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Nano-chitosan hydrogel alleviates *Candida albicans*-induced health alterations in Nile tilapia (*Oreochromis niloticus*): antioxidant response, neuro-behaviors, hepato-renal functions, and histopathological investigation

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Abstract

Background *Candida albicans* infection induces economic losses in aquaculture practices. Currently, the success of the nanotechnology field has gained more consideration in the aquaculture sector as it bestows favorable impacts in remedies in comparison to traditional practices.

Objective The present study was conducted to assess the role of nano chitosan gel (NCG) exposure via water in managing the deteriorating impacts triggered by *C. albicans* in Nile tilapia, *Oreochromis niloticus*. Hepato-renal function, behavioral and stress response, neurological function, hepatic antioxidant/oxidant status, and histopathological architectures were investigated.

Methods A total of 160 fish (average weight: 50.00 ± 6.30 g) were randomly assigned to four groups, each with four replicates: control, NCG, *C. albicans*, and NCG + *C. albicans*. The NCG was applied as bath treatment at a concentration of 75 µg/L for ten days.

Results The outcomes demonstrated that the *C. albicans* challenged fish exhibited obvious behavioral alterations including loss of equilibrium, surfacing, abnormal swimming and movement, and aggression. Infection with *C. albicans* caused an elevation in hepato-renal biomarkers (alanine and aspartate aminotransferases, alkaline phosphatase, urea, and creatinine), stress-related indices (glucose, cortisol, nor-epinephrine, and 8-hydroxy-2-

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deoxyguanosine), and lipid peroxides (malondialdehyde). Moreover, it caused a noticeable decline in the hepatic antioxidant indices (total antioxidant capacity and reduced glutathione content) and acetylcholinesterase activity. The hepatic, renal, and brain architectures were severely damaged by the *C. albicans* challenge, exhibiting significant fatty changes, necrosis, vacuolation, and congestion. Remarkably, the aqueous application of NCG in the *C. albicans*-challenged fish ameliorated all the aforementioned biomarkers and facilitated the regeneration of histopathological changes.

Conclusion Overall, the application of NCG in the aquatic environment is an effective tool for managing *C. albicans* infection in Nile tilapia. Moreover, it can be utilized in combating stress conditions in the aquaculture sector.

Keywords Antioxidant status, Biological stress, *Candida albicans*, Histopathology, Nano chitosan gel, Nile tilapia

Introduction

Recently, fungal infections have negatively altered fish health, resulting in many diseases and higher mortalities [1, 2]. Among them, an opportunistic fungal pathogen called *Candida albicans* typically lives in the mucosal areas and digestive tracts of birds and mammals [3]. *C. albicans* is sometimes isolated from aquatic environments that receive municipal wastewater. It serves as a sign of recent fecal contamination [4]. It can infect various fish species inducing skin ulcerations, and hemorrhages, and in turn, elevating the chance for secondary bacterial infections [5–7]. It is a pathogenic fungus known to be one of the causative agents of higher fish mortality potentiating economic losses [8]. Particularly, in Nile tilapia (*Oreochromis niloticus*), it severely destroys epithelial tissues of gills, inducing mortality because of respiratory failure [9]. This probably highlights the fact that *C. albicans* generate proteases, hemolysins, and esterase—components that contribute to its virulence [10, 11]. Also, It could disseminate in the larva of zebrafish (*Danio rerio*) through hindbrain route infection and proliferate inducing losses [12]. Moreover, It can pass through blood blood-brain barrier of mammals causing meningitis [13], however, no study focused on the neurotoxicity pathways of *C. albicans* in Nile tilapia.

Chitosan, a polymer of natural origin (chitin), possesses numerous advantageous properties such as being non-toxic, biocompatible, and biodegradable [14]. It has been frequently utilized to overcome fungal pathogens due to its features [15], particularly against drug-resistant *C. albican*. This is achieved through binding the higher molecular-weight chitosan with the cell membrane and biofilm, inducing obstruction of nutrient transport [16–18]. Lately, a novel approach has emerged, utilizing natural products or nanoparticles (NPs) loaded with them in fish diets or water, to minimize the rate of infections and sustain fish performance [19, 20]. Currently, developing NPs in aquaculture practices has been verified as more successful in boosting immune function and as antimicrobials [21–24]. Chitosan NPs are effectively utilized in fish because of their safety and effectiveness in provoking growth, and immune-antioxidant response, besides defeating microbial growth in farmed fish [25–27]. They have been used efficiently with *Ocimum basilicum* to boost the health of Nile tilapia [28]. Moreover, generating chitosan-derived NPs can boost the antifungal activity of chitosan even more. This was most likely brought about by the greater cellular absorption, larger surface area, nanosize, and higher surface-to-charge density [29]. In this regard, prior report exhibited their potent antifungal activity against *C. albicans* [30].

In a recent time, nano-gels (NGs) have been utilized as transporters for bioactive particles, drugs, proteins, and peptides, supplying them to the goal sites that can upkeep the revival of injured tissues [31]. The effectiveness of NGs as a vector is dominated by numerous features, including higher permeability [32], improved bioavailability [33], stability, power to pierce the small capillaries, and powerful drug release [34]. In addition, the presence of a 3D network of conjugated polymers allows precise distribution to the target site and treatment of diseases [35]. Nano chitosan gel (NCG) has been proven brilliant efficacy against (≥ 90% inhibition) biofilms of *C. albicans* [36]. In zebrafish, NCG did not trigger acute embryotoxicity up to 100 µg/mL [37]. Despite these benefits, its application in aquaculture is still scarce.

Nile tilapia is a universally cultivated fish species that is very prevalent among fish farmers. It is distinguished by a speedy rate of growth and higher palatability [38]. In advance, aqueous NCG has a promising role in enhancing blood indices, immune function, immune-associated genes, and disease resistance of Nile tilapia [39]. No studies are addressing the use of NCG in boosting the tissue antioxidants and architectures as well as its impact on behavior and neuro-stress-related biomarkers against fungal infection in fish. Subsequently, the present work is a pioneering step to investigate the promising role of NCG in Nile tilapia to alleviate the stress, hepato-renal, neuro-ethological, and histopathological alterations induced by *C. albicans.*

Materials and methods

NCG synthesis and characterization

For the synthesis of NCG, two steps have been carried out; the primary one is the separation of chitosan NPs as defined by Ismail et al. [40]. The other step is using chitosan NPs with a hybrid carbopol gel to produce NCG. Concisely, 50 mg chitosan was used to dissolve in 100 mL acetic acid and mixed for 120 min at 60 °C till obtaining a clear solution. Then, 20 mL of sodium tripolyphosphate was added until a white colloid was obtained. The other product of the synthesis protocol was washed out using a centrifuge at 20,000 rpm, where 20 mg of chitosan NPs was added to 20 mg carbopol, liquefied in 50 mL of deionized distilled water, and agitated to obtain a homogenous solution. Then, a drop-by-drop of trimethylamine (20%) was added until a thick gel was shaped. The characterization measures were separated into three classes: morphology, index, and identification based on a previous study [41].

Ethical approval and acclimation of fish

The experimental measures were accredited by Zagazig University's Animal Research Ethics Committee, Egypt (ZUIACUC-2-F-333-2022), and followed the NIH guidelines for experimental animal care. Fish (average body weight: 50.00 ± 6.30 g) were collected from Al-Abbassa Fish Hatchery in the Sharkia Governorate, Egypt, and transported in plastic bags. Upon arrival, they were maintained for ten days in 70 L aquaria (80 × 40 × 30 cm) for acclimatization. Compressed air was pumped into each aquarium using air stones for well aeration. Fish did not have any history of disease outbreaks and did not reveal any clinical abnormalities. Prior to the experiment, CCAC [42] guidelines were employed to perform a standard check of the fish's health. A daily partial water exchange (25%) was carried out and the fish were offered a basal diet (crude protein: 36.46%; Fat: 11.09%; crude fiber: 3.28%) at the rate of 3% of their body weight twice daily (8:00 and 4.00). A daily physicochemical assessment of the rearing water was performed throughout the acclimation and experimental period by taking water specimens from each aquarium. For monitoring water quality metrics [43], involving water temperature, dissolved oxygen, pH, and unionized ammonia, the portable oxygen meter (Jenway, London, UK), digital mini-pH meter (Fisher Scientific, Denver, USA), and multi-parameters ion Analyzer (HANNA Instruments, Rhode Island, USA) were employed, respectively. The recorded measurements were as follows: 25.00 ± 2.00 °C for temperature, 7.00 ± 0.1 for pH, 6.00 ± 0.40 mg/L for dissolved oxygen, and 0.01 ± 0.07 mg/L for unionized ammonia. These variables were within the acceptable limits for aquaculture [44].

Fungal strain (*C. albicans*)

The pathogenic *C. albicans* strain was isolated from naturally infected Nile tilapia in the Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt, and proved to be virulent. This isolate was recognized using standard biochemical traits and the VITEK® 2 system (Bio-M'erieux, Marcy l'Etoile, France). *C. albicans* was exposed to cultivation on sabaroud dextrose agar (Hi-media, India) and then incubated for 48 h at 24 °C [7]. The colonies were chosen and immersed in sterile phosphate buffer saline (PBS). The lethal dose (LD_{50}) of *C. albicans* was assessed and recorded at 6.4×10^8 CFU/mL for *C. albicans* [39]. A dose of 1.8×10^7 CFU/mL was designated for the experiment.

Experimental protocol

Prior to any treatment, fish were first anesthetized by being exposed to 100 mg/L of benzocaine solution to facilitate handling and decrease any stress exposure [45]. Fish ($n = 160$) were randomly chosen and divided into four groups in quadruplicate (40 fish/ group; 10 fish/ replicate) for ten days and each replicate was placed in a 70 L aquarium with adequate airflow. The first (control) group was not exposed to NCG or infected with *C. albicans.* The second (NCG) group was exposed to NCG and was not infected with *C. albicans.* The third (*C. albicans*) group was not exposed to NCG, but it was infected with *C. albicans.* The fourth (NCG + *C. albicans*) group was exposed to NCG and infected with *C. albicans*. The infection with *C. albicans* was applied by intraperitoneal inoculation of fish with 0.2 mL (1.8×10^7 CFU/mL) of *C. albicans* fungal suspension [7]. Moreover, the NCG solution was added at a dose of 75 µg/L to the aquarium water [39] after the appearance of clinical symptoms of *C. albicans* infection (lack of body reflexes, irregular swimming along with skin darkening, and some hemorrhages). To get rid of the excretory wastes, siphoning was done daily. After the full water change (three times per week), a freshly made NCG solution was introduced to keep their levels stable.

Behavioral assessment and sampling

Tracking of behavioral observations was done throughout 11 min for each aquarium twice a day, in the morning (8:00–10:00 am) and afternoon (1:00–3:00 pm) throughout the trial period [46]. The behavior of fish in each aquarium was recorded using the instantaneous sampling method through a direct visualization procedure by using a stopwatch and digital timer (NTT08). Each behavior was observed at 60-second intervals by one observer. The frequencies of behavior were recorded to calculate the number of fish that engaged to perform each behavior per unit of time (m^{-1}). Fish behavioral patterns included loss of equilibrium [47], surfacing [48], swimming [49],

resting [50], abnormal movement [51], and aggressive [52].

At the end of the study, fish were carefully chosen (9 fish/group) and anesthetized by immersing in benzocaine (100 mg/L) [45]. Then, blood was collected from the caudal blood vessels using sterile syringes without the addition of anticoagulant and reserved for 6 h at room temperature. Blood was exposed to centrifugation at 1750 ×*g* for 10 min, and serum samples were utilized for conducting biochemical assays. Moreover, the brain, liver, and kidney tissues of nine fish per group were used as samples for the neurological and antioxidant/oxidant assays as well as histopathological investigations.

Hepato-renal functions assay

Application of biochemical assessments for the activity of serum alanine and aspartate aminotransferases (ALT& AST) and alkaline phosphatase (ALP) enzymes as well as the levels of urea and creatinine using commercial kits of Biodiagnostic Co., Egypt (Cat. No.; AL 1031, AS 1061, AP 1020, UR 2110, CR 1250), respectively [53–56].

Stress-related assays

Following the protocol established by Teixeira et al. [57], blood glucose (GLU) levels were measured using the glucose oxidase/glucose peroxidase reaction Cortisol hormone and nor-epinephrine (NOR) were distinguished in fish serum by ELISA commercial kit (My-Biosource Inc., San Diego, California, USA) under Cat. No. of MBS704055 and MBS025809, respectively.

Neuro-related assays

Spectrophotometric measurements were made using brain tissue samples to determine the values of 8-hydroxy-2-deoxyguanosine (8-OHdG) and neurotransmitter (acetylcholine, AchE). The brain homogenates were subjected to centrifugation for 15 min at 3000 rpm at 5 °C after using 15 mL of 150 mM NaCl for homogenization. The value of 8-OHdG was estimated using a commercial ELISA kit (MyBiosource Inc., San Diego, California, USA) with a Cat. No. MBS1601729 following manufacturer's guidance. Firstly, all standards, reagents, and samples were prepared. The ELISA reagent and samples were added into each well and incubated for 1 h at 37 °C. After that, the plates were washed five times. Then, substrate solutions A and B were added and incubation occurred for 10 min at 37 °C. Finally, a stop solution was administered, the color was developed, and the optical density was determined within 10 min at 450 nm.

Furthermore, the level of AchE was estimated at 450 nm detection wavelength based on the protocol illustrated in the report of Ellman et al. [58] using a commercial kit (My-Biosource Inc., San Diego, California, USA) under Cat. No. MBS280290.

Hepatic antioxidant/oxidant assays

The hepatic tissues were splashed three times using a cold NaCl solution (0.9%) prior to homogenization in PBS (pH 7.5). Afterward, the homogenates were subjected to a 15-minute cold centrifugation at 3000 rpm. The supernatants were cautiously gathered in a sterile tube to assess antioxidant/oxidant indicators based on the Fernandez-Botran et al. [59] approach. These indicators were assayed using commercial kits (Biodiagnostic Co., Cairo, Egypt) based on the manufacturer's directions.

The colorimetric approach was used to evaluate the total antioxidant capacity (TAC; Cat. NO. TA 25 13) following Koracevic et al. [60] method. The reaction of antioxidants in the sample with a specified quantity of externally given hydrogen peroxide (H_2O_2), which results in the removal of a defined amount of H_2O_2, is used to quantify the anti-oxidative capacity. The conversion of 3, 5, dichloro-2-hydroxyl benzene-sulphonate to a colorful product in an enzymatic reaction provides a colorimetric measurement of the remaining H_2O_2. At first, H_2O_2 as a substrate was diluted and then, a working reagent was formed from the equivalent amount of enzyme buffer and chromogen. About 0.5 mL of sample and blank was thoroughly mixed with H_2O_2 and incubated at 37 °C for 10 min. Next, the working reagent was added to both, thoroughly mixed, and incubated for 5 min at 37 °C. The TAC was computed after the absorbance of the blank and sample were promptly measured at 505 nm.

To determine the amount of reduced glutathione (GSH; Cat. NO. GR 25 11) colorimetrically, the approach of Beutler et al. [61] was followed. It is based on the conversion of, 5′-dithiobis 2-nitrobenzoic acid into a stable yellow-colored compound (5-mercapto2-nitrobenzoic acid) by GSH. A mixture of 1.8 mL of distilled water, 0.2 mL of sample homogenate, 3 mL of the precipitating agent, and sulfosalicylic acid was formed. A 3000 rpm centrifugation was performed on this mixture for 4 min. After that, the supernatant was combined with 4.5 mL of Ellman's reagent in a 0.5 mL amount. Then, 4 mL of phosphate buffer, 0.5 mL of the diluted precipitating agent, and 0.5 mL of Ellman's reagent were coupled. The reaction mixture's absorbance at 412 nm was measured within 30 min of color formation.

Furthermore, malondialdehyde (MDA; Cat. NO. MD 25 29) was calculated by thiobarbituric acid approach [62]. The basis of this assay is the formation of a pink thiobarbituric acid reactive substance (colored final product) by the reaction of MDA with thiobarbituric acid in an acidic solution at 95 °C for 30 min. The absorbance of this final product was at 534 nm.

Histopathological investigation

Liver, kidney, and brain tissues were collected and fixed in neutral buffered formalin 10% for 24 h. Specimens were

dehydrated in graded ethanol (70%, 80%, 90 -100%) and cleared in xylene I followed by xylene II, then embedded in melted paraffin wax I followed by melted paraffin II. We obtained about 5 µm paraffin sections by using automated microtome then stained with Hematoxylin and Eosin (H & E) and examined microscopically for any histological alterations [63]. All section photos were taken using a Swift microscope associated with a Swift digital camera. The histopathological scoring was estimated by semiquantitative methods as follows: "0 = no, 1 = mild, 2 = moderate, and 3 = severe alterations" [64].

Data analysis

Prior to the statistical assay, the normality of the data was checked by using the Shapiro-Wilk normality test. Following that, a one-way analysis of variance (ANOVA) was employed to analyze the results using SPSS ver. 21 (IBM Corp., Armonk, USA). The Duncan test was implemented as a post-hoc analysis to check whether the means varied at a *P*-value of less than 0.05. The data were shown as mean ± standard error (*SE*).

Results

NCG characterization

The morphology of NCG appeared as spherical nanoparticles of 60–70 nm size as depicted by transmission electron microscopy (TEM, Fig. 1A) and 3D atomic force microscopy (AFM; Fig. 1B). High purity NCG's amorphous nature was demonstrated via X-ray diffraction (XRD; Fig. 1C). The measured zeta potential of +7 mV suggested a moderately positive surface charge on NCG (Fig. 1D). Moreover, a hydrodynamic diameter of approximately 68 nm of NCG was resulted by dynamic light scattering (DLS; Fig. 1E).

Impact on behavioral observation

The results demonstrated in Table 1 revealed that the *C. albicans* infection significantly ($P < 0.001$) altered the behavioral patterns. Fish of the *C. albicans* group showed significant ($P < 0.001$) high frequencies of loss of equilibrium, surfacing, abnormal swimming, and resting as well as abnormal movement (vertical and circular). Moreover, increasing the frequencies of most items of aggressive behavior as chasing, mouth pushing, approach, and

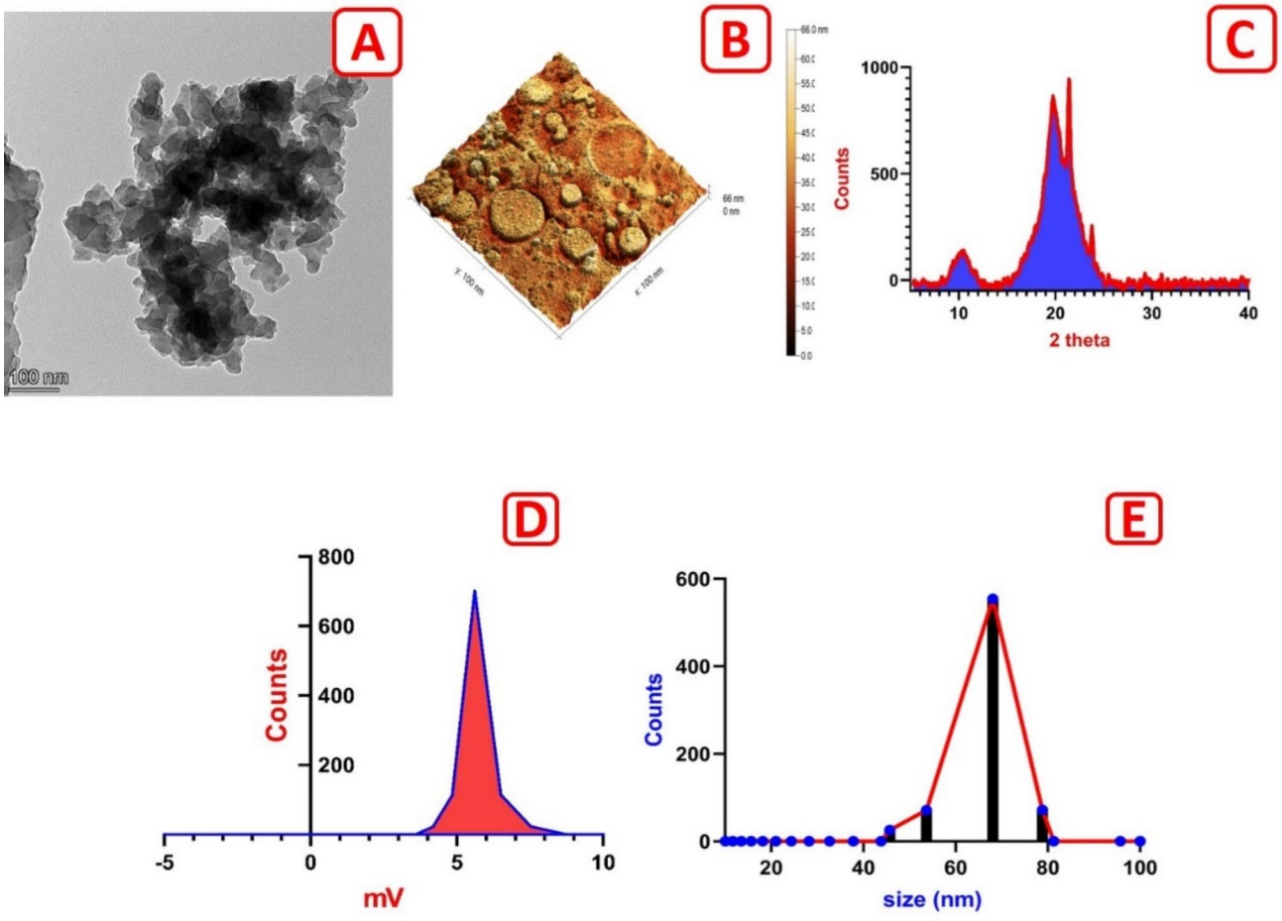

Fig. 1 Characterization images of nano chitosan gel. **A** TEM (100 nm). **B** 3D AFM. **C** XRD. **D** Zeta potential. **E** DLS

Table 1 Impact of nano chitosan gel (NCG) bath and/or *C. albicans* infection on frequencies of behavioral pattern (m^{-1}(of Nile tilapia for ten days

Behavior	Control	NCG	*C. albicans*	NCG + *C. albicans*	*P*-value
Loss of equilibrium	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	6.45 ± 0.04^{a}	3.31 ± 0.02^{b}	< 0.001
Surfacing	0.31 ± 0.02^{c}	0.38 ± 0.02^{c}	3.30 ± 007^{a}	2.08 ± 0.08^{b}	< 0.001
Abnormal swimming	1.14 ± 0.03^{c}	1.21 ± 0.02^{c}	9.85 ± 0.17^{a}	5.36 ± 0.09^{b}	< 0.001
Resting	1.34 ± 0.03^{c}	1.32 ± 0.07^{c}	3.77 ± 0.06^{a}	2.05 ± 0.03^{b}	< 0.001
Abnormal movement					
Vertical movement	0.00 ± 00^{c}	0.00 ± 0.00^{c}	3.04 ± 0.02^{a}	1.93 ± 0.04^{b}	< 0.001
Circular movement	0.00 ± 00^{c}	0.00 ± 00^{c}	3.37 ± 0.22^{a}	0.99 ± 0.10^{b}	< 0.001
Aggressive behaviour					
Chasing	0.15 ± 0.01^{c}	0.18 ± 0.01^{c}	3.29 ± 0.16^{a}	1.90 ± 0.06^{b}	< 0.001
Mouth pushing	0.00 ± 00^{c}	0.00 ± 0.27^{c}	2.10 ± 0.17^{a}	1.30 ± 0.17^{b}	< 0.001
Approach	0.55 ± 0.03^{c}	0.60 ± 0.11^{c}	4.09 ± 0.05^{a}	2.25 ± 0.02^{b}	< 0.001
Fin tugging	0.66 ± 0.02	0.73 ± 0.02	1.05 ± 0.09	1.00 ± 0.01	0.43
Fleeing	0.84 ± 0.02^{c}	0.89 ± 0.03^{c}	6.35 ± 0.20^{a}	3.25 ± 0.14^{b}	< 0.001
Butting	0.00 ± 00	0.00 ± 00	0.15 ± 0.14	0.10 ± 0.02	0.07

Values (means ± *SE*) that do not share similar superscripts in the same row differ substantially (One way ANOVA; $P < 0.05$)

Table 2 Impact of nano chitosan gel (NCG) bath on hepato-renal variables of Nile tilapia experimentally infected with *C. albicans* for ten days

Parameters	Control	NCG	*C. albicans*	NCG + *C. albicans*	*P*-value
ALT (U/mL)	7.12 ± 1.08^{c}	7.87 ± 0.50^{c}	32.25 ± 1.01^{a}	20.05 ± 0.54^{b}	< 0.001
AST (U/mL)	22.62 ± 0.94^{c}	23.50 ± 1.44^{c}	152.90 ± 1.67^{a}	74.75 ± 1.01^{b}	< 0.001
ALP (U/mL)	13.44 ± 0.32^{c}	15.76 ± 0.13^{c}	45.40 ± 1.96^{a}	30.06 ± 0.61^{b}	< 0.001
Urea (mg/dL)	8.91 ± 0.29^{c}	8.44 ± 0.25^{c}	18.02 ± 0.59^{a}	11.63 ± 0.37^{b}	< 0.001
Creatinine (mg/dL)	0.15 ± 0.01^{c}	0.18 ± 0.01^{c}	1.31 ± 0.05^{a}	0.88 ± 0.02^{b}	< 0.001

ALT: Alanine aminotransferase; AST: aspartate aminotransferase; ALP: Alkaline phosphatase. Values (means ± *SE*) that do not share similar superscripts in the same row differ substantially (One way ANOVA; $P < 0.05$)

Table 3 Impact of nano chitosan gel (NCG) bath on stress and neuro-related variables of Nile tilapia experimentally infected with *C. albicans* for ten days

Parameters	Control	NCG	*C. albicans*	NCG + *C. albicans*	*P*-value
GLU (mg/dL)	26.34 ± 0.65^{c}	25.45 ± 0.84^{c}	46.46 ± 1.46^{a}	33.99 ± 0.52^{b}	< 0.001
Cortisol (ng/mL)	18.65 ± 0.20^{c}	19.25 ± 0.66^{c}	119.50 ± 0.29^{a}	37.95 ± 1.12^{b}	< 0.001
NOR (pg/mL)	0.84 ± 0.02^{c}	0.89 ± 0.06^{c}	6.90 ± 0.52^{a}	2.90 ± 0.17^{b}	< 0.001
8-OHdG (ng/mg)	0.88 ± 0.01^{c}	0.91 ± 0.01^{c}	9.75 ± 0.43^{a}	4.00 ± 0.17^{b}	< 0.001
AchE (Pg/mg)	473.45 ± 1.99^{a}	476.14 ± 3.54^{a}	213.20 ± 7.62^{c}	285.61 ± 2.53^{b}	< 0.001

GLU: Glucose; NOR: nor-epinephrine; 8-OHdG: 8-hydroxy-2-deoxyguanosine, AchE: acetylcholinesterase. Values (means ± *SE*) that do not share similar superscripts in the same row differ substantially (One way ANOVA; $P < 0.05$)

fleeing induced by *C. albicans* infection. The fin tugging and butting did not alter significantly. On the other hand, using NCG as a treatment for *C. albicans* infection (NCG + *C. albicans*) significantly ($P < 0.001$) decreased these behavioral alterations relative to the non-treated group (*C. albicans*). There were no discernible differences were noted between NCG and control groups concerning behavioral traits.

Impact on hepato-renal function variables

Hepato-renal function levels (ALT, AST, ALP, urea, and creatinine) fluctuated in an indiscernible manner between the control and NCG groups (Table 2). *C. albicans* infection resulted in a noticeable increase ($P < 0.001$) in these variables as matched to the control. Treatment of the infected group with NCG (NCG + *C. albicans*) declined these biomarkers compared to the non-treated one (*C. albicans*).

Impact on stress and neuro-related variables

Table 3 reveals non-observable alterations in the value of stress-related variables (GLU, cortisol, and NOR) and neuro-related variables (8-OHdG and AchE) between control and NCG groups. A dramatic rise ($P < 0.001$) in GLU, cortisol, NOR, and 8-OHdG levels and a decline ($P < 0.001$) in the AchE value of the *C. albicans* group with respect to the control. On the contrary, these variables significantly declined ($P < 0.001$) in the treated

group (NCG + *C. albicans*) compared with the *C. albicans* group except for AchE activity that showed marked elevation ($P < 0.001$).

Impact on antioxidant/oxidant variables

Figure 2 (A–C) demonstrates a noteworthy rise ($P < 0.001$) in TAC and GSH levels and no significant change in the MDA level of the NCG group as matched to the control. *C. albicans* infection caused a considerable decline ($P < 0.001$) in the TAC and GSH and an increase in the MDA value relative to the control. Treatment of *C. albicans* with NCG improved these metrics relative to the non-treated group.

Histopathological outcomes

Normal histo-architectures were observed in the hepato-pancreatic tissues of the control and NCG groups including typical hepatic cords, pancreatic acini, sinusoids, and portal veins (Fig. 3A & B). While markedly distributed fatty changes represented by clear intra-cytoplasmic vacuole with peripherally located nuclei were obvious in the hepato-pancreas of the *C. albicans* group. As well, necrotic pancreatic acini with pyknotic nuclei were observed (Fig. 3C). These changes were declined in the NCG + *C. albicans* group, where less number of fatty degenerated cells and amelioration in pancreatic acinar epithelium was seen (Fig. 3D).

Additionally, the kidney tissues in the control and NCG groups demonstrated typical histological configurations of renal tubular epithelium, glomerular tufts, and vascular tissue (Fig. 4A & B). In contrast, necrosis of some renal epithelium with the presence of remnant cytoplasmic and nuclear material was obvious in the *C. albicans* group. In addition, congested renal blood vessels and hypo-cellular glomeruli were noted (Fig. 4C). Conversely, maintenance of renal tissue and few degenerative changes in some renal tubules were in the NCG + *C. albicans* group (Fig. 4D).

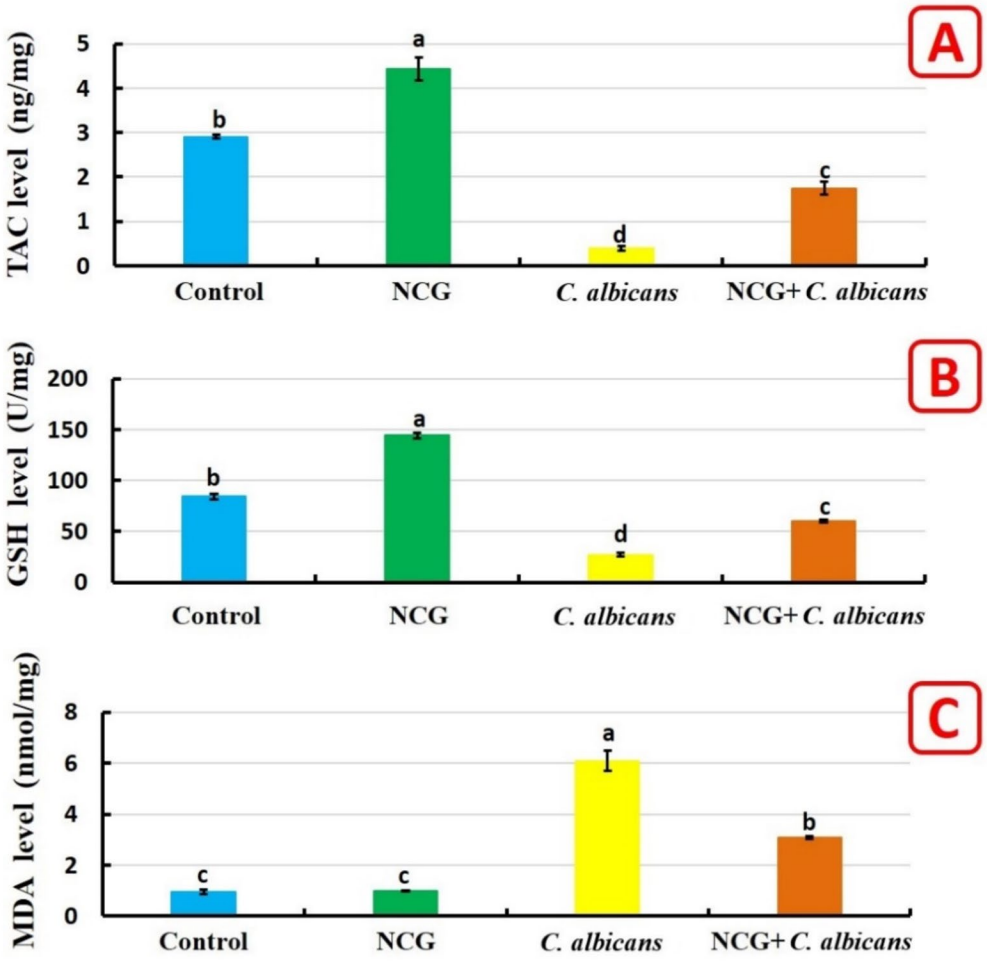

Fig. 2 Impact of nano chitosan gel (NCG) bath on hepatic antioxidant/oxidant variable of Nile tilapia experimentally infected with *C. albicans* for ten days. **A** Total antioxidant capacity (TAC; $P < 0.001$). **B** Reduced glutathione content (GSH; $P < 0.001$). **C** Malondialdehyde (MDA; $P < 0.001$). Bars (means ± *SE*) that do not share similar superscripts differ substantially (One way ANOVA; $P < 0.05$)

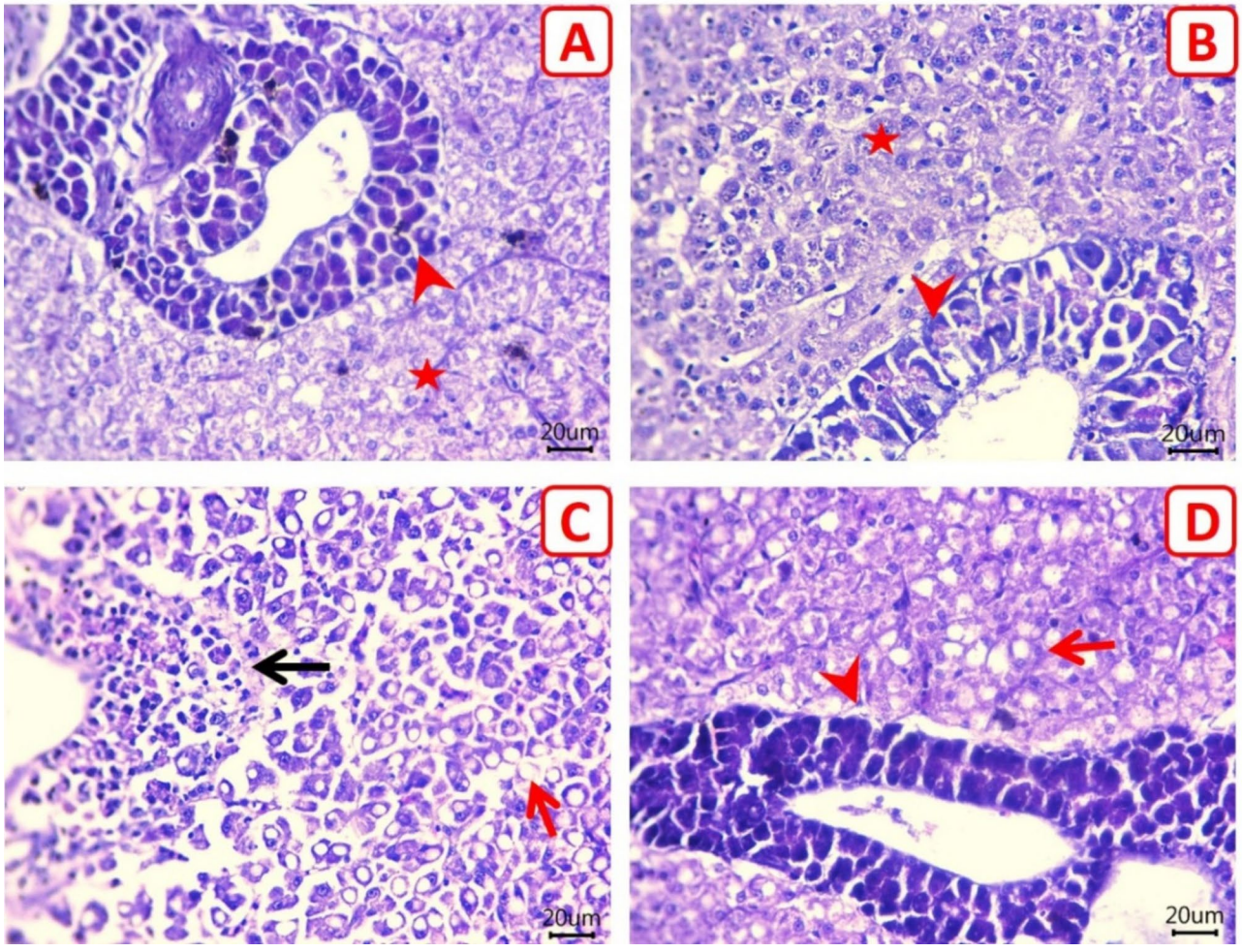

Fig. 3 Photomicrographs of hepato-pancreas sections (H&E). **A & B** Hepato-pancreases of the control and nano chitosan gel (NCG) groups reveal normal histological configurations of hepatic cords (stars) and pancreatic acini (arrowheads). **C** The hepato-pancreas of the *C. albicans* group reveals markedly distributed fatty changes (red arrow) and necrotic pancreatic acini with pyknotic nuclei (black arrow). **D** Hepato-pancreas of the NCG + *C. albicans* group reveals less number of fatty degenerated cells (arrow) and amelioration in pancreatic acinar epithelium (arrowhead). Scale bar 20 µm

Neurons, glial cells, and neuropil in histology of brain tissues from the control and NCG groups appeared normal (Fig. 5A & B). On the other side, marked areas of vacuolated neuropils and a large number of pyknotic neurons with perineuronal spaces were in the *C. albicans* group (Fig. 5C). An improvement in most neuronal structures was observed, accompanied by the presence of few areas of neuropil vacuolations and few numbers of pyknotic neurons in the NCG + *C. albicans* group (Fig. 5D).

In addition, the degree of histological damage in the liver, kidney, and brain tissues is shown in Table 4.

Discussion

In aquaculture practices, fungal diseases constitute a major obstacle in culturing fish [65]. *C. albicans* induces numerous skin ulcers and mortalities in Nile tilapia and African catfish (*Clarias gariepinus*) [8]. The usage of NGs has displayed aptitude in sustaining fish health besides its role in chelating toxicity [66]. Hence, the present work aims to investigate the influence of waterborne NCG in combating *C. albicans* in Nile tilapia.

The behavioral changes of an aquatic organism are a result of a variety of physiological and biochemical processes within the body [67]. Herein, we reveal the occurrence of behavioral alterations induced by *C. albicans* which may be linked to the decline in the brain indicator (AChE) as confirmed in our data. The lower AchE leads to acetylcholine accumulation at synaptic connections. This alters fish locomotor activity and behavior by throwing off the synchronization between neural and muscle connections [68]. Also, it is opined that *C. albicans* induced oxidative damage in brain tissue resulting in neurological alteration and consequently, behavioral changes. This result was confirmed in our findings by the high level of 8-OHdG which acts as an oxidative DNA damage biomarker. Snarr et al. [69] illustrated the ability

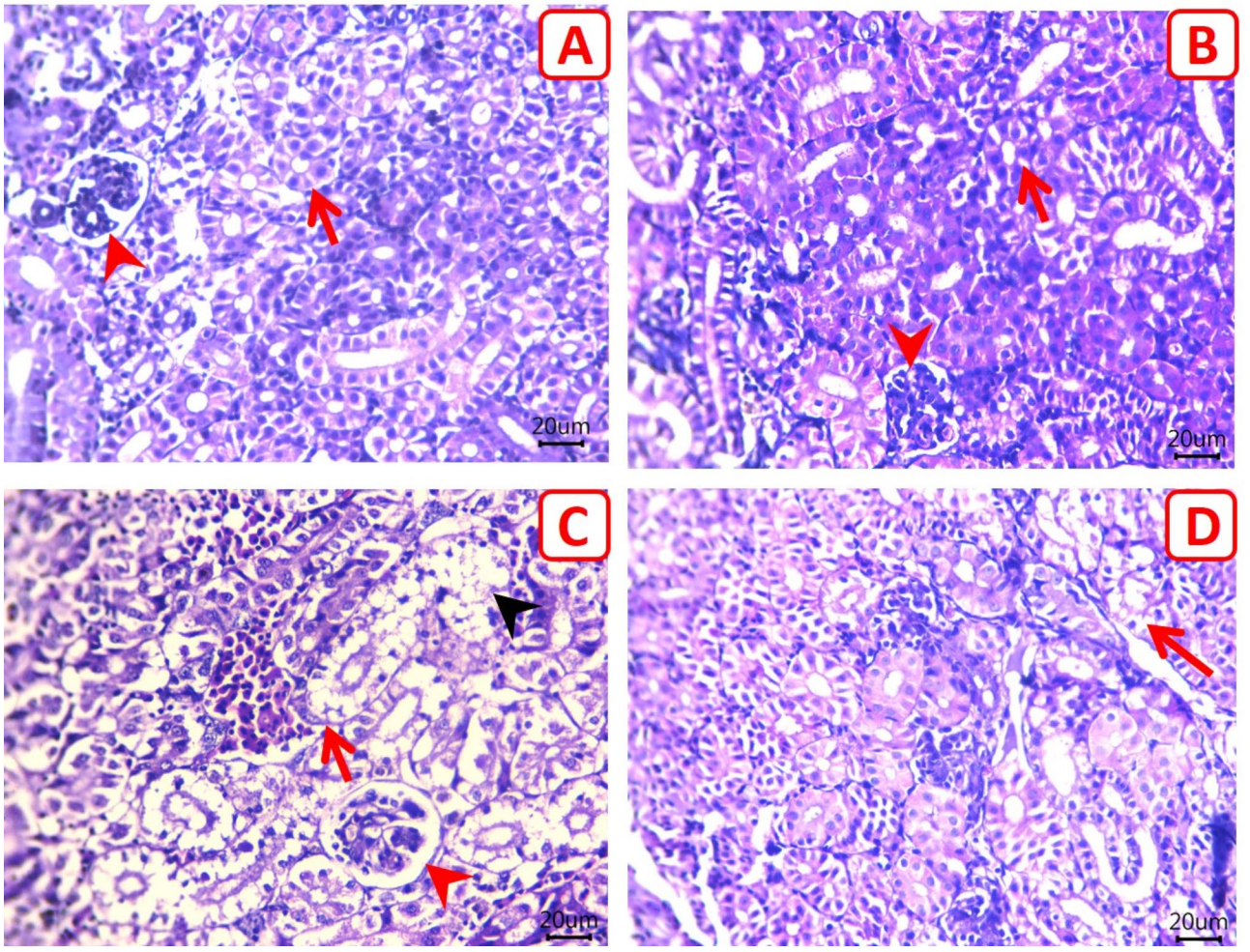

Fig. 4 Photomicrographs of kidney sections (H&E). **A & B** Kidneys of the control and nano chitosan gel (NCG) groups reveal normal histological configurations of renal tubular epithelium (arrows) and glomerular tufts (arrowheads). **C** Kidney of the *C. albicans* group reveals necrosis of some renal epithelium with the presence of remnant cytoplasmic and nuclear material (black arrowhead), congested renal blood vessels (red arrow), and hypo-cellular glomeruli (red arrowhead). **D** Kidney of the NCG + *C. albicans* group reveals maintenance of renal tissue with few degenerative changes in some renal tubules (arrow). Scale bar 20 µm

of *C. albicans* to invade the central nervous system by crossing the blood-brain barrier causing neuronal damage. Additionally, the disruption in behavior could be attributed to the negative impact of *C. albicans* on immune functions. Fish behavioral patterns are linked to the expression of particular cytokines in the brain. Fish with an immune suppression cytokine profile would decrease interaction in several areas of their social behavior and activity and become more vulnerable to predators [70]. Likely, Tartor et al. [5] supported our findings and described that *C. albicans* induced major clinical picture and post-mortem changes which in turn influence fish immune function and behavior. A study by Gibney and Drexhage [71] supported this assumption which reported that the majority of behavioral disorders are linked to immunosuppression, via altering cytokines levels, and in turn badly influence neuronal function.

Surprisingly, NCG has promise in modulating the neuro indicators and in turn, regenerating the behavioral alterations. It is assumed that the minute size of NCG can reach the brain and exert antioxidant activity which protects the brain tissue from oxidative damage produced by *C. albicans*. This was validated by the improvement of the histopathological picture of the brain tissues in our results. Concurrent with a recent report [34] describes that NGs have a potential role as nano-carriers for targeting the brain, via crossing the blood-brain barrier and efficiently delivering beneficial agents to the central nervous system. Nano chitosan exerted a neuroprotective potential by boosting the activity of glutathione peroxidase and superoxide dismutase enzymes which can scavenge the produced reactive oxygen species (ROS) hence, reducing oxidative stress [72]. Moreover, another study [73] explained that nano chitosan can protect against

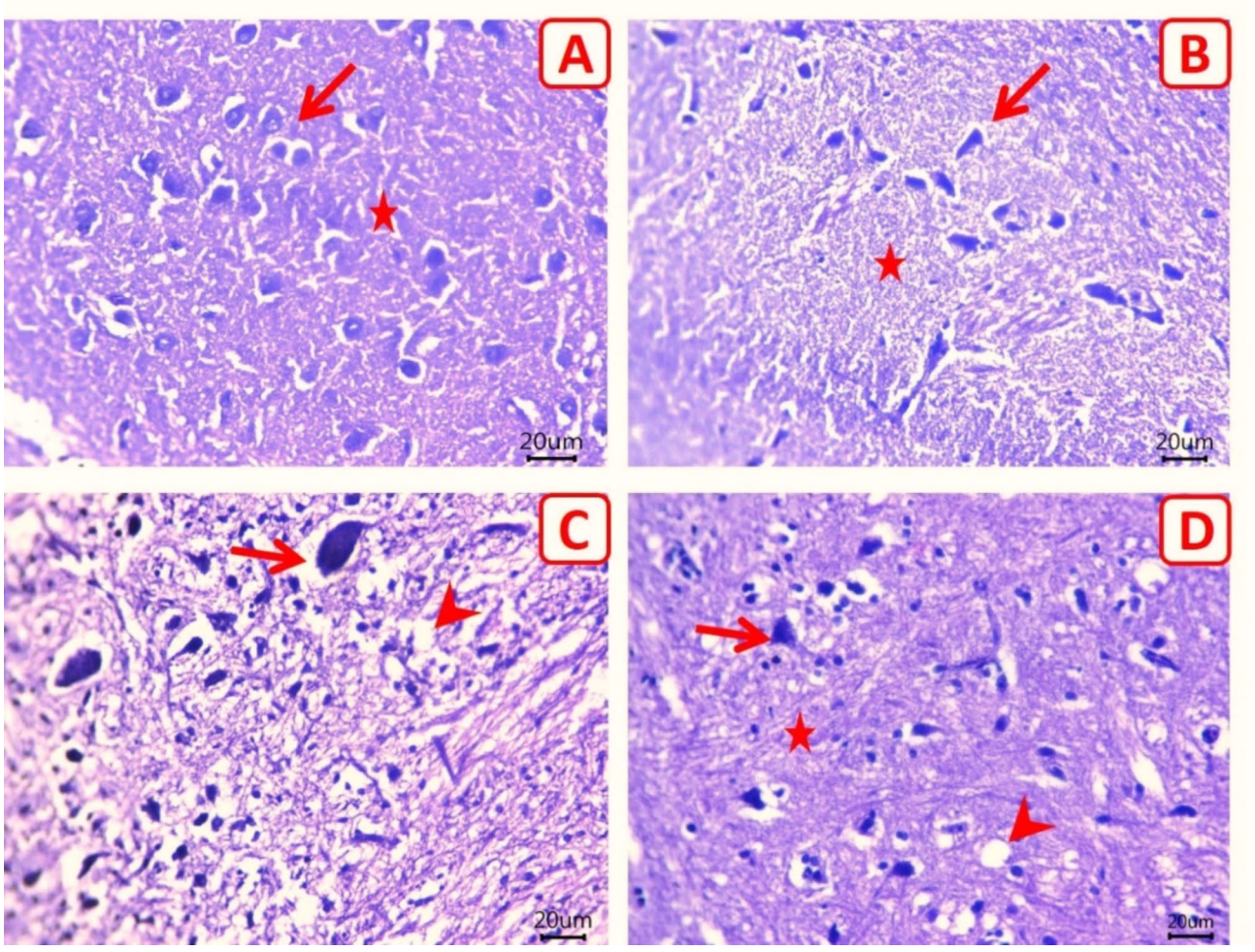

Fig. 5 Photomicrographs of brain sections (H&E). **A & B** Brains of the control and nano chitosan gel (NCG) groups reveal normal histological architectures of neurons (arrows), glia cells, and neuropil (stars). **C** Brain of the *C. albicans* group reveals a marked area of vacuolated neuropil (arrowhead) and a large number of pyknotic neurons with perineuronal space (arrow). **D** Brain of the NCG + *C. albicans* group reveals an improvement in the most neuronal cell body (arrow), neuropil (star) with the presence of few areas of vacuolated neuropil (arrowhead). Scale bar 20 µm

neurological damages via conjugation with nerve growth factor, plasmid DNA, and acteoside as well as reversing dopaminergic neuron loss led to amelioration of behavioral disorders.

Assessment of hepato-renal function biomarkers is essential to improve our knowledge about liver and kidney dysfunction in fish especially during infection [74]. Infection by *C. albicans* resulted in elevating hepatic (ALT, AST, and ALP) and renal indicators (urea and creatinine). This effect could be attributed to the effect of the virulence genes which alter the hepato-renal function via induction of oxidative damage [75]. Similarly, Oda et al. [9] reported an increase in hepato-renal indicators post-exposure of Nile tilapia to *C. albicans*. It is noteworthy that NCG has a modulating role in the hepato-renal parameters after the *C. albicans* challenge. Such modulation could be dominated by the antimicrobial role of NCG in eliminating *C. albicans* infection, and accordingly regenerating hepato-renal function. These findings were also confirmed via noticeable modulation and regeneration of the hepato-renal histological architecture in our findings. In line with previous studies, Ganan et al. [76] and Al-Zahrani et al. [77] described the antimicrobial activity of chitosan nanocomposites and suggested that the existence of amine groups (NH_3^+) in glucosamine which delivers chitosan its poly-cationic landscape. This nature gives chitosan the power to stick to the negatively charged surface of microorganisms, inducing deviations in the cell surface and consequent outflow of intracellular substances, then cell death.

Exposure of fish to fungal diseases alters the fish's biochemical traits, including stress indicators [78]. Cortisol, GLU, and NOR are vital stress biomarkers in fish [79]. We reported that GLU, cortisol, and NOR values were elevated in the exposed fish to *C. albicans* infection reflecting a potent stress response. A recent study by Lemos et

Table 4 Scoring system for evaluation of commonly observed lesions in the liver, kidney, and brain tissues of Nile tilapia experimentally infected with *C. albicans* and treated with nano chitosan gel (NCG) bath for ten days

Organ	Lesion	Control	NCG	*C. albicans*	NCG + *C. albicans*
Liver	degenerated hepatocytes	0	0	3	1
	necrotic pancreatic epithelium	0	0	2	0
	inflammatory cells infiltrates	0	0	2	1
Kidney	degenerated renal tubules	0	0	3	1
	necrotic renal tubules	0	0	2	0
	congested renal vasculatures	0	0	2	1
	hypo-cellular glomeruli	0	0	2	1
Brain	pyknotic neurons	0	0	3	1
	vacuolated neuropil				

Examined fish = three/group. Number of examined fields (10 random fields/group, 400X). The histopathological scoring was estimated by semiquantitative methods as follows: "0 = no, 1 = mild, 2 = moderate, and 3 = severe alterations"

al. [80] elucidated the mechanism of induction of stress conditions in fish. First, stress response in fish commences at the cellular level. Such a response may alter the individual homeostasis induce metabolic and physiological changes and possibly cause organismal functional weakening. Primary responses include endocrinological changes, such as increased concentrations of catecholamines (including NOR) and corticosteroids. Secondary responses include metabolic disturbances and elevated GLU levels. Finally, tertiary response involves changes in the behavioral pattern, which is verified in our findings. On the other hand, exposure to NCG modulated the stress indicator levels. It is possible that the minute-sized nano gel effectively diminished the fungal infection via its antimicrobial activity and gradually relieved the stress condition.

In the aquaculture industry, fish infections cause oxidative damage by producing excessive ROS and ultimately contribute to the pathogenesis of pathogens [81]. In the current work, the oxidative damage elicited by *C. albicans* recorded a notable decline in the activities of TAC and GSH and an increase in the oxidative indicators (MDA and 8-OHdG). This could be dominated by the ability of *C. albicans* to stimulate the release of more ROS, combined with the stress prompted by the pathogen itself, resulting in suppressing antioxidant activity and augmenting the production of ROS [82]. This finding was reinforced by a recent study that exhibited that *C. albicans* produced oxidative stress and declined values of catalase and superoxide dismutase in African catfish (*Clarias gariepinus*) [83]. Astonishingly, NCG applies a strong antioxidant capacity indicated by modifying TAC and GSH levels and reducing the values of MDA and 8-OHdG. This could be attributed to the power of chitosan to successfully hunt ROS and accordingly modulate the activity of antioxidant enzymes. Concurrently, Dizaj et al. [84] designated the antimicrobial activity of nanocomposites which involves the formation of ROS which prompts an elevation in oxidative damage in microbial cells and major damage to biomolecules, inducing genotoxic impacts. In the same instance, Abdel-Tawwab et al. [85] mentioned that the dietary administration of chitosan NPs fortified antioxidant activity in Nile tilapia.

C. albicans adversely altered the hepatic, renal, and brain histological pictures which could be attributed to its virulence capacity. Likewise, a study by Henriques and Silva [86] supported our findings and described the virulence of *C. albicans* factors which are accountable for the infection. These factors include adherence to the host, the formation of biofilm, and the release of hydrolytic enzymes. Furthermore, *C. albicans* can colonize on the epithelial surface and grow inducing deep piercing into tissues and severe injury in Zebrafish (*Danio rerio*) [87]. Similar histopathological changes were reported in the liver and kidney of Nile tilapia by *C. albicans* [7].

Focusing on the importance of NCG, the study outcomes implied that the NCG-exposed group has a defined improvement in the histological architecture following the challenge. The protecting activity of NCG is opined to its ability to easily penetrate tissues inducing antioxidant and antimicrobial activity which in turn protects tissues from the oxidative damage induced by *C. albicans*. Also, it could be attributed that NGs are characterized by effective encapsulation and binding activity with the microorganism as clarified by Jiang et al. [88]. Consistent with a recent report, Kuperkar et al. [89] revealed that the small-sized nano gel enables it to overpass the minute capillaries and then pierce tissues through a transcellular pathway. Chitosan can suppress microbial growth via binding with vital minerals and nutrients causing leakage of smaller particles such as phosphate and potassium, followed by larger particles such as DNA and RNA [90]. Furthermore, Chandrasekaran et al. [91] revealed that the antimicrobial action of chitosan nanocomposites is attributed to its metallic ion chelating property.

Based on the study outcomes, NCG has favorable effects on fish performance and can alleviate the negative impacts of *C. albicans* in Nile tilapia. These outcomes are because of its capability to relieve stress conditions and behavioral changes, modulate hepato-renal and neurological functions, and exert potent antioxidant activity in hepatic tissue, besides maintaining the histological architectures.

Conclusion

The findings of the current study imply the promising effects of the NCG (75 µg/L) in mitigating the hazardous effects of *C. albicans* infection in Nile tilapia. In addition, NCG is found to possess various privileges including potential neuro-behavioral and stress modulators, antioxidant activity, regenerating hepato-renal function, and histopathological changes induced by *C. albicans*. Therefore, using NCG as an antimicrobial substitute may favorably sustain the aquaculture sector. Further studies are required to test its efficacy on a broad spectrum of pathogens (both fungi and bacteria) as well as on other farmed aquatic species. In addition to investigating the long-term effects of NCG treatment on fish health and productivity.

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Author contributions
HHM, ANA-conceptualization, methodology, resources, data collection and analysis, visualization, validation, writing original draft, writing– review and editing; STA- conceptualization, investigation, data analysis, writing– review and editing, AAA, EMY-conceptualization, funding acquisition, writing– review and editing, MS, MSS, MMSG-methodology, data collection and analysis, visualization, validation, writing original draft, writing– review, and editing. MY, SJD: writing– review and editing. EKA-methodology, data collection, and analysis, visualization, validation, writing original draft; SHI-methodology, data collection and analysis, validation, writing the original draft. All authors have read and agreed to the published version of the manuscript.

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Data availability
All data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate
The Institutional Animal Care and Use Committee of Zagazig University, Egypt approved the experimental protocol (ZUIACUC–2-F–333–2022). All methods were performed in accordance with the relevant guidelines and regulations. Informed consent has been obtained from the private farm owners.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Dayana Senthamarai M, Rajan MR, Bharathi PV. Current risks of microbial infections in fish and their prevention methods: a review. Microb Pathog. 2023;185:106400.
2. Ibrahim RE, Elshopakey GE, Abd El-Rahman GI, Ahmed AI, Altohamy DE, Zaglool AW, Younis EM, Abdelwarith AA, Davies SJ, Al-Harthi HF, et al. Palliative role of colloidal silver nanoparticles synthetized by moringa against *Saprolegnia spp.* infection in Nile Tilapia: Biochemical, immuno-antioxidant response, gene expression, and histopathological investigation. Aquac Rep. 2022;26:101318.
3. Talapko J, Juzbašić M, Matijević T, Pustijanac E, Bekić S, Kotris I, Škrlec I. *Candida albicans*-the virulence factors and clinical manifestations of infection. J Fungi. 2021;7(2):79.
4. Kumamoto CA, Gresnigt MS, Hube B. The gut, the bad and the harmless: *Candida albicans* as a commensal and opportunistic pathogen in the intestine. Curr Opin Microbiol. 2020;56:7–15.
5. Tartor Y, Taha M, Mahboub H, El Ghamery M. Yeast species associated with diseased fish: occurrence, identification, experimental challenges and antifungal susceptibility testing. Aquaculture. 2018;488:134–44.
6. Younis GA, Esawy AEKM, Elkenany RM, Deen MMSE. Conventional identification of pathogenic fungi isolated from fresh water aquarium fish (*O. Niloticus and C. Gariepinus*) com-bined with molecular identification of *Saprolegnia parasitica* in Egypt. Adv Anim Vet Sci. 2020;8:77–88.
7. Zayed M, Soliman M, Kalil R, Saad T. Isolation of *Candida albicans* from naturally infected freshwater fish. Kafrelsheikh Vet Med J. 2016;14:21–45.
8. Eissa AE, Tharwat NA, Zaki MM. Field assessment of the mid winter mass kills of trophic fishes at Mariotteya stream, Egypt: Chemical and biological pollution synergistic model. Chemosphere. 2013;90(3):1061–8.
9. Oda SS, Tohamy HG, Massoud RG. Pathological alterations in nile tilapia experimentally infected with *Streptococcus iniae* and *Candida albicans*. Turkish J Fish Aquat Sci. 2016;16:779–88.
10. Gharaghani M, Shabanzadeh M, Jafarian H, Zarei Mahmoudabadi A. ABC typing and extracellular enzyme production of *Candida albicans* isolated from Candida Vulvovaginitis. J Clin Lab Anal. 2022;36:e24117.
11. Mroczyńska M, Brillowska-Dąbrowska A. Virulence of clinical Candida isolates. Pathogens. 2021;10:466.
12. Brothers KM, Newman ZR, Wheeler RT. Live imaging of disseminated candidiasis in zebrafish reveals role of phagocyte oxidase in limiting filamentous growth. Eukaryot Cell. 2011;10(7):932–44.
13. Jong AY, Stins MF, Huang SH, Chen SH, Kim KS. Traversal of *Candida albicans* across human blood-brain barrier in *vitro*. Infect Immun. 2001;69(7):4536–44.
14. Kluczka J. Chitosan: Structural and chemical modification, properties, and application. Inter J Mol Sci 2024;25:554.
15. Iber BT, Kasan NA, Torsabo D, Omuwa JW. A review of various sources of chitin and chitosan in nature. J Renew Mater. 2021;10(4):1097–123.
16. Muangsawat S, Chaiyosang P, Sinkanarak P, Sukted J, Thanyasrisung P, Matangkasombut O. Effects of efflux pumps on antifungal activity of chitosan against *Candida albicans*. J Oral Microbiol. 2024;16(1):2357976.
17. Srimaneepong V, Thanamee T, Wattanasirmkit K, Muangsawat S, Matangkasombut O. Efficacy of low-molecular weight chitosan against Candida albicans biofilm on polymethyl methacrylate resin. Aust Dent J. 2021;66(3):262–9.

18. Thanyasrisung P, Satitviboon W, Howattanapanich S, Matangkasombut O. Antifungal drug resistance in oral Candida isolates from HIV-infected and healthy individuals and efficacy of Chitosan as an alternative antifungal agent. Arch Oral Biol. 2023;147:105628.
19. Elabd H, Mahboub HH, Salem SMR, Abdelwahab AM, Alwutayd KM, Shaalan M, Ismail SH, Abdelfattah AM, Khalid A, Mansour AT, et al. Nano-curcumin/chitosan modulates growth, biochemical, immune, and antioxidative profiles, and the expression of related genes in Nile tilapia, Oreochromis niloticus. In: Fishes. 2023:8.
20. Ibrahim RE, Fouda MMS, Younis EM, Abdelwarith AA, Salem GA, Elkady AA, Ismail SH, Davies SJ, Abdel Rahman AN. The anti-bacterial efficacy of zinc oxide nanoparticles synthesized by *Nelumbo nucifera* leaves against *Clostridium perfringes* challenge in *Oreochromis niloticus*. Aquaculture. 2024;578:740030.
21. Fajardo C, Martinez-Rodriguez G, Blasco J, Mancera JM, Thomas B, De Donato M. Nanotechnology in aquaculture: applications, perspectives and regulatory challenges. Aquac Fish. 2022;7(2):185–200.
22. Kakakhel MA, Bibi N, Mahboub HH, Wu F, Sajjad W, Din SZU, Hefny AA, Wang W. Influence of biosynthesized nanoparticles exposure on mortality, residual deposition, and intestinal bacterial dysbiosis in *Cyprinus carpio*. Comp Biochem Physiol C Toxicol Pharmacol. 2023;263:109473.
23. Ferosekhan S, Gupta S, Singh AP, Rather MA, Kumari R, Kothari D, Pal AK, Jadhao SB. RNA-loaded chitosan nanoparticles for enhanced growth, immunostimulation and disease resistance in fish. Curr Nanosci. 2014;10:453–64.
24. Mahboub H, Elabd H, Kakakhel MA, Elshopakey GE, Abduljabbar MH, Alosaimi ME. Progressive immunomodulation through nanotechnology. In: Immunomodulators in Aquaculture and Fish Health, 1st edn. Edited by Iumalai P, Soltani, M., Lakshmi, S, editors. Boca Raton: CRC Press. 2023;13:11.
25. Abdel-Ghany HM, Salem ME-S. Effects of dietary chitosan supplementation on farmed fish; a review. Rev Aquac. 2020;12(1):438–52.
26. El-Naggar M, Salaah S, El-Shabaka H, El-Rahman FA, Khalil M, Suloma A. Efficacy of dietary chitosan and chitosan nanoparticles supplementation on health status of Nile tilapia, *Oreochromis niloticus* (L). Aquac Rep. 2021;19:100628.
27. Nuzaiba PM, Gupta S, Gupta S, Jadhao SB. Synthesis of L-methionine-loaded chitosan nanoparticles for controlled release and their in *vitro* and in *vivo* evaluation. Sci Rep. 2023;13(1):7606.
28. Abdel Rahman AN, Elshopakey GE, Behairy A, Altohamy DE, Ahmed AI, Farroh KY, Alkafafy M, Shahin SA, Ibrahim RE. Chitosan-*Ocimum basilicum* nanocomposite as a dietary additive in *Oreochromis niloticus*: effects on immune-antioxidant response, head kidney gene expression, intestinal architecture, and growth. Fish Shellfish Immunol. 2022;128:425–35.
29. Poznanski P, Hameed A, Orczyk W. Chitosan and chitosan nanoparticles: parameters enhancing antifungal activity. In: Molecules. vol. 2023;28: 2996.
30. Soussi S, Essid R, Karkouch I, Saad H, Bachkouel S, Aouani E, Limam F, Tabbene O. Effect of lipopeptide-loaded chitosan nanoparticles on *Candida albicans* adhesion and on the growth of *Leishmania major*. Appl Biochem Biotechnol. 2021;193(11):3732–52.
31. Kubeil M, Suzuki Y, Casulli M, Kamal R, Hashimoto T, Bachmann M, Hayashita T, Stephan H. Exploring the potential of nanogels: from drug carriers to radiopharmaceutical agents. Adv Healthc Mater. 2024;13(1):e2301404.
32. Shatsberg Z, Zhang X, Ofek P, Malhotra S, Krivitsky A, Scomparin A, Tiram G, Calder´on M, Haag R, Satchi-Fainaro R. Functionalized nanogels carrying an anticancer microRNA for glioblastoma therapy. J Control Release. 2016;239:159–60.
33. Ferrer MCC, Dastgheyb S, Hickok NJ, Eckmann DM, Composto RJ. Designing nanogel carriers for antibacterial applications. Acta Biomater. 2014;10(5):2105–11.
34. Manimaran V, Nivetha RP, Tamilanban T, Narayanan J, Vetriselvan S, Fuloria NK, Chinni S, Sekar M, Fuloria S, Wong LS, et al. Nanogels as novel drug nanocarriers for CNS drug delivery. Front Mol Biosci. 2023;10:1232109.
35. Gupta J, Sharma G. Nanogel: A versatile drug delivery system for the treatment of various diseases and their future perspective. Drug Deliv Transl Res 2025;15:455–482. https://link.springer.com/article/10.1007/s13346-024-01684-w
36. Raj V, Raorane C, Shastri D, Kim S, Lee S. Engineering a self-healing grafted chitosan-sodium alginate based hydrogel with potential keratinocyte cell migration property and inhibitory effect against fluconazole resistance *Candida albicans* biofilm. Int J Biol Macromol. 2024;261:129774.
37. Manivong S, Garcia Ac A, Patten SA, Fernandes JC, Benderdour M, Banquy X, Moldovan F, Roullin V. Chitosan-based nanogels: synthesis and toxicity profile for drug delivery to articular joints. Nanomater. 2022;12(8):1337.
38. El-Sayed A, Tilapia. culture, second edn. Cambridge, MA, USA: CABI, Academic Press, 2019;2006.
39. Mahboub HH, Eltanahy A, Omran A, Mansour AT, Safhi FA, Alwutayd KM, Khamis T, Husseiny WA, Ismail SH, Yousefi M, et al. Chitosan Nanogel aqueous treatment improved blood biochemicals, antioxidant capacity, immune response, immune-related gene expression and infection resistance of Nile tilapia. Comp Biochem Physiol B. 2024;269:110876.
40. Ismail SH, Hamdy A, Ismail TA, Mahboub HH, Mahmoud WH, Daoush WM. Synthesis and characterization of antibacterial carbopol/ZnO hybrid nanoparticles gel. Crystals. 2021;11:1092.
41. Ibrahim RE, Elshobaky G, ElHady M, Abdelwarith AA, Younis EM, Rhouma NR, Murad SK, Yassin EMM, Khamis T, Ismail SH, et al. *Nelumbo nucifera* synthesized selenium nanoparticles modulate the immune-antioxidants, biochemical indices, and pro/anti-inflammatory cytokines pathways in Oreochromis niloticus infected with *Aeromonas veronii*. Fish Shellfish Immunol. 2024;144:109287.
42. CCAC. Canadian Council on Animal Care Guidelines on: The Care and Use of Fish in Research, Teaching and Testing. Ottawa, ON, Canada: Canadian Council on Animal Care; 2005.
43. APHA. Standard Methods for Examination of Water and Wastewater, 21th, ed. American Public Health Association, American Water Works Association, Water Pollution Control Federation, New York. 2005.
44. Boyd CE, Tucker CS. Pond Aquaculture Water Quality Management. Springer Science & Business Media; 2012.
45. Neiffer DL, Stamper MA. Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. ILAR J. 2009;50:343–60.
46. Altmann J. Observational study of behavior: sampling methods. Behaviour. 1974;49:227–67.
47. Calfee R, Puglis H, Little E, Brumbaugh W, Mebane. Quantifying fish swimming behavior in response to acute exposure of aqueous copper using computer assisted video and digital image analysis. J Vis Exp 2016;108:e53477.
48. Frey DF, Miller RJ. The establishment of dominance relationships in the blue gourami, *Trichogaster trichopterus* (Pallas). Behaviour. 1972;42:8–62.
49. Anras MLB, Lagardère JP. Measuring cultured fish swimming behaviour: first results on rainbow trout using acoustic telemetry in tanks. Aquaculture. 2004;240:175–86.
50. Sekiguchi Y, Kohshima S. Resting behaviors of captive bottlenose dolphins (*Tursiops truncatus*). Physiol Behav. 2003;79:643–53.
51. Khalil F, Emeash H. Behaviors and stereotypies of nile tilapia (Oreochromis niloticus) in response to experimental infection with *Aeromonas hydrophila*. Aquat Sci Eng. 2018;33:124–30.
52. Farag MR, Zheng C, Gharib HS, El-Hady E, Mahdy EA, Abo-Elmaaty A, Abou-Zeid SM, Alagawany M, Di Cerbo A, Azzam MM, et al. Physiological and neurobehavioral disturbances induced by Al_2O_3 nanoparticle intoxication in nile tilapia fish: benefits of dietary chamomile essential oil. Aquac Nutr. 2023;3:6700708.
53. Reitman S, Frankel S. A colorimetric methods for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957;28:56–63.
54. Perakis N, Wolff C. Kinetic approach for the enzymic determination of creatinine. Clin Chem. 1984;30:1792–6.
55. Wenger C, Kaplan A, Rubaltelli F, Hammerman C. Alkaline phosphatase. Clin Chem. 1984;62:1094–8.
56. Chaney AL, Marbach EP. Modified reagents for determination of urea and ammonia. Clin Chem. 1962;8:130–2.
57. Teixeira RR, De Souza RC, Sena AC, Baldisserotto B, Heinzmann BM, Copatti CE. Essential oil of Aloysia triphylla is effective in Nile tilapia transport. Bol Inst Pesca. 2018;44:17–24.
58. Ellman GL, Courtney KD, Andres V Jr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88–95.
59. Fernandez-Botran R, Gorantla V, Sun X, Ren X, Perez-Abadia G, Crespo FA, Oliver R, Orhun HI, Quan EE, Maldonado C. Targeting of glycosaminoglycan-cytokine interactions as a novel therapeutic approach in allotransplantation1. Transplantation. 2002;74:623–9.
60. Koracevic D, Koracevic G, Djordjevi ´c V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human. J Clin Pathol. 2001;54:356–61.
61. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882–8.
62. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351–8.

63. Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques E-Book. Amsterdam, The Netherlands: Elsevier Health Sciences; 2018.
64. Gibson-Corley KN, Olivier AK, Meyerholz DK. Principles for valid histopathologic scoring in research. Vet Pathol. 2013;50(6):1007–10015.
65. Sarkar P, Stefi Raju V, Kuppusamy G, Rahman MA, Elumalai P, Harikrishnan R, Arshad A, Arockiaraj J. Pathogenic fungi affecting fishes through their virulence molecules. Aquaculture. 2022;548:737553.
66. Abdel Rahman AN, Mahboub HH, Ezz-Eldin RMM, Abdelwarith AA, Younis EM, Khamis T, Aziz EK, Basha WAA, Elmaghraby I, Davies SJ, et al. Lead toxicity in African catfish: promising role of magnetite nanogel against etho-neurological alterations, antioxidant suppression, gene toxicity, and histopathological/immunohistochemical disruptions. Aquaculture. 2025;594:741411.
67. Segarra A, Mauduit F, Amer NR, Biefel F, Hladik ML, Connon RE, Brander SM. Salinity changes the dynamics of pyrethroid toxicity in terms of behavioral effects on newly hatched delta smelt larvae. Toxics. 2021;9:40.
68. Bibi N, Zuberi A, Naeem M, Ullah I, Sarwar H, Atika B. Evaluation of acute toxicity of karate and its sub-lethal effects on protein and acetylcholinestrase activity in *Cyprinus carpio*. Int J Agric Biol. 2014;16:731–7.
69. Snarr B, Drummond R, Lionakis M. It's all in your head: antifungal immunity in the brain. Curr Opin Microbiol. 2020;58:41–6.
70. Kirsten K, Fior D, Kreutz LC, Barcellos LJG. First description of behavior and immune system relationship in fish. Sci Rep. 2018;8(1):846.
71. Gibney SM, Drexhage HA. Evidence for a dysregulated immune system in the etiology of psychiatric disorders. J Neuroimmune Pharmacol. 2013;8(4):900–20.
72. Ragusa A, Priore P, Giudetti AM, Ciccarella G, Gaballo A. Neuroprotective investigation of chitosan nanoparticles for dopamine delivery. In: Appl Sci. 2018;18:474.
73. Xue Y, Wang N, Zeng Z, Huang J, Xiang Z, Guan Y-Q. Neuroprotective effect of chitosan nanoparticle gene delivery system grafted with acteoside (ACT) in Parkinson's disease models. J Mater Sci Techol. 2020;43:197–207.
74. Ibrahim RE, Elshopakey GE, Abdelwarith AA, Younis EM, Ismail SH, Ahmed AI, El-Saber MM, Abdelhamid AE, Davies SJ, El-Murr A, et al. Chitosan neem nanocapsule enhances immunity and disease resistance in nile tilapia (Oreochromis niloticus). Heliyon. 2023;9(9):e19354.
75. Lopes JP, Lionakis MS. Pathogenesis and virulence of *Candida albicans*. Virulence. 2022;13:89–121.
76. Ganan M, Carrascosa AV, Martínez-Rodríguez AJ. Antimicrobial activity of chitosan against *Campylobacter spp.* and other microorganisms and its mechanism of action. J Food Prot. 2009;72(8):1735–8.
77. Al-Zahrani SS, Bora RS, Al-Garni SM. Antimicrobial activity of chitosan nanoparticles. Biotechnol Biotechnol Equip. 2021;35(1):1874–80.
78. Sadoul B, Geffroy B. Measuring cortisol, the major stress hormone in fishes. J Fish Biol. 2019;94(4):540–55.
79. Murugananthkumar R, Sudhakumari C-C. Understanding the impact of stress on teleostean reproduction. Aquac Fish. 2022;7(5):553–61.
80. Lemos LS, Angarica LM, Hauser-Davis RA, Quinete N. Cortisol as a stress indicator in fish: sampling methods, analytical techniques, and organic pollutant exposure assessments. Int J Environ Res Public Health. 2023;20(13):6237.
81. Baldissera MD, Souza CF, Zeppenfeld CC, Velho MC, Klein B, Abbad LB, Ourique AF, Wagner R, Da Silva AS, Baldisserotto B. Dietary supplementation with nerolidol nanospheres improves growth, antioxidant status and fillet fatty acid profiles in Nile tilapia: benefits of nanotechnology for fish health and meat quality. Aquaculture. 2020;516:734635.
82. Juan CA, de la Pérez JM, Plou FJ, Pérez-Lebeña E. The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. Inter J Mol Sci. 2021;22:4642.
83. Mahboub HH, Nada HS, Abdel-Ghany HM, Ghanem R, Ismail TA, Abdel Rahman AN. Detection, diagnosis, Koch's postulate, hepatorenal and antioxidant indicators for some systemic pathogenic fungi invading the liver and kidneys of African catfish (*Clarias gariepinus*) in Egypt with a histopathological approach. Aquac Res. 2022;53(7):2670–85.
84. Dizaj SM, Lotfipour F, Barzegar-Jalali M, Zarrintan MH, Adibkia K. Antimicrobial activity of the metals and metal oxide nanoparticles. Mater Sci Eng C Mater Biol Appl. 2014;44:278–84.
85. Abdel-Tawwab M, Razek NA, Abdel-Rahman AM. Immunostimulatory effect of dietary chitosan nanoparticles on the performance of Nile tilapia, *Oreochromis niloticus* (L). Fish Shellfish Immunol. 2019;88:254–8.
86. Henriques M, Silva S. *Candida albicans* virulence factors and its pathogenicity. In: Microorganisms.2021;9:704.
87. Chen YY, Chao CC, Liu FC, Hsu PC, Chen HF, Peng SC, Chuang YJ, Lan CY, Hsieh WP, Wong DS. Dynamic transcript profiling of *Candida albicans* infection in zebrafish: a pathogen-host interaction study. PLoS ONE. 2013;8(9):e72483.
88. Jiang L, Zhou Q, Mu K, Xie H, Zhu Y, Zhu W, Zhao Y, Xu H, Yang X. pH/temperature sensitive magnetic nanogels conjugated with Cy5.5-labled lactoferrin for MR and fluorescence imaging of glioma in rats. Biomaterials. 2013;34(30):7418–28.
89. Kuperkar K, Patel D, Atanase LI, Bahadur P. Amphiphilic block copolymers: their structures, and self-assembly to polymeric micelles and polymersomes as drug delivery vehicles. In: Polymers. 2022;14:4702.
90. Ke CL, Deng FS, Chuang CY, Lin CH. Antimicrobial actions and applications of chitosan. Polymers. 2021;13(6):904.
91. Chandrasekaran M, Kim KD, Chun SC. Antibacterial activity of chitosan nanoparticles: a review. In: Processes.; 2020;8: 1173.

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