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Effects of polysaccharide-based silver and selenium nanoparticles on growth performance, biochemical parameters, and immune response of *Cyprinus carpio*

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ABSTRACT

Avicennia marina mangrove leaves polysaccharide (AMLP) was used for the synthesis of polysaccharide-based selenium (AMLP-SeNPs) and silver nanoparticles (AMLP-AgNPs). The synthesized nanoparticles were further characterized by UV-Vis, DLS, FT-IR, X-ray diffraction, and HR-TEM analysis. A 60-day (8 weeks) feeding trial experiment was conducted to investigate the effects of AMLP, AMLP-SeNPs, and AMLP-AgNPs dietary supplementation on growth performance parameters, blood parameters, immunological and enzymatic profiles in *Cyprinus carpio*. The characterization results of AMLP-SeNPs and AMLP-AgNPs confirmed the formation of well-stabilized spherical nanoparticles with a mean particle size of 37.25 and 72.40 nm, respectively having a crystalline structure. The feeding experiment results demonstrated that 2 mg/kg of AMLP-SeNPs followed by 0.2 mg/kg of AMLP-AgNPs showed significantly ($p < 0.05$) higher final weight, weight gain (WG), specific growth rate (SGR%), protein and lipid efficiency, and lower food conversion ratio as compared to other groups. The catalase, superoxidase dismutase, and glutathione peroxidase activity were significantly ($p < 0.05$) higher in the group fed 2 mg/kg supplemented AMLP-SeNPs. Total protein and globulin contents were significantly ($p < 0.05$) higher and albumin concentration was significantly lower in fish that received 2 mg/kg of AMLP- SeNPs as compared to control. A significant increase in serum HDL and decrease in LDL and MDA concentrations were observed in the group supplemented with 2 mg/kg of nano selenium. The body's crude lipid, protein, moisture, and ash were not significantly different from the control. The AMLP-SeNPs showed significantly ($p < 0.05$) lower aspartate aminotransferase (AST), alanine aminotransferase (ALT), and higher alkaline phosphatase (ALP) activities compared to other test groups. The relative percentage survivability (RPS%) was higher in AMLP-SeNPs (84.6%) followed by AMLP-AgNPs (76.7%) after 8^{th} weeks of supplementary diets as compared to control groups. Overall, the finding of these studies revealed that the inclusion of AMLP-SeNPs improved the growth performance and antioxidant defense system, enhance immune response, and provide resistance against *Aeromonas hydrophila* in Common carp.

1. Introduction

Aquaculture is one of the major fast-growing food sectors all over the world which fulfill the consumer needs for higher protein, beneficial fats, and various micronutrients [1]. Although the aquaculture industry is threatened with various challenges such as several disease outbreaks, low production, high-cost feed, transportation, effluent pollution, and others [2]. In recent times nanotechnology has become an emerging field of research and due to its unique properties, that is suitable for novel applications purposes such as diagnostics, drugs, vaccination, nutrition, and aquatic health of animals which can improve the development of the animal production sector [3]. The fish-related disease is one of the major problems for the sustainable development of the aquaculture industry all over the world. Excessive use of antibiotics in fish culture management against various fish infections develops resistant strains which is a concern for humans and veterinary medicines [4]. *A. hydrophila* is a most common pathogenic gram-negative bacterium that can grow aerobically and anaerobically causing various infections

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in fresh fish populations [5]. It has been reported to be a predominant aquatic pathogen that causes septicemia and furuculosis in freshwater fishes and is responsible for gastrointestinal system infections in humans [6]. Recently, it has been reported that *A. hydrophila* develops resistance to several antibiotics such as amoxicillin, ampicillin, rifampicin, tetracycline, novobiocin, etc [7]. Therefore, due to the advancement of nanotechnology in the field of aquaculture, an efficient novel material has to be developed as an alternative to antibiotics against *A. hydrophila* which could be economically feasible for therapeutic applications. Among all the nanoparticles, silver nanoparticles (AgNPs) and selenium nanoparticles (SeNPs) have gained much attention in the aquaculture field to control various forms of bacterial, fungal, and viral diseases especially to combat antibiotic-resistant pathogens [8,9]. It has been previously reported that the AgNPs showed bactericidal activity against *Aeromonas* species, and *Vibrio harveyi* in various aquatic animals [10]. Previously, it was reported that fish's innate immune system and antioxidant enzyme activity could enhance the defensive mechanism against *A. hydrophila* infection [11].

Selenium is an important trace element essential for biological systems which exhibited a wide range of biological functions in both human and animal health including fish [12]. Selenium is the active site for many selenium-dependent enzymes such as glutathione peroxidase (GSH-Px) in cellular systems, which is involved in cellular protection by scavenging intracellular free radicals and provides an immunological function in various fish species [13]. Several reports have found that the *in vitro* and *in vivo* antioxidant capacity of selenium nanoparticles is exhibited through the activation of selenoenzymes, which includes selenium-dependent glutathione peroxidase (SeGSH-Px) and thioredoxin reductase which prevent cellular damages by scavenging free radicals [14–16]. Selenium is a vital dietary micronutrient essential for the normal body functions and metabolism of animals [17]. Supplementation of selenium plays a key role in the growth, fertility, and immune functions of humans and other vertebrates, including fishes [18]. Due to the higher bioavailability and low toxicity of selenium nanoparticles as compared to other forms, it has been suggested as an alternative to improve the selenium intake in the fish digestive system [19].

Polysaccharides are naturally the most abundant polymeric bioactive substances having different aspects of medicinal and nutrition values obtained from plants, animals, and microorganisms [20]. Previously, it has been reported that the polysaccharide obtained from the leaves of *Avicennia marina* mangrove plants showed potential bioactive properties such as antioxidant, and antibiofilm and can be used as natural antioxidants in food and pharmaceutical industries [21]. Several reports stated that advantageous properties of natural polysaccharides include biocompatibility, biodegradability, non-toxicity, and minimal side effects as compared to synthetic compounds [22,23,24]. Therefore, the combination of natural polysaccharides and nanoparticles through strategic functionalization may achieve some beneficial properties which reduce the limitations of nanoparticles and provide long-term stability [25].

Common carp (*Cyprinus carpio*) is one of the most widely cultivated freshwater fish globally and belongs to the largest family Cyprinidae. Majorly, it is one of the most important cultured fish in Asian and some European countries as it has highly adaptive capabilities to the environment, and higher marketing demands for commercial production [26,27]. Therefore, the present studies were undertaken to assess the comparative effects of polysaccharide-based SeNPs and AgNPs supplementary diets on the growth performance, hematological, biochemical parameters, and enzyme activities as well as examine the protective response against *A. hydrophila* infection in common carp, *C. carpio*.

2. Materials and methods

2.1. Synthesis of silver and selenium nanoparticles (AMLP-AgNPs and AMLP-SeNPs)

Avicennia marina leaves polysaccharides (AMLP) isolated from our previous studies were used for the synthesis of silver and selenium nanoparticle formation [21]. The synthesis of AMLP-AgNPs and AMLP-SeNPs was done according to the previously described method with some modifications [28,29]. For the synthesis, 0.03% of AMLP solution was stirred and 8 mM of $AgNO_3$ was added dropwise and the reaction solution was maintained at pH 10. The reaction was carried out for 30 min at 80 C on a magnetic stirrer. The AMLP-SeNPs were synthesized by mixing an aqueous solution of AMLP (1 mg/mL, 10 mL) and 10 mL of sodium selenite (0.01 M) and placed on a magnetic stirrer at room temperature and the mixture was treated with ultrasonication for 5 min. After adding 0.04 M of ascorbic acid (10 mL), the pH of the reaction mixture was adjusted immediately to pH 7 and the reaction was further carried out for 2 h. The synthesis of AMLP-AgNPs and AMLP-SeNPs was visually confirmed as the color of the reaction mixture was turned from pale yellow to dark brown and brick red in the case of AMLP-SeNPs. The resulting solution was centrifuged to isolate the formed nanoparticles which are free from uncapping ligand and any biomass residues.

2.2. Characterization of synthesized AMLP- AgNPs and AMLP-SeNPs

The UV-visible measurement of the synthesized AMLP-AgNPs and AMLP-SeNPs was recorded by using a UV-3600 spectrometer (Shimadzu, Japan) in the spectral range of 200 -700 nm. The particle size distribution and zeta potential analysis were measured by using a Zeta Sizer Nano S (Malvern, U.K). The morphological characteristics and size distribution analysis of synthesized AMLP-AgNPs and AMLP-SeNPs were determined by High-resolution Transmission Electron microscopy (Tecnai G2 F30 S-Twin, FEG-based TEM). The X-ray (XRD) diffraction patterns were analyzed using an X-ray diffractometer (X' Pert PRO, PAN-analytical) with Cu-kα radiation ($\lambda = 0.15406$ nm) in the range of 10-75°operating at 30 mA and 40 kV. The FT-IR measurement is carried out by KBr pressed disc method on Thermo Nicolet, Model; 6700, and data were recorded in the range of 4000-400 cm^{-1}

2.3. Experimental fish collection and rearing conditions

Approximately, five hundred freshwater common carp fish (*Cyprinus carpio*) were collected from Guru Fish Farm and Hatchery, Thukkanampakkam, Tamil Nadu (India). Fishes were transported safely in oxygenated plastic bags to the Marine Animal House (Pondicherry University, India), and transferred to 500 L capacity of FRP Fish Tanks containing fresh water with sufficient aeration throughout the acclimatization period *via* automatic air compressor (Suguna Air Compressors). The acclimatization of fish was done for two weeks to monitor the health status of the fish. During these periods, fish were fed three times per day with 5% of the body weight and the 80% FRP tank water was changed daily. During the experiment the basic water quality parameters were measured as follows: temperature of 27.0 ℃, pH 7.7, and dissolved oxygen (DO) of 6.20 mg/ml. The natural light cycle of 12 h and 12 h dark cycle was used.

2.4. Experimental design and diets preparation

After acclimation, 480 fish with an average initial weight (2.05 ± 0.43 g) were assigned to four experimental groups in triplicate and were reared into 12 FRP tanks containing freshwater (each one has a 500 L capacity and contains 40 fish). Fish were reared in these aquaria throughout the experimental studies. Two air stones connected with an air pump to maintain suitable aeration supported each aquarium. The

first group was controlled without nano addition and the other groups were dosed with AMLP (0.2 g/kg diet), AMLP-SeNPs (2 mg/kg diet), and AMLP-AgNPs (0.2 mg/kg diet). The concentrations of the nanoparticles selected in the present study were based on previous reports as the selected concentrations have minimum toxicity on fish and allowed them to investigate the effect of long-term exposure [30–32]. The water was exchanged every 24 h using a siphoning approach without severe disturbance to the fish and was appropriately aerated using an air compressor. The experimental fish were fed two times daily (6.00-20.00 h) for 60 days at a rate of 5% body weight. The unused feed and feces were removed daily by siphoning from the bottom of the tanks.

The experimental feed was prepared by grinding all of the ingredients and mixing them with vitamins and minerals, then adding the appropriate concentrations of AMLP, AMLP-AgNPs, and AMLP-SeNPs. Warm distilled water (35 ℃) was gently added until the feeds begin to clump, after which they were milled and dried in a forced-air oven before being stored in plastic containers at 20 ℃. The resulting pellet had a diameter of 0.2 mm and a length of 2 mm. Table 1 lists the contents and proximate chemical analyses of the various diets, as recommended by the Association of Official Analysis Chemists [33].

2.5. Growth parameters and feed utilization

At the end of the experiment, final body weight (FBW), weight gain (WG), specific growth rate (SGR% day^{-1}), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated using the following formulae; WG = final body weight (g) - initial body weight (g). SGR = 100 × (ln final weight - ln initial weight)/days. FCR = feed intake (g)/weight gain (g). PER = weight gain (g)/protein intake (g).

2.6. Whole-body proximate chemical composition

Following the final weighing, three fish were selected randomly from each tank and utilized to determine proximate composition. Protein, lipid, moisture, and ash were measured using the AOAC procedure [33]. The samples were kept at -20 ℃ until they were analyzed.

Table 1
Ingredients and proximate biochemical composition of basal diet.

Ingredients	Weight (g/kg)
Fish meal[a]	390
Soybean meal[b]	200
Wheat bran[b]	180
Corn meal[b]	150
Egg albumin[b]	30
Cod liver oil[b]	20
Vitamin mix[c]	10
Mineral mix[†]	10
Proximate composition (g/kg)	
Protein	440.29
Carbohydrate	290.32
Fiber	55.61
Lipid	65.78
Ash (%)	11.32
Moisture (%)	7.22
Energy (kJ/g)	14.76

*Vitamin mixture: Thiamine mononitrate IP 10 mg; riboflavin IP 10 mg; pyridoxine hydrochloride IP 3 mg; vitamin B12 (as tablets 1:100) 1 P 15 μg niacinamide 1 P 100 mg; calcium pantothenate IP 50 mg; biotin USP 100 μg; ascorbic acid IP 150 m.

† Mineral free mix contains: $CuSO_4$.$5H_2O$, 6 mg; $CaCO_3$,164 mg; NaH_2PO_4 $2H_2O$, 148 mg; KH_2PO_4 $2H_2O$, 337.6 mg; $CaCl_2$, 66.64 mg; $MgSO_4$, $7H_2O$ 80 mg; KCl, 22.40 mg; $AlCl_3.6H_2O$, 0.96 mg; $MnSO_4$ H_2O, 11.45 mg; $ZnSO_4$, 90 mg; $COCl_2$ $6H_2O$, 1.41 mg; KI 1.81 mg; cellulose, 69.74 mg/g.

[a] Ingredients was purchased from a local fisherman, Kalapet, Puducherry, India.

[b] Ingredients purchased from local Store, Kalapet, Puducherry, India.

[c] Ingredients purchased from Apollo Medicals, Muthiayalpet, Puducherry, India.

2.7. Blood samples

At the end of the 4th and 8th weeks of the trial, five fish were chosen randomly from each tank and were anesthetized by using a 2-phenoxyethanol (0.1-0.5 ml/L). The blood sample was drawn from the caudal vein of the fish using a heparinized syringe and collected in a plastic tube containing heparin as an anticoagulant. For a collection of the serum sample, separate blood portions were centrifuged at 2000g at 4 ℃ for 10 min and stored at -80 ℃ until further analysis.

2.8. Biochemical and Antioxidant enzymatic assay

The total protein and albumin contents were estimated by Biuret and Bromocresol green binding method [34]. Globulin content was quantified by subtracting serum albumin from total protein. Total serum cholesterol was measured according to the previously reported method [35]. The serum enzymatic activities that include aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were determined according to previously proposed methods [36]. The antioxidant enzyme activity such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities were determined according to previously described methods [37–39]. Malondialdehyde (MDA) or TBARS levels were estimated by the earlier developed colorimetric method [40].

2.9. Assessment of non-immune specific parameters

Myeloperoxidase activity was measured according to the proposed method [41]. The measurement of lysozyme activity was determined by the previously described method [42]. According to Rook et al. (1985), the respiratory burst was evaluated by reducing the nitro blue tetrazolium test [43].

2.10. Bacterial challenge experiment

The nutrient broth (NB) medium was used to grow *A. hydrophila* (MTCC 646) at 37 C for 24 h. The bacterial pellets were obtained by centrifugation at 3000 x g for 5 min at room temperature and bacterial cell suspension of 5×10^5 cells/mL was prepared using phosphate buffer saline (pH 7.4) by serial dilution. At the end of the 4th and 8th weeks of feeding trials, 10 fish from each treatment were selected randomly and divided into two duplicates in separate aquarium tanks and maintained with proper aeration. The bacterial challenge experiment was performed by intraperitoneal injection of *A. hydrophila* with a concentration of 0.1 mL. The death of the fish was counted daily and separated from each tank. The fish were observed for 14 days to note any abnormal clinical signs as well as the daily mortality rate. The relative percentage survival (RPS%) was determined as follows [44]; RPS = 100 [1 − (% mortality in treated fish/% mortality in the control fish)].

2.11. Estimation of total bacterial count

Aeromonas hydrophila count was determined at the end of the challenge for two weeks on the 4th and 8th weeks of challenge experiments according to a previous protocol [45]. For this, the blood was collected from the caudal vein and the muscles were dissected out aseptically from the challenged fish and plated in an Aeromonas agar media. After incubation at 37 ℃ for 48 h, the bacterial colonies were counted and expressed in $\log_{10}$ CFU.

2.12. Statistical analysis

Except for the mortality challenge, all data are provided by means ±

standard error of the means (SEM). Two-way analysis of variance (ANOVA) was used to determine the statistical significance of the data, followed by Duncan's multiple range tests (IBM SPSS 26.0, Inc., Chicago, IL). Significant differences between experimental groups were expressed at the significance level of $P < 0.05$.

3. Result and discussion

3.1. Synthesis and characterization of AMLP-SeNPs and AMLP-AgNPs

The results of the synthesis and characterization of AMLP-AgNPs and AMLP-SeNPs showed that the two polysaccharide-based nanoparticles exhibited brownish and brick red color in aqueous solution due to the reduction of silver ions and SeO_3^{2-} which could be attributed to the excitation of surface plasmon vibration of nanoparticles. UV-visible spectral of AMLP-AgNPs showed a characteristic absorption band centered at 417 nm while the maximum absorption peak for the synthesized AMLP-SeNPs appeared at 265 nm as shown in Fig. 1A and B. The results confirmed the formation of AMLP-AgNPs and AMLP-SeNPs *via* successful reduction of silver ions and SeO_3^{2-} using AMLP and ascorbic acid as reducing agents. The DLS analysis showed that the average particle size distribution of AMLP-AgNPs and AMLP-SeNPs was measured to be 37.25 and 72.40 nm, respectively (Fig. 1C and D). The polydispersity index (PDI) of AMLP-AgNPs and AMLP-SeNPs was found to be 0.545 and 0.248 indicating the polydispersity and monodisperse pattern of the nanoparticles, respectively. Earlier studies have reported that the PDI value of less than 0.3 indicates that the nanoparticles are present in the monodisperse form, while a higher PI value of nanoparticles indicates less homogenous size distribution [46]. A higher polydispersity was observed in the case of AgNPs synthesized from sulfated polysaccharide *Sargassum ailiquosum* with a broader size distribution of nanoparticles having PDI values > 0.5 [47]. According to previous reports, it was stated that the size calculated by DLS was found to be larger compared to HR-TEM analysis, which was explained by the fact that DLS calculated the overall size of hydrodynamic volumes associated with nanoparticles, while HR-TEM analysis observed only electron-rich particles [48]. It has been reported that the biological properties of the nanoparticles particularly depend on their size as smaller size particles tend to exhibit excellent biological capacity as compared to larger ones [49]. In one of the studies, it was reported that the small-sized SeNPs with an average size of 35 nm was found to be more effective to prevent the proliferation of cancerous cell *via* ROS-mediated process as compared to larger ones (91 nm) [50]. The lower zeta potential value leads to aggregation of particles due to Vander Waal interactions while the higher value of zeta potential prevents particles from agglomeration due to strong repulsive forces [51]. The result of the present studies shows that the Zeta potential of the AMLP-AgNPs and AMLP-SeNPs was about -18.9 and -24.6 mV (Fig. 1E

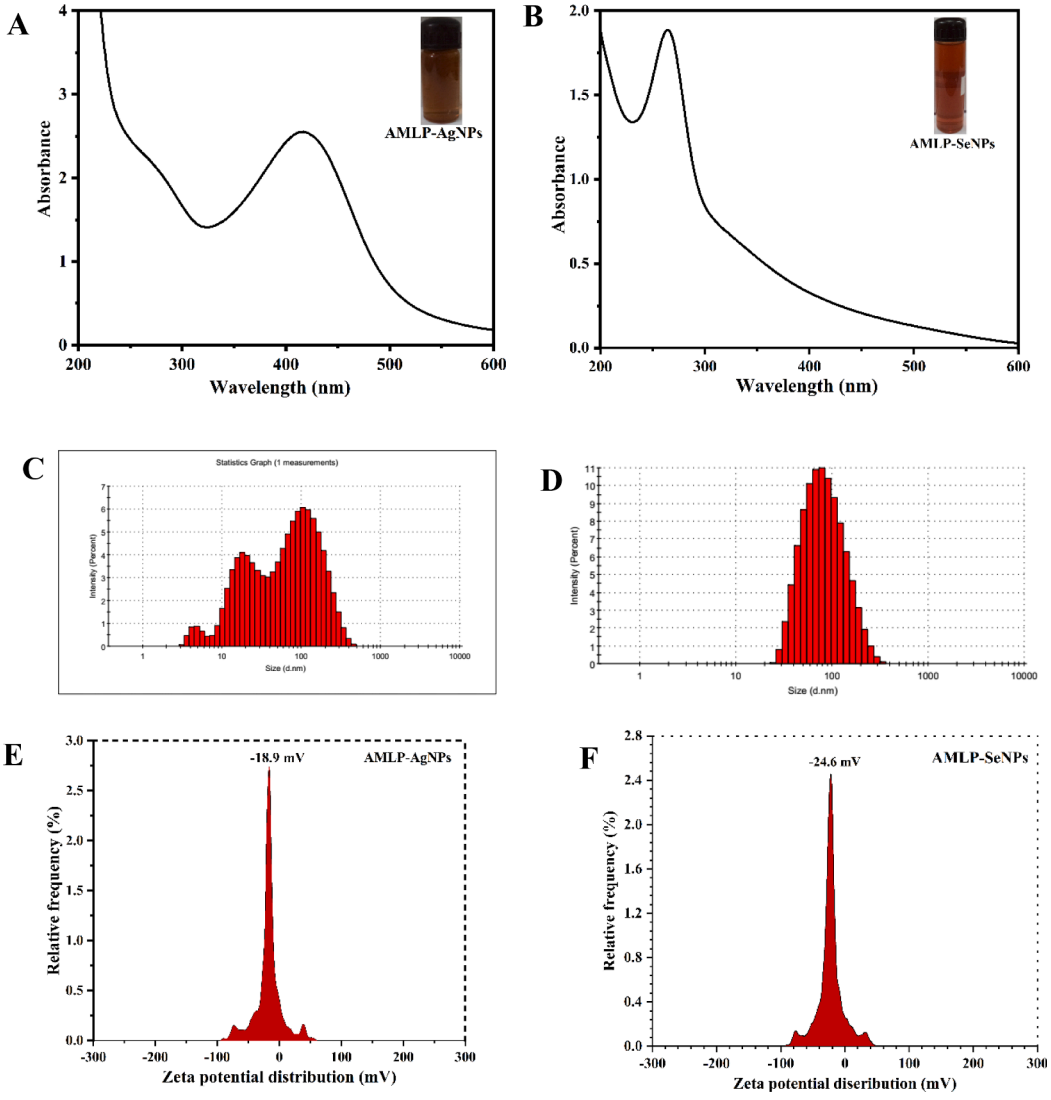

Fig. 1. UV-Visible absorption spectra, DLS analysis, and Zeta potential measurements of AMLP-AgNPs (A, C, and E); and AMLP-SeNPs (B, D, and F).

and F), which indicates that the higher negative charges present on the surface of the polysaccharides which facilitate the higher stability of synthesized nanoparticles.

The results from the HR-TEM images showed that the AMLP-AgNPs were observed to be polydisperse and spherical while the image of AMLP-SeNPs showed that the particles were monodispersed and spherical in structures having smooth surfaces (Fig. 2A and B). It has been reported that the molecular structure of polysaccharides contains reactive amino, hydroxyl, or carboxyl functional groups which affect the synthesis, stabilization, and growth of the nanoparticles [52]. Previously it has been reported that the morphology of AgNPs stabilized polysaccharides from marine macro alga (*U. faciata, P. capillacae, J. rubins,* and *C. sinusa*) and gum arabic-stabilized SeNPs was reported to be predominantly spherical having smaller size [28]. The SAED pattern of AMLP-AgNPs and AMLP-SeNPs indicates the crystalline nature of particles (supplementary Fig. 1A and B). The EDX spectra of synthesized AMLP-AgNPs, and AMLP-SeNPs confirmed the presence of silver (Ag) and selenium (Se) which showed the successful synthesis of silver and selenium nanoparticles (supplementary Fig. 1C and D). As shown in supplementary Fig. 2A and B, the histogram showed the particle size distribution of AMLP-AgNPs and AMLP-SeNPs acquired from HR-TEM images. The result demonstrates that the mean average size of the AMLP-AgNPs and AMLP-SeNPs was found to be 13.19 ± 11.14 and 39.28 ± 8.54 nm.

The results of the X-ray diffraction pattern of AMLP-AgNPs and AMLP-SeNPs are presented in Fig. 2C and D. The diffraction pattern of AMLP-AgNPs showed four distinct peaks at the 2θ position at 31.79° (122), 38.17° (111), 45.65° (200), 65.55° (220) planes which confirms the crystalline form of face-centered cubic structures (fcc) (JCPDS, file No. 04-0783) [53]. The Bragg reflection peaks present at 2θ values of 22.97° (100), 29.11° (101), 40.76° (110), 43.22° (102), 44.82° (111), 51.08° (201), 55.23° (112), 61.70° (202), 64.89° (210), and 68.16° (113) crystalline planes which suggest crystalline nature of the synthesized AMLP-SeNPs (JCPDS Card No. 06-0362) [54]. Debye-Scherrer equation (Dp = 0.94 X λ) / β X Cosθ), $\lambda = 1.54056$, β is a full width in radians at half maximum of the peak, is used to calculate the crystallite size from XRD data. The average crystallite size of the particle was calculated to be 12.58, and 14.71 nm for AMLP-AgNPs and AMLP-SeNPs, respectively.

FT-IR spectra are used to analyze the possible mechanism behind the functional groups involved in the synthesis of AMLP-AgNPs, and AMLP-SeNPs have been illustrated in supplementary Fig. 3A and B, respectively. The absorption peak at 3275.13 cm^{-1} was ascribed to the OH group in AMLP-AgNPs. The peak at 2920.23 and 2850.79 cm^{-1} has been assigned with symmetric and asymmetric C-H stretching groups, respectively. The sharp peak at 1637.56 cm^{-1} was attributed to the carbonyl stretching mode of the amide group of polysaccharides. The intense peak appeared at 1431.18 and 1396.46 cm^{-1} was ascribed to carboxylic functional groups. The peak at 1035.77, and 866.04 cm^{-1} corresponds to the S=O stretch and -C-O-SO_4 group of polysaccharides [28]. It can be inferred from the results that the carboxyl group of polysaccharides is involved in the bioreduction of silver and the sulfate group of polysaccharides AMLP has participated in the stabilization of AMLP-AgNPs. Similar results have been reported from the sulfated polysaccharides of *Porphyra vietnamensis* which was used for the synthesis of AgNPs [55]. As shown in Fig. 3D, the absorption peaks appeared at 3408, and 1639 cm^{-1} was assigned to the hydroxyl group (O-H stretching) and carboxyl group bending vibration in AMLP-SeNPs, respectively. The band at 2060.7 cm^{-1} was due to bound water. The formation of AMLP-SeNPs was confirmed by the stretching vibration peak of the hydroxyl group which is blue-shifted from 3448 to 3408 cm^{-1} which indicates the interaction of the hydroxyl group of AMLP and the surface of SeNPs. A similar interaction was reported in the case of *Lycium barbarum* polysaccharide capped selenium nanoparticles [56].

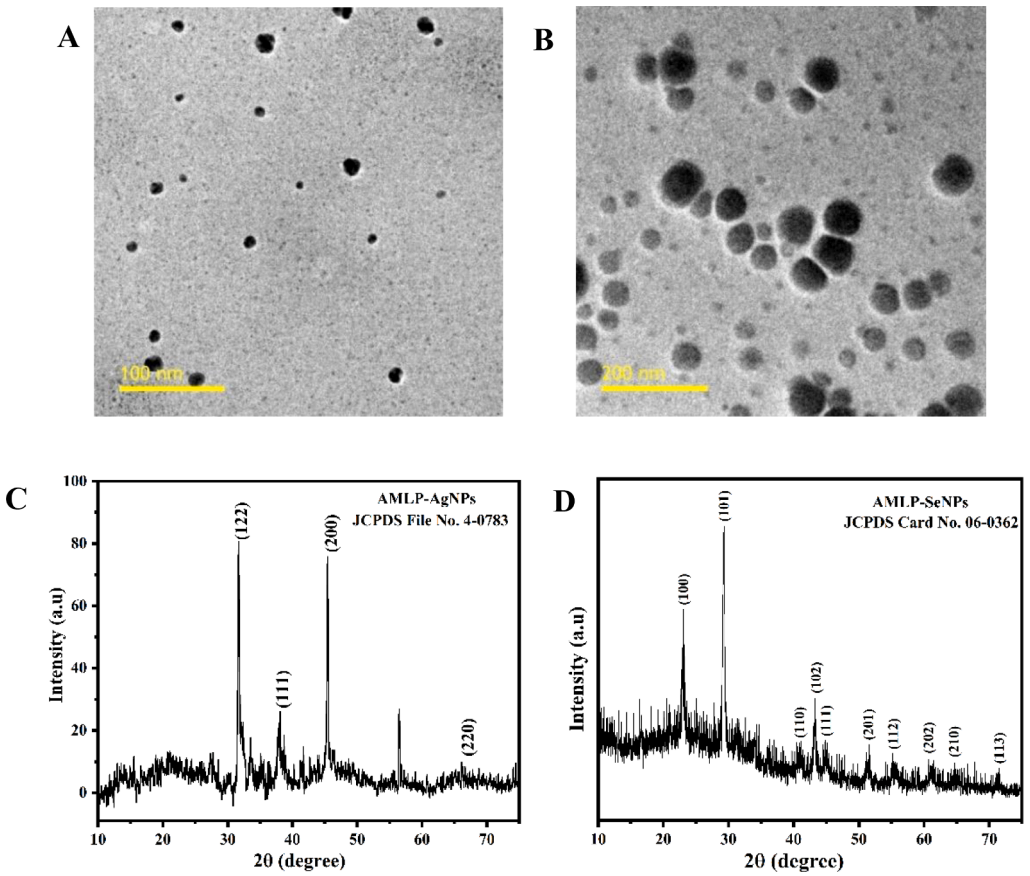

Fig. 2. HR-TEM images and X-ray diffraction pattern of AMLP-AgNPs (A and C); and AMLP-SeNPs (B, and D).

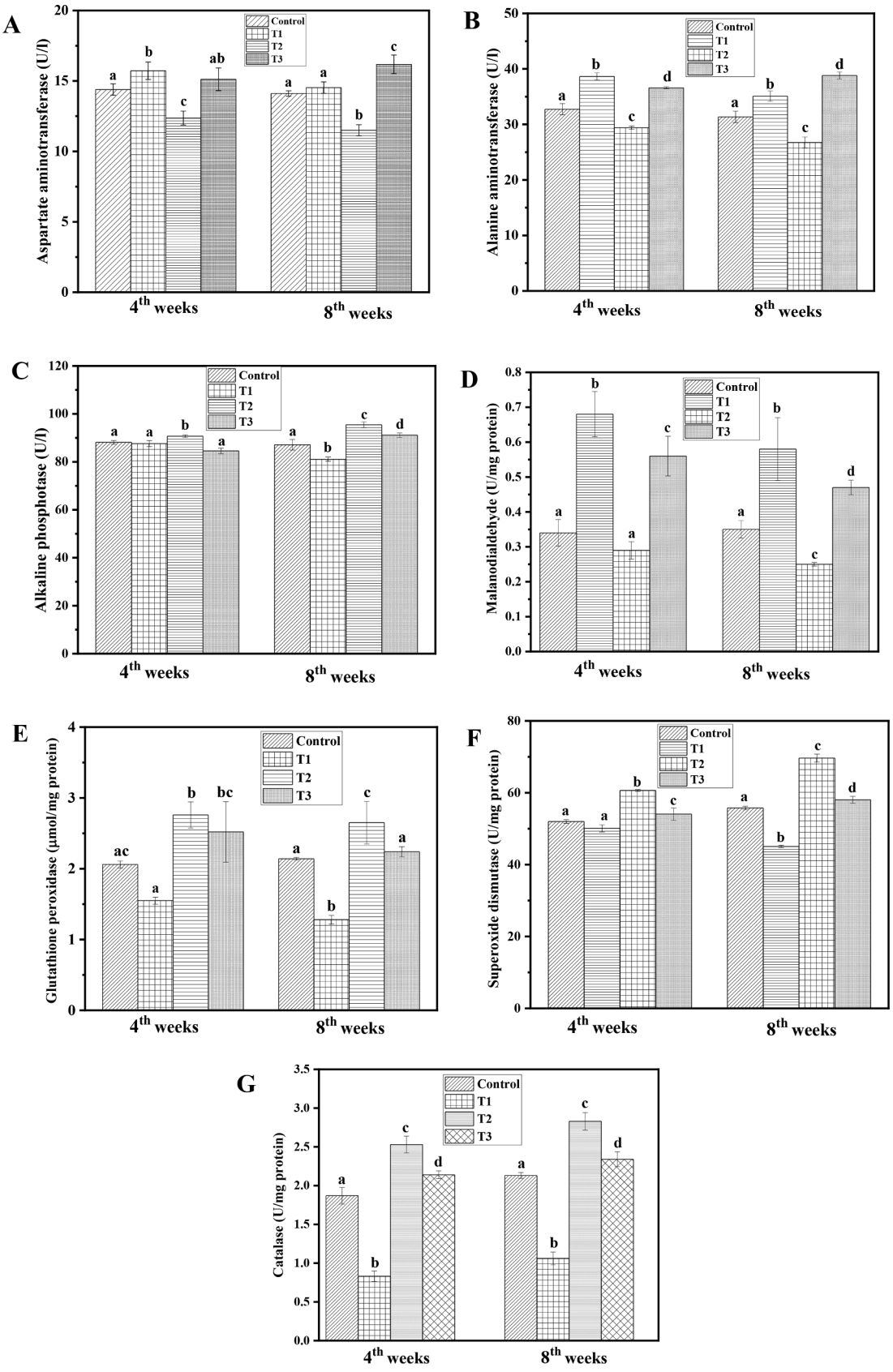

Fig. 3. Biochemical parameters and antioxidative enzyme activities of *Cyprinus carpio* in control and treatment groups after 4th and 8th weeks of supplemented diets. AST (A); ALT (B); ALP (C); MDA (D); GPx (E); SOD (F); and CAT (G).

3.2. Growth performance and whole-body chemical composition

Nanotechnology is an emerging area of research that can be employed for various purposes in the aquaculture industry to improve the production rate. Selenium is considered an essential element that participated in the important biological process for normal fish growth development and it maintain homeostatic functions when it is available in trace amount [57]. The growth performance and feed utilization of common carp fed different combinations of supplemented diets are illustrated in Table 2. The result demonstrates that the growth performance that includes final weight, weight gain, final length, and length gain was improved significantly ($p < 0.05$) after 8th weeks in the group fed AMLP-SeNPs supplementary diet followed by AMLP-AgNPs as compared to the control group. There was no mortality observed in different groups during the feeding trials. The results of SGR, FCR, LER, and PER are presented in Table 1. It indicates that the fish supplemented AMLP-SeNPs and AMLP-AgNPs diets had higher SGR, LER, and PER, but with lower FCR than those fed with AMLP and control diets. Overall, the fish fed supplementary AMLP-SeNPs diet (2 mg/kg) showed significantly ($p < 0.05$) higher final weight, weight gain (WG), and specific growth rate (SGR%) as compared to other groups (AMLP, AMLP-AgNPs, and control). Additionally, the results revealed that the group T2 supplemented with dietary AMLP-SeNPs showed improved PER and reduced FCR value. It indicates that dietary Se enhances protein synthesis in the gastrointestinal tract (GIT) through selenoproteins and improves protein utilization resulting in higher feed utilization thereby improving the growth performance [58]. In addition to this, selenium acts as a cofactor for digestive enzymes which is responsible for their activation and digestion to enhance nutrient absorption by intestinal epithelial cells [59]. Previous studies reported that the common carp (*C. carpio*) and Rohu (*Labeo rohita*) supplemented with dietary nano Se particles with dosages of 0.7–1 mg/kg, and 0.3 mg/kg, respectively exhibited improved growth performance and feed utilization [60,32,43]. Similarly, grass carp (*Ctenopharyngodon idella*) fed with diets of selenium nanoparticles (0.6-0.9 mg/kg) for ten weeks was observed to show improved growth performance and survival rate [61]. Earlier it has been reported that the variation in the amount of selenium requirement was dependent upon fish species, age, feeding duration, experimental conditions, chemical states of Se, and dietary factors [62]. Improved growth performance of fish fed with antioxidant supplemented diets (AMLP-SeNPs) might be due to the beneficial effects of antioxidants on the growth performance of fish. As previously reported selenium (Se) is an important component of the deiodinase enzyme which can stimulate the production of growth hormones indirectly from the pituitary gland in fish and other vertebrates leading to a higher growth rate [63]. The growth performance and feeding efficiency was improved due to increased thyroid hormone activity in fish fed with supplemented nano selenium. The growth performance, health status, and feed utilization have been reported to be improved in *Catla Catla* and *Oreochromis niloticus* supplemented with AgNPs because they exhibited antimicrobial activity and modified the aquatic microflora [64,31]. Earlier studies have also reported that exposure to AgNPs, could increase the population of beneficial microbes like lactic acid bacteria in the intestines which can enhance feed utilization and health improvement [65].

The whole-body proximate muscle composition of *C. carpio* fed AMLP, AMLP-SeNPs, and AMLP-AgNPs supplemented diets are presented in Table 3. The result shows that the proximate composition parameters of fish were not significant ($p < 0.05$) among the groups after 8 weeks of supplementary diets, which indicates that the inclusion of AMLP, AMLP-SeNPs, and AMLP-AgNPs did not affect the muscles composition of common carp. It was observed that the fish that received 2 mg/kg of AMLP-SeNPs showed higher protein and ash content compared to all other groups. However, the ash content of fish exposed to dietary AMLP, and AMLP-AgNPs groups were found to be lower. Therefore, the result suggests that the higher protein and lipid content of fish that received 2 mg/kg AMLP-SeNPs was efficiently utilized by the fish for better growth improvement. It has been observed that dietary selenium plays a key role in the enhancement of growth hormone production and protein synthesis in intestinal epithelial cells which improves the growth and metabolism in *Oncorhynchus mykiss* [66]. Similar observations have been made in *C. carpio* and *Seriola lalandi* fed different sources of selenium supplemented diets [67,68].

Table 2
Growth performance and feed utilization of common carp (*Cyprinus carpio*) fed AMLP, AMLP-SeNPs, and AMLP-AgNPs supplemented diets.

Parameters	Control	AMLP (T1)	AMLP-SeNPs (T2)	AMLP-AgNPs (T3)
Initial length (cm)	2.60 ± 0.22^{a}	2.60 ± 0.22^{a}	2.60 ± 0.22^{a}	2.60 ± 0.22^{a}
Final length (cm)	6.18 ± 0.69^{a}	7.93 ± 0.58^{b}	9.96 ± 0.76c	7.85 ± 0.64^{b}
Initial body weight (g)	2.05 ± 0.43^{a}	2.05 ± 0.43^{a}	2.05 ± 0.43^{a}	2.05 ± 0.43^{a}
Final body weight (g)	4.23 ± 0.22^{a}	6.04 ± 0.35^{b}	11.13 ± 0.45^{a}	8.35 ± 0.99^{c}
LG (cm)	3.58 ± 0.45^{a}	5.33 ± 0.65^{b}	7.36 ± 0.91^{c}	5.25 ± 0.70^{b}
WG (g)	2.18 ± 0.21^{a}	3.99 ± 0.74^{b}	9.07 ± 0.60^{c}	6.30 ± 0.74^{d}
SGR (%)	2.51 ± 0.14^{a}	3.01 ± 0.17^{b}	4.06 ± 0.08^{c}	3.42 ± 0.03^{d}
FCR (g)	2.28 ± 0.07^{a}	2.25 ± 0.08^{a}	1.98 ± 0.01^{b}	2.09 ± 0.04^{b}
PER	0.78 ± 0.07^{a}	1.14 ± 0.08^{b}	1.40 ± 0.01^{c}	1.33 ± 0.04^{d}
LER	2.39 ± 0.02^{a}	3.46 ± 0.16^{b}	4.19 ± 0.03^{c}	3.63 ± 0.08^{b}

Each value is presented as mean ± SD of triplicate analysis. Data with different alphabetical superscript letters are statistically significant ($P < 0.05$). AMLP, *Avicennia marina* leaves polysaccharide; SeNPs, selenium nanoparticle; AgNPs, silver nanoparticle; LG, length gain; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; LER, lipid efficiency ratio.

3.3. Biochemical parameters and antioxidant enzyme assay

The effect of dietary AMLP, AMLP-SeNPs, and AMLP-AgNPs supplementary diet on biochemical parameters of *C. carpio* for 4th and 8th weeks are presented in Table 4. The result shows that the fish supplemented with 2 mg/kg dietary AMLP-SeNPs showed significantly higher total protein and globulin contents but the concentration of albumin was lower as compared to other groups. However, the fish fed with AMLP, and AMLP-AgNPs were observed to decrease in albumin, globulin, and total protein compared to the control groups. It was stated that the total protein, albumin, and globulin levels were decreased because of higher

Table 3
Proximate composition of muscles of *Cyprinus carpio* fed AMLP, AMLP-SeNPs, and AMLP-AgNPs supplemented diets.

Parameters	Control	AMLP (T1)	AMLP-SeNPs (T2)	AMLP-AgNPs (T3)
Protein (%)	18.12 ± 0.93^{a}	19.28 ± 0.14^{a}	19.35 ± 0.085^{a}	19.31 ± 0.22^{a}
Lipid (%)	3.29 ± 0.20^{a}	3.56 ± 0.12^{ab}	3.91 ± 0.20^{a}	3.76 ± 0.31^{b}
Moisture (%)	79.0 ± 0.42^{a}	78.30 ± 0.99^{a}	79.03 ± 0.81^{a}	79.46 ± 0.58^{a}
Ash (%)	0.077 ± 0.002^{a}	0.075 ± 0.0006^{ab}	0.080 ± 0.00^{b}	0.074 ± 0.003^{b}

Each value is presented as mean ± SD of triplicate analysis. Data with different alphabetical superscript letters are statistically significant ($P < 0.05$). AMLP, *Avicennia marina* leaves polysaccharide; SeNPs, selenium nanoparticle; AgNPs, silver nanoparticle.

Table 4
Effect of AMLP, AMLP-SeNPs, and AMLP-AgNPs dietary supplementation on blood biochemical parameters of *Cyprinus carpio* for 8 weeks.

Parameters	Periods/ Treatments	Control	AMLP (T1)	AMLP-SeNPs (T2)	AMLP-AgNPs (T3)
Albumin (g/dl)	4 weeks 8 weeks	1.51 ± 0.11^{a}	1.09 ± 0.10^{b}	1.49 ± 0.36^{a}	0.80 ± 0.04^{b}
		1.59 ± 0.12^{a}	0.93 ± 0.07^{b}	1.56 ± 0.03^{a}	0.97 ± 0.04^{b}
Globulin (g/dl)	4 weeks 8 weeks	2.34 ± 0.15^{a}	1.14 ± 0.07^{b}	2.75 ± 0.27^{a}	2.70 ± 0.34^{a}
		2.81 ± 0.29^{a}	1.92 ± 0.16^{cb}	4.40 ± 0.15^{c}	2.79 ± 0.12^{a}
Total protein (g/dl)	4 weeks 8 weeks	3.85 ± 0.10^{ac}	2.24 ±0.03^{b}	4.24 ± 0.09^{c}	3.6 ± 0.43^{a}
		4.4 ± 0.45^{a}	2.85 ± 0.09^{b}	5.97 ± 0.17^{c}	3.75 ± 0.10^{d}
Cholesterol (mg/ml)	4 weeks 8 weeks	109.71 ± 1.07^{a}	110.99 ± 1.37^{a}	98.12 ± 0.76^{b}	111.02 ± 0.67^{a}
		102.45 ± 0.65^{a}	130.09 ± 0.30^{b}	93.40 ± 0.45^{c}	125.16 ± 3.44^{d}
Triglycerides (mg/ml)	4 weeks 8 weeks	127.77 ± 2.42^{a}	140.11 ± 1.79^{b}	121.64 ± 1.11^{c}	134.23 ± 1.94^{d}
		134.70 ± 2.48^{a}	151.19 ± 1.77^{b}	130.99 ± 1.42^{a}	145.02 ± 4.62^{c}
HDL (mg/ml)	4 weeks 8 weeks	49.22 ± 1.12^{a}	31.44 ± 1.11^{b}	54.21 ± 0.95^{c}	33.19 ± 1.66^{b}
		49.74 ± 0.91^{a}	34.44 ± 0.69^{b}	59.58 ± 0.96^{c}	39.83 ± 2.08^{d}
LDL (mg/mL)	4 weeks 8 weeks	34.12 ± 0.82^{a}	46.05 ± 0.24^{b}	26.17 ± 0.72^{c}	38.93 ± 0.67^{d}
		30.86 ± 0.64^{a}	58.44 ± 0.67^{b}	22.72 ± 055^{c}	36.76 ± 1.41^{d}

Each value is presented as mean ± SD of triplicate analysis. Data with different alphabetical superscripts letters are statistically significant at $P < 0.05$. AMLP, *Avicennia marina* leaves polysaccharide; SeNPs, selenium nanoparticle; AgNPs, silver nanoparticle; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

protein consumption which is used as an energy source or it may be leakage of proteins and amino acid absorption disorder due to damaged kidney and intestine [69,70]. The measurement of serum globulin content and albumin: globulin ratio is related to the immune status of the experimental animals [71]. It has been observed that the high molecular weight globulin protein has a key role in normal liver functions which prevents blood clotting and infections [72]. Therefore, the result indicates that dietary supplementation with AMLP-SeNPs exerted positive effects on the immune status of the common carp by improving the total protein, albumin, and globulin contents. It has been reported that the inclusion of Se improved the hematology and biochemical health of fish. In one of the studies, African catfish fed 0.5 g organic Se/kg was reported to increase in total protein, albumin, and globulin contents [73]. In other reports, it was reported that the concentration of albumin and globulin content increased in Nile tilapia infected with *S. iniae* fed with a 0.7 mg/kg SeNPs supplementary diet [74].

In the present study supplementation of 2 mg/kg of AMLP-SeNPs caused a reduction in total serum cholesterol and triglycerides levels. In addition to this, the cholesterol and triglycerides levels were registered significantly ($p < 0.05$) higher in groups fed AMLP, and AMLP-AgNPs. Elevation of cholesterol and triglycerides in fish is due to disturbance in the metabolism of lipids which is caused by exposure to stressors such as heavy metals [75]. It was reported from previous studies, that the addition of nano form of selenium (1 mg/kg) reduced the concentration of cholesterol and triglycerides [76]. Furthermore, a significant decrease in low-density lipoprotein (LDL) concentration was also observed in groups supplemented with dietary 2 mg/kg of AMLP-SeNPs, and the level of high-density lipoprotein (HDL) increased in *C. carpio,* as reported in previous studies that organic selenium could reduce total cholesterol and LDL levels and increase HDL concentrations [32]. Earlier studies have also demonstrated that the HDL has antioxidant proteins and enzymes due to its antioxidant capacity [77]. As apolipoprotein A-I (Apo-AI) has been reported to be an important factor in HDL it prevents lipid peroxidation in LDL [78]. Thus, in the present study, the fish group fed with 2 mg/kg RMLP-SeNPs increased the level of serum HDL and decreased LDL and MDA concentrations.

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) are the key liver enzymes that are used to measure liver function and their presence in blood plasma at high level indicate injury or damage to the hepatocytes. The results of liver enzymes that include AST, ALT, and ALP activities in *C. carpio* fed AMLP, AMLP-SeNPs, and AMLP-AgNPs dietary supplementation are shown in Fig. 3A, B, and C. In the present study, the AST, ALT, and ALP enzyme activities significantly ($p < 0.05$) increased in the group fed with a diet supplemented with AMLP-AgNPs and AMLP. However, the group fed with AMLP-SeNPs showed significantly lower AST and ALT levels and increased ALP enzymatic activity compared to all other groups after the 4th and 8th weeks of exposure. The result demonstrates that the inclusion of AMLP-SeNPs in fish fed 2 mg/kg nano Se normalized the levels of enzymes after the 4th and 8th weeks of exposure. In one of the studies, it was reported that the elevation of liver enzyme activities causes liver and kidney damage and higher ALP level in blood leads to alterations of enzymes causing skeletal disorders that include osteoporosis and hepatic cell ruptures [79]. It has been reported that the increase in ALP and AST activities in the blood resulted in necrosis of the liver and kidney which is similar to the results obtained by Monfared and Soltani in *Oncorhynchus mykiss* fed with AgNPs [80,81]. Therefore, the result indicates that there is a sign of infections in fish-fed AMLP, and AMLP-AgNPs diets which may cause some chronic stress leading to the impairment of innate and adaptive immune responses. Moreover, the lowest levels of AST and ALT were observed in the group supplemented with a 2 mg/kg diet AMLP-SeNPs which minimized the damage to liver function and injuries to red blood cells. Also, the level of serum ALP activities was found to be higher in AMLP-SeNPs group fish which is indicative of the fact that AMLP-SeNPs dietary supplements could enhance the activities and stimulates the innate immune response in fish. Hence, the result suggests that the supplementation of AMLP-SeNPs normalizes the levels of AST, ALT, and ALP enzymes as nano selenium can scavenge free radicals and protect tissues from oxidative stress. Previous reports have shown that the *C. carpio* fed 1 mg/kg, and 2 mg/kg supplemented SeNPs showed a significant ($p < 0.05$) increase in AST and ALT activities, whereas, ALP did not show any significant difference among groups [32]. In other studies, it was reported that the Asian seabass fed with 4 mg/kg of dietary SeNPs displayed reduced alanine aminotransferase (ALT) and aspartate transaminase levels (AST) activities [82].

Oxidative stress is a threat to aquaculture and it is mainly caused by excessive production of reactive oxygen species (ROS), hydrogen peroxide, and peroxide radicals which impair the oxidative balance at cellular levels. These free radicals triggered lipid peroxidation leading to cellular damage, modification of protein structure, and disease pathogenesis [83]. Selenium (Se) has been considered an essential strong antioxidant micronutrient that plays a key role in the activation of antioxidant defense mechanism through the formation of selenoproteins that helps in the synthesis of glutathione peroxidase enzymes thereby contributing to antioxidative and immune response [84]. During oxidative stress in fish antioxidant response would occur that includes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) which removes the free radicals such as reactive oxygen species [85]. As presented in Fig. 3D, the result revealed that the concentration of MDA was significantly ($p < 0.05$) found to be lower in the group fed 2 mg/kg of AMLP-SeNPs as compared to other groups. In addition to this, the serum GPx, CAT, and SOD activity were significantly ($p < 0.05$) higher in common carp fed 2 mg/kg of AMLP-SeNPs supplemented diets followed by AMLP-AgNPs compared to other groups (Fig. 3E, F, and G). Therefore, the result indicates that the *C. carpio* fed with 2 mg/ kg of AMLP-SeNPs showed improved GPx, CAT, and SOD antioxidants but the level of MDA was significantly lower as

compared to other groups. Moreover, the supplementation of AMLP-SeNPs to the diet is more effective than other groups for activation of antioxidant defense systems against adverse effects of oxidative stress. It has earlier been reported that the inclusion of SeNPs in the diet of fish species such as medaka, crucian carp, common carp, mahseer, striped catfish, red seabream, Nile tilapia, and European seabass enhanced GPx, SOD, and CAT activities while lower malondialdehyde (MDA) levels [74]. The size of the nanoparticles is an important factor in distribution within the organisms, as smaller particles tend to have better movement and absorption in the body. Since larger size nanoparticles (> 200) are detected as a foreign material by the body defense system and could be eliminated from the body. Therefore, the size and concentration of SeNPs used in fish nutrition are significant parameters to enhance growth and metabolism [86]. A similar study was conducted which showed *C. carpio*-fed dietary Se nanoparticles (2 mg/kg) enhanced GPx, SOD, and CAT activities and reduced MDA levels [32]. In another study, dietary SeNPs fed with 4 mg/kg and 1-2 mg/kg showed improved SOD, CAT, and GPx in Asian seabass and enhanced CAT activities in red Sea bream (*Pagrus major*), respectively [82,76].

3.4. Estimation of non-immune specific parameters

The non-specific immune parameters such as myeloperoxidase, respiratory burst, and lysozyme activity of fish serum after 4th and 8th weeks of supplemented AMLP, AMLP-SeNPs, and AMLP-AgNPs feeds are shown in Fig. 4A, B, and C. The results showed that the myeloperoxidase, respiratory burst, and lysozyme activities were significantly ($p < 0.05$) higher in AMLP-SeNPs and AMLP-AgNPs as compared to AMLP and control groups. However, fish fed with 2 mg/kg of AMLP-SeNPs was observed to be significantly ($p < 0.05$) increased in serum immune parameters after 8th weeks of nano selenium supplemented diets compared to other groups. Myeloperoxidase is related to the innate immune function and is used to detect the generation of oxidative radicals and provides a protective response against foreign bacterial pathogens through phagocytic activities of neutrophils [87]. Increased activity of myeloperoxidase indicates that the fish fed with nano form of selenium enhances the phagocyte ability which is responsible for the killing of invading pathogens. The production of B-lymphocytes plays an important role in the enhancement of lysozyme activity which improves the innate immune function of the fish [88]. The white blood cells such as neutrophils and macrophages in fish help in the production of serum lysozyme and provide immune defense from bacterial infection through lysis of the bacterial cells [89]. In the present study, AMLP-SeNPs fed fish showed the highest lysozyme activity which efficiently provides an immune response against bacterial infection. It was reported that the respiratory burst provides innate immunity by stimulation of immune cells and generates an excessive amount of reactive oxygen species (ROS) by phagocytes which can eliminate bacterial pathogens through phagocytic cells [90]. Therefore, in the current study, treatment groups (AMLP and AMLP-AgNPs) fed with supplemented diets provide a

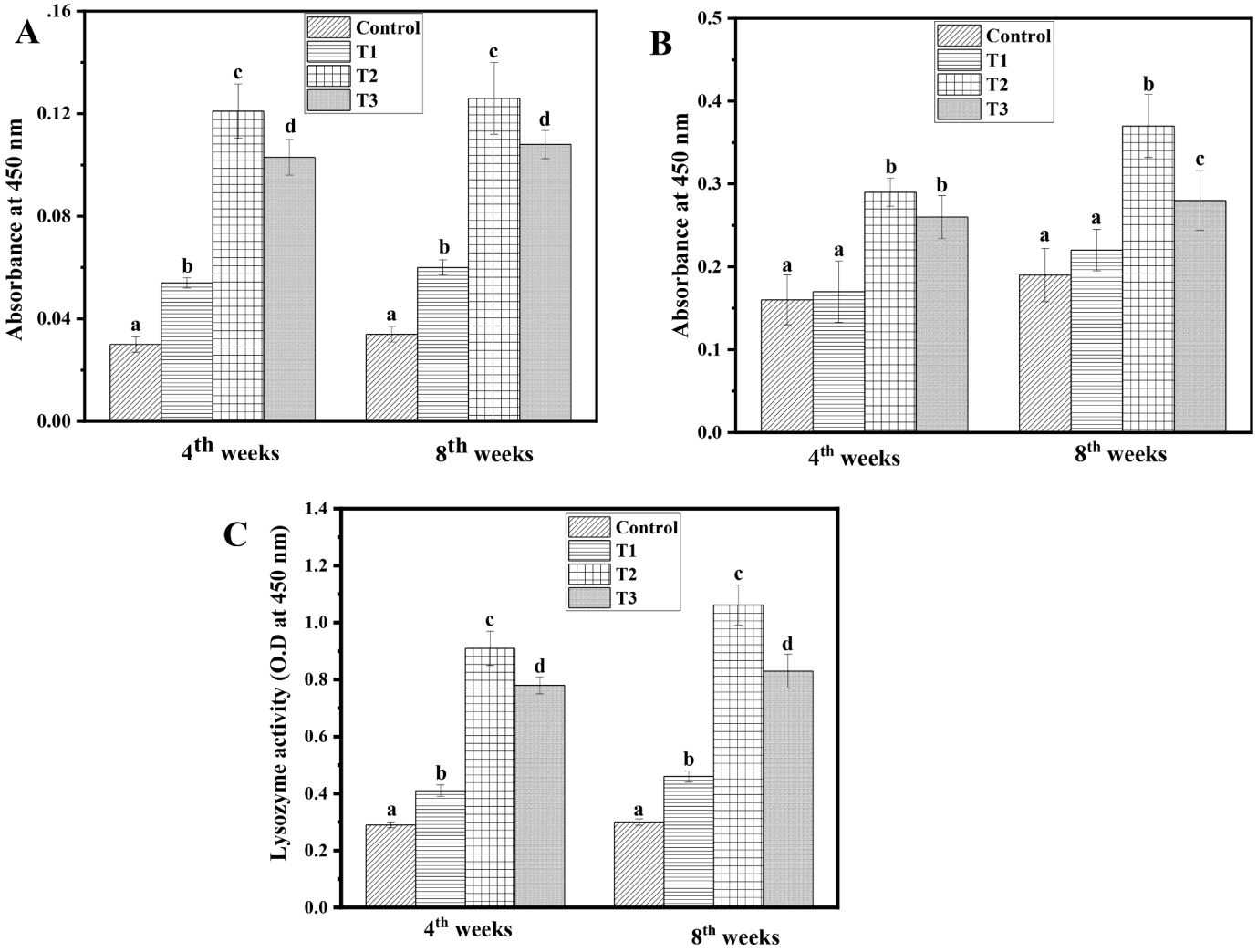

Fig. 4. Non-specific immune parameters of *Cyprinus carpio* in control and treatment groups after 4th and 8th weeks of supplemented diets. Myeloperoxidase activity (A); respiratory burst activity (B); and lysozyme activity (C).

non-specific immune defense system, however, the group fed with 2 mg/kg of AMLP-SeNPs exhibited a superior immune response against *A. hydrophila* infections as compared to other groups. It has been reported in earlier studies that *Labeo rohita*, and *Oreochromis niloticus* which received 0.3 mg/kg, and 1-2 mg/kg of SeNPs supplemented diets, respectively enhanced myeloperoxidase, respiratory burst, and lysozyme activity [43]. The dietary supplementation of 2 mg/kg SeNPs for 30 days in *Oreochromis mossambicus* significantly enhanced myeloperoxidase, respiratory burst, and lysozyme activity [91]. Moreover, the polysaccharides isolated from *Porphyra yezoensis*, and *Ficus carica* have also been reported to significantly ($p < 0.05$) improved serum lysozyme activity in grass carp and crucian carp, respectively [92,93]. In the case of *Oreochromis niloticus* exposed to AgNPs (10 μg/L) showed an increment of lysozyme and respiratory burst activity [31].

3.5. Bacterial challenge study

Various form of stressful conditions impacts aquaculture including sub-lethal ammonia level, high stocking density, and pathogenic infections that cause an imbalance in the physiological and metabolic functions of fish health [94]. The mortality rate, survivable percentage, and relative percentage survivability (RPS) of *C. carpio* after 4th and 8th weeks post-feeding trial challenged with *A. hydrophila* are represented in Table 5. A higher percentage of survivability was observed in fish-fed AMLP-SeNPs supplemented diets at the end of the 4th and 8th weeks of the challenge which was found to be 75 and 84% RPS, respectively. Whereas, the AMLP-AgNPs group showed 67 and 76% of RPS followed by the AMLP group having 42 and 50% RPS, respectively at the end of the 4th and 8th weeks of post-challenge. The lowest percentage of survivable rate was recorded in fish fed with control diets. The AMLP-fed group also showed significant RPS as compared to the control group which could be due to the enhancement of non-specific immune response and disease resistance towards *A. hydrophia.* It was evident from one of the studies, that *Carassius carassius* challenged with *A. hydrophila* for 14 days after 21 days of post-feeding with *Ficus carica* (FCPS), *Radix isatidis* (RIPS) and *Schisandra chinensis* (SCPS) polysaccharides supplemented diets showed 57.9, 47.4, and 42.1% of RPS against *A. hydrophila* infections [95]. Moreover, the fish-fed AMLP-SeNPs and AMLP-AgNPs supplemented diets, significantly ($p < 0.05$) reduced the *A. hydrophila* bacterial load after the 4th and 8th weeks of a challenge compared to AMLP, and control diets. As illustrated in Table 6, *A. hydrophila* counts in the blood of AMLP-SeNPs group fed dietary supplements were found to be 3.64 ± 0.09 and 3.16 ± 0.18 at the end of the 4th and 8th weeks of the challenge, whereas in the case of muscles it was found to be 2.42 ± 0.08 and 2.11 ± 0.13, respectively.

Table 5
Survival rate and relative percentage survival (%) of *Cyprinus carpio* fed AMLP, AMLP-SeNPs, and AMLP-AgNPs and subsequently challenged with *A. hydrophila.*

Groups	No. of challenged	Mortality (%)	Survivability (%)	Relative percentage of survival (%)
4th weeks				
Control	10	80	20	0
AMLP	10	46.67	53.33	42
AMLP-SeNPs	10	20	80	75
AMLP-AgNPs	10	26.67	73.33	67
8th weeks				
Control	10	100	0	0
AMLP	10	50	50	50
AMLP-SeNPs	10	13.33	86.67	84.6
AMLP-AgNPs	10	23.33	76.67	76.7

AMLP, *Avicennia marina* leaves polysaccharide; SeNPs, selenium nanoparticle; AgNPs, silver nanoparticle.

Table 6
Aeromonas hydrophila count in blood and muscles of *Cyprinus carpio* dietary supplemented with AMLP, AMLP-SeNPs, and AMLP-AgNPs after *A. hydrophila* infection at 4th and 8th weeks of challenge study.

Treatment groups	Aeromonas hydrophila count in log10 CFU			
	Blood (log10 CFU/mL)		Muscles (log10 CFU/g)	
	4th weeks	8th weeks	4th weeks	8th weeks
Control	7.49 ± 0.15^{a}	7.87 ± 0.06^{a}	6.73 ± 0.04^{a}	6.34 ± 0.06^{a}
AMLP	4.65 ± 0.07^{b}	4.25 ± 0.13^{b}	3.15 ± 0.13^{b}	2.59 ± 0.07^{c}
AMLP-SeNPs	3.67 ± 0.04^{c}	3.16 ± 0.18^{c}	3.39 ± 0.10^{b}	2.11 ± 0.13^{c}
AMLP-AgNPs	3.87 ± 0.07^{d}	3.64 ± 0.09^{d}	2.73 ± 0.03^{d}	2.42 ± 0.08^{d}

Each value is presented as mean ± SD of triplicate analysis. Data with different alphabetical superscripts letters are statistically significant at $P < 0.05$. AMLP, *Avicennia marina* leaves polysaccharide; SeNPs, selenium nanoparticle; AgNPs, silver nanoparticle.

The *A. hydrophila* counts in the AMLP-SeNPs group were significantly ($p < 0.05$) lower than control and AMLP groups. The present study shows that the highest percentage of survivability and the reduced bacterial count was recorded in group T2 (2 mg/kg AMLP-SeNPs) followed by group T3 (AMLP-AgNPs), which indicates that the inclusion of nano-selenium and silver nanoparticle supplemented diets significantly reduced the mortality rate by reducing the bacterial loads and develop resistance against *A. hydrophila* infections. AgNPs showed higher RPS because it inhibits bacterial cells through the binding of Ag^{+} ions to proteins, enzymes, DNA, RNA, and other micro and macromolecules leading to disruption of microbial metabolism and survival [96]. AgNPs are known to exhibit antimicrobial activity against several aquatic pathogens such as *A. salmonicida, A. hydrophila*, and *A. veronii* by decreasing total bacterial count and enhancing survivability in challenged fish [97,10,98]. Also, it was reported that the SeNPs used as an immunostimulant in fish could improve the innate immune response *via* regulating the redox-sensitive transcription factors and activating stress-related genes thus it prevents aquatic infection [99]. Moreover, an adequate amount of Se is required for stimulation of Th1-type cytokine response through intracellular signaling and to prevent cells against anti-inflammatory Th2-type humoral response [100]. Previously, it has been stated that the Nile-tilapia and rohu fed with 1-2 mg/kg and 0.3 mg/kg dietary Se nanoparticles exhibited higher resistance against *A. sobria*, and *A. hydrophila* infection respectively [91]. In other studies, it has been reported that the *P. hypophthalmus* treated with 1-2 mg/kg dietary nano selenium showed protective resistance against *A. veronii* biovar *sobaria* [18]. The present study also suggested that the nanoform of selenium is a highly effective immunostimulant to prevent infections in challenged fish compared to other groups. This is because *Avicennia marina* leaves polysaccharide (AMLP) exhibits potential biological activities such as antioxidant, antibacterial, and antibiofilm activity which enhances the bioactive properties of SeNPs.

4. Conclusion

In the present study, polysaccharide (AMLP) is used for the biosynthesis of AMLP-AgNPs, and AMLP-SeNPs, and it is further characterized by HR-TEM, XRD, and DLS. The result demonstrates that the synthesized nanoparticles were well-stabilized spherical, crystalline structures. Thereafter, the comparative effect of supplemented forms of AMLP, AMLP-AgNPs, and AMLP-SeNPs diets was investigated in a *C. carpio*. Overall, the results of the present study revealed that the dietary supplementation of nano selenium (AMLP-SeNPs) along with diets enhances the growth performance, and antioxidant enzyme activity, and enhances the immune response against *A. hydrophila* infection in *C. carpio.* However, AMLP-AgNPs supplemented diets exhibited a positive effect on the immune status of *C. carpio*, but the elevation of serum

AST and ALT activity might be indicative of the toxic effect. Therefore, AMLP-SeNPs enriched diets may be recommended for better improvement of biological, metabolic, and physiological functions of *C. carpio* due to their higher bioavailability, bioefficacy, and lower toxicity. The findings showed that using nanotechnology in aquaculture might open up new possibilities, such as minimizing feed nutrient losses, promoting faster growth, controlling diseases and potentially lowering production costs which increase the productivity of aquatic animals. In the food industry, the application of nanotechnology in aquaculture can make a significant contribution to the long-term provision of nutritious goods. The findings also showed that common carp developmental characteristics may be used to estimate the environmental danger of chemicals and nanomaterials.

Declaration of Competing Interest

All the authors of this manuscript declare that there were no conflicts of interest with funding agencies or any other associated research organizations.

There is no conflict of interest among the authors

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Supplementary materials

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