Interactive Effects of Curcumin and Silver Nanoparticles on Growth, Hemato-Biochemical Parameters, Digestive Enzymes Activity and Histology of Common Carp (*Cyprinus Carpio*)

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Research

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Abstract

Background: Promising physicochemical characteristics of nanoparticles (NPs) have encouraged their increasing synthesis and application in various industries which is accompanied with their leakage to the environment with inevitable effects on non-target animals including fish. Accordingly, developing strategies to protect fish against NPs contamination has gained attention. The aims of this study were to (a) explore the effects of feed born silver nanoparticles (AgNPs) in common carp (*Cyprinus carpio*), and (b) to examine whether dietary curcumin supplementation can ameliorate the impacts of AgNPs on growth, hemato-biochemical parameters, digestive enzymes activity and organ histology.

Methods: Nine experimental diets were prepared with three different levels of AgNPs (0, 0.05 and 0.15 g/kg) and curcumin (0, 0.75 and 1.5 g/kg) and fed to triplicate groups of common carp (4.82 ± 0.41 g) for 60 days.

Results: The results showed that AgNPs significantly ($P < 0.05$) reduces growth performance and enhances feed conversion ratio in a dose dependent manner. Supplementing 0.75 g/kg curcumin at lower AgNPs level improved growth rate while its inclusion at higher AgNPs level further hampered fish growth. A similar trend was observed for survival rate. The highest hematocrit and hemoglobin concentrations and white blood cells count were recorded in the group received 0.75 g/kg curcumin, and inclusion of the same dose of curcumin in the diet containing 0.05 g/kg AgNPs retrieved the reduction of these parameters. Serum aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase activities, and glucose, cholesterol and triglyceride concentrations were enhanced by increasing AgNPs level, and curcumin inclusion particularly at lower level of AgNPs significantly decreased their values. Activity of digestive enzymes including alkaline protease and lipase were progressively decreased by increasing AgNPs level and significant improvements were found by curcumin application at lower AgNPs level. In addition, severe pathological changes in liver and intestine were observed at high concentrations of AgNPs and curcumin.

Conclusions: The findings in this study demonstrated that dietary supplementation of curcumin at lower dose of AgNPs can restrain the toxic effects, however, its inclusion at higher dose of AgNPs exacerbated the negative impacts.

Background

Nowadays, nanoparticles (NPs) synthesis and usage have been considerably increasing because of their novel and unique physicochemical properties that differ substantially from bulk materials of the same composition [1]. Rapid development of the nanotechnology industry has been coupled with their inevitable discharge into the environment [2]. In aquatic systems, NPs can impact non-target animals such as fish and aquatic birds. The toxic effects of NPs might be physiological, biochemical or pathological causing complex damages at the level of cells, tissues, organs and ultimately the organism. A common approach to study the toxic effects of metal oxide NPs is through assessment of physiological status of different species [3]. Evaluation of physiological responses is used as a diagnostic tool in fish toxicology studies to identify their general health status, and the functionality of organs affected by toxicants [4, 5]. There are many studies regarding probable effects of NPs within a water body and their interactions with the aquatic organisms [6–10]. However, information regarding the effects of NPs on physiological responses (e.g., blood indices, gastrointestinal tract) especially via food/ feed, cosmetics and drug delivery systems is inconclusive [11].

Recently, some studies have been implemented to discover simple and adequate strategies (e.g., natural antioxidants) to decrease the adverse impacts of toxins on the health of aquatic organisms including fish [12–14]. Application of natural antioxidants is a promising area of interest due to their ameliorative effects on chronic disease treatment and other oxidative stress-related disorders [15, 16]. It has been shown that dietary antioxidants could alter carcinogen activation and detoxification mechanisms in various animals [17–19]. Curcumin is a naturally occurring phenolic secondary plant metabolite of the ginger family (Zingiberaceae) [20] which is commonly used as a food colorant, spice, traditional medicine and nutritional supplement [21]. It has a variety of biological and pharmacological activities including antioxidative, anti-inflammatory, anti-mutagenic, anticarcinogenic and anti-microbial properties [22–25]. It is believed that its antibacterial properties are mainly due to its mono-carbonyl and bioactive conjugated derivatives [26, 27]. Some studies have shown the beneficial effects of curcumin on aquatic animals especially on their hepatic oxidative enzymes activity [28] and gut microbiota [12].

In the completion of our previous study, the present study was designed to evaluate the probable effects of feed AgNPs contamination on growth performance, hemato-biochemical parameters as well as digestive enzymes activity of common carp (*Cyprinus carpio*). We also tried to elucidate whether dietary curcumin can prevent the potential damages of such dietary contamination.

Material And Methods

Experimental diets

Formulation and proximate composition of the basal diet are presented in Table 1. Silver nano-powder (Ag, 99.99 %, 20 nm, metal basis) was obtained from US Research Nanomaterial, Inc. [12]. All chemicals, solvents and culture media were of analytical grade and supplied by Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, USA) companies. The experiment was conducted in nine different treatments with three replicates as a completely randomized design. The treatments were consisted of different combinations of dietary AgNPs (0, 0.05 and 0.15 g/kg feed) and curcumin (0, 0.75 and 1.5 g/kg feed) inclusion levels in the basal diet (Table 2). After a thorough mixing and adding distilled water, the pellets were made, and oven dried at 45 °C overnight. Afterwards, they were crumbled, sieved to remove feed dusts and stored in plastic bags with hygroscopic gel at 4 °C [29].

Fish and experimental conditions

Common cap fingerlings with an average weight of 4.82 ± 0.41 g were obtained from a local farm. Six hundred seventy-five fingerlings were randomly allocated into 27 fiberglass tanks (containing 150 L water) and were acclimatized for 2 weeks prior to the experiment. During this period fish were fed 0.5 %

body weight (BW) once a day with the basal diet [30]. The feeding trial was lasted for 60 days under natural photoperiod. In the course of the experiment, fish were fed three times a day at the rate of 3 % BW [31]. The water pH (7.3), dissolved oxygen content (7.6 mg L^{-1}) and temperature (23 °C) of tanks were monitored daily. In addition, daily water exchange rate was 50 %.

Sample collection

The fish were fasted for 24 h before sampling and were anaesthetized with 200 ppm clove oil solution to minimize stress on fish. All the fish in each tank were counted and weighed collectively for calculation of survival and growth parameters. Blood was withdrawn from the caudal vein using 2-mL sterilized hypodermic syringes. The collected blood samples were divided into two portions. One portion was transferred into Eppendorf tubes containing heparin and immediately used for hematological examination. The second portion was transferred into Eppendorf tubes, left to clot at room temperature for 30 min, and centrifuged at 1500 ×*g* for 15 min. The collected serum was stored at –80 ºC for further biochemical analyses.

Hematology assays

The red blood cell (RBC) and white blood cell (WBC) counts were determined using Neubaeur hemocytometer [32]. Hematocrit (Ht) was measured using the standard microhematocrit method and hemoglobin concentration (Hb) was measured by the cyanomethemoglobin spectrophotometry method [33]. Differential leukocyte counts (monocyte, lymphocyte and neutrophil) were carried out using Giemsa staining method under light microscope [34]. The blood indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Seiverd [35].

Blood biochemistry assays

Albumin, cholesterol, glucose and triglyceride concentrations were measured using commercial kits (Pars Azmoon, Tehran, Iran) using an autoanalyzer (Labsystemphotic100, Japan) [36]. Serum total protein level was quantified using a BioRad Protein Assay Kit (No. 500 - 0006, Bio-Rad Laboratories, New Orleans, LA, USA) using bovine serum albumin as the standard, and following the method described by Bradford [37]. The activity of inflammatory enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), alkaline phosphatase (ALP) according to the Deutsche Gesellschaft fur Klinische Chemie (DGKC) method and lactate dehydrogenase (LDH) in compliance with DGKC (P-L) [38].

Digestive enzymes activity

After removing visceral fat on ice, intestine was dissected out and washed by cold normal saline solution and stored at −80 °C until extraction [39]. The samples were homogenized in 1:3 (w/v) cold 50 mM tris-HCl buffer (pH = 7.5), using Polytron PT 1300 D homogenizer with a 7 mm generator at a setting of 10 for 3 × 30 s. The homogenate was centrifuged at 10,000 ×*g* for 20 min at 4 °C and the supernatant was collected and stored at −80 °C until analyses [40].

Amylase activity was assayed by means of a starch-iodine detection following Metais and Bieth [41]. Briefly, 50 µL of enzymatic extract was mixed with the substrate (3 g L^{-1} starch in 66 mM Na_2PO_4) and incubated for 20 min at 25 °C. The reaction was stopped with 20 µL of 1 N HCl, and after addition of 2 mL of 0.33 mM iodine solution the absorbance was read at 580 nm. One unit of α-amylase activity was defined as the mg starch hydrolyzed per min at 25 °C.

Lipase activity was determined by hydrolysis of nnitrophenyl myristate. Each assay (0.5 ml) contained 0.53 mM nnitrophenyl myristate, 0.25 mM 2-methoxyethanol, 5 mM sodium cholate and 0.25 M Tris–HCl (pH 9.0). Incubation was carried out for 15 min at 30 °C, and the reaction was terminated by adding 0.7 ml of acetone/n-heptane (5:2, v/v). The reaction mixture was vigorously mixed and centrifuged at 6080 g for 2 min. The absorbance at 405 nm in the resulting lower aqueous layer was measured. The extinction coefficient of n-nitrophenol was 16,500 $M^{-1}cm^{-1}$ l^{-1}. One unit of enzyme activity was defined as 1 µmol of n-nitrophenol released per min [42].

Alkaline protease activity was assayed as described by García-Carreño and Haard [43] using Azocasein 2% in Tris-HCl, pH = 7.5 as substrate. The specific enzyme activity was reported as unit activity per mg protein per min.

Histological studies

The intestinal, hepatic and gill tissue samples were fixed in Bouin's solution for 72 h. Afterwards, samples were submitted to standard tissue passage procedures for histological examinations by 70%, 80%, 90% and 100% ethanol (Razi, Iran) and finally were embedded in paraffin blocks (Merck, Germany). Sections of 4 µm thickness were prepared and stained with hematoxylin and eosin (Merck, Germany). The slides were analyzed under light microscope (Olympus, Germany). Tissue pathological changes including atrophic or elongated nuclei of hepatocells, morphologically abnormal lamellar body, necrosis and hyperplasic lamellas of gill tissue along with increased goblet cell population and epithelial hyperplasia of intestinal tissue were observed and graded as normal (0), mild (+1), moderate (+2), severe (+3) and very severe (+4).

Statistical analysis

The homoscedasticity of variance of the dependent variables was checked by Levene's test. Standard normality test of Kolmogorov-Smirnov was applied to determine normality of data set. Two-way ANOVA was used to elucidate whether or not there were significant differences among various experimental groups. Tukey's HSD test at $P<0.05$ was used to assess significant differences among treatments. All statistical analyses were carried out using SPSS statistical software (version 19, SPSS, Inc., Chicago, IL).

Results

Growth performance

Two-way ANOVA results revealed that all growth indices were affected by two-way interaction of dietary AgNPs and curcumin supplementation (Table 3, $P < 0.05$). Dietary inclusion of AgNPs led to the significant reduction ($P < 0.05$) of final body weight, weight gain and specific growth rate in a dose dependent manner. Curcumin supplementation in diets contacting 0.05 g/kg AgNPs improved growth performance but its inclusion at higher level of AgNPs aggravated the growth depression. A progressive increase in feed conversion ratio (FCR) was observed by increasing AgNPs level and supplementing 0.75 g/kg curcumin at lower level of AgNPs decreased FCR. A similar trend was observed for fish survival rate. Curcumin supplementation did not influence the abovementioned parameters compared to the control group.

Hemato-biochemical Parameters

All hemato-biochemical parameters were interactively affected by dietary AgNPs and curcumin contents ($P < 0.05$). Ht and Hb concentrations, and WBC and RBC counts were significantly decreased by AgNPs level, and curcumin inclusion in diets containing 0.05 g/kg AgNPs significantly enhanced their values (Table 4). Moreover, the highest Ht, Hb, WBC and RBC values were detected in fish fed the diet containing 0.75 g/kg curcumin. Higher MCV values were found in groups received AgNPs containing diets and curcumin supplementation reduced its value. MCHC was remarkably decreased by AgNPs inclusion and a significant improvement was found by supplementing 0.75 g/kg curcumin in the diet containing lower level of AgNPs.

Serum glucose, cholesterol and triglyceride concentrations, and activities of AST, ALT and LDH were notably increased by AgNPs inclusion and curcumin supplementation at lower AgNPs level decreased their values. Serum protein content significantly decreased at both levels of AgNPs and inclusion of curcumin in diets containing 0.05 g/kg AgNPs significantly improved the protein concentration. The significant reduction of ALP activity at lower level of AgNPs was retrieved by curcumin application (Table 5).

Histology

Hepatocyte nucleus narrowing and atrophy were observed in fish fed diets containing different concentrations of AgNPs and curcumin (Fig. 1). The highest liver tissue destruction was detected in treatments containing high concentrations of AgNPs and curcumin (Table 7). No lesions were observed in liver tissue of fish fed with 0.75 g/kg curcumin. Gill blades deformation, necrosis and hyperplasia as well as sticking of the end of the gill fibers were observed in fish fed diets containing different concentrations of AgNPs and curcumin (Fig. 2). The highest gill tissue destruction was detected in treatments containing high concentrations of AgNPs and curcumin (Table 7). No lesions were observed in gill tissue of fish fed with 0.75 g/kg curcumin. Increased number of goblet cells and intestinal epithelial hyperplasia were observed in fish fed diets containing different concentrations of AgNPs and curcumin (Fig. 3). The severe pathological changes in intestine tissue were observed in treatments containing high concentrations of AgNPs and curcumin (Table 7). Similarly, no changes or lesions were observed in intestine of fish fed diet contained 0.75 g /kg of curcumin.

Discussion

Pollutants including nano-sized materials discharged into water bodies affect aquatic ecosystems via their uptake by the aquatic organisms and their accumulation in animal tissues over time leading to subsequent gastrointestinal toxicity [44]. Our results showed that feed born AgNPs hampers growth performance of common carp, and curcumin supplementation at lower inclusion level could partially prevent suppressive effect of AgNPs. Moreover, no adverse effect of curcumin was found on fish growth performance which is consistent with the results of studies by Lee et al. [45], Jang et al. [46] and Imani et al. [14] indicating no impacts of plant extracts on animal feed intake, weight gain and feed conversion ratio. Dose dependent reduction of growth performance by AgNPs in this study agrees with depression of growth performance in *Clarias batrachus* [47] and *Epinephelus coioides* [48] by AgNPs and copper nanoparticles, respectively. However, Ramsden et al. [49] showed that titanium dioxide nanoparticles had no effects on growth of rainbow trout (*Oncorhynchus mykiss*) but resulted in subtle biochemical disturbances in the brain.

All hematological parameters (with exception of hematocrit) were beneficially influenced by application of curcumin in AgNPs containing diets. Our results are in accordance with findings of Shaluei et al. [50] in silver carp (*Hypophthalmichthys molitrix*) and Laban et al. [51] in fathead minnow (*Pimephales promelas*). The reduction of RBC count, and Hb and Ht levels by AgNPs in this study could be due to following reasons: (a) erythropoiesis disorder and the formation of RBC, (b) the conditions of confinement or stress induced by the lack of food and (c) lysing of RBC due to toxicant stress [52, 53].

Blood parameters (biochemical and hematology) are used as useful diagnostic tools for monitoring health status, detecting illnesses, and following the progress of disease and response to therapy in organisms [54]. For instance, blood enzymes (e.g., ALT, AST, LDH) are often used in diagnosis of fish diseases and detection of tissue damages caused by environmental pollutants [55]. Large quantities of ALT and AST are released into blood stream mostly during hepatocytes damage, so analysis of their concentrations in serum can contribute to the monitoring of liver function and health status [56]. In the present study, increased AST, ALT and LDH activities were detected in fish fed AgNPs containing diets probably indicating cellular membrane damage and increased enzymes leakage. This is consistent with the results of previous studies on rainbow trout [54] and Caspian kutum (*Rutilus kutum*) [57]. Furthermore, our results showed that curcumin inclusion in AgNPs containing diets can remarkably reduce serum AST, ALT and LDH activities indicating that its application can protect hepatocytes against AgNPs induced damages. The protective effects of curcumin on blood biochemical parameters could be attributed to the presence of various compounds found in plant extracts (e.g., antioxidants and flavonoids) which beneficially influence cellular physiological functions (e.g., improving antioxidant capacity and membrane stability, and preventing leakage of intracellular enzymes into the blood during oxidative stress) [7, 58].

Digestive enzymes have been widely used for assessment of physiological status including growth phase of fish, and been implicated in monitoring water bodies [59]. It has been shown that fish experiencing different environmental conditions would express different digestive enzymes activity and they can regain their digestive capacity after removal of constraints [60–62]. In the present study, dietary inclusion of AgNPs suppressed lipase and alkaline protease activities and increased the amylase activity. Likewise, Le Bihan et al. [63] showed that silver exposure results in lower protease activity in cuttlefish (*Sepia officinalis*). Also, the results of another study by Wang et al. [48] revealed that exposure to copper nanoparticles and copper sulphate in grouper (*E. coioides*) results in depressed digestive enzymes activity (protease, amylase and lipase). In contrast, Samanta et al. [64] reported enhancement of digestive enzymes activity in three teleost species including *Anabas testudineus*, *Heteropneustes fossilis* and *Oreochromis niloticus* following herbicide Almix exposure. These authors suggested that fish might respond to increased energy requirement through regulating digestive enzymes profile. However, such elevation in digestive enzymes activity might be also an indication of pancreatitis [65, 66] which remains to be elucidated in the future. In the present study, the sole curcumin supplementation led to significant reduction of lipase and amylase activities compared to the control. Furthermore, its inclusion in diets containing 0.05 g/kg AgNPs retrieved the reduction of lipase and protease activities. Similarly, Imani et al. [14] showed that cinnamon oil supplementation in diets for rainbow trout lowers digestive enzymes activity. Also, Nazdar et al. [13] showed that dietary silymarin inclusion could partially prevent toxic effects of NiO-NPs in rainbow trout. However, in this study curcumin application could not provide enough protection at higher concentration of AgNPs. This finding implies that supplemental effect of plant metabolites is variable depending on the duration of feeding trial, sampling time, feed composition, and toxicant moiety and type.

Fish histology could serve as a model for studying the interactions between environmental factors and organs structures and functions [67]. Depositions and toxicity of NPs in various organs and its histopathological changes such as atrophy, deformation, necrosis and hyperplasia were previously reported in different fish species [68–71]. In this regards, dietary additives can influence fish physiology and protect fish against harmful agents [72]. In the present study, histological alterations in different tissues (gill, liver and intestine) of the fish fed on diets supplemented with or without curcumin, were investigated after exposing fish with AgNPs. According to our results, the highest tissues destruction was observed in fish fed on diets containing high concentrations of AgNPs. While, dietary supplementation of curcumin at lower concentration of AgNPs had restrained the toxic effects. Our finding was in accordance with the finding of Murali et al. [73] who has studied the chronic exposure effects of AL_2O_3 NPs in *Oreochromis mossambicus*. In another study, Alkaladi et al. [69] investigated the chronic exposure effects of ZnONPs in *O. niloticus*. Their results showed that the supplementation of a mixture of vitamin C and E has a beneficial effect against the ZnONPs toxicity, which leads to reduction in abnormal tissues changes. Hajirezaee et al. [71] also reported that supplementation of diet with vitamin C at a level of 500 to 1000 mg/kg of feed could effectively prevent undesirable effects of TiO_2-NPs in liver of *C. carpio*. In addition, histological analysis revealed the altered morphology in studied tissues following the exposure of fish to NPs. These histological changes might occur due to various biochemical and molecular level changes as a result of NPs induced stress. Therefore, further studies especially at the molecular level are needed.

Conclusions

In summary, the results of this research suggested that curcumin supplementation in diet containing lower concentration of AgNPs can retrieve the adverse effects of AgNPs on fish growth performance, feed utilization and digestive enzymes activity.

Declarations

Ethics approval

The experimental fish were used after approval of the experimental protocol by the ethics committee of Faculty of Veterinary Medicine, Urmia University.

Consent for publication

Not applicable.

Availability of data and materials

The datasets produced and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

There are no conflicts to declare.

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Authors contribution

Conceptualization, Validation, Visualization, Methodology: ZK, KS, AI, SR. Investigation: ZK KS, AI. Formal analysis, collection and interpretation of data: ZK, KS, AI, SB. Manuscript drafting, review & editing: ZK, KS, AI, SB, TP, SR. Funding acquisition, Project administration, Resources: KS, AI, TP.

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Tables

Table 1. Formulation and proximate composition of the basal diet.

Ingredients (% dry matter)		Proximate composition (%)	
Soybean meal	46	Dry matter	92
Wheat meal	19.5	Protein	40
Wheat flour	16	Lipid	11
Kilka fish meal	5	Fiber	5
Kilka fish oil	2.5	Ash	12
Soybean oil	3	Nitrogen-free extract	24
Calcium di-phosphate	2.5	Crude energy [b] (MJ/kg)	18.8
Vitamin premix	0.5		
Mineral premix	0.5		
Vitamin C (stable)	0.23		
Choline chloride	0.22		
Methionine	0.2		
BHT [a]	0.1		
Binder	0.3		
Cellulose	3.35		

[a] Butylated hydroxytoluene

[b] Crude energy content of the diet was calculated according to protein (5.64 kcal/g), ether extract (9.44 kcal/g) and carbohydrate (4.11 kcal/g) energy contents according to NRC (1983).

Table 2. Specifications of the experimental diets.

Treatments	Nomenclature	Diets specification
1	Ctrl	Basal diet
2	Cur0.75	Basal diet + 0.75 g/kg curcumin
3	Cur1.5	Basal diet + 1.5 g/kg curcumin
4	AgNPs0.05	Basal diet + 0.05 g/kg AgNPs
5	AgNPs0.15	Basal diet + 0.15 g/kg AgNPs
6	Cur0.75/AgNPs0.05	Basal diet + 0.75 g/kg curcumin + 0.05 g/kg AgNPs
7	Cur0.75/AgNPs0.15	Basal diet + 0.75 g/kg curcumin + 0.15 g/kg AgNPs
8	Cur1.5/AgNPs0.05	Basal diet + 1.5 g/kg curcumin +0.05 g/kg AgNPs
9	Cur1.5/AgNPs0.15	Basal diet + 1.5 g/kg curcumin + 0.15 g/kg AgNPs

Table 3. Growth performance, feed utilization and survival of common carp fed the experimental diets for 60 days.

Treatments	FBW[a]	WG[b]	SGR[c]	FCR[d]	Survival (%)
Ctrl	21.1±0.13^{a}	16.4±0.45^{a}	2.49±0.12^{ab}	2.26±0.10^{f}	98.7±2.31^{a}
Cur0.75	21.1±0.26^{a}	16.3±0.30^{a}	2.48±0.13^{ab}	2.31±0.10^{f}	98.7±2.31^{a}
Cur1.5	21.0±0.18^{a}	16.5±0.12^{a}	2.56±0.08^{a}	2.34±0.10^{f}	90.7±2.31^{a}
AgNPs0.05	16.5±0.49^{c}	11.6±0.60^{c}	2.04±0.16^{cd}	3.04±0.15^{d}	61.3±6.11^{c}
AgNPs0.15	15.5±0.18^{d}	10.6±1.80^{c}	1.93±0.13^{cde}	3.32±0.10^{c}	45.3±2.31^{d}
Cur0.75/AgNPs0.05	17.5±0.23^{b}	12.7±0.53^{b}	2.16±0.07^{bc}	2.73±0.05^{e}	77.3±2.31^{b}
Cur0.75/AgNPs0.15	13.5±0.10^{e}	8.77±0.35^{d}	1.75±0.12^{de}	3.71±0.10^{b}	24.0±4.01^{e}
Cur1.5/AgNPs0.05	16.1±0.11^{c}	11.5±0.40^{c}	2.09±0.12^{cd}	3.01±0.10^{d}	62.7±2.31^{c}
Cur1.5/AgNPs0.15	12.0±0.14^{f}	7.36±0.35^{e}	1.58±0.15^{e}	4.34±0.10^{a}	20.0±4.01^{e}

Values in the same column having different superscripts are significantly different ($P < 0.05$).

[a] Final body weight (g).

[b]Weight gain (g) = (Final mean body weight – initial mean body weight).

[c]Specific growth rate (%) = [(ln final body weight − ln initial body weight) / days] × 100.

[d]Feed conversion ratio = dry feed fed / wet weight gain.

Table 4. Hematological parameters of common carp fed the experimental diets for 60 days.

Treatments	Hct[a]	Hb[b]	MCV[c]	MCHC[d]	WBC[e]	RBC[f]
Ctrl	25.47±1.16^{a}	5.80±0.30^{ab}	210.75±15.30^{bcd}	22.77±0.20^{ab}	4.77±0.35^{b}	1.21±0.04^{b}
Cur0.75	26.43±1.07^{a}	6.23±0.30^{a}	183.71±10.50^{d}	23.64±2.10^{ab}	6.23±0.31^{a}	1.43±0.04^{a}
Cur1.5	25.13±0.80^{ab}	5.73±0.31^{ab}	183.51±7.49^{d}	22.84±1.70^{ab}	4.87±0.31^{b}	1.37±0.02^{a}
AgNPs0.05	20.77±1.11^{c}	3.73±0.20^{cd}	241.49±11.40^{ab}	18.03±1.70^{d}	3.47±0.25^{cd}	0.86±0.02^{d}
AgNPs0.15	18.23±0.61^{d}	3.43±0.21^{d}	244.16±4.00^{a}	18.83±1.10^{cd}	3.23±0.15^{cd}	0.74±0.02^{e}
Cur0.75/AgNPs0.05	22.87±1.07^{bc}	5.63±0.20^{ab}	183.40±6.10^{d}	24.68±1.80^{a}	4.63±0.15^{b}	1.24±0.03^{b}
Cur0.75/AgNPs0.15	21.27±0.61^{c}	4.27±0.20^{cd}	200.91±12.40^{cd}	20.06±0.10b^{cd}	3.53±0.28^{cd}	1.06±0.04^{c}
Cur1.5/AgNPs0.05	21.27±0.35^{c}	4.84±0.31^{bc}	217.29±11.40^{abc}	22.72±1.00^{ab}	3.77±0.15^{c}	0.98±0.04^{c}
Cur1.5/AgNPs0.15	17.17±0.71^{d}	3.83±0.20^{cd}	197.59±13.9^{cd}	22.34±0.90^{abc}	3.07±0.15^{d}	0.87±0.03^{d}

Values in the same column having different superscripts are significantly different ($P < 0.05$).

[a]Hematocrit (%)

[b]Hemoglobin (g l^{-1})

[c]Mean corpuscular volume (fl).

[d]Mean corpuscular hemoglobin concentration (%).

[e]White blood cells count (×10^3 cell ml^{-1}).

[f]Red blood cells count (×10^6 cell ml^{-1}).

Table 5. Blood biochemical parameters of common carp fed the experimental diets for 60 days.

Treatments	Protein (g dl^{-1})	Albumin (g dl^{-1})	Glucose (mg dl^{-1})	Cholesterol (mg dl^{-1})	Triglyceride (mg dl^{-1})	AST[a] (IU l^{-1})	ALT[b] (IU l^{-1})	ALP[c] (IU l^{-1})	LD (IU
Ctrl	3.58±0.23^{a}	0.59±0.06^{ab}	77.33±1.60^{bc}	66.67±0.80^{c}	97.83±1.50^{d}	88.83±1.40^{fg}	21.87±1.30^{d}	74.47±1.41^{a}	21
Cur0.75	3.54±0.02^{a}	0.60±0.05^{a}	74.33±1.61^{c}	56.33±1.31^{d}	86.93±1.31^{e}	83.77±1.51^{gh}	16.57±0.71^{e}	77.33±1.02^{a}	20
Cur1.5	3.46±0.11^{ab}	0.46±0.09^{abc}	60.43±0.90^{d}	53.67±0.80^{d}	79.23±1.40^{f}	82.87±1.81^{h}	17.53±0.71^{e}	77.97±1.01^{a}	21
AgNPs0.05	2.83±0.09^{cd}	0.44±0.09^{abc}	82.33±1.71^{a}	83.67±1.12^{a}	116.63±1.20^{b}	134.37±1.70^{d}	39.57±2.00^{b}	48.93±1.80^{b}	26
AgNPs0.15	2.57±0.13^{d}	0.45±0.10^{abc}	83.57±2.21^{a}	85.33±0.60^{a}	123.67±2.11^{a}	203.73±1.31^{a}	61.43±1.82^{a}	44.37±0.81^{b}	30
Cur0.75/AgNPs0.05	3.54±0.11^{a}	0.61±0.06^{a}	73.33±1.52^{c}	67.01±1.01^{c}	100.13±1.41^{d}	91.30±1.60^{f}	22.57±1.01^{d}	76.33±2.01^{a}	22
Cur0.75/AgNPs0.15	2.64±0.15^{d}	0.39±0.07^{bc}	53.33±1.40^{e}	48.67±0.61^{e}	72.67±2.10^{g}	186.47±2.81^{b}	62.17±1.31^{a}	34.13±1.37^{c}	28
Cur1.5/AgNPs0.05	3.14±0.11^{bc}	0.39±0.07^{bc}	80.33±1.50^{ab}	73.01±0.91^{b}	111.87±2.30^{c}	101.93±1.92^{e}	27.97±2.51^{c}	78.47±1.20^{a}	24
Cur1.5/AgNPs0.15	2.61±0.07^{d}	0.38±0.03^{c}	54.01±1.30^{e}	45.33±1.80^{f}	63.07±1.91^{h}	177.63±1.41^{c}	61.46±1.10^{a}	38.13±1.38^{c}	31

Values in the same column having different superscripts are significantly different ($P < 0.05$).

[a]Aspartate aminotransferase activity.

[b]Alanine aminotransferase activity.

[c]Alkaline phosphatase activity.

[d]Lactate dehydrogenase activity.

Table 6. Digestive enzymes activity in common carp fed the experimental diets for 60 days.

Treatments	Protease (U/mg pro)	Lipase (U/mg pro)	Amylase (U/mg pro)
Ctrl	86.66±2.18^{ab}	43.73±2.04^{a}	35.86±1.22^{cd}
Cur0.75	82.91±2.20^{ab}	37.10±1.41^{c}	31.20±0.91^{e}
Cur1.5	85.66±2.73^{ab}	34.73±2.03^{c}	36.81±1.50^{cd}
AgNPs0.05	59.93±2.43^{d}	37.91±1.35^{bc}	44.53±1.25^{b}
AgNPs0.15	50.83±2.18^{cd}	35.73±1.68^{c}	49.76±1.38^{a}
Cur0.75/AgNPs0.05	86.90±1.93^{a}	44.63±2.21^{a}	33.36±1.49^{de}
Cur0.75/AgNPs0.15	35.81±2.40^{bcd}	23.31±1.90^{d}	21.36±1.11^{f}
Cur1.5/AgNPs0.05	80.23±2.11^{ab}	42.63±1.91^{ab}	38.66±1.78^{c}
Cur1.5/AgNPs0.15	38.81±2.25^{abc}	25.26±1.28^{d}	27.11±1.17^{g}

Values in the same column having different superscripts are significantly different ($P < 0.05$).

Table 7. Histological changes of common carp fed the experimental diets for 60 days.

Treatments	Liver		Gill			Intestine	
	HNN	HNA	GBD	GBN	GBH	Gc	IEH
Ctrl	-	-	-	-	-	-	-
Cur0.75	-	-	-	-	-	-	-
Cur1.5	+	++	+	++	+++	++	++
AgNPs0.05	++	+++	+++	++	++++	+++	+++
Cur0.75/AgNPs0.05	+	+	+	-	+	+	+
Cur1.5/AgNPs0.05	++	++++	++++	+++	++++	++	++
AgNPs0.15	++	++++	+++++	+++	+++++	+++	++++
Cur0.75/AgNPs0.15	+++	+++++	+++++	++	+++++	++++	++++
Cur1.5/AgNPs0.15	+++	+++++	+++++	++++	+++++	++++	+++++

HNN, Hepatocyte nucleus narrowing; HNA, Hepatocyte nucleus atrophy; GBD, Gill blade deformation; GBN, Gill blade necrosis; GBH Gill blade hyperplasia; Gc, Goblet cells; IEH, intestinal epithelial hyperplasia. (+) indicates the degree and severity of tissue changes and lesions.

Figures

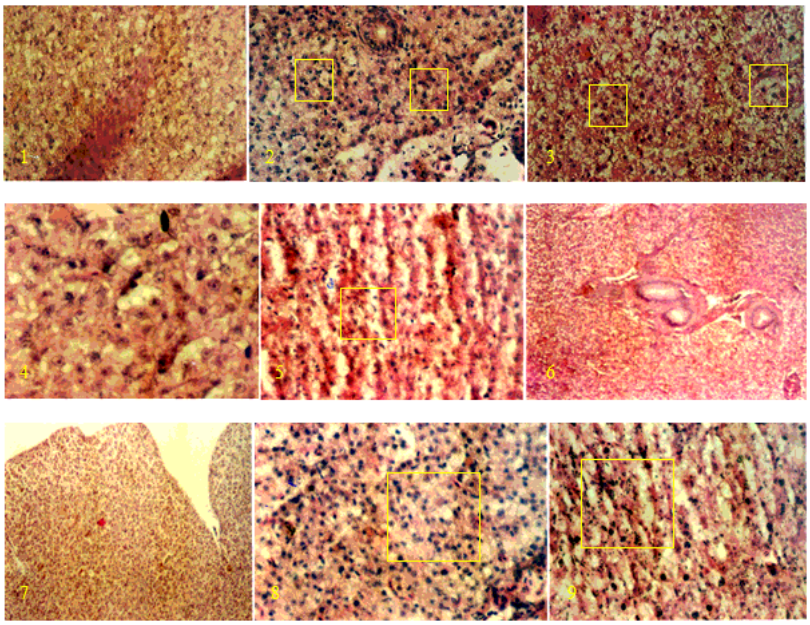

Figure 1

Liver cross-section of common carp fed the experimental diets: (1) basal diet or control; (2) diet containing 0.05 g/kg AgNPs; (3) diet containing 0.15 g/kg AgNPs; (4) diet containing 0.75 g/kg curcumin; (5) diet containing 0.05 g/kg AgNPs and 0.75 g/kg curcumin; (6) diet containing 0.15 g/kg AgNPs and 0.75 g/kg curcumin; (7) diet containing 1.5 g/kg curcumin;(8) diet containing 0.05 g/kg AgNPs and 1.5 g/kg curcumin and (9) diet containing 0.15 g/kg AgNPs and 1.5 g/kg curcumin for 60 days. Square □ indicates hepatocyte nucleus narrowing and atrophy. All pictures are magnified ×100.

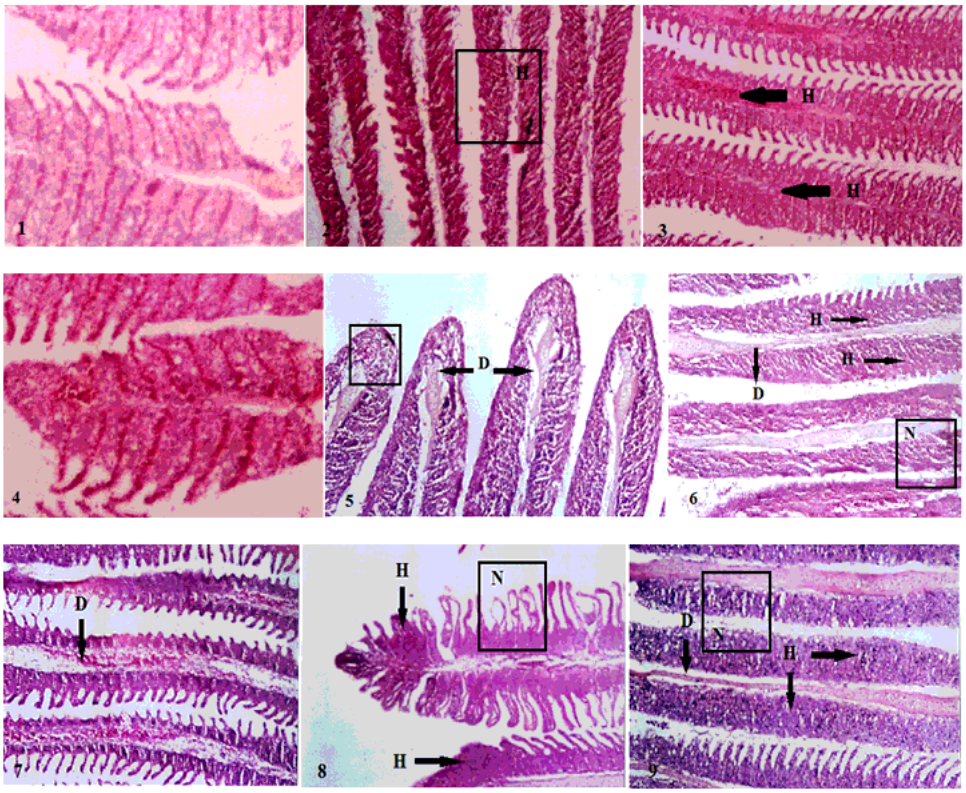

Figure 2

Gill cross-section of common carp fed the experimental diets: (1) basal diet or control; (2) diet containing 0.05 g/kg AgNPs; (3) diet containing 0.15 g/kg AgNPs; (4) diet containing 0.75 g/kg curcumin; (5) diet containing 0.05 g/kg AgNPs and 0.75 g/kg curcumin; (6) diet containing 0.15 g/kg AgNPs and 0.75 g/kg curcumin; (7) diet containing 1.5 g/kg curcumin;(8) diet containing 0.05 g/kg AgNPs and 1.5 g/kg curcumin and (9) diet containing 0.15 g/kg AgNPs and 1.5 g/kg curcumin for 60 days. The letters D, N and H indicate deformation, necrosis and hyperplasia of gill blades, respectively. All pictures are magnified ×40.

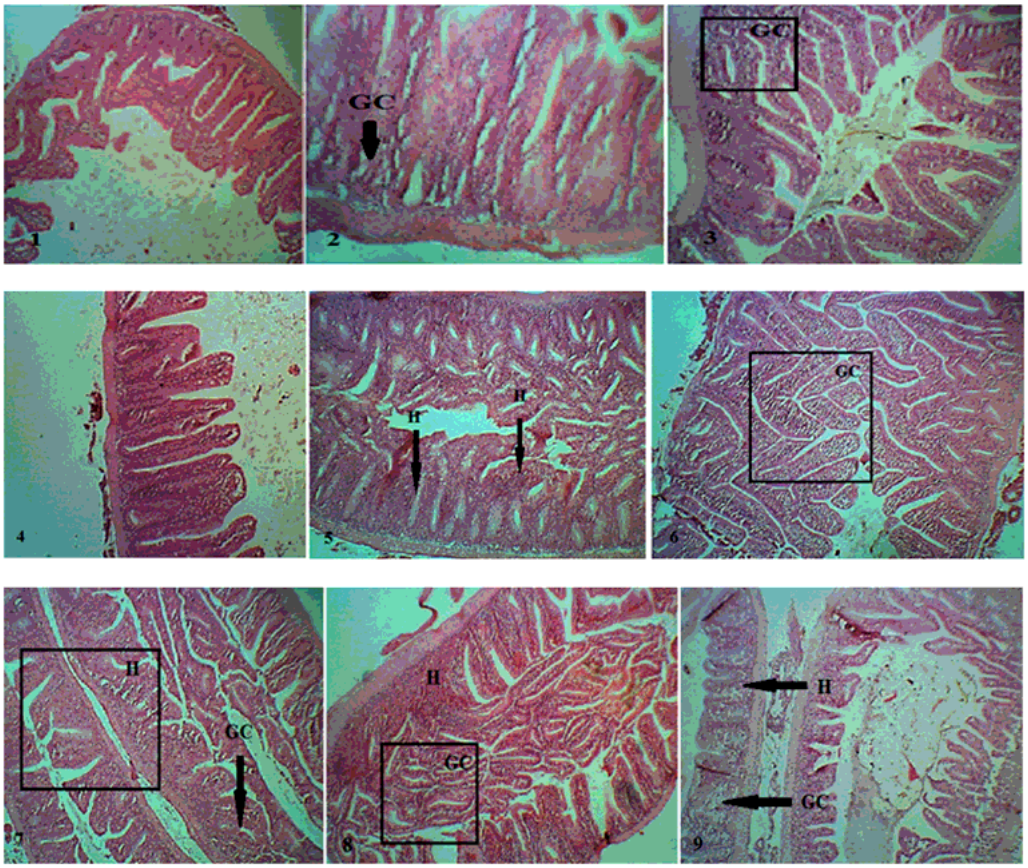

Figure 3

Intestine cross-section of common carp fed the experimental diets: (1) basal diet or control; (2) diet containing 0.05 g/kg AgNPs; (3) diet containing 0.15 g/kg AgNPs; (4) diet containing 0.75 g/kg curcumin; (5) diet containing 0.05 g/kg AgNPs and 0.75 g/kg curcumin; (6) diet containing 0.15 g/kg AgNPs and 0.75 g/kg curcumin; (7) diet containing 1.5 g/kg curcumin;(8) diet containing 0.05 g/kg AgNPs and 1.5 g/kg curcumin and (9) diet containing 0.15 g/kg AgNPs and 1.5 g/kg curcumin for 60 days. The letters GC and H indicate an increase in goblet cells and intestinal epithelial hyperplasia, respectively. All pictures are magnified ×40.