	Nickel Oxide	Aluminium Oxide	Nickel Oxide
1.	Superoxide dismutase	-1.1	- 2.9	95	65
2.	Catalase	- 1.7	- 4.0	3338	1506
3.	Glutathione S-transferase	-1.8	-3.4	186	175

Table 2. Summary of core proteins contact amino acid residues with aluminium oxide

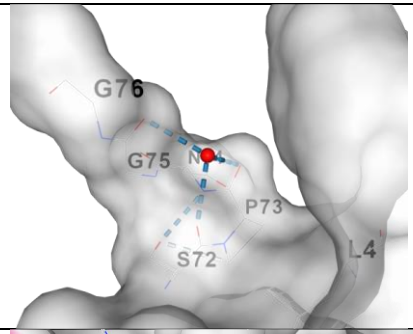
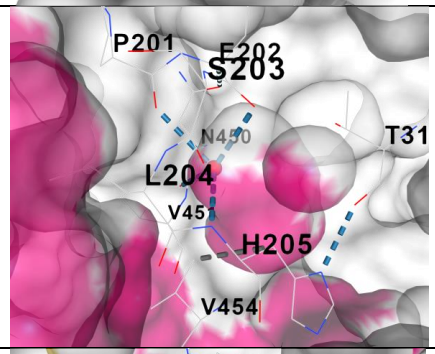
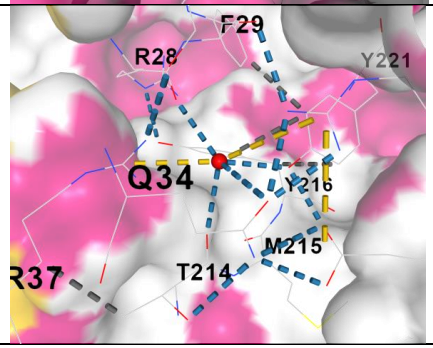
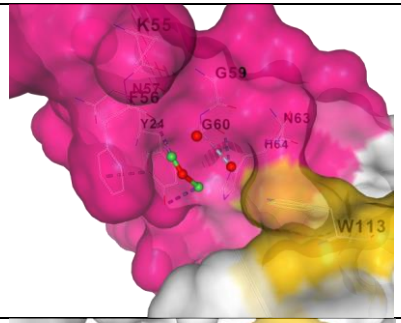
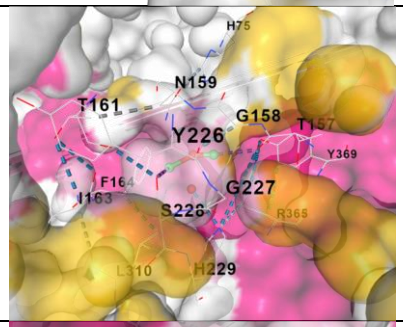
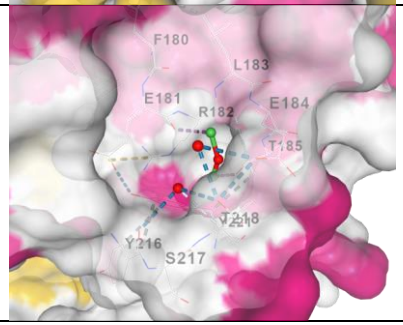
S.No.	Protein Names	Interacted Amino Acid Residues	Interaction Between Aluminium oxide Cluster with Core Proteins
1.	Superoxide dismutase	**Chain A**: LEU4 SER72 PRO73 ASN74 GLY75 GLY76	
2.	Catalase	**Chain A**: PRO201 GLU202 SER203 LEU204 HIS205 THR311 ASN450 VAL451 VAL454	
3.	Glutathione S-transferase	**Chain A:** ARG28 PHE29 CYS30 GLN34 ARG37 GLU181 ARG182 THR214 MET215 TYR216 TYR221	

Table 3. Summary of Core Proteins Contact Amino acid Residues with Nickel Oxide

S.No.	Protein Names	Interacted Amino Acid Residues	Interaction Between Nickel Oxide Cluster with Core Proteins
1.	Superoxide dismutase	**Chain A:** TYR24 LYS55 PHE56 ASN57 GLY59 GLY60 ASN63 HIS64 TRP113	
2.	Catalase	**Chain A:** HIS75 THR157 GLY158 ASN159 THR161 ILE163 PHE164 TYR226 GLY227 SER228 HIS229 LEU310 ARG365 TYR369	
3.	Glutathione S-transferase	**Chain A:** PHE180 GLU181 ARG182 LEU183 GLU184 THR185 TYR216 SER217 THR218 TYR221	

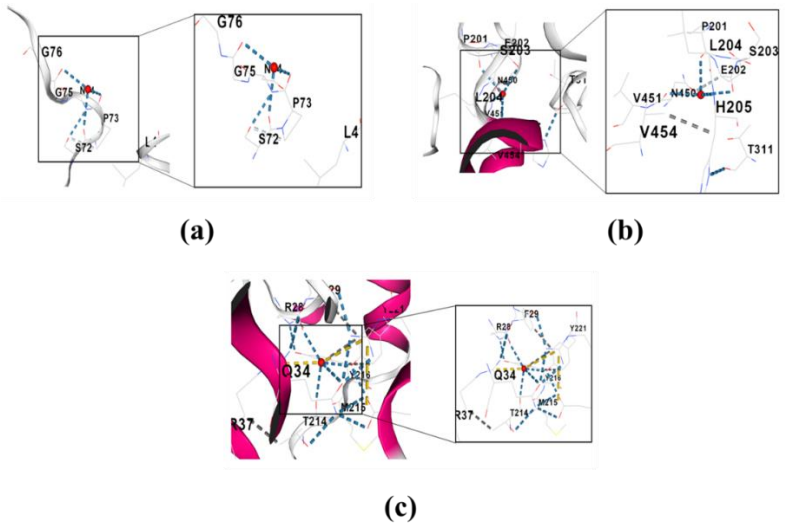


Fig. 2. Docked complex of (a) Superoxide dismutase (b) Catalase (c) Glutathione S- transferase with Al_2O_3 NPs. Hydrogen-bond between strong donor and acceptor atoms were represented in blue lines. Hydrogen-bond between a carbon donor atom and an acceptor, or a Pi group and a donor atom were represented in light blue lines, and hydrophobic contacts were represented in grey lines. Ionic interactions were represented in yellow lines

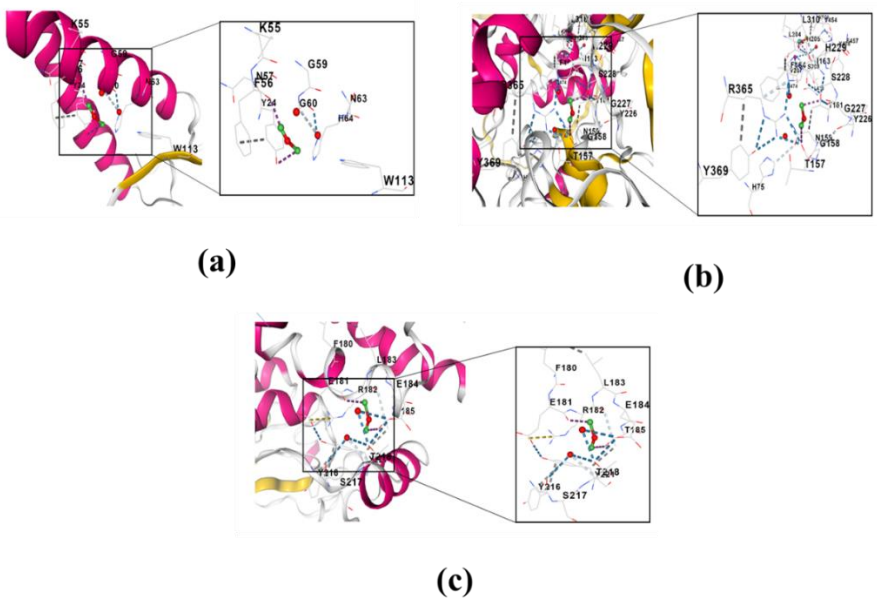


Fig. 3. Docked complex of (a) Superoxide dismutase (b) Catalase (c) Glutathione S- transferase with NiO NPs. Hydrogen-bond between strong donor and acceptor atoms were represented in blue lines. Hydrogen-bond between a carbon donor atom and an acceptor, or a Pi group and a donor atom were represented in light blue lines, and hydrophobic contacts were represented in grey lines. Metal coordination interactions were represented in purple lines

4. DISCUSSION

In silico structure-based method known as molecular docking is frequently utilized to predict the two molecules interactions. Docking approaches provide insights about the interactions between proteins and ligands at molecular level. [22] Ferreira et al. stated that, Molecular docking is one of the most popular and accurate technique for predicting the interaction

between the two molecules in structure based drug design.

The interaction between the molecules (ligands - Al_2O_3 and NiO NPs) and target proteins (Enzymes) could predict the activation and inhibition of enzymes. The outcome data of the docking can be used to study binding energy and stability of complexes. The objective of docking is to attain optimized ligand - protein complex conformation with possess less binding energy. Mainly the binding free energy is revealed through hydrogen bonds and other parameters.

Three dimensional structures of fish *O.mossambicus* antioxidant enzymes (SOD, CAT and GST) were used for molecular docking with Al_2O_3, NiO NPs to study their toxicity potential. The targeted proteins SOD (UniProt ID: F5CT17), CAT (UniProt ID: A0A669DJW6) and GST (UniProt ID: A0A669DBR6) were retrieved from Alpha fold database , the ligands Al_2O_3, NiO NPs was drawn from ACD Chemsketch software and converted into PDB format. The energy minimization was done by SPDB viewer software. Antioxidant enzymes proteins were separately docked against Al_2O_3, NiO NPs. The docking binding affinities with Al_2O_3 were reported that SOD showed -1.1 kcal/mol, CAT -1.7 kcal/mol and GST -1.8 kcal/mol respectively. Likewise binding affinity of SOD reported -2.9 kcal/mol, CAT -4.0 kcal/mol and GST -3.4 kcal/mol against NiO NPs. Among all CAT with NiO exhibited best binding energy followed by GST and SOD. Binding energy of CAT and GST are closer to each other, whereas SOD reported minimum binding affinity with Al_2O_3.

In biological systems, hydrogen bonds (HB) are the most common motif. They are crucial in determining the selectivity and affinity of protein–ligand binding. Hydrogen bonds are critical to the binding of ligands with proteins. The molecular docking of Superoxide dismutase (SOD) revealed that the amino acids GLY 75, SER 72 and PRO 73 formed hydrogen bond with Aluminium oxide nanoparticles (Table 2 & Fig.2a). Similarly hydrogen bonds are formed while docking the catalase with Al_2O_3 NPs, amino acids HIS 205, GLU 202, ASN 450, and PRO 201 were involved to form hydrogen bond (Table 2 & Fig.2b). Glutathione S – transferase reported ionic interactions with ARG 37, ARG 182 and GLN 34, THR 214, TYR 221, ARG 28 amino acids formed hydrogen bonds when docked with Al_2O_3 NPs (Table 2 & Fig.2c).

We identified metal coordination interactions between amino acids TYR 24 and PHE 56 with the ligand, there is no hydrogen bond formation when NiO NPs docked against Super oxide dismutase (Table 3 & Fig.3a). The molecular docking of catalase revealed the formation of two different types of interactions such as hydrogen bond and metal coordination interactions, amino acid HIS 229 formed the Hydrogen bond with ligand and THR 157, SER 228 formed metal coordination interactions with NiO NPs (Table 3 & Fig.3b). Likewise Glutathione S-transferase was reported hydrogen bonds with ligands by THR 218, GLU 184 and THR 185, GLU 181 formed metal coordination interactions (Table 3 & Fig.3c).

Aquatic organisms, especially fishes are most susceptible to various NPs exposure, several studies were reported the toxicity of NPs exhibiting the alterations in antioxidant enzyme activities in fishes. Antioxidant enzymes are applied as biomarkers for nanoparticles that cause oxidative stress in aquatic organisms by producing ROS and free radicals. Most of the organisms develop complex antioxidant defence system that protects the cells from overproduction of ROS generated by oxidative stress including SOD, CAT and Glutathione reductase [23]. Many studies reported alterations of antioxidant enzymes activities in the vital tissues of fishes [24]. The docking analysis showed that the Al_2O_3, NiO NPs are capable of interacting with the key amino acids residues, the interactions of this metal oxide NPs with different antioxidant enzymes proteins lead to inhibition of enzymes activities which affects the organisms defence mechanism against ROS and free radicals. By interfering with the intermolecular connections that maintain both secondary and tertiary structures contaminants such as ligands have the potential to cause structural instability and disturb the conformation of proteins [25]. Binding of amino acids in the active site causes structural changes in the heme groups and ultimately affects the enzyme activity.

The results of this study showed that the catalase significantly inhibited by NiO, the key amino acids such as HIS 229, THR 157 and SER 228 interact through metal coordination interactions. Previous investigation has demonstrated that amino acid His 74 which is located in the active site of CAT is important for enzyme's catalytic activity [26]. Even though SOD exhibited the binding energy -2.9 kcal/mol, it did not stabilized by hydrogen bonding and

possesses metal co-ordination with the key amino acid residues TYR 24 and PHE 56.

From the molecular docking analysis, Al_2O_3, NiO NPs effectively interact with antioxidant enzymes and reduce their activity and also evident that the key amino acids were associated with the interactions. The inhibition of Superoxide dismutase, Catalase and Glutathione S-transferase will directly resultant into anti-oxidant activity. Superoxide dismutase is an enzymatic anti-oxidant agent that catalyzes the transformation of O_2^- to H_2O_2 and keeps up with the redox balance by diffusing the superoxide. Superoxide dismutase comprises a vital anti-oxidant activity against oxidative stress in the cells. Catalase is a significant anti-oxidant enzyme that controls the degree of oxidative stress [27]. Catalase as a fundamental H_2O_2 scavenger changes over hydrogen peroxide to oxygen and water. Reduced CAT activity would cause the higher accumulation of H_2O_2 *in vivo*, prompting oxidative damage in proteins and nucleic acids [28]. Similarly, the Glutathione S-transferase (GST) is important anti-oxidant enzyme that regulates stress-induced signalling pathways. Glutathione S-transferase (GSTs) can reduce lipid hydroperoxides through their Se-independent glutathione-peroxidase action [29]. In recent years [30] Esraa et al*.,* reported antioxidant protein docking with manganese dioxide nanoparticles, [31] Mostafa Y. Morad et al*.* reported antioxidant enzymes of SOD and GST docking against selenium NPs in snails and [32] Ibrahim et al. documented the tissue damaging enzymes AST and ALT with fungal mediated selenium nanoparticles in snails and [33] Sutha et al*.,* 2022 reported the TCEP -Tris (2 chloroethyl) phosphate possessed binding affinity with the estrogen receptor in zebra fish by docking studies. [34] Harsha Thummala et al*.* reported reduced GST activity with ZnO NPs in snails when subjected to molecular docking.

In addition to that, [35] documented molecular docking of chlorpyrifos, a organophosphate insecticide against crystal structure of human peroxiredoxin 5 , Bovine xanthine oxidase, and crystal structure of antibacterial FabH with docking score of -2.67, -3.76 and -3.16 respectively, after evaluating the antioxidant enzymes activities in gill, kidney and liver tissues of freshwater fish *Capoeta umbla,* the result found that Chlorpyrifos has a negative correlation with the activity of the SOD, CAT, GPx, and GR enzymes and [36]addressed the effect of heavy metals on G6PD enzyme activity from kidney, liver and gill tissues of fish *Capoeta trutta* by using spectrophotometric method, the G6PD molecule activity was further calculate with the protein of (PDB ID: 5JYU and 2BH9) with the highest activity being calculate in the 0-100ns range, this study explore the physiological function and environmental sensitivity of G6PD in fish [37-39].

From this study, we studied the interaction and binding affinity of Aluminium oxide and nickel oxide with Superoxide dismutase, Catalase and glutathione S-transferase retrieved from *Oreochromis species* (Tilapia).

5. CONCLUSION

Our docking study showed that Aluminium oxide nanoparticles interact with the cell antioxidant enzymes (Superoxide dismutase, Catalase and Glutathione S-transferase) and repress their activity. Consequently, it would be vital for check, whether binding sites are accessible for Aluminium oxide nanoparticles. In the current study, the three-dimensional structures of antioxidant enzymes, Superoxide dismutase Catalase and Glutathione S-transferase were downloaded from AlphaFold database and docked with Aluminium oxide and Nickel Oxide NPs. Docking study uncovered that Aluminium oxide nanoparticles have bound with the binding pockets in the antioxidant enzymes. Among the antioxidant enzymes, Catalase was maximally inhibited (lowest minimum binding energy = -4.0 Kcal/mol) and Superoxide dismutase was insignificantly inhibited (Minimum binding energy = -2.9 kcal/mol) by Nickel oxide nanoparticles. Similarly, Catalase and Glutathione S-transferase was moderately inhibited (lowest minimum binding energy = -1.7 Kcal/mol and -1.8 Kcal/mol respectively) and Superoxide dismutase (SOD) was insignificantly inhibited (Minimum binding energy = -1.1 kcal/mol) by Aluminium oxide nanoparticles. As a conclusion, the molecular docking study shown that Nickel oxide is inhibited Catalase and Glutathione S-transferase significantly than the Aluminium oxide. Moreover, Aluminium oxide showed moderate lowest binding affinity with all anti-oxidant enzymes. Hence, from this study we suggest Nickel oxide nanoparticles could possibly inhibit Superoxide dismutase, Catalase, and Glutathione S-transferase than the Aluminium oxide nanoparticles. This work provided baseline information about the interaction between the nanoparticles and key amino acids in the antioxidant enzymes, further

research is needed to evaluate the toxicity mechanism of metal oxide nanoparticles and to elucidate their toxicity *in vivo* and *in vitro* as well to protects the aquatic ecosystem at this moment.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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