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Impacts of Biosynthesized Manganese Dioxide Nanoparticles on Antioxidant Capacity, Hematological Parameters, and Antioxidant Protein Docking in Broilers

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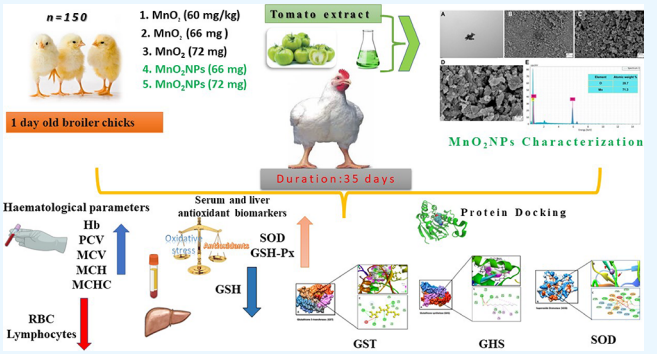
ABSTRACT: Using green tomato extract, a green approach was used to synthesize manganese oxide nanoparticles (MnO_2NPs). The synthesis of MnO_2NPs was (20.93−36.85 nm) confirmed by energy-dispersive X-ray (EDX), scanning and transmission electron microscopy (SEM and TEM), Fourier transform infrared spectroscopy (FTIR), and UV−visible spectroscopy (UV−vis) analyses. One hundred fifty-day-old Arbor Acres broiler chicks were randomly divided into five groups. The control group received a diet containing 60 mg Mn/kg (100% NRC broiler recommendation). The other four groups received different levels of Mn from both bulk MnO_2 and green synthesized MnO_2NPs, ranging from 66 to 72 mg/kg (110% and 120% of the standard level). Each group comprised 30 birds, in three replicates of 10 birds each. Generally, the study's results indicate that incorporating MnO_2NPs as a feed additive had no negative effects on broiler chick growth, antioxidant status, and overall physiological responses. The addition of MnO_2NPs, whether at 66 or 72 mg/kg, led to enhanced superoxide dismutase (SOD) activity in both serum and liver tissues of the broiler chicks. Notably, the 72 mg MnO_2NPs group displayed significantly higher SOD activity compared to the other groups. The study was further justified through docking. High throughput targeted docking was performed for proteins GHS, GST, and SOD with MnO_2. SOD showed an effective binding affinity of −2.3 kcal/mol. This research sheds light on the potential of MnO_2NPs as a safe and effective feed additive for broiler chicks. Further studies are required to explore the underlying mechanisms and long-term effects of incorporating MnO_2NPs into broiler feed, to optimize broiler production and promote its welfare.



INTRODUCTION

Nanotrace elements and their biosynthesis have gained widespread attention recently due to the advent of nanotechnology.[1−3] Nanotrace elements have demonstrated encouraging outcomes in poultry diets. Studies have indicated that nanoparticles of critical minerals including calcium, zinc, copper, selenium, and chromium have been examined for their potential advantages in growth performance, feed consumption, and the health condition of animals.[4] Nanoparticles contain increased physical activity and chemical neutrality, which may boost the bioavailability of trace minerals and improve their effectiveness in animal feeding.[5] Research has demonstrated that feeding trace minerals in the form of nanoparticles may have strong antibacterial activity against important poultry diseases and can control gut health by boosting the population of beneficial microorganisms.[6] Furthermore, the usage of green nanozinc and market nanozinc has been reported to increase meat quality, antioxidant status, mineral deposition, and bone growth in broiler chickens.[7] These results show that nanotrace elements have the potential to be efficient feed additives for poultry, giving benefits over inorganic or organic mineral sources.[8] Manganese is a trace mineral that is commonly employed in chicken production. It holds particular significance in broilers due to its crucial involvement in various physiological processes, including metabolism, skeletal development, enzyme functioning, and immune system responses.[9] The National Research Council (NRC) has recommended a 60.00 mg/kg manganese diet for broilers, highlighting its nutritional significance.[10] Manganese is essential for the sustained activity

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of superoxide dismutase which plays a critical role in the antimicrobial function of immune cells.[11] However, excessive intake of manganese can be toxic, generating free radicals, inactivating antioxidant enzymes, and impairing immune function and other trace elements' bioavailability.[12] Therefore, it is essential to investigate the possible effects of nanotrace elements at different levels to avoid toxicity.

Nanotrace elements are known to express characteristics of the novel substance including a significant surface area, elevated surface activity, efficient catalytic properties, pronounced adsorption capabilities, and little toxicity, resulting in suitable metabolic signaling and/or antioxidant activity.[13,14] The heightened activity and efficacy of nanotrace elements possess a dual nature, as their overprovision beyond biological necessities might result in toxicity. However, their use in poultry diets has shown promising results Matuszewski et al.[15] found that the application of manganese oxide nanoparticles ($NanoMn_2O_3$) had no deleterious effects on chicken growth and development but decreased Mn excretion, indicating that $NanoMn_2O_3$ may substitute commercial Mn_2O_3 in broiler diets. Hassan et al.[5] explored the possible uses of nanotrace minerals, including manganese, in various poultry species, emphasizing their impacts on the performance and health of birds, such as antibacterial activity and modification of gut health. However, nanomaterials enable logical engineering concepts such as size control, surface modification, crystalloid alteration, and stimuli-responsive functionalization for battling resistant microorganisms.[16] These properties frequently provide unique killing mechanisms as well as extraordinary antimicrobial qualities such as broad-spectrum activity, long-lasting effects, and resistance-independent effects.[17] Also, nanomaterials may have long-term stability and do not result in detectable secondary resistance, suggesting that they can prevent antimicrobial resistance evolution. However, more studies should be done regarding the antibacterial resistance of nanomaterials as mentioned by Xie et al.[18] They explored the potential of antimicrobial nanomaterials in limiting the spread of antibiotic resistance. In mammals, nanomaterials exhibit different adsorption, distribution, metabolism, and excretion behaviors. On the one hand, further efforts are needed to standardize antimicrobial nanomaterial biosafety assessments. However, the low selectivity of antimicrobial nanoparticles may result in unavoidable harm to animals.[18] Hameed[19] underlined the potential of nanomaterials, especially nanomanganese, as feed additives in animal diets, aiming to boost production efficiency and meet human requirements for quality poultry and animal products. Hence, it is imperative to do thorough research on the impacts of nanotrace elements to enhance their efficacy in poultry nutrition and mitigate any potential adverse consequences on birds' health. MnO_2 nanoparticles have been shown to mimic antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase as well as exhibit oxidase-mimicking activity.[20] Additionally, manganese dioxide nanoparticles have been engineered to have physicochemical properties that allow them to penetrate cartilage and mitigate oxidative stress in chondrocytes.[21] The interactions between proteins, which are fundamental biological molecules, form the basis of many biological systems. Any size of protein can interact with another protein; therefore, any protein oligomer has the potential to interact. Because of the specificity of their interactions, alterations at interaction interfaces pose a greater threat than at noninterface regions.[22] Pose generation and scoring are the two steps involved in the laborious process of protein−protein docking in general. In recent years, molecular docking has emerged as a crucial component of in silico drug development. This method entails forecasting the atomic-level interactions between a small molecule and a protein. This makes it possible for scientists to investigate how tiny compounds, like nanominerals, behave within a target protein's binding site and comprehend the basic biochemical mechanism underlying this interaction.[23] The method is structure-based and needs a high-resolution three-dimensional image of the target protein, which can be obtained using methods such as cryo-electron microscopy, nuclear magnetic resonance spectroscopy, or X-ray crystallography.[24] Nevertheless, there is a scarcity of studies examining the potential impacts of nanomanganese-based supplements on broiler chickens' antioxidant state and physiological responses. Hence, the current study sought to assess the impact of administering nanomanganese at varying levels (66 and 72 mg/kg diet) on the antioxidant capacity, hematological parameters, and antioxidant protein docking in broilers.

MATERIALS AND METHODS

The research was done under the ethical norms established by the ethics committee of the Faculty of Agriculture at Minia University, with approval number MU/FA/013/12/22.

Preparation of Magnesium Oxide Nanoparticles (MnO_2NPs). The preparations of MnO_2NPs were prepared eco-friendly (green synthesis) using green tomato aqueous extracts as follows:

Preparation of Green Tomato Extract. The green tomato water-based extraction was made by obtaining 1000 g of fresh green tomatoes. The fruits were decontaminated through a washing process using deionized water (DI) to eliminate any impurities and dust. Subsequently, they were sliced and subjected to hot air drying at a temperature of 50 °C until a stable weight was achieved. The dehydrated slices were finely ground using a blender, and the resulting powder of green tomatoes was afterward placed into a 500 mL beaker. Subsequently, a volume of 200 mL of deionized water was introduced into the mixture, which was then subjected to stirring at a temperature of 60 °C for 60 min. Subsequently, the extraction of green tomato was allowed to cool to ambient temperature and subsequently subjected to filtration. The collected filtrate was thereafter held at a temperature of 4 °C in a glass bottle that was sealed to prevent air exposure, to facilitate its future utilization. The green tomato water-based extraction was subjected to double filtration using Whatman no. 1 filter paper, following which it was directly utilized for the synthesis of MnO_2 nanoparticles.

Synthesis of MnO_2NPs. The synthesis of MnO_2NPs was conducted using the procedure outlined by Ogunyemi et al.,[25] with a few modifications applied. Concisely, a concentration of 1 mM (mM) of Manganese dioxide (MnO_2) was introduced into a 25 mL (ml) extract solution of green tomato. The resultant mixture was subjected to heating at a temperature of 40 °C for a duration of 90 min while maintaining continuous stirring at a pH value of 7.7. Following the completion of the reaction, the produced manganese dioxide nanoparticles were isolated using centrifugation at a speed of 10,000 rpm for 10 min. Following the centrifugation process, the acquired nanoparticles were subjected to three rounds of washing using deionized water. Subsequently, the particles were dried in an oven maintained at a temperature of 40 °C. Further

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treatment involved calcination in a muffle furnace at a temperature of 200 °C for 3 h. Subsequently, the produced manganese dioxide nanoparticles with a green coloration were carefully preserved within a glass container to facilitate subsequent characterization processes.

Characterization of MnO_2NPs. *UV−vis Spectroscopy Analysis.* The UV−vis spectroscopy technique was employed to measure the maximum absorption levels of MnO_2NPs, with a 300−550 nm wavelength range. The measurements were conducted using a T80 UV−vis spectrometer from the UK, with a path length of 1 cm, as previously described in the literature.[26]

MnO_2 band gap energy estimation. Using Tauc's method (where the absorption coefficient, α, obtained from $\alpha = 2.3A/d$, where (A) is the measured absorbance and (d) is the cuvette path length), the UV−vis absorption spectra were used to estimate optical band gaps, E_g. The absorption coefficient is plotted as a function of photon energy, $h\nu$, as follows: $\alpha h\nu \sim (h\nu - E_g)n$, where n is the transition coefficient; values of 1/2 or 2 for permitted direct and indirect shifts.

Consequently, in the Tauc plots, we used $n = 1/2$. The bandgap energy may be obtained by extrapolating the linear area on the graph of $(\alpha h\nu)^2$ vs photon energy to the abscissa (Figure 1, inset).

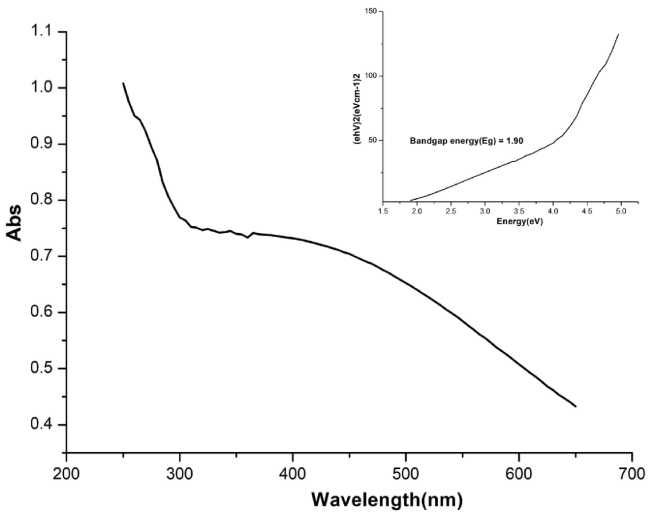

Figure 1. UV−Vis absorption peak intensity of MnO_2NPs. Inset: estimation of direct band gap energies from Tauc plot analysis of MnO_2NPs.

TEM and SEM (Transmission and Scanning Electron Microscope) Observation. The morphology of the MnO_2 nanoparticles (NPs) was checked through transmission electron microscopy (TEM) using (JEM-1230, JEOL, Akishima, Japan). Scanning Electron Microscopy (SEM) was utilized using (JSM IT 200, Hitachi, Japan) according to the method of Brodusch et al.[27] In summary, a film was formed on a grid composed of carbon-coated copper through the immobilization of a small quantity of the sample onto the grid. The SEM grid was subjected to a drying process by exposing it to a mercury lamp for 5 min.

EDS (Energy-Dispersive X-ray Spectroscopy). The chemical composition of synthesized MnO_2NPs was performed with an energy-dispersive spectrum (EDS), which was attached with SEM. The chemical composition analysis of the produced manganese dioxide nanoparticles (MnO_2NPs) was conducted using an energy-dispersive spectrum (EDS) that was integrated with scanning and transmission electron microscopy (SEM and TEM).

Analysis of FTIR (Fourier Transform Infrared Spectroscopy). The determination of the functional group of MnO_2NPs was conducted following the methodology outlined by Ogunyemi et al.[25] This involved analyzing the dried powder of the biosynthesized MnO_2NPs using a Fourier transform infrared spectrometer (Vector 22, Bruker, Germany). The analysis was performed within the range of 500−4000 cm^{-1}, with a resolution of 4 cm^{-1}.

Effects of Synthesized MnO_2NPs in Broiler Chicks. *Experimental Design, Birds, Management, and Diets.* The current study was carried out at the Animal and Poultry Production Farm, Faculty of Agriculture at Mina University, El-Minya, Egypt. A total of 150 Arbor Acres broiler chicks 1 day old, with an average beginning weight of 44.40 ± 1.15 g, were housed individually in pens on a floor litter system until they reached 35 days of age. The study employed a design comprising five experimental groups, each consisting of 30 birds. There were three repetitions for each group. The treatment groups were provided with the following dietary regimens: The first group was provided with a basal diet with manganese (Mn) at a level equivalent to 100% of the recommended dosage (60 mg/kg diet), serving as the control group. The second group received the basal diet along with an additional 66 mg of Mn from MnO_2 per kilogram of diet. Similarly, the third group received the basal diet supplemented with 72 mg of Mn from MnO_2 per kilogram of diet. The fourth and fifth groups were administered the basal diet along with 66 or 72 mg of Mn from MnO_2 nanoparticles per kilogram of diet, which corresponded to 110% and 120% of the recommended dosage, respectively. The formulation of the basal diet was designed to meet the specified nutritional needs of broiler chicks as outlined by the National Research Council.[10] The light cycle was 23 h per day for the whole duration of the experiment, from day 1 to day 35. Water and feed were available *ad libitum*. Table 1 displays the constituents and the proximate chemical analysis of the experimental diets.

Growth Performance Parameters. The initial body weight (BW) at day one, the final BW at 35 days of age, feed consumption (FC), body weight gain (BWG), and feed conversion ratio (FCR) were recorded weekly from 1 to 35 days of age.

Blood Sampling and Analyses. On day 35, six chicks per treatment were selected, and slaughtered by severing the jugular vein. The blood sample/bird was collected in two separate tubes. The first contained an anticoagulant for whole blood collection to determine packed cell volume (PCV), hemoglobin (Hb), red blood cells (RBCs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Additionally, the total and differential counts of white blood cells were manually determined. The second tube without anticoagulant was centrifuged at 3,000 rpm for 10 min for serum separation by utilizing sterile tubes and stored at a temperature of −80 °C until the biochemical determinations were conducted.

Analysis of Biochemical Properties. The serum biochemical components were analyzed using colorimetric techniques following the instructions provided by the manufacturer (Biodiagnostic company, Dokki, Giza, Egypt).

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Table 1. Ingredients and Proximate Chemical Analyses of the Experimental Diets

ingredient (g/kg)	starter (1−21 d)	finisher (22−35 d)
yellow corn	552.3	543.0
soybean meal, 44% crude protein	328.2	366.10
corn gluten meal	50.00	0
sunflower oil	30.50	60.00
dicalcium phosphate	16.50	10.50
sodium chloride	2.50	2.50
limestone	11.50	12.00
DL-methionine	1.50	1.400
lysine	2.50	2.00
cystine	2.00	0
vitamin−mineral premix[a]	2.50	2.5
analytical composition		
crude protein	22.98	20.96
metabolizable energy (kcal/kg)	3050	3172.65
crude fiber	3.78	3.76
calcium	0.91	0.75
available phosphorus	0.45	0.38
methionine + cystine	1.0	0.8
Mn[b]	0.001	0.001

[a]Each kg of minerals and vitamins mixture contains pantothenic acid − 4.00 mg; nicotinic acid − 8.00 mg; folic acid − 400 mg; biotin − 20 mg; chorine − 200 mg; copper − 4.00 mg; iodine − 4.00 mg; iron − 120.00 mg; manganese − 22.000 mg; zinc − 22.000 mg and selenium − 40 mg; vitamin D3 of 4800.000 IU; vitamin E acetate − 4.000 mg; vitamin K3 − 800 mg; vitamin B1 − 40 mg; vitamin B2 − 1600 mg; vitamin B6 − 600 mg; vitamin B12 − 4.00 mg. [b]To adjust the Mn contents in the diets, extra Mn from MnO_2 was added to the diets after Mn calculation.

Collection of Liver Samples. At the age of 35 days, a group of 30 bird subjects participating in the experiment were sacrificed, 6 birds assigned to each treatment, 2 birds each replicate, utilizing jugular vein bleeding following a 12 h fasting period. Liver samples were collected and rinsed twice with PBS ice-cold to avoid contamination in blood. The samples were then dried with filter paper and stored at −80 °C for analysis of SOD, GSH, GSH-px, and GST in liver tissues.

Prediction of Target Proteins. *Molecular Docking of MnO_2NPs.* PDB was utilized to retrieve the targeted proteins GST (PDB ID: 1VF1), GHS (PDB ID: 2HGS), and SOD (PDB ID: 2JLP). The molecular docking investigations were conducted utilizing the AutoDock and AutoDock Vina software tools.[28] To conduct targeting docking investigations, the receptor molecule was appropriately prepared through the addition of polar hydrogen atoms. Using UCSF chimera, the protein structures were refined by removing extra chains and attaching ligands. A total of 1000 steepest descent step runs were conducted for both the selected proteins to run the molecular docking experiment.[29] The Castp 3.0 server was used for identifying the active residues.[30,31] Molecular docking was performed through the PyRx server. On active residues, the grid size was employed along the *x*-, *y*-, and *z*-axes for molecular docking investigations, respectively. The 2D and 3D results were visualized through UCSF Chimera and Discovery Studio.

ADMET Analysis. For notable toxicological and pharmacological characteristics prediction, AdmetSAR and Swiss ADME online servers were used to compute the Adsorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) features for the MnO_2 compound. The SwissADME server was used for calculating physiochemical properties such as Lipinski

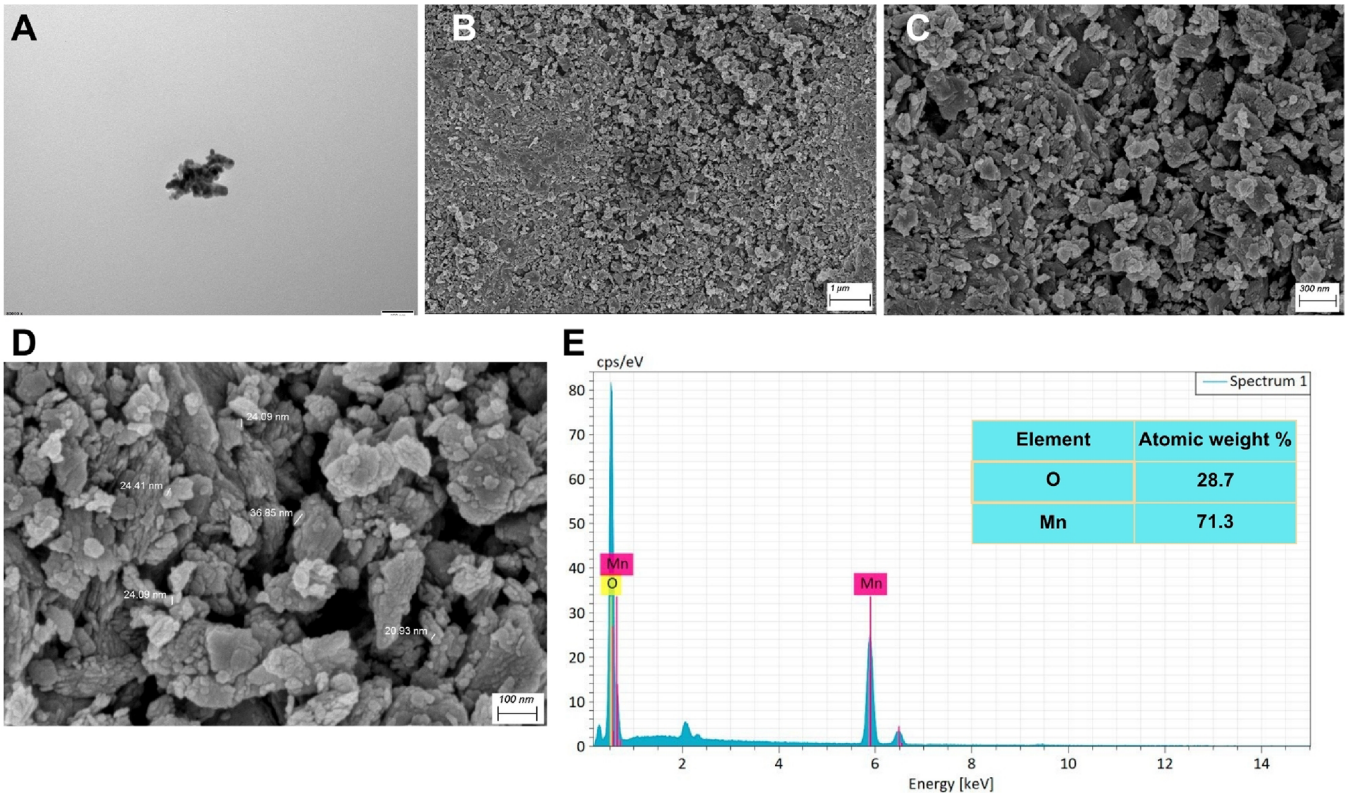

Figure 2. TEM (A), SEM (B, C, D) images, and EDS (E) of MnO_2 nanoparticles.

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properties including molecular weight, number of heavy atoms, number of hydrogen bond donors and acceptor, etc. The AdmetSAR server was used for calculating blood/brain barrier permeability, $Caco^{-2}$ cell permeability, acute oral toxicity, human intestine absorption, and carcinogenicity.[32]

Statistical Analysis. The study investigated the impact of different amounts of manganese (66 and 72 mg/kg diet) and its forms (MnO_2 or MnO_2NPs) as well as the interaction between these factors (levels × forms), using a two-way analysis of variance (ANOVA). The significance of changes in means across groups was determined using Duncan's multiple range test, implemented through SAS 9.4 software (SAS, 2013).

RESULTS

Biosynthesis of MnO_2NPs. The determination and quantification of MnO_2 nanoparticles (NPs) were performed utilizing the intensity of the UV−vis absorption peak. The strength of the absorption peak for the synthesized material is depicted in Figure 1. A wide absorption peak was seen at a wavelength of 350 nm. The emergence of the peak of absorption at a wavelength of 340 nm signifies the existence of MnO_2 nanoparticles. Our findings indicate that the band gap energy of the sample is 1.9 eV.

SEM and TEM are used to analyze the surface features' morphology. Figure 2A,B,C,E shows SEM and TEM images of MnO_2 nanoparticles from microscopic examinations. This suggests that the spherical-like structures of nanomaterials were produced. The coprecipitation method allows for the exact assessment of the relative atomic concentration of various outer surface layer elements synthesized nanomaterials with chemical compositions characterized by EDS analysis. This demonstrates that oxygen and manganese are present and have weight, as determined by SEM. Manganese and oxygen are the chemical composition peak of this material's characteristics. As can be seen in Figure 2D, samples of MnO_2 nanoparticles were indicated by the EDS of spectrum feature having atomic weight ratios of 71.3 and 28.7 for manganese to oxygen, respectively.

Fourier transform infrared spectroscopy (FTIR) was utilized to conduct a more detailed analysis of the MnO_2 nanoparticles. The absorption of Mn−O at 526 cm^{-1} is clear in the spectrum. The spectral bands detected at a wavenumber of 3432 cm^{-1} can be attributed to the stretching vibrations of primary and secondary amines, the stretching of hydroxyl groups in alcohols, and the stretching of carbon−hydrogen bonds in alkanes. The observed peaks at 1637 and 1400 cm^{-1} can be attributed to the amide (I−II) areas, respectively, which are distinctive features associated with proteins and enzymes (Figure 3).

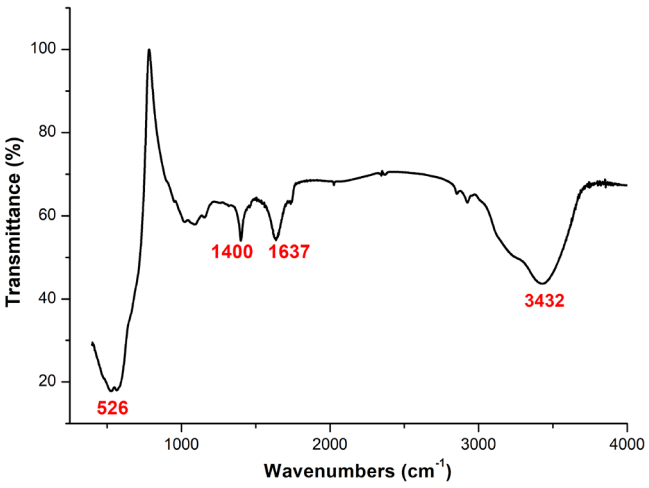

Figure 3. FTIR of MnO_2 nanoparticles.

Effects of Synthesized MnO_2NPs in Broiler chicks. *Effect of Manganese Form and Level.* The impact of different forms of manganese on the productive performance of broiler chicks is illustrated in Table 2. Interestingly, both bulk and nanomanganese exhibited the highest values in terms of body weight, body weight gain, and the most efficient feed conversion ratio when compared to the control group. Furthermore, when we consider the interaction between form and level of manganese, the group that received the highest level of MnO_2NPs demonstrated some remarkable results. This group showed a significantly heavier final body weight (p = 0.001), greater body weight gain (p = 0.001), and a better (lowest) feed conversion ratio (p = 0.03) compared to the other groups. Whenever, when comparing feed intake values, there were no significant differences observed among all groups at either day 21 (p = 0.09) or day 35 (p = 0.78) of the experiment. This result suggests that the form and level of manganese did not have a notable influence on the chicks' feed intake. These findings provide valuable insights into the effects of different manganese forms on broiler chicks' productive performance.

It appears that both bulk and nanomanganese can positively impact body weight, body weight gain, and feed conversion ratio. Additionally, the study highlights the potential advantage of using MnO_2NPs at higher levels (72 mg/kg diet) in achieving even better results. Table 2 shows the impact of varying levels of manganese on the productive performance of broiler chicks. It is interesting to note that both levels of manganese resulted in the highest values for body weight (p = 0.02), body weight gain (p = 0.01), feed consumption (p = 0.05), and feed conversion ratio (p = 0.01) when compared to the control group. The group that received the highest level of MnO_2NPs showed the most remarkable results in terms of final body weight (p = 0.001), body weight gain (p = 0.001), and the lowest feed conversion ratio (p = 0.03) compared to the other groups. However, there were no significant differences in feed intake values observed among all groups at either day 21 (p = 0.09) or day 35 (p = 0.78) of the experiment. These findings suggest that both levels of nanomanganese can have a positive impact on broiler chicks' productive performance. Moreover, the study highlights the potential advantage of using higher levels of MnO_2NPs (72 mg/kg diet) to achieve even better results. Overall, these results provide valuable insights into the effects of different manganese levels on broiler chicks' productive performance.

The physiological characteristics of blood (Table 3), such as red blood cell count (RBC's), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), demonstrate that the consumption of manganese (Mn) in either bulk or nano form has a notable impact on the physical attributes of blood in broiler chicks, except for Hb concentrations, which remained unaffected by the addition of Mn in any form. Interestingly, the results reveal that the broiler chicks receiving the highest level of Mn in bulk form exhibited the highest values of RBC count, PCV, and MCV. Following this group, the control group

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Table 2. Effects of Green-Synthesized MnO_2NPs on Productive Performance of Broiler Chickens (n = 30)[C]

	body weight (g/b/day)		body weight gain (g/b/p/day)		feed intake (g/b/p/day)		feed conversion (g F /g BWG/p/day)	
parameter classification	initial	Day 35	d0-d21	d0-d35	d0-d21	d0-d35	d0-d21	d0-d35
(A) effect of Mn Form								
control	45.15	1949[b]	763[b]	1904[b]	1098	3289	1.44	1.73[a]
MnO_2	44.38	2130[a]	835[a]	2086[a]	1150	3356	1.38	1.61[b]
MnO_2NPs	44.04	2184[a]	838[a]	2140[a]	1192	3339	1.42	1.56[c]
SEM	0.59	35	26	35	26	80	0.03	0.02
significance	0.58	0.01	0.03	0.05	0.06	0.84	0.65	0.01
(B) effect of concentration of Mn								
control	45.15	1949[b]	763	1904[c]	1098[b]	3289	1.44	1.73[a]
66[A] mg/kg diet	44.88	2112[a]	852	2067[b]	1183[a]	3325	1.39	1.61[b]
72[B] mg/kg diet	43.55	2202[a]	821	2159[a]	1159[a]	3370	1.41	1.56[c]
SEM	0.59	35.28	26	34	26	80	0.03	0.02
significance	0.06	0.02	0.28	0.01	0.05	0.58	0.92	0.01
(A×B) effect of interaction between Mn form and concentrations								
control	45.15	1949 [c]	763	1904[d]	1098	3289	1.44	1.73[a]
MnO_2 (66 mg/kg diet)	44.71	2107 [b]	838	2062[c]	1153	3294	1.37	1.60[c]
MnO_2 (72 mg/kg diet)	44.06	2153 [b]	832	2109[b]	1147	3417	1.38	1.62[c]
MnO_2NPs (66 mg/kg diet)	45.05	2118 [b]	866	2072[c]	1213	3356	1.40	1.62[c]
MnO_2NPs (72 mg/kg diet)	43.03	2251[a]	809	2208[a]	1171	3323	1.39	1.66[b]
SEM	0.59	35.28	26	35	26	80	0.03	0.02
significance	0.15	0.001	0.18	0.001	0.09	0.78	0.74	0.003

[A]= 10% of Mn above NRC recommendation of Mn in broiler's diet. [B]= 20% of Mn above NRC recommendation of Mn in broiler's diet. [C]Means (a−c) with different letters in the same column are significantly different at (P < 0.05). SEM, standard error of the mean.

Table 3. Effects of Green-Synthesized MnO_2NPs on Broiler Chickens' Blood Physical Characteristics[C]

parameter classification	RBC's $n \times 10^6$/mL	hemoglobin (%)	peaked cell volume (%)	MCV × 10^{-5} (fl)	MCH × 10^{-5} (pg)	MCHC (g/dl)
(A) effect of Mn form						
control	6.89[a]	12.53	36.09[a]	52.37[b]	18.18[b]	34.71[b]
MnO_2	7.28[a]	13.38	39.06[a]	53.58[a]	18.34[b]	34.24[b]
MnO_2NPs	6.08[b]	12.65	31.31[b]	51.46[b]	20.87[a]	40.55[a]
SEM	0.24	0.43	1.28	0.36	0.21	0.43
significance	0.0006	0.12	0.0001	0.0002	0.0001	0.0001
(B) effect of concentration of Mn						
control	6.89	12.53	36.09	52.37	18.18[c]	34.71[c]
66[A] mg/kg diet	6.64	12.70	34.81	52.36	19.13[b]	36.57[b]
72[B] mg/kg diet	6.72	13.33	35.56	52.68	20.08[a]	38.22[a]
SEM	0.24	0.43	1.28	0.36	0.21	0.43
significance	0.76	0.17	0.57	0.38	0.001	0.003
(A×B) effect of interaction between Mn form and concentrations						
control	6.89[b]	12.53[b]	36.09[b]	52.37[bc]	18.18[c]	34.71[c]
MnO_2 (66 mg/kg diet)	6.81[b]	12.31[b]	29.21[c]	51.30[c]	18.17[c]	34.22[c]
MnO_2 (72 mg/kg diet)	7.75[a]	12.40[b]	36.21[b]	53.10[ab]	18.52[c]	34.26[c]
MnO_2NPs (66 mg/kg diet)	6.47[b]	12.99[ab]	33.41[b]	51.61[c]	20.09[b]	38.92[b]
MnO_2NPs (72 mg/kg diet)	5.69[c]	14.36[a]	41.91[a]	54.06[a]	21.65[a]	42.19[a]
SEM	0.24	0.43	1.28	0.36	0.21	0.43
significance	0.002	0.04	0.0006	0.0001	0.0001	0.0001

[A]= 10% of Mn above NRC recommendation of Mn in broiler's diet. [B]= 20% of Mn above NRC recommendation of Mn in broiler's diet. [C]Means (a−c) with different letters in the same column are significantly different at (P < 0.05). SEM, standard error of the mean.

displayed comparatively lower values, while the lowest values were observed in the group supplemented with MnO_2 and the group receiving low levels of MnO_2 nanoparticles (MnO_2NPs). Conversely, the birds that received the highest levels of nano Mn displayed the lowest values of RBC count, PCV, and MCV when compared to the other groups. Conversely, when examining the influence on MCH and MCHC levels, it was observed that the addition of the highest level of MnO_2 nanoparticles resulted in increased values of MCH and MCHC. This increase was evident regardless of the form of Mn utilized or the interaction between Mn form and concentration, ultimately yielding higher MCH and MCHC values in comparison to the other experimental groups.

The results demonstrated that the consumption of Mn in either low or high levels had a significant effect on the physical attributes of blood in broiler chicks, except for Hb concentrations, which remained unaffected by the addition of Mn at any level. Interestingly, it was observed that the broiler

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Table 4. Effects of Green-Synthesized MnO_2NPs on Total and Differential Counts of White Blood Cells of Broiler Chickens[C]

parameter classification	WBC's $n \times 10^3$/mL	lymphocyte %	monocyte %	heterophils %	H/L ratio
(A) effect of Mn form					
control	15.20	50.71[b]	6.49[b]	40.79	0.80
MnO_2	15.05	51.71[a]	6.71[b]	39.57	0.76
MnO_2NPs	14.91	50.24[b]	7.43[a]	40.31	0.82
SEM	0.07	0.33	0.24	0.47	0.01
significance	0.12	0.001	0.01	0.15	0.06
(B) effect of concentration of Mn					
control	15.20	50.71	6.49	40.79	0.80
66[A] mg/kg diet	14.98	51.18	7.05	39.76	0.77
72[B] mg/kg diet	14.98	50.77	7.10	40.12	0.79
SEM	0.07	0.33	0.24	0.47	0.01
significance	0.95	0.24	0.84	0.46	0.37
(A×B) effect of interaction between Mn form and concentrations					
control	15.20	50.71[bc]	6.49	40.79	0.80
MnO_2 (66 mg/kg diet)	15.00	52.05[a]	6.71	39.24	0.75
MnO_2 (72 mg/kg diet)	15.10	51.38[ab]	6.71	39.91	0.77
MnO_2NPs (66 mg/kg diet)	14.96	50.32[bc]	7.39	40.28	0.80
MnO_2NPs (72 mg/kg diet)	14.87	50.16[c]	7.48	40.34	0.82
SEM	0.07	0.33	0.24	0.47	0.01
significance	0.10	0.01	0.06	0.27	0.12

[A]= 10% of Mn above NRC recommendation of Mn in broiler's diet. [B]= 20% of Mn above NRC recommendation of Mn in broiler's diet. [C]Means (a−c) with different letters in the same column are significantly different at ($P < 0.05$). SEM, standard error of the mean.

Table 5. Effects of Green-Synthesized MnO_2NPs on Broiler Chickens' Serum Oxidative Stress Biomarkers in Serum and Liver Tissues[C]

	serum				liver tissues		
parameter classification	GST (IU/L)	SOD (IU/L)	GSH (IU/L)	GSH-Px (IU/L)	SOD (μmol/g protein)	GSH (μmol/g protein)	GSH-Px (μmol/g protein)
(A) effect of Mn form							
control	254.16	20.02[b]	4.16[a]	12.96[b]	502.10[b]	919.10	11.42[b]
MnO_2	258.00	34.91[a]	5.12[a]	17.06[a]	518.82[ab]	944.9	18.16[ab]
MnO_2NPs	272.06	37.43[a]	5.23[b]	17.10[b]	535.58[a]	895.0	20.45[a]
SEM	33.04	4.31	0.21	0.65	13.11	41.46	3.23
significance	0.67	0.05	0.001	0.0001	0.05	0.84	0.04
(B) effect of concentration of Mn							
control	254.16	20.02[b]	4.16	12.96[c]	502.10[b]	919.10	11.42[b]
66[A] mg/kg diet	260.81	32.47[a]	4.56	15.28[b]	517.30[ab]	915.41	18.79[ab]
72[B] mg/kg diet	269.25	39.87[a]	4.72	17.74[a]	531.10[a]	924.50	19.82[a]
SEM	33.04	4.31	0.21	0.65	13.11	41.46	3.23
significance	0.80	0.05	0.47	0.02	0.05	0. 84	0.04
(A×B) effect of interaction between Mn form and concentrations							
control	254.16	20.02[b]	5.23[a]	12.10[c]	502.10c	919.10[a]	11.42[c]
MnO_2 (66 mg/kg diet)	246.05	38.95[a]	4.97[a]	16.09[ab]	499.17[b]	960.32[b]	15.78[b]
MnO_2 (72 mg/kg diet)	269.95	30.87[ab]	5.28[a]	18.03[a]	500.47[b]	929.53[b]	19.10[ab]
MnO_2NPs (66 mg/kg diet)	275.57	40.79[a]	4.16[b]	14.47[b]	527.42[a]	870.53[b]	20.54[a]
MnO_2NPs (72 mg/kg diet)	268.54	34.07[ab]	4.18[b]	17.45[a]	538.47[a]	919.50[b]	21.79[a]
SEM	33.04	4.31	0.21	0.65	13.11	41.46	3.23
significance	0.96	0.04	0.006	0.003	0.04	0.005	0.02

[A]= 10% of Mn above NRC recommendation of Mn in broiler's diet. [B]= 20% of Mn above NRC recommendation of Mn in broiler's diet. [C]Means (a−c) with different letters in the same column are significantly different at ($P < 0.05$). SEM, standard error of the mean.

chicks that were administered the highest level of Mn in bulk form exhibited the highest values of RBC count, PCV, and MCV. The control group displayed relatively lower values, while the lowest values were observed in the group supplemented with MnO_2 and the group receiving low levels of MnO_2 nanoparticles (MnO_2NPs). Conversely, the birds that received the highest levels of nano Mn displayed the lowest values of RBC count, PCV, and MCV compared to the other groups. On the other hand, the study also found that the addition of the highest level of MnO_2 nanoparticles led to increased values of hemoglobin, PCV, MCV, MCH, and MCHC and decreased RBC counts compared with other groups. These findings reflect the distinct impact of Mn supplementation, both in bulk and nano form, on the physical

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blood characteristics of broiler chicks. However, the total and differential counts of white blood cells in broiler chickens (Table 4) showed no significant effects when fed either bulk or Nano forms of Mn, except for an increase in the percentage of lymphocytes and monocytes. Specifically, the highest percentage of lymphocytes was observed in broiler chicks fed bulk MnO_2 compared to other groups. Additionally, the monocyte percentage increased when fed MnO_2 in its Nano form. When considering the interaction between the form and levels of MnO_2, there were no remarkable differences among all groups in terms of white blood cell total and differential counts, except for lymphocytes, which showed a significant increase when broiler chicks were fed the lowest level of MnO_2. Interestingly, MnO_2NPs did not affect lymphocyte percentage compared to the control groups and the highest level of bulk MnO_2.

The study also revealed that the total and differential counts of white blood cells in broiler chickens remained unaffected when fed either bulk or nano forms of Mn (Table 4), except for a rise in the percentage of lymphocytes and monocytes. Notably, broiler chicks fed bulk MnO_2 had the highest percentage of lymphocytes. Similarly, the monocyte percentage increased when fed MnO_2 in its nano form. When examining the interaction between the form and levels of MnO_2, there were no significant differences among all groups concerning white blood cell total and differential counts, except for lymphocytes, which had a significant increase when broiler chicks were fed the lowest level of MnO_2. Interestingly, MnO_2NPs did not affect lymphocyte percentage compared to the control groups and the highest level of bulk MnO_2.

Findings from Table 5 offered intriguing insights into the impact of different forms of Mn supplementation on serum and liver GST, SOD, GSH, and GSH-px activities. It was observed that the consummations of MnO_2NPs, whether in high or low levels, led to an elevation in the activities of SOD, GSH, and GSH-px when compared to its bulk form or control groups. Also, the was a nonsignificant impact on GST activities.

Table 5 provides intriguing insights into the impact of different levels of Mn supplementation on serum and liver GST, SOD, GSH, and GSH-px activities. The consumption of MnO_2NPs, whether in high or low levels, led to an elevation in the activities of SOD, GSH, and GSH-px compared to its low or high level or control groups. Surprisingly, the group consuming the lowest level of nano Mn also demonstrated elevated levels of these. Furthermore, when studying the interaction between Mn form and levels, the group of birds consuming the highest level of nano Mn exhibited the highest activities of SOD, GSH, and GSH-px.

This was followed by the group consuming the lowest level of nano Mn, surpassing the levels observed in the other groups. These results shed light on the intricate relationship between Mn supplementation and oxidative stress biomarkers, highlighting the potential benefits of utilizing nano Mn in broiler diets.

Protein Docking. The targeted proteins GHS (PDB ID: 2HGS) and SOD (PDB ID: 2JLP) were separately docked against MnO_2 (Figure 4). The interaction exhibiting the highest interaction energy score between the ligand and protein was considered the most favorable interaction. Through an analysis of docking binding affinities, it is reported that GST showed −2.7, GHS −3.5 kcal/mol, and SOD −2.3 kcal/mol, respectively (Figures 4, 5, and 6). The capability of GHS was more with high binding affinity compared to SOD.

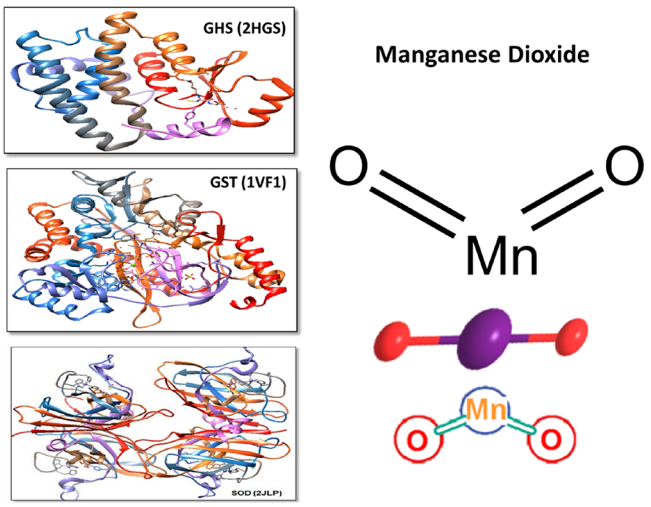

Figure 4. Targeted proteins and ligands are shown above. The comprehensive three-dimensional (3D) structure of both proteins is labeled to provide detailed information appropriately. The ligand two-dimensional (2D) and three-dimensional (3D) structures are also shown separately.

The observed differences indicate that MnO_2 has effective energy scores for both proteins. These results allow us to examine and interpret the protein's behavior on binding with MnO_2, thereby fostering a more profound comprehension of its functionality and possible associations with the molecule. The 2D/3D visualization of protein and ligand along with binding pockets is shown (Figures 4−7).

MnO_2NPs Exhibit Promising AMDET Results. The solubility of the MnO_2NPs molecule was observed, and it successfully complied with the standard Lipinski properties as indicated in Table 6. These results are completely supported by the calculation of several ADMET parameters. The wet lab results were further supported by dry lab work. The in -silico results MnO_2 has been proven to be a promising, safe, and effective feed additive for broiler chicks.

DISCUSSION

Biosynthesis of MnO_2NPs. The present study demonstrates the successful synthesis of MnO_2 nanoparticles through the combination of MnO_2 and green tomato extract. This achievement serves as a fundamental step toward future investigations concerning the physical and biochemical characterization of these nanoparticles. Additionally, the confirmation of the green synthesis of MnO_2 nanoparticles has been achieved through the identification of the unique absorption peak observed in the UV−vis spectra. Consistent with the findings of the work conducted by Mounika et al.,[33] it was seen that MnO_2 nanoparticles exhibit a distinct peak in the UV−vis spectrum at a wavelength of 365 nm. The band gap of MnO_2 nanoparticles varies depending on the specific conditions. In this case, the band gap was found to be 1.9 eV. Furthermore, we have conducted a comprehensive literature review to compare our results with previously reported values. The band gap energy reported in the literature ranges from typically reported to be in the range of approximately 1.8 to 2.7 eV., in similar materials or systems.[34−36] Our determined value falls within this range, indicating consistency with previous studies. However, in the case of $MnFe_2O_4$ nanoparticles, the band gap energy was

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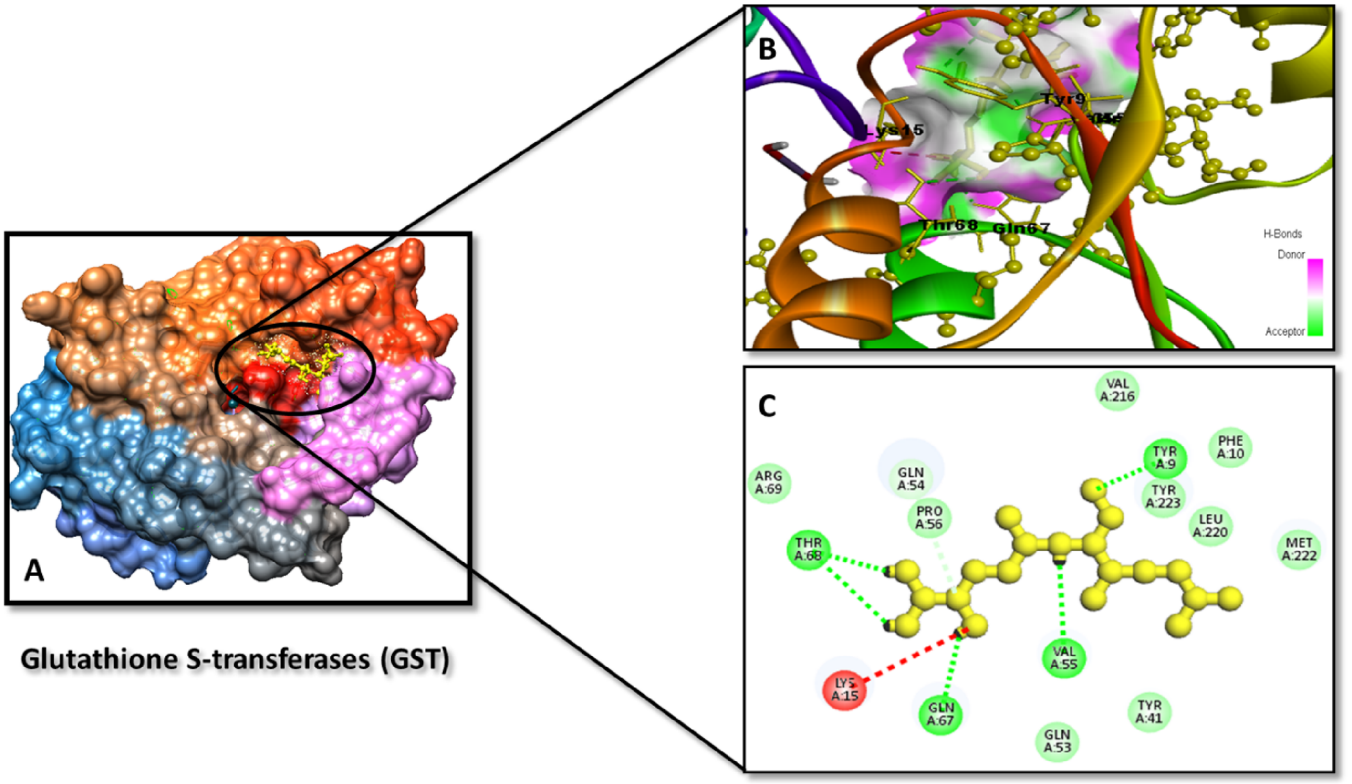

Figure 5. 3D visualization of GST − MnO_2 docking results. (A) UCSF chimera full complex visualization. (B) Binding pockets and ligand attraction and (C) indication of active binding residues includes TYR 9, PHE 10, ARG 13, GLY 14, LYS 15, GLU 17, SER 18, TYR 41, GLN 54, VAL 55, PRO 56, THR 68, ARG 69, LEU 72, ASP 100, MET 103, GLY 104, LEU 106, LEU107, PHE 109, PRO 110, PHE 111, GLU 162, MET 166, LYS 170, ILE 207, SER 208, TYR 212, VAL 216, LEU 220.

calculated using a modified Heisenberg model and was found to either decrease or increase depending on the specific ion doping.[37] This observation was made based on a comprehensive investigation of their morphology, structure, size, and properties, which were assessed by the integrated utilization of scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), and Fourier-transform infrared spectroscopy (FTIR).

Effects of Green Synthesized MnO_2NPs of Broiler chicks. Our results suggest that both bulk and nanomanganese can have a positive impact on the weight of the body, feed conversion, and body weight gain ratio. Additionally, the study highlights the potential advantage of using MnO_2NPs at higher levels (72 mg/kg diet) in achieving even better results. Our study is consistent with other research on the benefits of manganese in animal nutrition. Manganese is an important mineral that plays an essential role in many biological processes, including bone development[38,39] and immune function.[40] Several studies highlight that manganese dietary supplementation can enhance the growth rate, and feed efficiency in poultry.[2,40] Al-Qubaisi et al.[41] found that 100 mg/kg manganese dietary supplementation significantly improved weight gain of body and conversion of feed ratio for broiler chicks. Despite several studies on the Mn supplementation effects, there appears to be a prevailing agreement among academics that there is a lack of observable enhancement in production outcomes, irrespective of the mode of delivery or quantity. For instance, Collins and Moran[42] found no effect on body weight, feed intake, and broiler chickens FCR when Mn was administered at a rate of 180 mg/kg of feed. The findings of previous studies conducted by researchers[43−46] yielded comparable outcomes, indicating that there was no observed impact on growth performance irrespective of the quantity and origin of manganese. Even studies involving various forms of Mn, such as nanoparticles, by Lofti et al.[47] and studies using various combinations of Mn with Zn, by Sunder et al.[48] failed to find any effect on production parameters. However, one study on Japanese quails found that Mn supplementation at 60 mg/kg affected the final body weight and FCR, but not the feed intake.[49] It is worth noting that exceptionally high doses of Mn, such as 1500[44] and 1600 mg/kg and more,[45] significantly reduced BW. Conductorly, several studies have investigated the effects of Mn supplementation on broiler chicken's growth performance using various Mn sources and levels. Despite these variations, the results have consistently shown that there were no significant differences in body weight, feed efficiency, or mortality among the treatments.[43−45,50] This suggests that the amount of Mn in the control diet was sufficient for optimal performance of the birds. Additionally, Jankowski et al.[50] found that reducing the contents of Cu, Mn, and Zn addition in the feed of slaughtered turkeys did not harm their growth performance. However, the optimal level of manganese supplementation can vary depending on the form and source of the mineral, as well as the animal species and age.[50] Excessive manganese supplementation can also lead to toxicity and adverse effects on animal health, such as impaired growth and skeletal abnormalities.

Manganese (Mn) plays several important roles in broiler health and development, including its impact on blood parameters. Manganese is involved in the production of red blood cells and hemoglobin.[51] Based on the provided results, it seems that the consumption of manganese (Mn) has a

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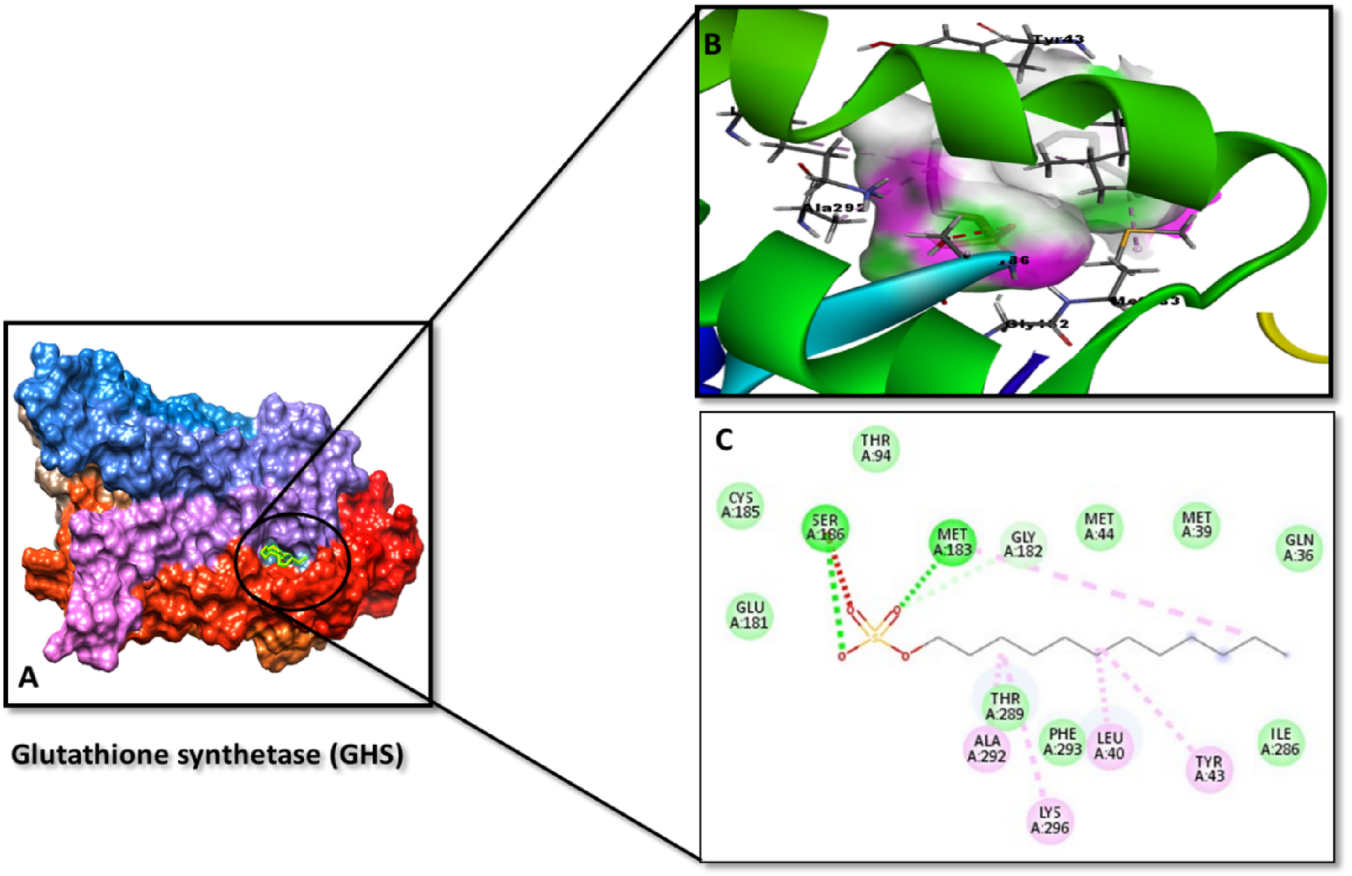

Figure 6. 3D visualization of GSH − MnO_2 docking results. (A) UCSF chimera full complex visualization. (B) Binding pockets and ligand attraction and (C) indication of active binding residues including PHE 9, THR 100, LEU 103, ARG 125, MET 129, ILE 143, GLU 144, ILE 145, ASN 146, THR 147, ILE 148, SER 149, SER 151, PHE 152, GLU 214, ASN 216, GLN 220, ARG 267, TYR 270, ARG 273, GLN 300, GLY 303, THR 304, LYS 305, VAL 362, LYS 364, GLN 366, ARG 367.

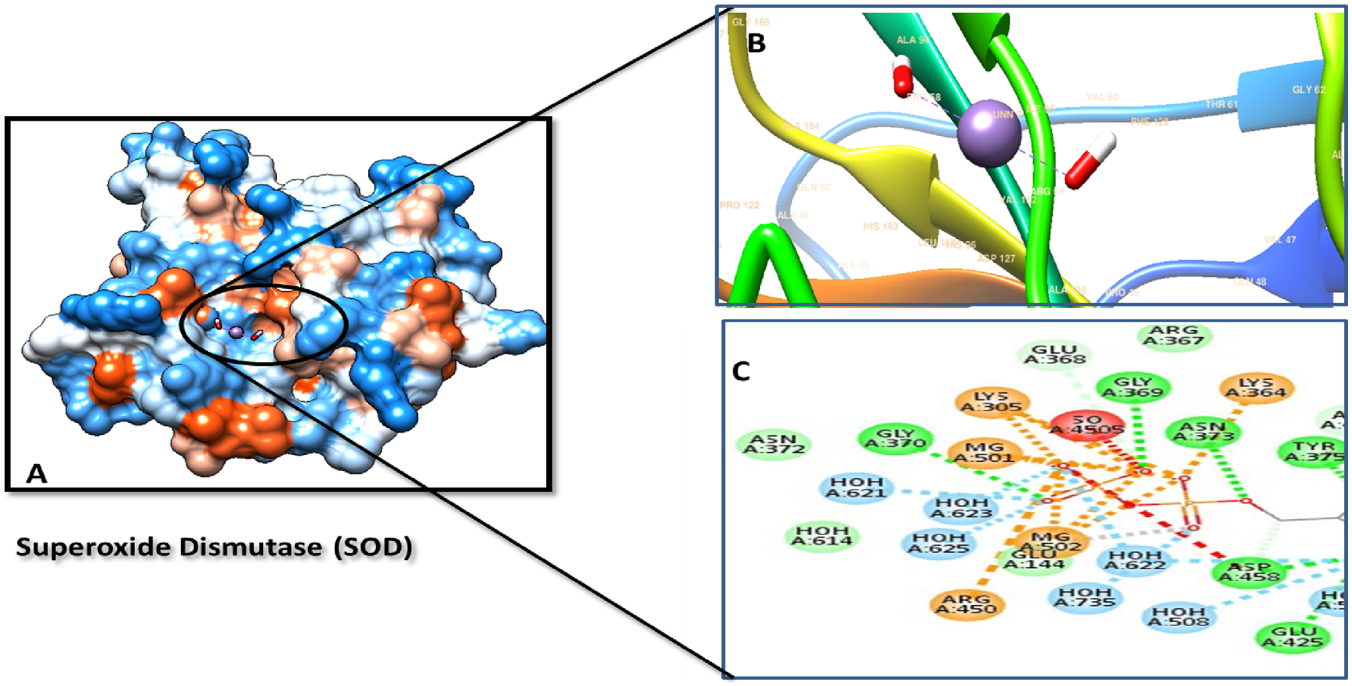

Figure 7. 3D visualization of SOD − MnO_2 docking results. (A) UCSF chimera full complex visualization. (B) Binding pockets and ligand attraction and (C) indication of active binding residues include SER 92, ASN 130, PHE 131, ALA 132, ARG 134, ARG 140, ARG 142, GLU 166, ARG 171.

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Table 6. Numerous ADMET Characteristics of the MnO_2 Molecule and Toxicity Prediction

MnO_2	Lipinski properties	
molecular Weight	86.94	
A log *P*	−0.24	
H-bond acceptor	2	
H-bond donor	0	
rotatable bonds	0	
heavy atoms	3	
TPSA	34.14 Å^2	
ADMET properties	results	probability
blood−brain barrier	BBB+	0.9822
human intestinal absorption	HIA+	0.9700
Caco-2 permeability	Caco2+	0.5575
P-glycoprotein Substrate	Nonsubstrate	0.8987
P-glycoprotein Inhibitor	Noninhibitor	0.9576
mitochondrial toxicity	Mitochondria	0.5500
CYP inhibitory promiscuity	Low CYP Inhibitory Promiscuity	0.9609
acute oral toxicity	II	0.6631
carcinogenicity (three-class)	Nonrequired	0.5346
rat acute toxicity (LD50, mol/kg)	2.4357	

significant effect on the physical attributes of blood in broiler chicks, except for hemoglobin (Hb) concentrations. The study found that the group administered the highest level of Mn in bulk form had the highest values of Packed Cell Volume (PCV), red blood cell (RBC) count, and mean corpuscular volume (MCV), while the birds that received the highest levels of Nano Mn displayed the lowest values of RBC count, compared to the other groups. Additionally, the addition of the highest level of MnO_2 nanoparticles led to increased values of mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), regardless of the form of Mn utilized or the interaction between Mn form and concentration. One of the key benefits of manganese supplementation in broiler diets is its ability to enhance hemoglobin levels.[51] This ultimately yielded higher MCH and MCHC values in comparison to the other experimental groups. These findings highlight the significant impact of Mn supplementation, in both bulk and nano form, on the physical blood characteristics of broiler chicks. The results emphasize the importance of understanding the potential effects of manganese supplementation on broiler blood parameters. Further research is necessary to delve into the underlying mechanisms behind these observed changes and their implications for the overall health and well-being of broiler chicks. The obtained data underscores the importance of understanding the potential effects of manganese supplementation on blood parameters in avian species. Further research is warranted to explore the underlying mechanisms behind these observed changes and their implications for broiler chicks' overall health and well-being.

Manganese is known to play a role in immune function. It contributes to the production and activation of immune cells, such as lymphocytes and macrophages, The immune system of broilers is crucial for disease resistance and overall performance. Manganese has been shown to play a pivotal role in modulating immune responses, particularly with broiler blood parameters. Studies have revealed that Mn supplementation positively influences white blood cell counts, lymphocyte proliferation, and antibody production, thereby enhancing the immune system's ability to combat pathogens.[52,53] Furthermore, Mn has been found to promote the synthesis of immunoglobulin, which contributes to the humoral immune response.[54]

The recent research on the effects of manganese on broiler blood parameters highlights its significant benefits. Manganese supplementation has been found to enhance hemoglobin levels,[52,53] and boost immune function.[54] These findings underscore the importance of incorporating adequate manganese levels in broiler diets to optimize blood health and overall performance. Further studies are warranted to explore the optimal dosage and duration of manganese supplementation in broiler production systems.

Manganese is a microelement that is involved in several cellular metabolic activities. It is crucial for immunological defenses, bone formation, blood coagulation, nervous system function, control of blood sugar levels, and defense against reactive oxygen species (ROS).[55−57] Numerous biochemical processes involving enzymes, such as glutamine synthetase, alkaline phosphatase, pyruvate carboxylase, glycosyltransferase, and particularly mitochondrial manganese superoxide dismutase (Mn-SOD), involve manganese. Manganese is a crucial element in many biochemical reactions, playing a vital role in both metalloenzymes and enzyme activators. It serves as an Mn-SOD enzymatic cofactor, which helps cell protection against free oxygen radical damage. Mn-SOD catalyzes the one-electron reduction of peroxide anion to hydrogen peroxide, which decreases the risk of oxidative stress on cells. Research has shown that a decrease in the amount of manganese present in the cell may lead to a reduction in SOD activity, which can affect the performance of other antioxidant enzymes such as GPx and CAT in the catalytic triad. Our study revealed that the addition of MnO_2NPs led to enhance the activities of SOD, GSH, and GSH-px, these results agreed with the results of the study conducted by Asaikkuttia et al.[57] discovered that there was a positive correlation observed between the Augmentation of manganese oxide nanoparticles dosage in the dietary intake of fish and the subsequent elevation of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutamic oxaloacetate transaminase, and glutamic pyruvate transaminase, inside the cellular composition. Furthermore, studies have shown that during the induction of oxidative stress in cells because of ROS, levels of reduced GSH and MDA increase, as well as an increase in methylated DNA, as reported by Campos et al.[58] The effect of MnO_2NPs on antioxidant status was confirmed with protein docking analysis, which showed that MnO_2NPs have a high ability to band with specific proteins of SOD, GSH, and GST with low toxic effects. In addition, future research is needed to deeply understand the mechanisms and effects of MnO_2NPs on broiler antioxidant status.

CONCLUSIONS

In conclusion, the present study was to evaluate the impacts of nanomanganese on antioxidant status, hematological alteration, and protein docking in broiler chickens. The observed findings suggested that nanomanganese at 66 and 72 mg/kg levels have some positive impacts on the productive, antioxidant status, and hematological alteration of broiler chicks. The addition of 72 mg Mn as MnO_2NPs represented better effects for most studied parameters with non-negative effects on broiler chicks. The mechanism of this effect remains to be further studied.

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Notes

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