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The Effect of Green Synthesized Zinc Oxide Nanoparticles Using *Allium cepa* Extracts on Triton X-100 Induced Hyperlipidemia in Rats

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ABSTRACT

Background: The rise in hyperlipidemia cases is still alarming, worldwide. This study determined the effect of zinc oxide nanoparticles (ZnO NPs) on hyperlipidemia in experimental rats.

Methods: Rats were assigned to six equal groups of five rats. Hyperlipidemia was induced by 7 days intraperitoneal injection of Triton X-100. Group 1 (Sham) received standard diet, Group 2 (Negative control) received 100 mg/kg Triton X-100, Group 3 (Standard) received 100 mg/kg Triton X-100 and 10 mg/kg atorvastatin, Group 4 received 100 mg/kg Triton X-100 and 100 µg/kg ZnO NPs, Group 5 received 100 mg/kg Triton X-100 and 300 µg/kg ZnO NPs and Group 6 received 100 mg/kg Triton X-100 and 500 µg/kg ZnO NPs, orally.

Results: ZnO NPs displayed a light crystalline phase with fine peaks of ZnO wurtzite structure. ZnO showed lower antioxidant activity at concentrations of 60 and 100 µg/mL. A significant decrease in serum total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL) levels and atherogenic index with a significant increase in high density lipoproteins (HDL) level were noticed in groups received ZnO NPs in a dose dependent manner. There was a significant decrease in alanine transaminase (ALT) level, with no significant difference in aspartate transaminase (AST) and alkaline phosphatase (ALP) levels at the highest dose. There was a significant decrease in serum antioxidant enzymes and cardiovascular biomarkers. Histologically, there was a healing in cardiac tissue following administration of ZnO NPs.

Conclusion: ZnO NPs were shown to have antihyperlipidemic and cardiovascular protective effects by regulating lipid metabolism-related biochemical parameters.

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Introduction

The rise in the number of cases of hyperlipidemia is generating an alarming condition, all over the world. Hyperlipidemia-related cardiovascular disease (CVD) continues to be a leading cause of early death and rising health-care costs (1). The World Health Organization (WHO) revealed that CVDs are still globally the leading cause of death. An estimated number of about 17.9 million people died from CVDs in 2019, which represents approximately 32% of all prevalent global deaths, while about 85% of these deaths were due to heart attack and stroke. Low- and middle-income nations have been disproportionally affected, while more than 83% of CVDs deaths took place in low- and middle-income countries and arise virtually and equally in men and women. However, by 2030, it was estimated that nearly 27.3 million people will die from CVDs, primarily from CVDs and these are propelled to continue to remain the single leading cause of death (2).

Hyperlipidemia is characterized by elevated levels of total cholesterol (TC), triglycerides (TG), and low density lipoprotein cholesterol (LDL) in blood and it initiates the hardening of coronary arteries or atherosclerosis which reduces the blood flow and induces oxygen deficiency, and this silently-produced situation eventually leading to heart failure (2, 3). Hyperlipidemia is a major risk factor for CVDs, and statin-based lowering LDL cholesterol indicates a considerable reduction in hyperlipidemic related CVDs events. However, in some cases who have achieved low LDL cholesterol levels continued to remain at high residual CVDs risk, particularly if TG level was elevated. This may result due to other risk factors beyond LDL cholesterol developing in this situation. In the past few decades, age-adjusted LDL cholesterol has decreased significantly following the increased use of statins, and a significant increase in TG levels has become increasingly common throughout the world (4).

Triton X-100 has been used as a surfactant which suppresses the activity of lipase enzymes to prevent the uptake of lipoproteins from the circulation by extra-hepatic tissue resulting into high blood lipid levels. Triton X-100 is used to induce hyperlipidemia in some animals too (5). Nanomedicine has recently appeared as a rapidly expanding field that has shown to be a potent and active therapy for numerous diseases. Nanomedicine is concerned with physiological processes at the nanoscale level, as well as their application in the development of non-invasive technologies such as diagnostic tools, delivery carriers, and other clinically relevant devices. The synthesis and design of nanomaterials are being studied in order to increase their safety and efficiency. During administration, when nanoparticles (NPs) are introduced into the bloodstream, they pass via the reticuloendothelial system and disperse across the extracellular matrix. In addition, NPs move towards the targeted cells in the tissue and enter the cell through endocytosis (6). Inorganic NPs have a lot of potential in medicine, especially in terms of illness detection, diagnosis, and treatment. Gold, iron oxide and silica have been widely investigated for their medicinal applications (6). In this study, Zinc oxide (ZNO) NPs will be used to investigate its anti-hyperlipidemic potential against Triton X-100 induced hyperlipidemia in rats.

The *Allium cepa* (Onion bulb) belongs to the *Liliaceae* family and is one of the most important Nigeria's monocotyledonous and cool-season vegetable crops. Onion has long been utilized as a dietary supplement due to its inexpensive and readily availability. Although the onion bulb is low in calories, it causes an increase in metabolic rate, which in turn causes a boost in appetite (7). For the first time, plants are being used for nanoparticle synthesis; it is a more cost-effective and environmentally beneficial alternative for chemical and physical synthesis. Furthermore, the use of plants for large-scale synthesis may be quickly scaled up without the use of hazardous chemicals or the need for high energy, or temperature (8). Therefore, this study aimed to investigate the effect of green synthesized ZnO NPs using *A. cepa* extracts on triton X-100 induced hyperlipidemia in experimental rats.

Materials and Methods

Fresh onion bulbs were purchased from Jimeta vegetable market. Identification and authentication were done in the herbarium of the Department of Botany, Modibbo Adama University, Yola, Nigeria. To prepare and synthesize ZnO NPs, the onion bulbs were rinsed with sterile distilled water and the outer casing of the bulb was physically shed off, while the fleshy part of the onion was washed again with sterile distilled water. The onion bulb was slashed into smaller pieces and 50 g of onion bulb was also grounded using pestle and mortar with a small volume of distilled water. The extract was filtered by means of muslin cloth and then filtered by Whatmann No.1 filter paper (460×570 mm), which was then used as reducing agent and stabilizer.

Zinc nitrate was used as precursor for the synthesis of ZnO NPs. For ZnO NPs synthesis, the reaction mixture was prepared by mixing of 10 mL of onion bulb extract and 90 mL of 1 mM concentration of zinc nitrate in a 250 mL conical flask. The mixture

was maintained in a darkroom at 37°C for 24 hours. Finally, the reaction mixture was centrifuged at 2,500 rpm for 45 minutes. After centrifugation, the pellet was allowed to air dry which was then used for further studies. The NPs thus procured was purified by repeating the centrifugation at 2,500 rpm within 25°C for another 45 minutes, followed by re-diffusion of the pellet in deionized water to throw away any undesirable organic molecules.

To characterize ZnO NPs by scanning electron microscopy (SEM), the synthesized ZnO NPs were dispersed in water and the resultant suspension was homogenized using ultra sonicator for one to two hours. A drop of the ZnO NPs suspension was placed on a piece of micro glass slide attached to a metal grid coated with carbon film and was dried gradually at room temperature. The sample was then sputter coated with gold and visualized using scanning electron microscope (SEM, JEOL JSM-6480 LV) to assess the particles shape.

To conduct Fourier transform infrared spectroscopy (FTIR), the solution containing ZnO NPs were centrifuged at 2,500 rpm for 45 minutes. The pellets were purified and allowed to be dried at room temperature which was then loaded in to FTIR. The FTIR spectra were recorded at 1 cm^{-1} resolution by FTIR spectrophotometer (FTIR-8400S SHIMADZU) using KBr pellet technique. The frequency range was measured as wave numbers typically over the range 4000-1000 cm^{-1}.

To assess *in vitro* scavenging activity of ZnO NPs against 2,2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO) was used to make different amount of ZnO NPs which was made up to 3 mL with DPPH (0.1 M). The reaction mixture was kept at room temperature in dark conditions for 30 min and the absorbance was measured at 517 nm. Ascorbic acid was used as standard. The % radical scavenging activity was calculated using the formula:

$$\% RSA = \frac{Abs._{control} - Abs._{sample}}{Abs._{contro}} \times 100$$

To undertake animal experiments, thirty healthy male Wistar rats weighing 120±10g were enrolled. The rats were purchased from Veterinary Research Institute, Vom-Jos, Plateau State, Nigeria. These rats were kept to acclimatize for fourteen days in the animal house of the Department of Biochemistry, Modibbo Adama University, Yola, Nigeria. The animals were fed with standard pellet diet (vital feed Jos) and water was administered *ad libitum* for a period of two weeks before experiments. Experimental animals were handled in accordance with the international guiding principles of Biochemical research involving animals (9).

Induction of hyperlipidemia was done by the intraperitoneal (IP) injection of a solution of Triton X-100 (100 mg/kg) dissolved in phosphate-buffered saline (pH: 7.4) in a single dose/day for 7 days. After two week of acclimation, experimental rats were randomly divided in six equal groups of five rats per group. The rats were treated with single dose/day of Triton X-100 and test drugs were grouped as Group I (Sham group) received standard diet, Group II (Hyperlipidemic control group) received Triton X-100 (100 mg/kg/day), Group III (Standard group) were administered with Triton X-100 (100 mg/kg/day)+Atorvastatin (10 mg/kg/day), Group IV (Test group) took Triton X-100 (100 mg/kg/day)+ZnO NPs (100 µg/kg/day), Group V (Test group 2) received Triton X-100 (100 mg/kg/day)+ZnO NPs (300 µg/kg/day), and finally Group VI (Test group 3) was administered with Triton X-100 (100 mg/kg/day)+ZnO NPs (500 µg/kg/day).

The examinations were carried out at the end of the administration period after overnight fasting. The rats were anesthetized with urethane. Blood samples were collected via cardiac puncture into test tubes containing heparin. Serum was obtained after centrifuged at 1000 rpm for 10 minutes using centrifuge machine (Hettich, Germany). Heart tissues were collected from individual groups and fixed in 10% neutral buffered formalin. The tissues were kept and dehydrated with ethanol and then embedded in paraffin which were cut into 5-µm sections. The tissue sections were deparaffinized and stained with Mayer hematoxylin and eosin stains for photomicroscopic capture. Assays were performed in accordance with the manufacturer's instructions.

For biochemical assay regarding lipid profile, serum total cholesterol (TC) was evaluated based on CHOD-PAP enzyme colorimetric; serum triglycerides (TG) were examined based on the GPO/PAP enzyme colorimetric method; serum low density lipoprotein (LDL) was measured based on the PEG/CHOD-PAP colorimetric method; serum high density lipoprotein (HDL) was investigated by direct measurement method; and atherogenic index (AI) was determined according to the method explained by (10).

Regarding liver enzymes, serum aspartate aminotransferase (AST) was determined by the colorimetric method previously explained (11); serum alanine aminotransferase (ALT) was evaluated by the method described before (11); serum alkaline phosphatase (ALP) was measured by the method mentioned before (12). For antioxidant biomarkers, serum superoxide dismutase (SOD) was examined

by colorimetric method; serum catalase (CAT) activity was investigated by colorimetric method; glutathione peroxidase (GPx) activity was evaluated using Ultra-Violet (UV) method.

Regarding cardiac biomarkers, creatine kinase isoenzyme-MB (CK-MB) was quantitatively determined based on the fluorescence immunoassay (FIA) for CK-MB activity in serum by sandwich immunodetection method utilizing ichroma™ test instrument; Cardiac troponin-i (c-TnI) was quantitatively determined based on the FIA for the cT-i activity in serum by sandwich immunodetection method applying ichroma™ test instrument; myoglobin was quantitatively measured based on FIA for the myoglobin activity in serum by sandwich immunodetection method using ichroma™ test instrument.

Statistical analysis was done using the Statistical Package for Social Science (SPSS software Incorporation, version 24.0, Chicago, Illinois, USA). All numerical data were reported as mean±standard error of mean (SEM). Differences between and within group means were analyzed using One-way analysis of variance (ANOVA) followed by Tukey post hoc test. A $p<0.05$ was considered statistically significant.

Results

An immediate color change was observed following the addition of 90 mL of zinc nitrate into 10 mL of a pale red onion bulb extract. The metal ions of zinc nitrate were reduced following exposure to onion bulb extract in a dark condition after 24 hours. Color development from pale red to yellow was visually confirming the presence of ZnO NPs. SEM characterizations of the synthesized ZnO NPs were shown in Figure 1 revealing crystal nature of ZnO NPs in a number of aggregates at a much lesser diameter. FTIR spectra of the untreated onion bulb extract with zinc nitrate were presented in Figure 2. FTIR analysis of the onion bulb extract confirmed the possible compounds to be responsible for the reduction of zinc metal to ZnO NPs.

FTIR spectra of the synthesized ZnO NPs was shown in Figure 3 verifying several bands indicating to several characteristics absorption of the ZnO NPs. Table 1 demonstrates the *in vitro* antioxidant activity of ZnO NPs against DPPH. The DPPH radical scavenging activities of the synthesized ZnO NPs were considerably lower ($p<0.05$); when compared to ascorbic acid at the same concentration. It was observed that the radical scavenging activity of ZnO NPs was dose dependent.

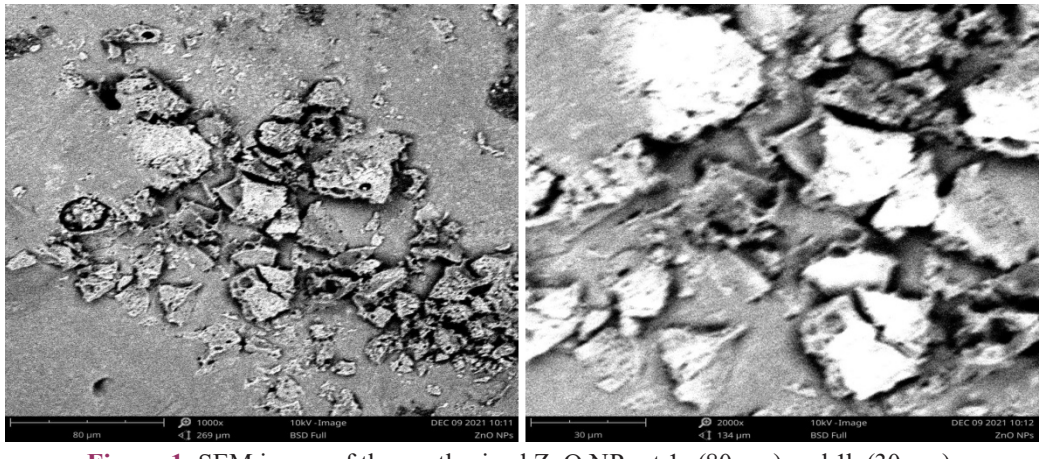

Figure 1: SEM image of the synthesized ZnO NPs at 1a (80 µm) and 1b (30 µm).

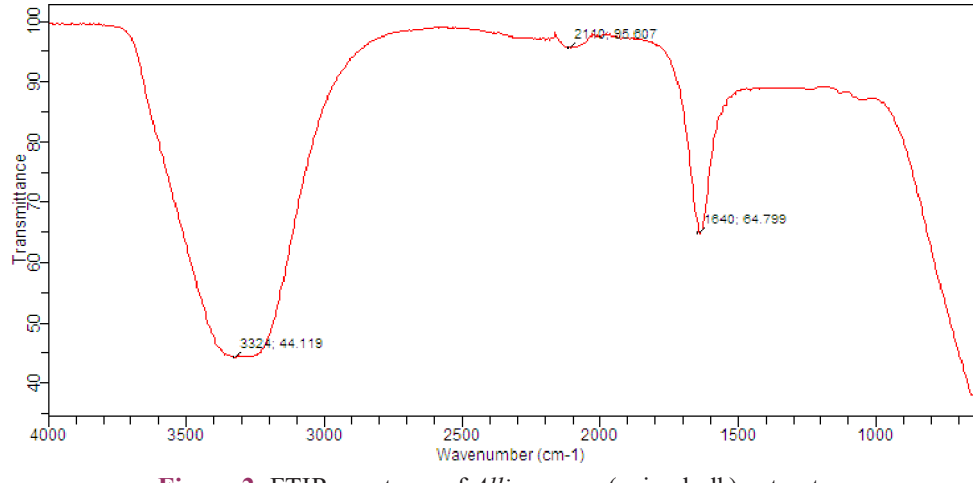

Figure 2: FTIR spectrum of *Allium cepa* (onion bulb) extract.

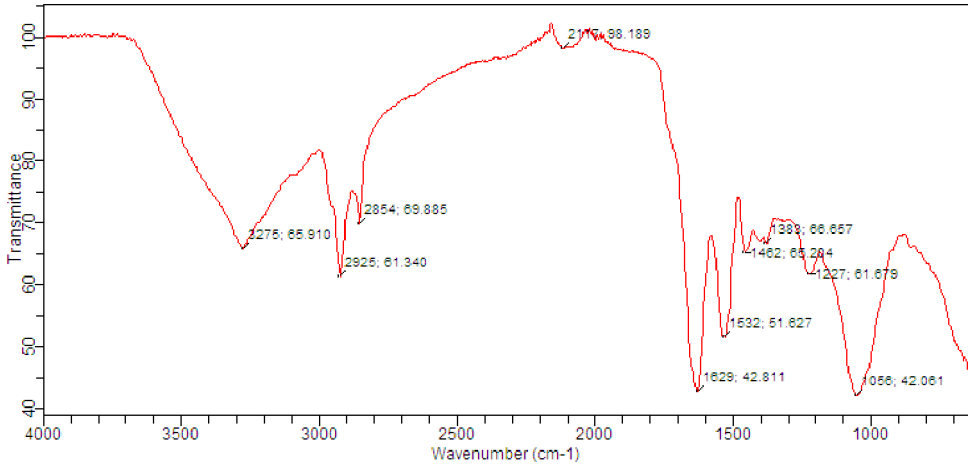

Figure 3: FTIR spectrum of synthesized ZnO NPs.

Table 1: DPPH radical scavenging activity of ZnO NPs (% Inhibition).

Concentration (µg/mL)	ZnO NPs	Ascorbic acid
20	45.15±0.007	45.52±0.005
40	46.78±0.003[a]	58.76±0.008
60	50.18±0.003[a]	72.64±0.001
80	57.50±0.008[a]	77.05±0.002
100	61.41±0.003[a]	88.02±0.005

Values were displayed as mean±SEM (n=3). a: Significantly (p<0.05) lower than ascorbic acid at the same concentration.

Table 2: Effect of ZnO NPs on serum lipid profile in Triton X-100 induced hyperlipidemic rats.

Group	TC	TG	HDL	LDL	AI
Sham	118.76±1.4[bf]	65.33±1.6[bf]	74.85±1.30[e]	33.83±0.40[bf]	0.58±0.04[b]
Negative	237.53±2.43[a]	214.54±2.33[ac]	31.37±1.93[df]	158.44±1.00[ac]	6.57±0.032[ac]
Atorvastatin (10 mg/kg)	124.19±2.0[ab]	85.28 ±0.78[ab]	55.74±1.91[e]	51.70±0.27[ab]	1.22±0.22[b]
ZnO NPs (100 µg/kg)	208.93±5.7[abcg]	176.66±3.5[abcg]	37.70±1.82[dfh]	135.22±2.92[abcg]	4.64±0.21[acg]
ZnO NPs (300 µg/kg)	191.58±2.6[abc]	169.16±2.11[ac]	43.01±1.00[f]	116.89±2.31[abc]	3.38±0.01[bc]
ZnO NPs (100 µg/kg)	153.38±3.0[bh]	152.32±2.78[bh]	52.00±0.57[fg]	68.52±2.20[abh]	1.93±0.05[bh]

TC: Total Cholesterol, TG: Triglycerides, HDL: High density lipoproteins, LDL: Low density lipoproteins, AI: Atherogenic index, Values were exhibited as mean±SEM (n=5). a: Significantly (p<0.05) higher than sham group, b: Significantly (p<0.05) lower than negative control, c: Significantly (p<0.05) higher than standard control, d: Significantly (p<0.05) lower than normal control, e: Significantly (p<0.05) higher than negative control, f: Significantly (p<0.05) lower than standard control, g: Significantly (p<0.05) higher compared to test groups, h: Significantly (p<0.05) lower compared to test groups.

Table 2 illustrates the serum lipid profile parameters and atherogenic index in hyperlipidemic rats. Administration of ZnO NPs caused significant decrease (p<0.05) of TC, TG, LDL cholesterol levels and AI values, thus HDL cholesterol level remained significantly higher in all levels (p<0.05). The total cholesterol level in the hyperlipidemic rats reduced in a dose dependent manner with the highest reduction (93.38±3.0) obtained at 500 µg/kg of ZnO NPs. Increase in HDL cholesterol level was dose dependent with the highest observed in standard drug and 500 µg/kg (46.74±1.91 and 43.00±0.57), respectively. TC, TG and LDL cholesterol levels were positively correlated with AI.

Table 3 reveals the result of the effect of ZnO NPs on liver enzymes. Administration of Triton X-100 to induce hyperlipidemia in rats showed a significant increase (p<0.05) in serum hepatic enzyme levels of ALT, AST and ALP in comparison to sham control group. Treatment with ZnO NPs caused a significant reduction (p<0.05) in ALT level at the highest dose (500 µg/kg). No significant difference was visible in AST level in treatment groups (p<0.05); when compared to sham control group. There was no significant difference in ALP level (p<0.05) at the highest dose (500 µg/kg); when compared to experimental control group.

Table 4 presents the result of the effect of ZnO NPs on SOD, CAT and GPx in Triton X-100 induced hyperlipidemic rats. The findings indicated

Table 3: Effect of ZnO NPs on serum liver function enzymes in Triton X-100 induced hyperlipidemic rats.

Group	ALT	AST	ALP
Sham	25.12±0.57^{b}	26.73±0.53^{b}	92.89±2.2^{b}
Negative control	81.34±0.97^{ac}	57.09±1.8^{ac}	105.87±2.2^{ac}
Atorvastatin (10 mg/kg)	24.94±0.47^{b}	29.71±0.82^{b}	95.73±1.7^{b}
ZnO NPs (100 µg/kg)	69.48±1.3^{ac}	51.01±2.1^{c}	101.97±1.4^{a}
ZnO NPs (300 µg/kg)	54.61±2.3^{ac}	38.54±2.70^{c}	99.74±0.0^{b}
ZnO NPs (500 µg/kg)	33.65±0.44^{b}	31.04±0.83^{b}	97.16±0.56^{b}

ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, Values were demonstrated as mean±SEM (n=5). a: Significantly (p<0.05) higher than normal control, b: Significantly (p<0.05) lower than negative control, c: Significantly (p<0.05) higher than standard control.

Table 4: Effect of ZnO NPs on serum antioxidant enzymes in Triton X-100 induced hyperlipidemic rats.

Group	SOD (U/mL)	CAT (µmol/H$_2$O$_2$/min/mL)	GPx (U/mL)
Sham	2.28±0.02^{b}	54.94±1.48^{b}	37.60±0.48^{b}
Negative control	1.72±0.11^{a}	32.37±1.96^{ac}	24.48±0.25^{ac}
Atorvastatin (10 mg/kg)	2.22±0.10^{b}	52.76±2.22^{b}	35.71±1.65^{b}
ZnO NPs (100 µg/kg)	2.35±0.20^{b}	24.17±0.24^{ac}	25.46±1.04^{ac}
ZnO NPs (300 µg/kg)	2.34±0.01^{b}	25.29±0.01^{ac}	31.93±0.39^{ab}
ZnO NPs (500 µg/kg)	2.37±0.01^{b}	31.87±2.19^{acd}	33.38±0.93^{bd}

SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, Values were shown as mean±SEM (n=5). a: Significantly (p<0.05) lower than normal control, b: Significantly (p<0.05) higher than negative control, c: Significantly (p<0.05) lower than standard control, d: Significantly (p<0.05) higher compared to other treatment groups.

Table 5: Effect of ZnO NPs on serum cardiac markers in Triton X-100 induced hyperlipidemic rats.

Group	CK-MB (ng/mL)	c-TnI (ng/mL)	Myoglobin (ng/mL)
Sham	2.65±0.16^{b}	0.37±0.52^{b}	35.81±3.16^{bc}
Negative	7.72±0.27^{ac}	1.27±0.03^{ac}	61.86±1.86^{ac}
Atorvastatin (10 mg/kg)	2.85±0.09^{b}	0.52±0.31^{b}	27.14±1.25^{bd}
ZnO NPs (100 µg/kg)	6.22±0.23^{abce}	1.17±0.07^{abce}	55.61±0.13^{abc}
ZnO NPs (300 µg/kg)	5.40±0.20^{abc}	1.04±0.09^{abc}	54.58±0.70^{abc}
ZnO NPs (500 µg/kg)	3.20±0.15^{bf}	0.87±0.15^{abcf}	48.97±0.88^{abcf}

CK-MB: Creatine kinase, c-TnI: Cardiac troponin-I, Values were presented as mean±SEM (n=5). a: Significantly (p<0.05) higher than normal control, b: Significantly (p<0.05) lower than negative control, c: Significantly (p<0.05) higher than standard control, d: Significantly (p<0.05) lower than normal control, e: Significantly (p<0.05) higher compared to test groups, f: Significantly (p<0.05) lower compared to test groups.

a significant decrease (p<0.05) in SOD, CAT and GPx in hyperlipidemic rats; when compared to sham group. There were significant increases (p<0.05) within levels of SOD and GPx with little effect on CAT level following the administration of ZnO NPs at the highest dose (500 µg/kg).

Table 5 displays the findings of serum cardiac biomarkers of CK-MB, c-TnI and myoglobin in Triton X-100 induced hyperlipidemic rats. The results denoted to a significant increase (p<0.05) in serum CK-MB, c-TnI and myoglobin levels in hyperlipidemic rats as compared to normal control group. There was a significant decrease (p<0.05) in levels of CK-MB, c-TnI and myoglobin in the treatment groups following the administration of ZnO NPs in different concentrations.

Histopathological studies of the heart tissue of sham control and treated groups indicated the cardioprotective effect of ZnO NPs in Triton X-100 induced hyperlipidemia in experimental rats. The result of the histological architecture of the heart sections was unable to significantly protect myocardium from lipoma and ectopic fatty infiltration following the administration 100 µg/kg and 300 µg/kg of ZnO NPs. However, at the highest dose of 500 µg/kg of ZnO NPs, myocardium showed a significant improvement from histological benign cardiac lesion characterized by excessive fat deposition in the region of the interatrial septum that spared the fossal ovalis (Figure 4).

Discussion

In SEM of synthesized ZnO NPs using onion extracts of *A. cepa*, the micrographs recorded at different magnifications of 80 µm and 30 µm showed that ZnO NPs were clustered together and surface

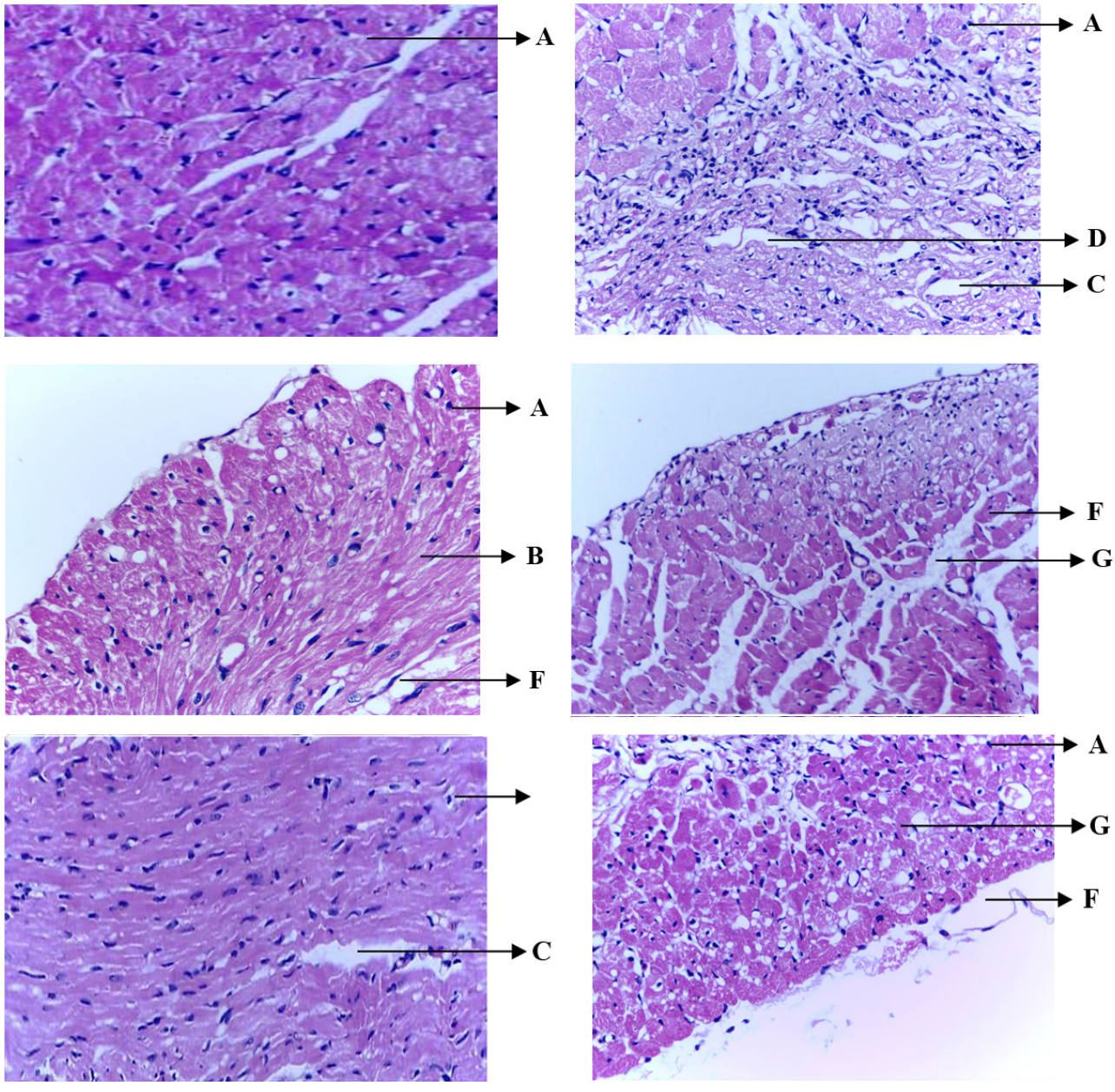

Figure 4: Histopathological analysis of the heart. a: Sham group showing normal histological pattern of cardiac tissue. Myocardium with multiple peripheral nuclei and branching straited muscle fibers. A: Fat nuclei (×40, H&E). b: Hyperlipidemic rats (negative control group) showing ectopic accumulation of fat and myocardial steatosis associated visceral obesity with multiple fatty degeneration and very less fragmentation of myocardial muscle fibers. A: Fat nuclei, C: Ectopic fat depots, D: Cardiac steatosis (×40, H&E). c: Test group treated with ZnO NPs (300 µg/kg) showing fatty infiltration and associated cardiac muscle bundle fibers in the anterior wall of the right ventricle. A: Fat nuclei, B: Muscle fibers, F: Cardiac lipoma (×40, H&E). d: Test group treated with ZnO NPs (500 µg/kg) showing cardiomyocytes restoring toward normal with mild fatty infiltration. F: Cardiac lipoma, G: Normal cardiomyocytes (×40, H&E). e: Standard control group treated with atorvastatin (10 mg/kg) showing mild necrosis and very mild myocardial ectopic fat depots in the myocardial wall of the right ventricle. A: Fat nuclei, C: Ectopic fat depots (×40, H&E). f: Test group treated with ZnO NPs (100 µg/kg) showing lipomatous hypertrophy of the right atrium, characterized by fat deposition. A: Fat nuclei, F: Cardiac lipoma, G: Normal cardiomyocytes (×40, H&E).

of the aggregates was rough. Similar findings were reported before (13). They have used a biological approach and the milky latex of the *A. cepa* plant as a reducing agent and surface stabilizer for the first time in the synthesis of spherical ZnO NPs. The SEM study of ZnO NPs using *Ocimum tenuiflorum* leaf extract has been previously provided (14). The hexagonal ZnO NPs were generated with a diameter range of 11 to 25 nm, according to the SEM measurement. *Trifolium pratense* flower extract was reported to be utilized to create ZnO NPs, and SEM pictures of the particles showed that they were agglomerated and ranged in size from 100 to 190 nm (15).

The ZnO NPs decreased, capped, and effectively stabilized using *A. cepa* (onion bulb) extract. In FTIR spectrum measurements to identify the potential biomolecules of zinc nitrate-free onion bulb extract, several absorption peaks ranged from 3324 cm^{-1} to 1640 cm^{-1} were noticed. The spectrum showed absorption band at 3340 cm^{-1} reflecting N-H stretching class of aliphatic primary amine, illustrated absorption band at 21103340 cm^{-1} revealing C≡C stretching of alkyne compounds and the region of absorption band at 1640 cm^{-1} presented C=N of amine and C=C of alkene compounds. Similar results were obtained (16) revealing that the FTIR spectrums of *A. cepa* extract showed strong absorption peaks that were observed at 3300 and 1620 cm^{-1} and were assigned to O–H stretch and N–H bend functional groups. Weaker bands observed at 2955, 777 and 633 cm^{-1} were assigned to C–H stretch (alkanes), C–H (aromatics) and C=C–H (alkynes). The absorption peaks at 1520 and 1431 cm^{-1} were attributed to the C=C bending and C–C stretching of aromatic rings, respectively (16).

In FTIR spectrum of the synthesized ZnO NPs, the presence of bands at 3275, 2925, 2854, 2117, 1629, 1532, 1462, 1388, 1227 and 1056 cm^{-1} were demonstrated. These bands were indicating the presence of N-H band of primary amines, C-O stretching alcohols, carboxylic acids, ester and ethers. FTIR spectrum of ZnO NPs revealed that the peak at 2925 cm^{-1} was the characteristic absorption of ZnO bond and the broad absorption peak at 3275 cm-1 attributed to the characteristic absorption of hydroxyl group. A similar report confirmed the FTIR spectrum of ZnO NPs derived from *A. cepa* showing the fundamental mode of vibration at 3458.04 cm^{-1} which corresponded to the O-H stretching vibration, 1625.35 cm^{-1} corresponded to the N-H bend, 1418.86 cm^{-1} corresponded to C-C stretching vibration of alcohol, carboxlic acid, ether and ester (17). The bond at 1148.10 cm^{-1} corresponded to C-N symmetric stretching vibration, while the O-H bending of the hydroxyl group at 3458.04 cm^{-1} was observed. The development of zinc tetrahedral coordination was the cause of the absorption at 875.23 cm^{-1}. The bond at 835.61cm^{-1} was due to the C-C stretching vibration. The peak at 668.29 cm^{-1} suggested the ZnO NPs stretching vibrations, which was similar with those previously described (18).

Antioxidant capacity of synthesized ZnO NPs using *A. cepa* extract has been measured by DPPH assay to examine the radical scavenging activity of the synthesized ZnO NPs (19). We depicted the decrease in absorbance at 517 nm as the concentrations of ZnO NPs increased from 20 µg/mL to 100 µg/mL; which indicated radical scavenging activity of ZnO NPs. In higher concentrations, the radical scavenging capability of ZnO NPs synthesized by *A. cepa* had marginally been less than ascorbic acid. The manufactured ZnO NPs' radical-scavenging abilities were found to increase with a rise in concentration. The DPPH activity on the produced ZnO NPs demonstrated improved antioxidant activity at higher concentrations (60-100 g/mL) as compared to ascorbic acid in terms of radical scavenging activity. When oxidants and free radicals accumulated in the body to a point that they can no longer be eliminated, it can result in oxidative stress (20). Antioxidants are very important in the treatment of cardiovascular diseases (21). Inhibiting chain initiation, binding of transition metal ion catalysts, the breakdown of peroxides, reductive capacity, and radical scavenging activity are a few examples of the mechanisms that contribute to antioxidant activity (22). In the present study, DPPH was used as a source of free radicals for evaluation of ZnO NPs antioxidant activity. ZnO NPs significantly exhibited lower radical scavenging activity when compared to ascorbic acid at the higher concentrations. The radical scavenging activity of ZnO NPs was found to be dose dependent as an antioxidant.

In animal models, Triton X-100 was shown to cause acute hyperlipidemia and to prevent the clearance of TG-rich lipoproteins (23). Triton X-100 injection can lead to an increase in plasma TG and cholesterol levels. In the current study, Triton X-100 significantly increased the serum TC, LDL cholesterol, TG, AST, ALP, AST and AI levels, while decreased HDL cholesterol level similar to a previous study (24). In the current study, ZnO NPs were able to reverse the Triton X-100-induced levels of serum TC, LDL cholesterol, TG, AI, AST, ALP and ALT and increased HDL cholesterol level toward normal state. The effects of alendronate on the markers of hyperlipidemia were comparable to atorvastatin, which is mostly prescribed as a standard antihyperlipidemic drug (25). Thus, based on the markers of hyperlipidemia, it may be concluded from the present study that ZnO NPs may inhibit the synthesis of cholesterol via the mevalonate pathway, and its efficacy was similar to atorvastatin. In this study, it was established that AI increased in Triton X-100 administered rats as reported in a previous study (24). AI, which is a measure of the extent of atherosclerotic lesion, was significantly reduced by the oral treatment of ZnO NPs and atorvastatin, thus confirming previous findings.

Comparing Triton X-100-induced hyperlipidemic rats to the healthy control group, there was a significantly decreased activity of serum antioxidant enzymes (SOD, CAT, and GPx). Due to their extensive

use in scavenging the free radicals produced by Triton X-100-induced hyperlipidemia, which is reflected in the high degree of lipid peroxidation, the activity of these antioxidant enzymes have decreased. While the results of Muthuraman *et al.* (26) are consistent with those of ZnO NPs administration that significantly increased the SOD level in Triton X-100-induced hyperlipidemic rats at doses of 500 g/kg, 300 g/kg, and 100 g/kg, but there was little change in the activity of catalase and glutathione peroxidase. This difference may be attributed to the sensitivity of SOD to Zinc as SOD plays a vital role in modulating ROS generation and is highly sensitive to Zinc (26).

Harikumar et al. (27) reported that the SOD is a major antioxidant enzyme that protects cells from the harmful effects of superoxide by accelerating the dismutating superoxide. They have also illustrated that the ZnO nanoparticle increased SOD enzyme activity in keratinocytes. Glutathione peroxidase catalyzed the reaction of hydrogen peroxides with GSH with the production of oxidized glutathione and the reduction product of the hydrogen peroxide, while catalase decomposed it into water and oxygen (28). Catalase is an antioxidant enzyme expressed in the various tissues, and it protects cells against potentially harmful effects of the hydrogen (26). Okuno et al. revealed that the adipose tissues highly expressed catalase enzyme comparable to the liver and kidney. Results from this study exhibited that the ZnO NPs had small increases in the catalase activity compared to the increase in the SOD and GPx activities (29).

Serum CK-MB, c-TnI and myoglobin are well known diagnostic markers of myocardial damage. Myocardial cells ruination due to insufficient oxygen supply, rupture of cardiac membrane can lead to enzyme leakage and their entrance into blood stream (30). The results of this study illustrated significant increase in serum CK-MB, c-TnI and myoglobin activities in Triton X-100 induced hyperlipidemic rats when compared to rats of normal control group. These results are nearly similar to those previously noted that hepatic and cardiac functions were significantly reduced by a high cholesterol diet as shown by an augmentation of serum levels of AST, ALT, LDH and CK-MB activities (31). Increased activities of these enzymes in serum are indicative of cellular damage, loss of functional integrity, and/or permeability of cell membrane (32).

A well-known diagnostic enzyme marker of myocardial injury is serum CK-MB. Serum CK-MB increase was seen in this investigation. The cardiac membrane permeability abnormalities of Triton X-100-induced hyperlipidemic rats were supported by this elevated enzyme level in the serum. In both humans and animals, c-TnI is regarded as a prognostic marker of drug-induced cardiac cell malfunction (30). Increased troponin-I levels are crucial for the assessment of infarct size in myocardial infarction, increased coronary disease, inadequate coronary flow, and reduced left ventricular systolic performance (33). According to our reports, the primary cause of the release of cardiac troponin into the bloodstream may be related to loss of membrane integrity brought on by cardiac myocyte injury (Priscilla). However, administration of ZnO NPs in Triton X-100 induced hyperlipidemia in rats resulted in a significant decrease in serum CK-MB, c-TnI and myoglobin.

The heart sections of hyperlipidemic rats showed marked degeneration of myocardial muscles with ectopic and lipomatous cardiomyocytes. This result is consistent with other studies showed the relationship between hyperlipidemia and pathological alteration of heart in rats. Our results showed that, treatment with 100 µg/kg and 300 µg/kg of the synthesized ZnO NPs in hyperlipidemic rats insignificantly reduced the ectopic and myocardial steatosis. However, administration of 500 µg/kg ZnO NPs in hyperlipidemic rats significantly improved the heart tissue and nearly restored them to their normal state. This result is in agreement which reported the lipomas as the neoplasms, being encapsulated masses of adipose tissue typically composed of mature fat cells (34). Moreover, the current study suggested that ZnO NPs improved the adverse conditions in hyperlipidemic rats which restored the supply of the heart with oxygen rich blood to nearly normal as shown in the heart histological results.

Conclusion

In this study, the oral administration of the synthesized ZnO NPs ameliorated the state of Triton X-100 induced hyperlipidemia in rats by significantly lowering the TC, TG and LDL and significantly increase of HDL level toward normal, improvement in liver enzymes activities, reforming of antioxidants status and ameliorating markers of coronary heart diseases which provided scientific evidences for antihyperlidemic effect of ZnO NPs. The improvements of these parameters were dose dependent and the maximal effect was seen in higher doses of ZnO nanoparticle. The histological changes brought about by Triton X-100 induced hyperlipidemic rats were reverted by oral administration of ZnO NPs at 500 µg/kg as confirmed by H&E staining results. Further studies are warranted for the possible mechanism for action of ZnO NPs.

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Conflict of Interest

None declared.

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