Aquaculture 584 (2024) 740684

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Artichoke *(Cynara scolymus)* leaf extract abates the neurotoxic and neurobehavioral outcomes of fluoride in Nile tilapia (*Oreochromis niloticus*) via balancing oxidative stress, inflammation, apoptosis, and acetylcholinesterase activity

Asmaa Elsayyad [a,b], Yasmin A. Reyad [c], Basma A. Elshafey [d], Enas K. Aziz [d], Mohamed M. M. Metwally [e,f], Yasmina M. Abd-Elhakim [g,*], Abdel-Wahab A. Abdel-Warith [h], Elsayed M. Younis [h], Simon J. Davies [i], Walaa El-Houseiny [j,*], Ahmed H. Arisha [k,l], Hanan A. Ghetas [m]

[a] *Department of Biochemistry, Physiology, and Pharmacology, Faculty of Veterinary Medicine, King Salman International University, South of Sinaa 46612, Egypt*
[b] *Department of Pharmacology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt*
[c] *Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Mansoura University, Egypt*
[d] *Department of Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, University of Sadat City, Egypt*
[e] *Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, King Salman international University, Ras Sidr, Egypt.*
[f] *Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt*
[g] *Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt*
[h] *Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia*
[i] *Aquaculture Nutrition Research Unit ANRU, Carna Research Station, Ryan Institute, College of Science and Engineering, University of Galway, H91V8Y1 Galway, Ireland*
[j] *Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt*
[k] *Department of Animal Physiology and Biochemistry, Faculty of Veterinary Medicine, Badr University in Cairo (BUC), Badr City, Cairo, Egypt*
[l] *Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt*
[m] *Department of Aquatic Animals Medicine and Management, Faculty of Veterinary Medicine, University of Sadat City, Egypt*

A R T I C L E I N F O

Keywords:
Fluoride
Cynara scolymus leaf extract
Aggression
Pro-inflammatory cytokines
Heat shock protein 70

A B S T R A C T

This study assessed the effects of fluoride (FLO) on neurobehavioral function, brain oxidative status and inflammatory response, acetylcholinesterase activity, and histopathological picture in Nile tilapia (*Oreochromis niloticus*) with a mitigative trial using Artichoke *(Cynara scolymus)* leaf extract (AE). To achieve this, 240 *O. niloticus* fish (30 ± 1.5 g average initial weight) were distributed into four groups, each with four replicates in a 60-day feeding trial. A basal diet without supplements was given to the control group. A 300 mg/kg AE supplement was included in the 2nd group's basal diet (AE). A sub-lethal concentration of FLO (6.1 mg/L) was administered to the 3rd group of tilapias. Meanwhile, the last group was fed an AE-containing diet and exposed to FLO. The findings exhibited that supplementing AE to the *O. niloticus* diets significantly resolved the FLO-induced decrease in the frequency of middle swimming, feeding, and middle crossing. Additionally, AE food supplementation significantly ($P < 0.05$) attenuated the FLO-induced-aggression. The significant exhaustion in enzymatic and non-enzymatic antioxidant brain content documented in FLO-exposed fish was significantly reverted by AE incorporation into their diets. Also, cortisol, glucose, cholesterol, triglycerides, 8-OHdG, and MDA displayed detectable increases in FLO-exposed groups, but the level of brain acetylcholinesterase was significantly reduced. Pro-inflammatory cytokines (*il-1β* and *tnf-α*), apoptotic (*caspase-3* and *p53*), and stress-related (*hsp70*) genes were noticeably elevated in the brains of fish treated with FLO, whereas the expression of anti-oxidative (*sod* and *cat*) genes was dramatically downregulated. Yet, incorporating AE in the diet repaired the alterations that FLO elicited in most of the indicators mentioned above. Further, AE dietary supplementation significantly minimized the FLO-induced histopathological alterations in the fish brain tissue. These findings suggested that AE would be an effective dietary supplement to lessen the harmful effects of FLO on the Nile tilapia's behavior and brain health.

* Corresponding authors.
E-mail addresses: yasmina_forensic@hotmail.com (Y.M. Abd-Elhakim), drwalaaelhouseiny@yahoo.com (W. El-Houseiny).

https://doi.org/10.1016/j.aquaculture.2024.740684
Received 13 November 2023; Received in revised form 11 February 2024; Accepted 16 February 2024
Available online 17 February 2024

1. Introduction

The aquatic ecosystem is a sump for numerous environmental pollutants (El-Bouhy et al., 2023). One of the most prevalent substances in the environment is fluorine (Davison and Weinstein, 2006). In soil and rock minerals, organic and inorganic fluorine compounds prevail. Fluoride (FLO) is crucial for the growth and development of living things (Mahmoud et al., 2020). Freshwater FLO values in unpolluted areas range from 0.01 to 0.3 mg/L (Camargo, 2003). Nevertheless, both human and natural activities like FLO-containing agro-pesticides and fertilizers use, swift industrialization, the release of fluoridated municipal water, FLO-mineral weathering, and volcanic eruptions assist in the increment in FLO burdens in groundwater and surface water. According to an investigation of industrial effluent, the FLO level ranged from 0.1 g/L to 3–5 g/L (Wu et al., 2006). Fish are the primary targets of FLO pollution in aquatic ecosystems as they can get FLO from the water (Cao et al., 2015).

Fluorosis is a significant clinical and public health issue worldwide (Kaur et al., 2017). Enzymatic poisons like FLO ions hinder metabolic processes, including glycolysis and protein synthesis, by interfering with enzyme activity (Camargo, 2003). Excessive FLO in fish may have a range of detrimental consequences. The hematoencephalic barrier is permeable to FLO, which can settle in different brain parts (Ottappilakkil et al., 2023), leading to aberrant behavior (Bajpai et al., 2009). Exposure to FLO induces a delay in the maturation of sensorimotor responses, a decline in the nociceptive reflex reaction, a spike in locomotor activity, and inhibition of acetylcholinesterase (AChE) activity (Dominguez et al., 2021). Moreover, FLO has been linked to several health risks, including alteration to the key biochemical pathways (Guo et al., 2018), triggering oxidative stress (Gupta and Poddar, 2014), damaging the head kidney structure, and impaired immune defenses (Ling et al., 2017), hindering the expression of pro-inflammatory cytokines and destroying the host's resistance against bacteria (Singh et al., 2017).

There is currently no method to avert fluorosis; phytochemical-based remediation of the condition's identifying symptoms has been postulated as a potential cure. Oxidative stress is viewed as FLO's main action (El-Houseiny et al., 2022a; Miranda et al., 2021). The well-known Fenton reaction, which leads to radical production, may be triggered by FLO (Agalakova and Gusev, 2012). Consequently, natural supplementation with antioxidants might effectively alleviate the detrimental impacts of FLO on fish (Cao et al., 2015). The artichoke, *Cynara scolymus* L., is a mediterranean native plant and a member of the *Asteraceae* family. It is grown worldwide due to its beneficial nutritional and therapeutic characteristics (Cavini et al., 2022). Officially, items made from therapeutic artichoke are just dried leaves. In addition to the polysaccharide inulin, fibers, and minerals, it is an excellent source of polyphenolic chemicals, primarily caffeoylquinic acids, and flavonoids, which were extracted from the plant's polar extracts (Ibrahim et al., 2022). The phenolic compounds in artichokes protect against oxidative harm to biological components like proteins, lipids, and DNA by scavenging reactive oxygen species (ROS) and free radicals (Ceccarelli et al., 2010). According to Gebhardt (2001), flavonoids and 1,3-dicaffeoylquinic acid were thought to be choleretic, anti-cholestatic, and diuretic, lowering cholesterol. The plant and its derivatives are frequently used to treat dyspepsia, but they are also traditionally used to avert and cure atherosclerosis and renal malfunction (diuretic) (Mahady, 2009). It has a neuroprotective effect against aflatoxin in rats (Ibrahim et al., 2022). Unfortunately, little is known regarding the impact of AE as a supplement in the fish diet. To demonstrate the effects of chronic FLO exposure, we selected the common and economically significant freshwater fish Nile tilapia (*Oreochromis niloticus*) in the current study. Due to it being affordable, a good source of protein and micronutrients, fast-growing, and tolerant to a wide range of environmental factors, tilapia farming has gained the attention of fish farmers (Mounes et al., 2024; Surachetpong et al., 2020). Also, it has been employed effectively in numerous xenobiotic ecotoxicological experiments and is capable of withstanding extreme environmental stress (Abd El-Hakim et al., 2020; El-Houseiny et al., 2022b; El-Houseiny et al., 2023; El Basuini et al., 2020; Mohamed et al., 2019).

In the early phases of growth and development, the central nervous system is far more vulnerable to FLO intoxications (Dominguez et al., 2021). Considering the rising demand for FLO on a global scale and the possibility of expanding anthropogenic supplies, it is crucial to create a nutritional plan to counteract its adverse impacts on fish. Furthermore, the addition of AE to the diets of cultured fish may be beneficial depending on their biological processes. Hence, behavioral, biochemical, molecular, and histological studies were used in the current investigation to evaluate the hazards of FLO exposure as a neurotoxic agent and the ameliorative impact of AE in Nile tilapia (*O. niloticus*).

2. Material and methods

2.1. Reagents

As a white crystalline powder, sodium fluoride (99% purity) was procured from Thomas Baker Chemical Industries Pvt. Ltd. (Mumbai, India). Commercial AE (super artichoke) capsules were manufactured by Western Pharmaceutical Industries in Cairo, Egypt, and used in this investigation. HPLC was employed to define the biologically active components of the AE in the earlier study of Ibrahim et al. (2022). Seventeen elements were found in the artichoke capsule during analysis. The chlorogenic acid (28.00 mg/g) in the artichoke has the highest component content, followed by several substances, including pyrocatechol (1.86 mg/g), gallic acid (1.24 mg/g), catechin (1.39 mg/g), naringenin (1.29 mg/g), and ferulic acid (1.07 mg/g).

2.2. Fish and experimental circumstances

The male Nile tilapia fingerlings, which had an average body weight of 30 ± 1.5 g, were acquired from the Fish Research Centre, Zagazig University, Egypt. Before the experiment began, the fish were raised and maintained in a lab environment on a basal diet in glass aquariums with 70 L of dechlorinated tap water to minimize the influence of other detrimental factors. The following values were recorded across the course of the study: 27.3 ± 0.05 °C, 6.5 ± 0.5 pH, 0.03 ± 0.01 mg ammonia/L, and 6.80 ± 0.5 mg dissolved oxygen /L. The water parameters were within the varieties required for fish growth during the trial (Boyd and Tucker, 2012). In the laboratory, the photoperiod was adjusted to 12 h of light and 12 h of darkness. Under the supervision of Zagazig University's Animal Use in Research Committee, the experiment was conducted per the National Institutes of Health's (NIH) ethical standards for the Use and Care of Laboratory Animals in Scientific Investigations (ZU-IACUC/2/F/274/2023).

2.3. Diet formulation and experiment layout

Consistent with Nutrient Requirements of Fish and Shrimp (Jobling, 2012), the experimental diet ingredients displayed in Table 1 were created to meet the fish's nutritional demands to the greatest extent possible. The components of the diet were well combined before being mechanically pelletized, dried in the air at room temperature for 24 h, and then put into storage at 4 °C until use. Following the guidelines of AOAC (2006), the moisture, crude protein, crude fiber, crude fat, and total ash contents of each studied diet were examined by the forced-air oven, macro-Kjeldahl, ether extraction method, and muffle furnace, respectively. Fish were fed at a rate of 5% of their live weight three times per day, at 9:00, 12:00, and 16:00 and any extra feed and feces were siphoned out. Post acclimation, 240 fish were allocated into four groups, each consisting of four replicates (15 fish per replica, a total of 60 fish in each group). A basic meal without any supplementation was supplied to the normal control group (CON), and the 2nd group (AE) received a basal diet with 300 mg AE /kg diet. The AE dose was chosen based on a

Table 1
Ingredients and proximate chemical analysis of the experimental diets.

Ingredients (g /kg)	Experimental diets	
	C	Artichoke leaf extract
Fish meal 66%	190	190
Ground corn	250	249.7
Soybean meal 44%	340	340
Corn oil	30	30
Wheat bran	100	100
Cod liver oil	20	20
starch	40	40
Artichoke leaf extract	0	**0.3**
Vitamin premix[a]	10	10
Mineral premix[b]	20	20
Total	1000	1000
Chemical analysis		
Crude protein (N × 6.25)	310.1	312.1
Crude lipids	74.5	73.3
Crude fiber	54.5	53.3
Ash	55.6	56.8
Nitrogen free extract[c]	505.4	504.6
Gross energy (kcal/kg)[d]	4533.1	4530.1

[a] Vitamin premix (per kg of premix): vitamin A,8000000 IU; vitamin E, 7000 mg; vitamin D_3, 2,000,000 IU; vitamin K_3,1500 mg; biotin, 50 mg; folic acid, 700 mg; nicotinic, 20,000 mg; pantothenic acid,7000 mg; vitamin B_1, 700 mg; vitamin B_2, 3500 mg; vitamin B_6, 1000 mg; vitamin B_{12}, 7 mg.

[b] Mineral premix (per kg of premix): zinc sulfate, 4.0 g; iron sulfate, 20 g; manganese sulfate,5.3 g; copper sulfate, 2.7 g; calcium iodine, 0.3 g; sodium selenite, 70 mg; cobalt sulfate, 70 mg, and $CaHPO_4{\cdot}2H_2O$ up to 1 kg.

[c] Calculated by difference (1000 –protein% + lipids% + ash% + crude fiber %).

[d] Gross energy (GE) was calculated as 5.65, 9.45, and 4.11 kcal/g for protein, lipid, and NFE, respectively (NRC, 1993).

preliminary analysis in which the effect of various AE doses (100, 300, and 500 mg/Kg diet) on growth performance indices (final body weight, body weight gain, and specific growth rate), fish survival, and physiological parameters of Nile tilapia including hepatic and kidney function were assessed. The results revealed that adding 300 and 500 mg/Kg diet of AE to the Nile tilapia diet significantly improved their survival and growth without adversely affecting hepatic and renal function. No significant difference was detected between the effects of the two doses, 300 and 500 mg/Kg diet. Hence, the medium dose of 300 mg/Kg diet of AE was chosen to be tested. In the third group of tilapias (FLO), 1/10 of the calculated c-LC_{50} (6.1 mg/L) of FLO was administered (Ahmed et al., 2020). The water was entirely changed every 48 h by evolving the fish to freshly made FLO solutions. The last group (AE + FLO) received AE while also being exposed to FLO, as previously discussed. FLO exposure and AE supplementation were maintained for two months. Mortality rate (MR, %) = 100- (Fish number at the end of feeding trial/ Initial fish number) × 100.

2.4. Behavioral assays

As described by Altmann (1974), behavior metrics were executed across the investigation between (09:00 AM) and (04:00 PM) through the approach of direct observation with a stopwatch and a video camera. Throughout the testing time, all behavior attitudes were observed and documented every (15) minutes for six hours. These were the behavior patterns that were observed:

Feeding behavior refers to the actual fish meal consumption during feeding time following Bond (1979) methodology.

Surfacing behavior: documented as the frequency of fish air piping near the water's surface due to insufficient dissolved oxygen in aquariums (El-Hawarry et al., 2018).

Swimming behavior implies monitoring fish swimming in a fast or slow manner without engaging in any behavioral activity at the top, middle, or bottom of the aquarium, according to the procedure set by Chen et al. (2001).

Body shaking is described as a quick series of two or three lateral shakes of the entire fish body (Myrberg Jr, 1972).

Aggressive behavior: In compliance with the protocols of De Boer (1980); Frey and Miller (1972), various patterns were tracked to evaluate fish aggression (the movement of one fish in the direction of another), fin tugging (one fish biting another fish), chasing (the frequency with which a fish swims vigorously after another), fleeing (fish escaping the enemy), and mouth pushing (the number of times two fish stand face to face, mouths wide against each other).

Laterality refers to the quantity of fish observed at the bottom of an aquarium moving side to side for more than three minutes each day (Ismail et al., 2009).

Crossing test: a midline externally divided the aquarium, and the number of fish that crossed it within three minutes for each aquarium was recorded (Scott et al., 2003).

2.5. Blood and brain collection

The fish were anesthetized at the end of the experimental period with tricaine methanesulfonate (MS-222, Argent Chemical Labs, Redmond, Washington, USA) to collect blood and reduce handling stress. Twelve blood samples from each group (3/replicate) were extracted from the caudal vessels without using an anticoagulant, left to cool at room temperature, and then centrifuged for 15 min at 3000 ×*g* to separate the serum, which was stored at −20 °C until a physiological profile evaluation. After blood samples were gathered, specimens of the brain from 30 fish from each group were retrieved, freed of adherent fat and connective tissues, rinsed in cooled saline solution (0.9%), and dried on tissue paper. After that, brain samples were collected from twelve fish randomly taken from all tanks per group and then homogenized in 10 volumes of phosphate-buffered saline (pH 7.4), centrifuged at 664 ×*g* at 4 °C for 15 min, and the supernatant samples were maintained at −80 °C for biochemical analysis. To evaluate the expression of apoptotic and antioxidant genes, some tissues (12/group) were treated with TRIzol (Thermo Scientific) reagent and deposited in a deep freezer (−80 °C) for RNA separation. The remaining brain tissue samples were obtained and fixed in a 10% neutral formalin buffer for histopathological analysis.

2.6. Biochemical assays and DNA damage indicator

We measured serum cortisol levels using an ELISA commercial kit from MyBioSource Inc., San Diego, CA, USA (Cat. No. MBS704055). Glucose levels were assessed using MyBioSource colorimetric kit (Catalog No. MBS841763). 8-hydroxy-2-deoxyguanosine (8-OHdG) activity was assessed by an ELISA commercial kit from My-Biosource (San Diego, CA, USA, Catalog No. MBS1601729), complying with the manufacturer's guidelines, as recommended by Setyaningsih et al. (2015). Colorimetric diagnostic kits (Spectrum Diagnostics, Egyptian Company for Biotechnology, Cairo, Egypt) were utilized for assessing serum total cholesterol (Catalog No. 230002) and triglycerides (Catalog No. 314002) using the approaches of Allain et al. (1974) and McGowan et al. (1983), respectively.

2.7. Evaluation of brain homogenate oxidative stress and lipid peroxidation markers and acetylcholinesterase activity

Following compliance with the instructions provided by the manufacturer, the reduced glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), glutathione S-transferases (GST), and malondialdehyde (MDA) concentrations in the brain homogenate were identified by colorimetric commercial kits of Biodiagnostic Co., Cairo, Egypt (Catalog No. GR 25 11, GP 2524, CA 25 17, SD 25 21, GT 2519, and MD 25 29). The level of AChE activity in brain tissue was determined using an ELISA commercial kit from MyBioSource Inc., San Diego, CA, USA (Cat. No. MBS705766).

2.8. Transcriptional study of genes linked to stress, apoptosis, and antioxidants

To get the total RNA, the frozen brain samples were subjected to TRIzol treatment (easyREDTM, iNtRON Biotechnology, Korea). The first-strand cDNA was generated from the extracted RNA using a Quantitect® Reverse Transcription kit from Qiagen (Germany). The primer's forward and reverse sequences are presented in Table 2. The Rotor-Gene Q instrument was applied for the qPCR analysis via the QuantiTect® SYBR® Green PCR kit (Qiagen, Germany) under the following thermocycler conditions: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. All RT-qPCR experiments were performed in accordance with the minimum information for the publication of quantitative real-time PCR experiments (MIQE) guidelines (Bustin et al., 2009). The Schmittgen and Livak (2008) comparative $2^{-\Delta\Delta}$CT method determined each gene's relative mRNA expression pattern. The expression of reference genes *gapdh*, *β-actin,* and *EF1α* were studied for gene normalization.

2.9. Histopathological study

The whole brain of twelve arbitrarily chosen fish per group was sampled and trimmed as specified by Meyers (2009) and fixed in a 10% neutral buffered formalin solution for 48 h. After fixation, the brain tissue specimens were dehydrated in ethyl alcohol. They were cleared in Histo-Choice® (Sigma-Aldrich, St. Louis, USA), infiltrated and blocked in paraffin-beeswax mixture (90% paraffin and 10% beeswax), sectioned by Leica RM2125 RTS manual rotary microtome at sections five μm thick, stained with Harris hematoxylin and eosin Y solutions as specified by Suvarna et al. (2018) and examined by light microscope. The histological alterations in the brain tissue were evaluated and quantitatively scored following the protocol suggested by Bernet et al. (1999) for assessing aquatic pollution with few modifications. Briefly, for each fish snapshots of ten randomly chosen non-duplicated high-power microscopic fields (×40) were made using the AmScope CMOS C-Mount microscope camera (100 images per group) attached to a Nikon light microscope (Nikon Inc., New York, USA). Next, these images were interpreted where the encountered encephalopathic changes were classified into three reaction patterns (1) circulatory; congestion, edema, and hemorrhages, (2) inflammatory; intracerebral, and meningeal inflammatory cell infiltration, gliosis, and perivascular leukocytic cuffing, and (3) regressive; neuronal pyknosis, and necrosis, perineuronal vacuolation and neuropil microcavitation. The frequency of a specific encephalopathic alteration within a group was calculated by dividing the number of images displayed by this alteration (lesion) by the total number of images in the group (100). Next, the brain index (the greater the index, the worse the pathological condition of the brains within a group) was calculated from the formula:

$$\text{Brain index} = \Sigma_{rp}\ \Sigma_{alt}\ \left(a_{org\ rp\ alt} \times w_{org\ rp\ alt}\right)$$

where: rp (reaction pattern), alt (histopathological alteration), a is a score value signifies the degree of the alteration and its value ranged between zero (absence of the changes) and six (diffuse lesion), and w is an importance factor denotes the alteration seriousness and its value ranged between 1 (least importance) and 3 (great importance).

2.10. Statistical analysis

The data's normality and variance homogeneity were assessed by the Kolmogorov-Smirnov and Levene's tests. Once the normality assumptions were satisfied, one-way Analysis of Variance (ANOVA) was used to statistically analyze the data by SPSS (version 16.0, SPSS Inc., USA). The Tukey's multiple comparisons post hoc test was used to compare the means of the various groups. Statistical significance was considered at $P < 0.05$. The analysis's findings were presented as means and standard error (±SE). Furthermore, GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA) was used for constructing the graph representing the study findings.

3. Results

3.1. Fish mortalities and behavioral changes

As displayed in Table 3, no significant differences in the mortality rate was detected among the different experimental fish groups. The impact of AE food supplementation on *O. niloticus* neurobehavioral performance parameters after 60 days of FLO exposure was summarized in Table 3. Compared to fish raised in unpolluted water, there were significant ($P < 0.05$) deficits in the frequency of eating, middle swimming behavior, and middle crossings in the FLO-exposed fish. Relative to control fish, FLO-exposed fish had considerably ($P < 0.05$) more body shaking frequency, surface and bottom swimming behavior, surfacing

Table 2
Oligonucleotide primer sequences and real-time PCR conditions.

Gene name	Primer sequences		NCBI accession no.	PRODUCT SIZE (BP.)
hsp70	F	TTCAAGGTGATTTCAGACGGAG	NM_001311332.1	111
	R	CTTCATCTTCACCAGGACCATG		
caspase-3	F	GGCTCTTCGTCTGCTTCTGT	NM_001282894.1	80
	R	GGGAAATCGAGGCGGTATCT		
p53	F	TTTTCTCCTCCCTGTTCGTGG	XM_025905405.1	125
	R	CGGGAACCTCATGCTTCACT		
cat	F	CCCAGCTCTTCATCCAGAAAC	XM_019361816.2	103
	R	GCCTCCGCATTGTACTTCTT		
sod	F	GGTGCCCTGGAGCCCTA	XM_003449940.5	99
	R	ATGCGAAGTCTTCCACTGTC		
tnf-α	F	CCAGAAGCACTAAAGGCGAAGA	NM_001279533.1	82
	R	CCTTGGCTTTGCTGCTGATC		
il-1β	F	TGGTGACTCTCCTGGTCTGA	XM_005457887.3	86
	R	GCACAACTTTATCGGCTTCCA		
gapdh	F	CCGATGTGTCAGTGGTGGAT	NM_001279552.1	82
	R	GCCTTCTTGACGGCTTCCTT		
EF-1α	F	GCTTCAACGCTCAGGTCATC	NM_001279647.1	87
	R	TGTGGGCAGTGTGGCAATC		
β-actin	F	TGACCTCACAGACTACCTCATG	XM_003443127.5	89
	R	TGATGTCACGCACGATTTCC		

hsp70: Heat shock protein 70, *p53*: Tumor protein P53, *cat*: catalase, *sod*: superoxide dismutase, *tnf-α*: tumor necrosis factor alpha, *il1-β*: interleukin 1 beta, *gapdh*: Glyceraldehyde-3-phosphate dehydrogenase, *EF-1α*: Elongation factor-1 alpha.

Table 3
Effect of artichoke *(Cynara scolymus)* leaf extract (AE) supplementation on mortality rate and the behavioral responses of *O. niloticus* exposed to fluoride (FLO) for 60 days.

Items		Experimental groups			
		CON	AE	FLO	AE + FLO
Mortality rate		2.22 ± 2.22	2.22 ± 0.00	6.66 ± 3.85	2.22 ± 2.22
Behavioral patterns					
Feeding frequency		0.850^{a} ± 0.028	0.923^{a} ± 0.014	0.280^{c} ± 0.015	0.486^{b} ± 0.020
Surfacing frequency		0.123^{bc} ± 0.017	0.086^{c} ± 0.014	0.450^{a} ± 0.028	0.200^{b} ± 0.011
Swimming	Surface	0.410^{c} ± 0.032	0.416^{c} ± 0.024	1.700^{a} ± 0.057	0.816^{b} ± 0.044
	Middle	3.466^{a} ± 0.176	3.683^{a} ± 0.130	1.533^{c} ± 0.202	2.300^{b} ± 0.115
	Bottom	0.926^{c} ± 0.053	0.856^{c} ± 0.048	1.356^{a} ± 0.023	1.103^{b} ± 0.014
Body shaking frequency		0.040^{bc} ± 0.005	0.016^{c} ± 0.008	0.120^{a} ± 0.011	0.060^{b} ± 0.005
	Approach	0.150^{b} ± 0.011	0.113^{b} ± 0.008	0.416^{a} ± 0.060	0.226^{b} ± 0.017
	Chasing	0.183^{c} ± 0.012	0.140^{c} ± 0.005	0.870^{a} ± 0.017	0.340^{b} ± 0.023
Aggressive	Fin tugging	0.050^{bc} ± 0.005	0.016^{c} ± 0.008	0.306^{a} ± 0.023	0.090^{b} ± 0.005
	Fleeing	0.103^{c} ± 0.008	0.086^{c} ± 0.012	0.533^{a} ± 0.044	0.226^{b} ± 0.014
	Mouth Pushing	0.030^{c} ± 0.005	0.010^{c} ± 0.005	0.346^{a} ± 0.14	0.173^{b} ± 0.014
Laterality		0.010^{c} ± 0.005	0.000^{c} ± 0.000	1.133^{a} ± 0.088	0.350^{b} ± 0.028
No. of midline crossing		2.316^{ab} ± 0.116	2.433^{a} ± 0.076	1.106^{c} ± 0.023	2.000^{b} ± 0.057

Values are represented as the mean ± SE. The means within the same row carrying different superscripts are significant at $P < 0.05$. (N = 12/group) **CON**: Fish fed a basal diet without any supplementation. Artichoke leaf extract (**AE)** group: Fish fed a basal diet supplemented with 300 mg AE /kg diet. Fluoride group (**FLO**): Fish fed on a basal diet and exposed to 6.1 mg/l. AE + FLO group: Fish fed a basal diet containing 300 mg AE and exposed to 6.1 mg/L FLO.

Table 4
Effect of artichoke (*Cynara scolymus*) leaf extract (AE) supplementation on the biochemical parameters and brain AChE of *O. niloticus* exposed to fluoride (FLO) for 60 days.

Items		Experimental groups			
		CON	AE	FLO	AE + FLO
Serum	Cholesterol (mg/dl)	91.50^{b} ± 2.565	72.66^{c} ± 1.201	125.83^{a} ± 0.927	96.00^{b} ± 1.154
	Triglycerides (mg/dl)	82.33^{c} ± 1.452	75.33^{d} ± 0.881	111.66^{a} ± 2.027	91.66^{b} ± 0.881
	Cortisol (ng/mL)	5.73^{bc} ± 2.603	5.03^{c} ± 0.881	8.25^{a} ± 2.557	6.44^{b} ± 1.097
	Glucose (mg/dl)	72.83^{c} ± 0.927	60.16^{d} ± 1.166	106.33^{a} ± 1.452	81.00^{b} ± 1.154
	8-OHdG (ng/mL)	22.63^{c} ± 1.329	22.58^{c} ± 1.087	75.33^{a} ± 1.452	33.00^{b} ± 1.154
	AChE (ng/mg)	8.58^{a} ± 0.495	8.50^{a} ± 0.519	3.10^{c} ± 0.152	6.36^{b} ± 0.277
Brain homogenate	SOD (U/g tissue)	4.07^{a} ± 0.050	4.23^{a} ± 0.037	1.99^{c} ± 0.037	3.35^{b} ± 0.104
	CAT (U/g tissue)	1.86^{b} ± 0.051	2.40^{a} ± 0.056	1.00^{d} ± 0.063	1.52^{c} ± 0.043
	GPX (U/g tissue)	142.83^{a} ± 1.481	148.00^{a} ± 2.081	92.73^{c} ± 1.545	123.00^{b} ± 2.309
	GSH (mmol/g tissue)	2.37^{a} ± 0.014	2.40^{a} ± 0.011	1.07^{c} ± 0.050	2.08^{b} ± 0.046
	GST (U/ g tissue)	2.21^{a} ± 0.011	2.30^{a} ± 0.017	1.07^{c} ± 0.038	2.02^{b} ± 0.037
	MDA (nmol/g tissue)	11.23^{bc} ± 0.260	10.26^{c} ± 0.2407	36.35^{a} ± 3.336	18.16^{b} ± 0.726

Values are represented as the mean ± SE. The means within the same row carrying different superscripts are significant at $P < 0.05$. (N = 12 / group) CON: Fish fed a basal diet without any supplementation. Artichoke leaf extract (AE) group: Fish fed a basal diet supplemented with 300 mg AE / kg diet. Fluoride group (FLO): Fish fed on a basal diet and exposed to 6.1 mg/l. AE + FLO group: Fish fed a basal diet containing 300 mg AE and exposed to 6.1 mg FLO /L.

frequency, laterality, and aggression signs (approach, fleeing, fin tugging, chasing, and mouth pushing). Instead, compared to fish-fed non-fortified diets, adding AE to *O. niloticus* diets significantly ($P < 0.05$) enhanced all neurobehavioral signs. Likewise, AE supplementation to the FLO-exposed group positively impacts all behavioral variables ($P < 0.05$).

3.2. Lipid profile, stress, and DNA damage indicator

Regarding lipid profile, a significant ($P < 0.05$) decline in cholesterol and triglycerides was noticed in the fish after receiving AE treatment for 60 days (Table 4). Meanwhile, FLO exposure significantly raised the lipid profile's variables compared to other groups. Surprisingly, FLO exposure and AE medication (AE + FLO group) decreased cholesterol and triglyceride levels near the levels of the CON group. Regarding glucose and cortisol levels, different reactions were induced by exposure to either AE or FLO alone. Fish in the FLO group had considerably higher blood glucose and cortisol levels than fish in any other group. Additionally, fish exposed to FLO had a much higher 8-OHdG than the other groups. On the contrary, compared to fish-fed basal diets without any supplements, diets with AE supplements added resulted in considerably ($P < 0.05$) reduced glucose, cortisol, and 8-OHdG levels. The treatment of FLO-exposed groups with AE restored glucose, cortisol, and DNA damage indices (Table 4).

3.3. Indicators of oxidative stress and lipid peroxidation in brain tissue

As apparent in Table 4, fish exposed to FLO had significantly lower levels of enzymatic (GPx, CAT, SOD, and GST) and non-enzymatic (GSH) antioxidants than control fish, but significantly higher levels of MDA, a byproduct of lipid peroxidative destruction. Interestingly, when compared to fish fed a diet not supplemented with AE, brain tissues from the fish-fed supplemented diet displayed a significant improvement in CAT content but a significant decrease in MDA level. Compared to the FLO-exposed group, the variables mentioned above were better in the AE co-supplemented groups but remained considerably lower than those reported in the control group.

3.4. Alterations to AChE levels in brain tissue

Table 4 shows a significant ($P < 0.05$) decline in the brain AChE content in *O. niloticus* grown in FLO-polluted water compared to those produced in non-polluted water. Contrarily, fish fed an AE-enriched diet concomitant with FLO exposure significantly ($P < 0.05$) augmented AChE activity in their brain tissue.

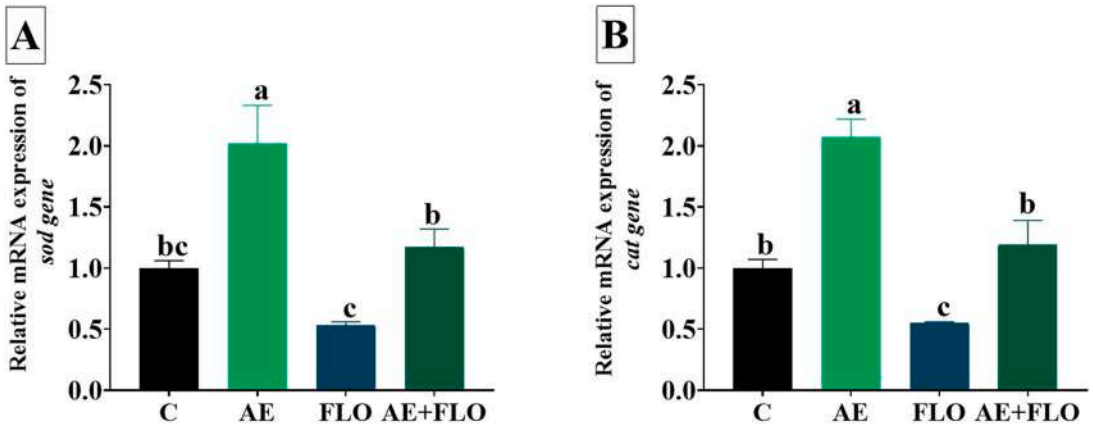

Fig. 1. Effect of fluoride (FLO) exposure and/or artichoke leaf extract (AE) supplementation for 60 days on mRNA expression of (A) superoxide dismutase (*sod*) and (B) catalase (*cat*) in the brain of *Oreochromis niloticus*. Data expressed as mean ± SE, $N = 12$ for each group. Each bar carrying different letters (a, b, and c) significantly differed at $P < 0.05$.

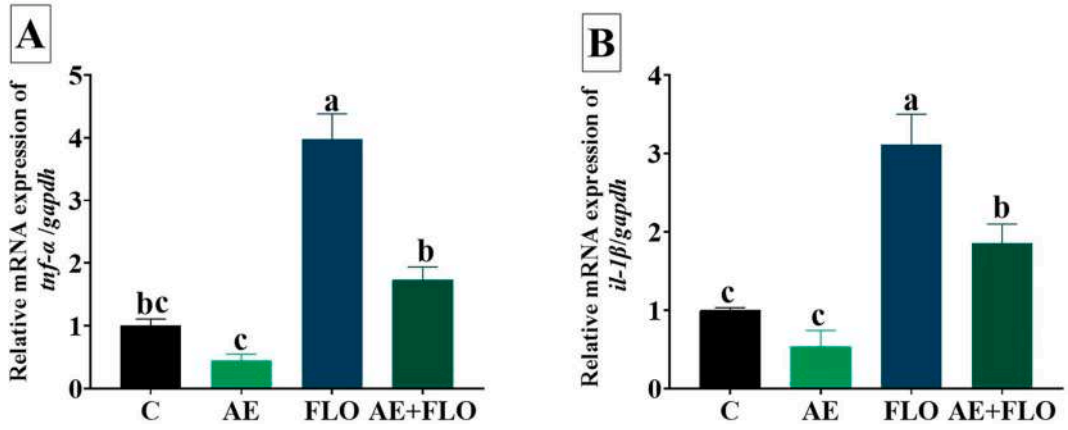

Fig. 2. Effect of fluoride (FLO) exposure and/or artichoke leaf extract (AE) supplementation for 60 days on mRNA expression of (A) tumor necrosis factor-alpha (*tnf-α*) and (B) interleukin 1 beta (*il1- β*) genes in the brain of *Oreochromis niloticus*. Data expressed as mean ± SE, N = 12 for each group. Each bar carrying different letters (a, b, and c) significantly differed at $P < 0.05$.

3.5. Gene expression profile

The expression of genes implicated in antioxidants (*cat, sod*) was significantly ($P < 0.05$) up-regulated but the apoptotic *casp-3 gene* was significantly ($P < 0.05$) down regulated in the brain of tilapias fed the AE-enriched diet than those in the CON group (Figs. 1 and 3). In contrast, the expression of genes correlated to inflammation (*tnf-α* and *il-1β*), stress (*hsp70*), and apoptosis (*p53*) showed a trend toward down-regulation, but non-significant (Figs. 2 and 3). Yet, in fish exposed to FLO, the inflammatory and apoptotic genes were significantly ($P < 0.05$) upregulated compared to the control group. Furthermore, (*cat* and *sod*) genes were diminished in the FLO-exposed group. Meanwhile, the AE + FLO group showed improvements in the previously stated criteria to the FLO-exposed group.

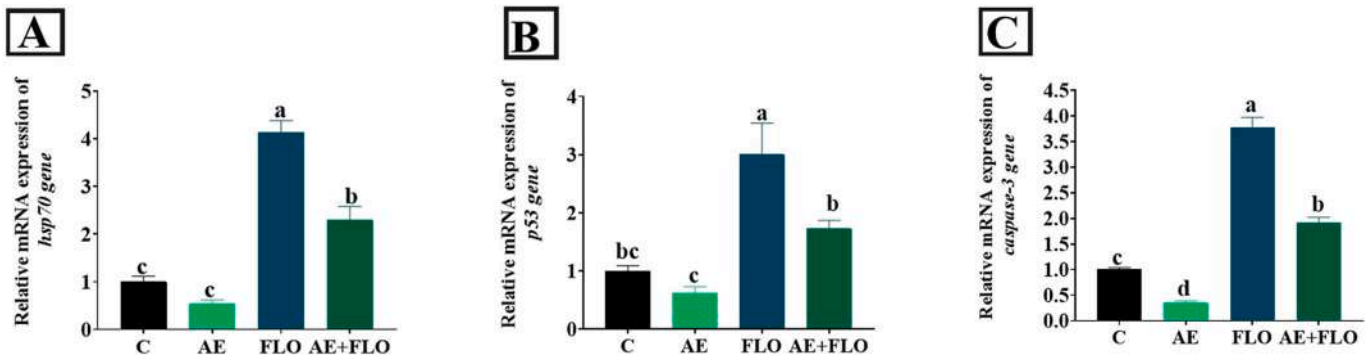

Fig. 3. Effect of fluoride (FLO) exposure and/or artichoke leaf extract (AE) supplementation for 60 days on mRNA expression of (A) heat shock protein 70 (*hsp70*), (B) tumor suppressor gene (*p53*), and (C) *caspase-3* in the brain of *Oreochromis niloticus*. Data expressed as mean ± SE, N = 12 for each group. Each bar carrying different letters (a, b, and c) significantly differed at $P < 0.05$.

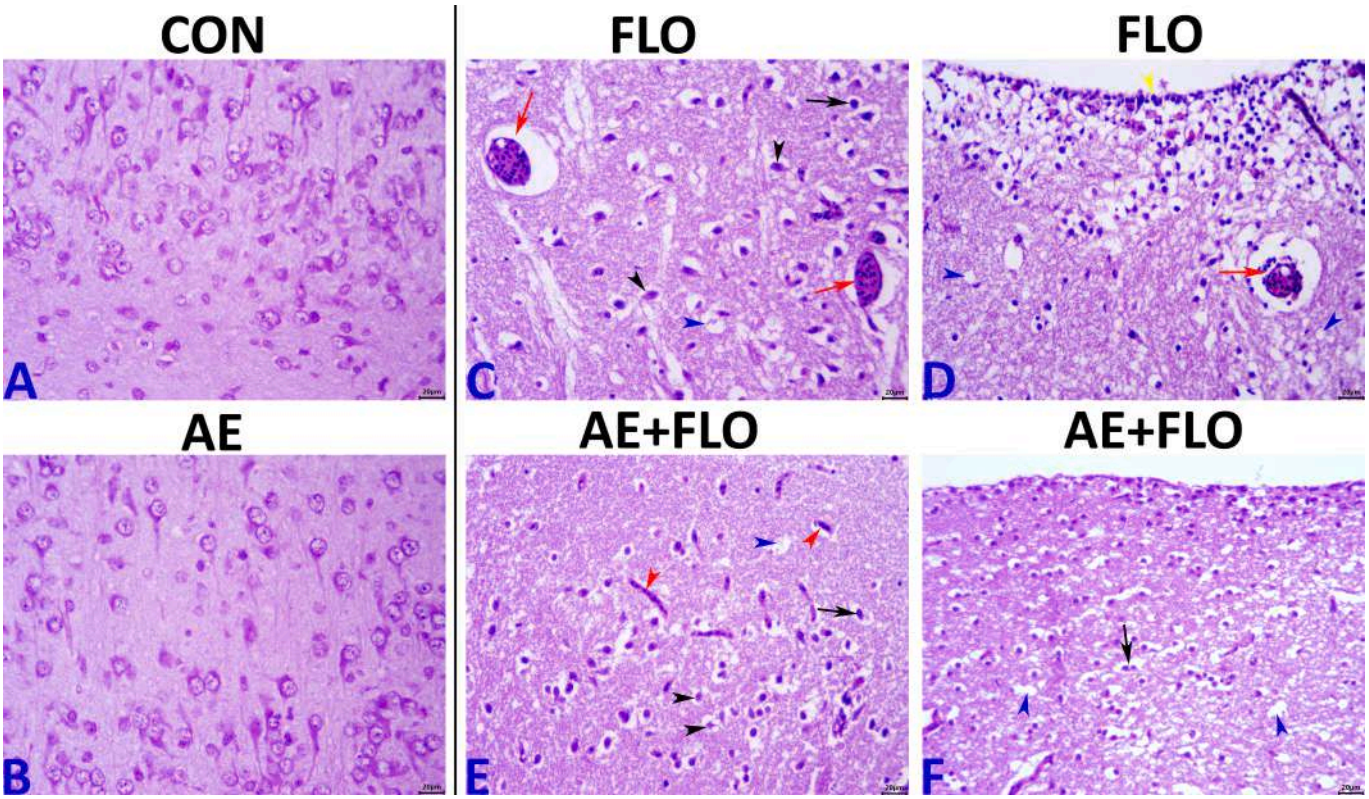

Fig. 4. Representative light micrographs of the H&E-stained cerebral tissue sections show a normal histological picture in the CON (A) and AE groups (B). The cerebral tissues of the FLO group show notable encephalopathic alterations: vascular congestion (red arrows), neuronal pyknosis (black arrow), neuronal necrosis (black arrowheads), neuropil microcavitation (blue arrowheads), and meningeal infiltration with mononuclear cells (yellow arrowhead) (C and D). The cerebral tissues of the AE + FLO group show significant reductions in the fluoride-induced cerebral and meningeal changes, with the persistence of capillary congestion (red arrowheads), neuronal pyknosis (black arrow), neuronal necrosis (black arrowheads), and neuropil microcavitation (blue arrowheads). The scale bar equals 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.6. Histopathological findings

The light microscopic examination and the image analysis of the cerebral tissue sections declared normal histological pictures with nil encephalopathic alterations in the CON and AE groups (Fig. 4, A, and B). Fluoride exposure induced a vast array of encephalopathic morphological changes, including cerebral and meningeal vascular congestions, leukocytic infiltrations, neuronal pyknosis, necrosis with perineuronal vacuolations, and neuropil microcavitation (Fig. 4, C and D). Supplementation with artichoke leaf extract significantly lowered the frequency and lessened the severity of the fluorine-induced encephalopathic changes but did not normalize the brain tissue in the AE + FLO group. The basic alterations in this group were retrogressive, such as neuronal pyknosis, necrosis, and neuropil microcavitation, with almost the absence of FLO-induced meningeal changes (Fig. 4, E, and F). The encephalopathic alterations and brain index of all groups were summarized in Table 5.

4. Discussion

The abundance of substantial FLO concentrations in water seriously threatens fish health. Despite this, there is no conclusive proof of its neurotoxicity in tilapia fish. Over the past decade, scientific and public attention on the welfare of farmed tilapia has intensified (Ayyat et al.,

Table 5
Effect of fluoride exposure (FLO) and artichoke leaf extract (AE) supplementation for 60 days on the histology of the brain tissue of *O. niloticus*.

Histopathological alteration (HA)			CON		AE		FLO		AE + FLO	
Reaction pattern	Type	W	FQ (%)	Index	FQ (%)	Index	FQ (%)	Index	FQ (%)	Index
Inflammatory alterations	Leukocytic infiltration	2	0.00	$0.00^{a} \pm 0.00$	0.00	$0.00^{c} \pm 0.00$	14	$2.6^{a} \pm 0.30$	7	$1.4^{b} \pm 0.30$
	Perivascular cuffing	2	0.00	$0.00^{b} \pm 0.00$	0.00	$0.00^{b} \pm 0.00$	6	$1.2^{a} \pm 0.32$	3	$0.6^{ab} \pm 0.30$
	Gliosis	2	0.00	$0.00^{c} \pm 0.00$	0.00	$0.00^{c} \pm 0.00$	16	$3.2^{a} \pm 0.32$	8	$1.6^{b} \pm 0.26$
Circulatory alterations	Congestion	1	0.00	$0.00^{c} \pm 0.00$	0.00	$0.00^{c} \pm 0.00$	63	$6.2^{a} \pm 0.20$	32	$3.2^{b} \pm 0.32$
	Hemorrhages	1	0.00	$0.00^{a} \pm 0.00$	0.00	$0.00^{a} \pm 0.00$	4	$0.40^{a} \pm 0.26$	0	$0.00^{a} \pm 0.00$
	Edema	1	0.00	$0.00^{b} \pm 0.00$	0.00	$0.00^{b} \pm 0.00$	6	$1.2^{a} \pm 0.32$	2	$0.40^{b} \pm 0.16$
Regressive alterations	Neuronal pyknosis	2	0.00	$0.00^{c} \pm 0.00$	0.00	$0.00^{c} \pm 0.00$	46	$9.0^{a} \pm 0.68$	19	$3.4^{b} \pm 0.30$
	Neuronal necrosis	3	0.00	$0.00^{c} \pm 0.00$	0.00	$0.00^{c} \pm 0.00$	21	$6.40^{a} \pm 0.60$	11	$3.30^{b} \pm 0.30$
	Perineuronal vacuolation	2	0.00	$0.00^{c} \pm 0.00$	0.00	$0.00^{c} \pm 0.00$	64	$13.2^{a} \pm 0.80$	31	$6.00^{b} \pm 0.00$
	Neuropil vacuolation	1	0.00	$0.00^{c} \pm 0.00$	0.00	$0.00^{c} \pm 0.00$	36	$3.6^{a} \pm 0.40$	19	$1.9^{b} \pm 0.10$
Brain index				$0.00^{c} \pm 0.00$		$0.00^{c} \pm 0.00$		$47.00^{a} \pm 0.96$		$21.80^{b} \pm 1.17$

FQ (lesion frequency), W (importance factor), The values are shown in means ± SE. The means within the same row carrying different superscripts are significant at $P < 0.05$. CON: Fish fed a basal diet without any supplementation. Artichoke leaf extract (AE) group: Fish fed a basal diet supplemented with 300 mg AE /kg diet. Fluoride group (FLO): Fish fed on a basal diet and exposed to 6.1 mg/l. AE + FLO group: Fish fed a basal diet containing 300 mg AE and exposed to 6.1 mg FLO /L.

2024; van de Vis et al., 2020). Behavior reflects the responses of fish to their surroundings and is thus a crucial aspect of fish welfare (Cooke, 2016). Aquaculture stressors have been associated with deviations in foraging behavior, aggressiveness, and swimming behavior, which makes them potential indicators of poor welfare (Khalil et al., 2022; Martins et al., 2012). Fish exposed to FLO in the current experiment showed an abrupt decrease in feeding frequency and midline crossing, reflecting diminished food consumption and activity. As enzymatic poisons, the FLO ions hinder protein synthesis, enhance glycolysis, and produce a substantial loss of body weight (Shalini and Sharma, 2015). Behavioral modifications such as breathing abnormalities and rapid and aggressive movement, which could affect fish metabolism, may be associated with decreasing feeding habits and growth rates (Martins et al., 2012). Moreover, fish under stressful circumstances frequently demonstrate reduced appetite (Conde-Sieira et al., 2018). Also, FLO deposition in the exoskeleton or bones can negatively impact development or other physiological processes (Shi et al., 2009). It could considerably partake in their reduced activity and feeding behavior alongside the related oxidative stress. In this regard, the growth-retarding consequences of FLO on many fish species were validated by multiple earlier research (El-Houseiny et al., 2022a; Yoshitomi and Nagano, 2012). Likewise, Chen et al. (2013) demonstrated that carp fish developed anorexia and decreased the effectiveness of food assimilation if exposed to FLO. On the other hand, fish-fed diets, including artichoke leaf extract, exhibited elevated feeding frequency and midline crossings. Following these findings, adding AE to the Sea Bream diet showed a growth-promoting impact (Gökçe et al., 2015). Consuming AE can boost the activity of digestive enzymes and promote growth (Gökçe et al., 2015). Besides, antioxidant, choleretic, hepatoprotective, bile-enhancing and lipid-lowering effects which may effectively improve fish performance (Moglia et al., 2008; Salem et al., 2015).

The fish in the present investigation that had been exposed to FLO had a variety of neurobehavioral imperfections, such as increased body shaking, surfacing swimming, aggressive behavior, and laterality. A number of underlying biological mechanisms might drive FLO-induced neurobehavioral impairments. FLO exposure alters the circadian cycle, induces anxiety-like behaviors, and reduces melatonin and brain antioxidant enzyme levels (Karaman et al., 2023). Brain tissue is a target for FLO (Kalisinska et al., 2014), and the capacity of FLO to disrupt ion transport and alter the physiological and morphological characteristics of neurons across the blood-brain barrier has been identified (Dec et al., 2017). It is known that FLO exposure reduces the amounts of norepinephrine and epinephrine in the hippocampus and neocortex of exposed animals, impairing their ability to become acclimated to new environments, which is similar to fish exploration behavior (Vorhees et al., 2021). Furthermore, the shift in oxygen consumption demonstrated a substantial negative connection with erratic swimming and loss of body balance, and the fish use several physiological methods to deal with hypoxia, including changes in respiration rate and activity (such as surfacing swimming) (Kishore et al., 2022). Another potential explanation for fish's FLO-induced neurobehavioral performance is the decreased AChE activity in the brain. AChE is a crucial enzyme in the regulation of the neural action potential. Continuous nerve firing is inhibited by AChE activity, which is crucial for properly operating the sensory and neuromuscular systems (Payne et al., 1996). Additionally, AChE is vital for various physiological processes such as cell adhesion, synaptogenesis, neurogenesis, stimulation of thrombopoiesis, hematopoiesis, actions of dopamine neurons, and amyloid fiber association (Aswani and Trabucco, 2019). Fish behavioral alterations might arise from interrupted AChE activity (Oliveira et al., 2015). In this regard, Oliveira et al. (2012) displayed that fish swimming performance declined when AChE activity was inhibited. AChE activity and swimming behavior are also closely connected, according to a recent study by Yang et al. (2017). Moreover, there has already been evidence linking cholinesterase inhibitors to aggression (Devinsky et al., 1992). Likewise, it has been noted that AChE inhibition alters fish perception, decreasing the probability of escaping (Kamrin, 1997). Similar neurobehavioral issues have been found in *Clarias batrachus* fish following FLO exposure (Kishore et al., 2022; Sahu and Kumar, 2021). Furthermore, rats exposed to FLO have been shown to develop neurobehavioral issues; such problems have been linked to lower AChE activity (Dominguez et al., 2021; Shalini and Sharma, 2015). In the present study, AE food supplementation to FLO-exposed fish dramatically restored AChE activity, reducing the detrimental impacts of FLO. By influencing pro-inflammatory proteins, the phenolic acid-rich artichoke extract exerts neuroprotective benefits (Abd El-Aziz et al., 2021). Our findings support earlier research by Ibrahim et al. (2022), which revealed that AE restored AChE activity and antioxidant enzymes in mice brains treated with aflatoxin B1. Additionally, El-Nashar et al. (2022) showed that AE has the ability to protect the mouse brain from streptozotocin induced neurotoxicity, and inflammation due to flavonoid glycosides of apigenin, luteolin, kaempferol, and quercetin, as well as caffeoylquinic acids in its phytochemical profiling.

The current study concluded that fish exposed to FLO had higher blood glucose levels, which is a reliable indicator of environmental stress. By secreting excessive levels of glucocorticoids and catecholamines in reaction to stress, it has been suggested that glycogenolysis will increase while the glycolytic pathway will drop (Uddin et al., 2018). Further, the observed hyperglycemia may be linked to the synthesis of glucose from protein and amino acids (Almeida et al., 2001). Fluoride can negatively affect insulin levels, deteriorate pancreatic health, and cause aberrant glucose tolerance (Skórka-Majewicz et al., 2020). Increased blood glucose was formerly noted in rats exposed to FLO (McGown and Suttie, 1977). Our results indicated that fish treated with FLO had significantly higher serum cortisol levels. To sustain disrupted homeostasis under stressful situations, the hypothalamus-pituitary-interrenal (HPI) axis is induced to produce cortisol and other corticosteroids (Gagnon et al., 2006). Also, FLO-exposed fish revealed increments in the cholesterol and triglyceride levels, representing a stressful condition. With chronic FLO exposure, the plasma levels of cholesterol and triglycerides increased continuously and significantly in *Channa punctatus* fish (Guru and Behera, 2015). It is interesting to note that the fish exposed to FLO and supplemented with AE showed apparent regaining of their serum glucose, cortisol, cholesterol, and triglycerides levels, demonstrating AE's capacity to lessen the stress caused by FLO. In line with our observations, supplementing with artichoke extract was linked to a considerable drop in triglycerides and total and low-density lipoprotein cholesterol (Sahebkar et al., 2018). Also, it has the antihypercholesterolemic and anti-hyperglycemic effects (Fallah Huseini et al., 2012). By inhibiting the activity of hydroxymethylglutaryl-CoAreductase, the AE components, including cynaroside, luteolin, and chlorogenic acid, suppressed the synthesis of cholesterol in primary rat hepatocytes (Gebhardt and Therapeutics, 1998). As antihyperlipidemic molecules, the sesquiterpenes cynaropicrin, aguerin B, and grosheimin were discovered (Bhutani et al., 2007).

FLO has been demonstrated to pass through the blood-brain barrier in zebrafish, causing detrimental effects on neural cells and triggering the production of ROS (Dondossola et al., 2022). Here, fish exposed to FLO showed a significant reduction in the antioxidant content of GST, SOD, GPx, CAT, and GSH but an extreme rise in MDA. The transcription of the brain antioxidant genes CAT and SOD was proved to be identical. Also, a vast array of encephalopathic morphological changes, including cerebral and meningeal vascular congestions, leukocytic infiltrations, neuronal pyknosis, necrosis with perineuronal vacuolations, and neuropil microcavitation were recorded in FLO-exposed fish's brain. The evidence for FLO's ability to create ROS and reduce the activity of antioxidant enzymes in fish tissues is evolving (Ahmed et al., 2020; El-Houseiny et al., 2022a). Fluoride can quickly enter cells and generate detrimental impacts on various tissues owing to its tiny ionic radius and potent biological activity (Devi and Piska, 2006). Additionally, some metals, including FLO, are renowned for their potent oxidizing effects

and can deplete the body's primary antioxidants, especially enzymes that include thiols (Pinto et al., 2003). The direct competitive suppression of enzyme activity by FLO may also be to blame for the observed decrease in SOD and CAT activity in fish exposed to FLO (Zhan et al., 2006). Furthermore, fish exposed to FLO reported elevations in the 8-OHdG level. This biomarker suggests oxidative damage to DNA, which eventually leads to tissue damages; it is also indicated that oxidative stress is a major mechanism of FLO toxicity (Oliveira et al., 2010). Importantly, the concurrent use of AE significantly improved the antioxidative status and has a genoprotective effect on experimental fish. Similarly, Mehmetçik et al. (2008) demonstrated that using artichoke extract effectively prevented oxidative stress-induced hepatotoxicity in rats. Also, AE is used as a chelating agent for lead toxicity in rats by reduction of MDA level and increased ferric reducing/antioxidant power (FRAP) due to the impacts of the artichoke extract flavonoids (Heidarian and Rafieian-Kopaei, 2013). By boosting TAC levels, artichokes reduced the increase in MDA concentrations and enhanced the antioxidant defense mechanism (Cicek et al., 2022). Leaf extract from wild artichokes may function through various mechanisms to effectively counteract the oxidative stress triggered by free fatty acid (FFA)-induced lipotoxic liver damage. These include elevating RSH levels, decreasing ROS production and lipid peroxidation, and regulating cytoprotective Nrf2/ARE-regulated genes to minimize inflammatory response (Acquaviva et al., 2023). Decrease of pro-inflammatory (*tnf-α* and *il-1β*) gene transcription here supports this theory. Other researchers have indicated that phenolic chemicals, particularly chlorogenic acid, have neuroprotective effects through boosting antioxidant defense as evidenced by increased activity of SOD, CAT, GPx, GST, and GR. This was confirmed for aluminum (Wang et al., 2018) or arsenic-induced neurotoxicity in mice (Metwally et al., 2020).

The molecular approach implemented in this work demonstrated that FLO elevated *tnf-α* and *il-1β* gene expression. Likewise, the stress and apoptotic genes *hsp70, caspase-3,* and *p53* are upregulated. FLO elicited similar upregulation of IL-1β and TNF-α, two pro-inflammatory cytokines in *Clarias gariepinus* (Singh et al., 2017) and in rats (Caglayan et al., 2021). Oxidative stress is a key variable in tissue inflammatory responses (Jadeja et al., 2017). Fluoride substantially boosted the expression of many genes associated with inflammation, mostly by activating the NF-κB signaling system (Li et al., 2021; Luo et al., 2017). Environmental pollution causes a rise in P53 activity, which is a warning indication of water contamination (Dong et al., 2009). The expression of p53 and Caspase-3 was significantly enhanced in *C.gariepinus* (Singh et al., 2017), Zebrafish (Mukhopadhyay and Chattopadhyay, 2014), and rats (Caglayan et al., 2021; Tu et al., 2018) treated with FLO due to apoptosis. During stressful situations, hsp70 becomes activated (Arambašić et al., 2013). Environmental toxins that disrupt cellular integrity trigger the creation of Hsp70 (Hahn et al., 1985). HSP70 expression was elevated in the liver of female zebrafish (Mukhopadhyay and Chattopadhyay, 2014) and kidneys of mice (Chattopadhyay et al., 2011) receiving FLO. In contrast to the FLO-treated group, administration of FLO + AE provoked a substantial drop in the expression of the genes mentioned above, indicating cytoprotective, anti-apoptotic, anti-inflammatory, and antioxidant effects (Acquaviva et al., 2023; Bogavac-Stanojevic et al., 2018). In this regard, AE metabolites can protect chondrocytes from an IL-1β stimulus miming the inflammatory situation in osteoarthritis (Wauquier et al., 2021). Also, AE exerted neuroprotection against diethylnitrosamine-induced brain toxicity in rats by hampering caspase-3 and oxidant indicators (Cicek et al., 2022).

5. Conclusion

Based on the findings presented here, it can be deduced that chronic FLO levels in water cause neurological consequences in fish. Numerous biological processes, including gene expression, apoptosis/necrosis, inflammation, and oxidative status, can be disrupted by FLO. AE supplementation conveys a neuroprotective impact against FLO-induced neurobehavioral aberrations in Nile tilapia. The anticipated underlying mechanisms of AE could be its antioxidant activity and improvement in AChE.

CRediT authorship contribution statement

Asmaa Elsayyad: Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. **Yasmin A. Reyad:** Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. **Basma A. Elshafey:** Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. **Enas K. Aziz:** Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. **Mohamed M.M. Metwally:** Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. **Yasmina M. Abd-Elhakim:** Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. **Abdel-Wahab A. Abdel-Warith:** Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. **Elsayed M. Younis:** Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. **Simon J. Davies:** Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. **Walaa El-Houseiny:** Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. **Ahmed H. Arisha:** Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. **Hanan A. Ghetas:** Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the Researchers Supporting Project (RSP2024R36), King Saud University, Riyadh, Saudi Arabia. Also, the authors thank all staff of the Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, for their support during the work.

References

Abd El-Aziz, N.M., Awad, O.M.E., Shehata, M.G., El-Sohaimy, S.A., 2021. Antioxidant and anti-acetylcholinesterase potential of artichoke phenolic compounds. Food Biosci. 41, 101006.

Abd El-Hakim, Y.M., El-Houseiny, W., Abd Elhakeem, E.-M., Ebraheim, L.L., Moustafa, A. A., Mohamed, A.A.R., 2020. Melamine and curcumin enriched diets modulate the haemato-immune response, growth performance, oxidative stress, disease resistance, and cytokine production in *Oreochromis niloticus*. Aquat. Toxicol. 220, 105406.

Acquaviva, R., Malfa, G.A., Santangelo, R., Bianchi, S., Pappalardo, F., Taviano, M.F., Miceli, N., Di Giacomo, C., Tomasello, B., 2023. Wild artichoke (*Cynara cardunculus* subsp. *sylvestris, Asteraceae*) leaf extract: phenolic profile and oxidative stress inhibitory effects on HepG2 cells. Molecules 28 (6), 2475.

Agalakova, N.I., Gusev, G.P., 2012. Molecular Mechanisms of Cytotoxicity and Apoptosis Induced by Inorganic Fluoride, vol. 2012. International Scholarly Research Notices.

Ahmed, N.F., Sadek, K.M., Soliman, M.K., Khalil, R.H., Khafaga, A.F., Ajarem, J.S., Maodaa, S.N., Allam, A.A., 2020. *Moringa oleifera* leaf extract repairs the oxidative misbalance following sub-chronic exposure to sodium fluoride in nile tilapia *Oreochromis niloticus*. Animals 10 (4), 626.

Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W., Fu, P.C., 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. 20 (4), 470–475.

Almeida, J., Novelli, E., Silva, M.D.P., Júnior, R.A., 2001. Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus*. Environ. Pollut. 114 (2), 169–175.

Altmann, J., 1974. Observational study of behavior: sampling methods. Behaviour 49 (3–4), 227–266.

AOAC, 2006. Official Methods of Analysis, 18th edn. USA, Washington,DC.

Arambašić, J., Mihailović, M., Uskoković, A., Dinić, S., Grdović, N., Marković, J., Poznanović, G., Bajec, D., Vidaković, M., 2013. Alpha-lipoic acid upregulates antioxidant enzyme gene expression and enzymatic activity in diabetic rat kidneys through an O-GlcNAc-dependent mechanism. Eur. J. Nutr. 52, 1461–1473.

Aswani, V., Trabucco, D., 2019. Biochemical adaptation in brain acetylcholinesterase during acclimation to sub-lethal temperatures in the eurythermal fish *Tilapia mossambica*. Sci. Rep. 9 (1), 19762. https://doi.org/10.1038/s41598-019-56066-x.

Ayyat, M.S., Ayyat, A.M.N., Abdel-Rahman, M.S., Al-Sagheer, A.A., 2024. Appraisal of leaf protein concentrate derived from sugar beet and carrot as a novel fish meal substitute for juvenile Nile tilapia (*Oreochromis niloticus*). Anim. Feed Sci. Technol. 307, 115833 https://doi.org/10.1016/j.anifeedsci.2023.115833.

Bajpai, S., Tewari, S., Tripathi, M., 2009. Evaluation of acute toxicity levels and behavioural responses of *Hetropneustes fossilis* (Bloch) to sodium fluoride. Aquaculture 10 (1), 37–43.

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., Wahli, T., 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish Dis. 22 (1), 25–34.

Bhutani, K.K., Birari, R., Kapat, K., 2007. Potential anti-obesity and lipid lowering natural products: a review. Nat. Prod. Commun. 2 (3), 1934578X0700200316.

Bogavac-Stanojevic, N., Kotur Stevuljevic, J., Cerne, D., Župan, J., Marc, J., Vujic, Z., Crevar-Sakac, M., Sopic, M., Munjas, J., Radenkovic, M., 2018. The role of artichoke leaf tincture (*Cynara scolymus*) in the suppression of DNA damage and atherosclerosis in rats fed an atherogenic diet. Pharm. Biol. 56 (1), 138–144.

Bond, C.E., 1979. Biology of Fishes. W. B. Saunders Company.

Boyd, C.E., Tucker, C.S., 2012. Pond Aquaculture Water Quality Management. Springer Science & Business Media.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., 2009. The MIQE Guidelines: M Inimum I Nformation for Publication of Q Uantitative Real-Time PCR E Xperiments. Oxford University Press.

Caglayan, C., Kandemir, F.M., Darendelioğlu, E., Küçükler, S., Ayna, A., 2021. Hesperidin protects liver and kidney against sodium fluoride-induced toxicity through anti-apoptotic and anti-autophagic mechanisms. Life Sci. 281, 119730.

Camargo, J.A., 2003. Fluoride toxicity to aquatic organisms: a review. Chemosphere 50 (3), 251–264.

Cao, J., Chen, J., Xie, L., Wang, J., Feng, C., Song, J., 2015. Protective properties of sesamin against fluoride-induced oxidative stress and apoptosis in kidney of carp (*Cyprinus carpio*) via JNK signaling pathway. Aquat. Toxicol. 167, 180–190. https://doi.org/10.1016/j.aquatox.2015.08.004.

Cavini, S., Guzzetti, L., Givoia, F., Regonesi, M.E., Di Gennaro, P., Magoni, C., Campone, L., Labra, M., Bruni, I., 2022. Artichoke (*Cynara cardunculus* var. *scolymus* L.) by-products as a source of inulin: how to valorise an agricultural supply chain extracting an added-value compound. Nat. Prod. Res. 36 (8), 2140–2144. https://doi.org/10.1080/14786419.2020.1841188.

Ceccarelli, N., Curadi, M., Picciarelli, P., Martelloni, L., Sbrana, C., Giovannetti, M., 2010. Globe artichoke as a functional food. Mediterr. J. Nutr. Metab. 3 (3), 197–201.

Chattopadhyay, A., Podder, S., Agarwal, S., Bhattacharya, S., 2011. Fluoride-induced histopathology and synthesis of stress protein in liver and kidney of mice. Arch. Toxicol. 85, 327–335.

Chen, J., Cao, J., Wang, J., Jia, R., Xue, W., Li, Y., Luo, Y., Xie, L., 2013. Effects of fluoride on growth, body composition, and serum biochemical profile in a freshwater teleost, *Cyprinus carpio*. Environ. Toxicol. Chem. 32 (10), 2315–2321.

Chen, T.C., Ormond, R., Mok, H.K., 2001. Feeding and territorial behaviour in juveniles of three co-existing triggerfishes. J. Fish Biol. 59 (3), 524–532.

Cicek, B., Genc, S., Yeni, Y., Kuzucu, M., Cetin, A., Yildirim, S., Bolat, I., Kantarci, M., Hacimuftuoglu, A., Lazopoulos, G., 2022. Artichoke (*Cynara Scolymus*) Methanolic leaf extract alleviates Diethylnitrosamine-induced toxicity in BALB/c mouse brain: involvement of oxidative stress and apoptotically related klotho/PPARγ signaling. J. Personal. Med. 12 (12), 2012.

Conde-Sieira, M., Chivite, M., Míguez, J.M., Soengas, J.L., 2018. Stress effects on the mechanisms regulating appetite in teleost fish. Front. Endocrinol. 9, 631. https://doi.org/10.3389/fendo.2018.00631.

Cooke, M., 2016. Animal welfare in farmed fish. Bus. Benchmark Farm Anim. Welf. Invest. Brief 23, 1–16.

Davison, A.W., Weinstein, L.H., 2006. Chapter 8: Some problems relating to fluorides in the environment: Effects on plants and animals. In: Tressaud, A. (Ed.), Advances in Fluorine Science. Elsevier, pp. 251–298.

De Boer, B.A., 1980. A causal analysis of the territorial and courtship behaviour of *Chromis cyanea* (*Pomacentridae*, Pisces). Behaviour 73 (1–2), 1–50.

Dec, K., Łukomska, A., Maciejewska, D., Jakubczyk, K., Baranowska-Bosiacka, I., Chlubek, D., Wąsik, A., Gutowska, I., 2017. The influence of fluorine on the disturbances of homeostasis in the central nervous system. Biol. Trace Elem. Res. 177, 224–234.

Devi, A., Piska, R., 2006. Effect of fluoride on tissue proteins of fresh water cat fish *Clarias batrachus* (Lin.). J. Aquat. Biol. 21 (1), 184–187.

Devinsky, O., Kernan, J., Bear, D.M., Neurosciences, C., 1992. Aggressive behavior following exposure to cholinesterase inhibitors. J.Neuropsych. 4 (2), 189–194.

Dominguez, S., Lencinas, I., Bartos, M., Gallegos, C., Bras, C., Mónaco, N., Minetti, A., Gumilar, F., 2021. Neurobehavioral and neurochemical effects in rats offspring co-exposed to arsenic and fluoride during development. NeuroToxicology 84, 30–40.

Dondossola, E.R., Pacheco, S.D., Visentin, S.C., Mendes, N.V., Baldin, S.L., Bernardo, H. T., Scussel, R., Rico, E.P., 2022. Prolonged fluoride exposure alters neurotransmission and oxidative stress in the zebrafish brain. NeuroToxicology 89, 92–98.

Dong, X., Zhu, L., Wang, J., Wang, J., Xie, H., Hou, X., Jia, W., 2009. Effects of atrazine on cytochrome P450 enzymes of zebrafish (*Danio rerio*). Chemosphere 77 (3), 404–412.

El Basuini, M.F., Teiba, I.I., Zaki, M.A.A., Alabssawy, A.N., El-Hais, A.M., Gabr, A.A., Dawood, M.A.O., Zaineldin, A.I., Mzengereza, K., Shadrack, R.S., Dossou, S., 2020. Assessing the effectiveness of CoQ10 dietary supplementation on growth performance, digestive enzymes, blood health, immune response, and oxidative-related genes expression of Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol. 98, 420–428. https://doi.org/10.1016/j.fsi.2020.01.052.

El-Bouhy, Z.M., Mohamed, F.A., Elashhab, M.W., El-Houseiny, W., 2023. Toxicity bioassay and sub-lethal effects of profenofos-based insecticide on behavior, biochemical, hematological, and histopathological responses in grass carp (*Ctenopharyngodon idella*). Ecotoxicology 32 (2), 196–210.

El-Hawarry, W.N., Mohamed, R.A., Ibrahim, S.A., 2018. Collaborating effects of rearing density and oregano oil supplementation on growth, behavioral and stress response of Nile tilapia (*Oreochromis niloticus*). Egypt. J. Aquatic Res. 44 (2), 173–178. https://doi.org/10.1016/j.ejar.2018.06.008.

El-Houseiny, W., Abd El-Hakim, Y.M., Metwally, M.M., Ghfar, S.S.A., Khalil, A.A., 2022a. The single or combined Silybum marianum and co-enzyme Q10 role in alleviating fluoride-induced impaired growth, immune suppression, oxidative stress, histological alterations, and reduced resistance to *Aeromonas sobria* in African catfish (*Clarias gariepinus*). Aquaculture 548, 737693.

El-Houseiny, W., Algharib, S.A., Mohamed, E.A., Metwally, M.M., Mahmoud, Y.K., Alghamdi, Y.S., Soliman, M.M., Abd-Elhakim, Y.M., El-Murr, A.E., 2022b. Dietary parsley seed mitigates methomyl-induced impaired growth performance, hemato-immune suppression, oxidative stress, hepato-renal damage, and *pseudomonas aeruginosa* susceptibility in *Oreochromis niloticus*. Antioxidants 11 (6), 1185.

El-Houseiny, W., Arisha, A.H., Metwally, M.M., Abdel-Warith, A.-W.A., Younis, E.M., Davies, S.J., Hassan, B.A., Abd-Elhakim, Y.M., 2023. Alpha-lipoic acid suppresses gibberellic acid nephrotoxicity in Nile tilapia (*Oreochromis niloticus*) via modulating oxidative stress, inflammation, cytokine production, and apoptosis. Pestic. Biochem. Physiol. 196, 105598.

El-Nashar, H.A., Abbas, H., Zewail, M., Noureldin, M.H., Ali, M.M., Shamaa, M.M., Khattab, M.A., Ibrahim, N., 2022. Neuroprotective effect of artichoke-based nanoformulation in sporadic Alzheimer's disease mouse model: focus on antioxidant, anti-inflammatory, and amyloidogenic pathways. Pharmaceuticals 15 (10), 1202.

Fallah Huseini, H., Kianbakht, S., Heshmat, R., 2012. *Cynara scolymus* L. in treatment of hypercholesterolemic type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. J. Med. Plants 11 (41), 58–65.

Frey, D.F., Miller, R., 1972. The establishment of dominance relationships in the blue gourami, *trichogaster trichopter* us (pallas). Behaviour 42 (1–2), 8–60.

Gagnon, A., Jumarie, C., Hontela, A., 2006. Effects of cu on plasma cortisol and cortisol secretion by adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 78 (1), 59–65.

Gebhardt, R., 2001. Anticholestatic activity of flavonoids from artichoke (*Cynara scolymus* L.) and of their metabolites. J. Med. Sci. Monitor: Intern. Med. J. Exper. Clin. Res. 7, 316–320.

Gebhardt, R., Therapeutics, E., 1998. Inhibition of cholesterol biosynthesis in primary cultured rat hepatocytes by artichoke (*Cynara scolymus* L.) extracts. J. Pharmacol. 286 (3), 1122–1128.

Gökçe, M.A., Ündağ, E., Erbaş, C., Taşbozan, O., Özcan, F., 2015. Effect of artichoke leaves extract on growth and digestive enzyme activity of sea bream *Sparus Aurata* (*Linneaus*, 1758). Aquaculture 164.

Guo, S.-N., Zheng, J.-L., Yuan, S.-S., Zhu, Q.-L., 2018. Effects of heat and cadmium exposure on stress-related responses in the liver of female zebrafish: heat increases cadmium toxicity. Sci. Total Environ. 618, 1363–1370.

Gupta, S., Poddar, A.N., 2014. Sodium fluoride toxicity in the fresh water cat fish *Clarias batrachus* (Linn.): effects on the erythrocyte morphology and antioxidant enzymes. Res. J. Environ. Toxicol. 8 (2), 68–76.

Guru, S.K., Behera, R., 2015. Changes in some haematological parameters induced on exposure to a sub-lethal dose of fluoride (NaF) in the freshwater fish, *Channa punctatus* (BLOCH). Biolife 3 (4), 778–782.

Hahn, G.M., Shiu, E.C., West, B., Goldstein, L., Li, G.C., 1985. Mechanistic implications of the induction of thermotolerance in Chinese hamster cells by organic solvents. Cancer Res. 45 (9), 4138–4143.

Heidarian, E., Rafieian-Kopaei, M., 2013. Protective effect of artichoke (*Cynara scolymus*) leaf extract against lead toxicity in rat. Pharm. Biol. 51 (9), 1104–1109.

Ibrahim, E.A., Yousef, M.I., Ghareeb, D.A., Augustyniak, M., Giesy, J.P., Aboul-Soud, M. A., El Wakil, A., 2022. Artichoke leaf extract-mediated neuroprotection against effects of aflatoxin in male rats. Biomed. Res. Int. 2022, 4421828.

Ismail, M., Ali, R., Ali, T., Waheed, U., Khan, Q.M., 2009. Evaluation of the acute toxicity of profenofos and its effects on the behavioral pattern of fingerling common carp (*Cyprinus carpio* L., 1758). Bull. Environ. Contam. Toxicol. 82, 569–573.

Jadeja, R.N., Devkar, R.V., Nammi, S., 2017. Oxidative stress in liver diseases: pathogenesis, prevention, and therapeutics. Oxidative Med. Cell. Longev. 2017.

Jobling, M., 2012. National Research Council (NRC): nutrient requirements of fish and shrimp. Aquac. Int. 20 (3), 601–602. https://doi.org/10.1007/s10499-011-9480-6.

Kalisinska, E., Bosiacka-Baranowska, I., Lanocha, N., Kosik-Bogacka, D., Krolaczyk, K., Wilk, A., Kavetska, K., Budis, H., Gutowska, I., Chlubek, D., 2014. Fluoride concentrations in the pineal gland, brain and bone of goosander (*Mergus merganser*) and its prey in Odra River estuary in Poland. Environ. Geochem. Health 36, 1063–1077.

Kamrin, M.A., 1997. Pesticide Profiles: Toxicity, Environmental Impact, and Fate. CRC Press.

Karaman, M., Toraman, E., Sulukan, E., Baran, A., Bolat, İ., Yıldırım, S., Kankaynar, M., Ghosigharehagaji, A., Budak, H., Ceyhun, S.B., 2023. Fluoride exposure causes behavioral, molecular and physiological changes in adult zebrafish (*Danio rerio*) and their offspring. Environ. Toxicol. Pharmacol. 97, 104044.

Kaur, R., Saxena, A., Batra, M., Biotechnology, 2017. A review study on fluoride toxicity in water and fishes: current status, toxicology and remedial measures. Intern. J. Environ. Agricul. 2 (1), 456–466.
Khalil, A.A., Abd-Elhakim, Y.M., Said, E.N., Moselhy, A.A., Abu-Elsaoud, A.M., El-Houseiny, W., 2022. Milk thistle and co-enzyme Q10 fortified diets lessen the nickel chloride-induced neurotoxic and neurobehavioral impairments in *Oreochromis niloticus* via regulating the oxidative stress response, acetylcholinesterase activity, and brain nickel content. Aquaculture 553, 738102.
Kishore, D., Shubhajit, S., Chukwuka, A.V., Chandra, S.N., 2022. Behavioural toxicity and respiratory distress in early life and adult stage of walking catfish *Clarias batrachus* (*Linnaeus*) under acute fluoride exposures. Toxicol. Environ. Heal. Sci. 1–14.
Li, L., Lin, L.M., Deng, J., Lin, X.L., Li, Y.M., Xia, B.H., 2021. The therapeutic effects of *Prunella vulgaris* against fluoride-induced oxidative damage by using the metabolomics method. Environ. Toxicol. Chem. 36 (9), 1802–1816.
Ling, L.-B., Chang, Y., Liu, C.-W., Lai, P.-L., Hsu, T., 2017. Oxidative stress intensity-related effects of cadmium (Cd) and paraquat (PQ) on UV-damaged-DNA binding and excision repair activities in zebrafish (*Danio rerio*) embryos. Chemosphere 167, 10–18.
Luo, Q., Cui, H., Deng, H., Kuang, P., Liu, H., Lu, Y., Fang, J., Zuo, Z., Deng, J., Li, Y., 2017. Sodium fluoride induces renal inflammatory responses by activating NF-κB signaling pathway and reducing anti-inflammatory cytokine expression in mice. Oncotarget 8 (46), 80192.
Mahady, G.B., 2009. Medicinal plants for the prevention and treatment of coronary heart disease. Ethnopharmacology 2, 75–99.
Mahmoud, N., Mutchnick, S.A., Svider, P.F., McLeod, T.M., Fribley, A.M., 2020. Chapter 10 - fluorine in human metabolism, health and disease. In: Prasad, A.S., Brewer, G.J. (Eds.), Essential and Toxic Trace Elements and Vitamins in Human Health. Academic Press, pp. 153–162.
Martins, C.I., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M.T., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J.-C., Carter, T., Biochemistry, 2012. Behavioural indicators of welfare in farmed fish. Fish Physiol. 38, 17–41.
McGowan, M.W., Artiss, J.D., Strandbergh, D.R., Zak, B., 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin. Chem. 29 (3), 538–542.
McGown, E., Suttie, J., 1977. Mechanism of fluoride-induced hyperglycemia in the rat. Toxicol. Appl. Pharmacol. 40 (1), 83–90.
Mehmetçik, G., Özdemirler, G., Koçak-Toker, N., Çevikbaş, U., Uysal, M., 2008. Effect of pretreatment with artichoke extract on carbon tetrachloride-induced liver injury and oxidative stress. Exp. Toxicol. Pathol. 60 (6), 475–480.
Metwally, D.M., Alajmi, R.A., El-Khadragy, M.F., Yehia, H.M., AL-Megrin, W.A., Akabawy, A.M., Amin, H.K., Moneim, A.E.A., 2020. Chlorogenic acid confers robust neuroprotection against arsenite toxicity in mice by reversing oxidative stress, inflammation, and apoptosis. J. Funct. Foods 75, 104202.
Meyers, T.R., 2009. Standard necropsy procedures for finfish. In: NWFHS Laboratory Procedures Manual, 5th ed. US Fish Wildlife Service, Washington, pp. 64–74.
Miranda, G.H.N., Ferreira, M.K.M., Bittencourt, L.O., Lima, L.A.D.O., Puty, B., Lima, R.R., 2021. Chapter 17 - the role of oxidative stress in fluoride toxicity. In: Patel, V.B., Preedy, V.R. (Eds.), Toxicology. Academic Press, pp. 157–163.
Moglia, A., Lanteri, S., Comino, C., Acquadro, A., de Vos, R., Beekwilder, J., 2008. Stress-induced biosynthesis of dicaffeoylquinic acids in globe artichoke. J. Agric. Food Chem. 56 (18), 8641–8649.
Mohamed, W., El-Houseiny, W., Ibrahim, R., Abd-Elhakim, Y., 2019. Palliative effects of zinc sulfate against the immunosuppressive, hepato- and nephrotoxic impacts of nonylphenol in Nile tilapia (*Oreochromis niloticus*). Aquaculture 504, 227–238.
Mounes, H.A.M., Abd-El Azeem, Z.M.A., Abd El-Bary, D.A., Al-Sagheer, A.A., Abd-Elhakim, Y.M., Hassan, B.A., Sadek, S.S., Ahmed, K.M., 2024. Effect of substituting soybean meal in Oreochromis niloticus diets with pumpkin (*Cucurbita maxima*) seed cake on water quality, growth, antioxidant capacity, immunity, and carcass composition. Animals 14 (2), 195.
Mukhopadhyay, D., Chattopadhyay, A., 2014. Induction of oxidative stress and related transcriptional effects of sodium fluoride in female zebrafish liver. Bull. Environ. Contam. Toxicol. 93 (1), 64–70.
Myrberg Jr., A.A., 1972. Ethology of the bicolor damselfish, *Eupomacentrus partitus* (Pisces: *Pomacentridae*): a comparative analysis of laboratory and field behaviour. Anim. Behav. Monogr. 5, 197-IN192.
Oliveira, H.H., Liebel, S., Rossi, S.C., Azevedo, A.C., Barrera, E.A., Garcia, J.R.E., Grötzner, S.R., Neto, F.F., Randi, M.A., Ribeiro, C.A., 2015. Mixtures of benzo (a) pyrene, dichlorodiphenyltrichloroethane and tributyltin are more toxic to neotropical fish *Rhamdia quelen* than isolated exposures. J. Ecotoxicol. Environ. Safety 122, 106–115.
Oliveira, M., Ahmad, I., Maria, V.L., Ferreira, C., Serafim, A., Bebianno, M.J., Pacheco, M., Santos, M., 2010. Evaluation of oxidative DNA lesions in plasma and nuclear abnormalities in erythrocytes of wild fish (*Liza aurata*) as an integrated approach to genotoxicity assessment. Mutat. Res./Genet. Toxicol. Environ. Mutagen. 703 (2), 83–89.
Oliveira, M., Gravato, C., Guilhermino, L., 2012. Acute toxic effects of pyrene on Pomatoschistus microps (*Teleostei, Gobiidae*): mortality, biomarkers and swimming performance. Ecol. Indic. 19, 206–214.
Ottappilakkil, H., Babu, S., Balasubramanian, S., Manoharan, S., Perumal, E., 2023. Fluoride induced neurobehavioral impairments in experimental animals: a brief review. J. Biol. Trace Element Res. 201 (3), 1214–1236.
Payne, J., Mathieu, A., Melvin, W., Fancey, L., 1996. Acetylcholinesterase, an old biomarker with a new future? Field trials in association with two urban rivers and a paper mill in Newfoundland. Mar. Pollut. Bull. 32 (2), 225–231.
Pinto, E., Sigaud-kutner, T.C., Leitao, M.A., Okamoto, O.K., Morse, D., Colepicolo, P., 2003. Heavy metal–induced oxidative stress in algae 1. J. Phycol. 39 (6), 1008–1018.
Sahebkar, A., Pirro, M., Banach, M., Mikhailidis, D.P., Atkin, S.L., Cicero, A.F., 2018. Lipid-lowering activity of artichoke extracts: a systematic review and meta-analysis. Crit. Rev. Food Sci. Nutr. 58 (15), 2549–2556.
Sahu, G., Kumar, V., 2021. The toxic effect of fluoride and arsenic on behaviour and morphology of catfish (*Clarias batrachus*). Nat. Environ. Pollut. Technol. 20 (1), 371–375.
Salem, M.B., Affes, H., Ksouda, K., Dhouibi, R., Sahnoun, Z., Hammami, S., Zeghal, K.M., 2015. Pharmacological studies of artichoke leaf extract and their health benefits. Plant Foods Hum. Nutr. 70, 441–453.
Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. 3 (6), 1101–1108.
Scott, G.R., Sloman, K.A., Rouleau, C., Wood, C.M., 2003. Cadmium disrupts behavioural and physiological responses to alarm substance in juvenile rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 206 (11), 1779–1790.
Setyaningsih, Y., Husodo, A.H., Astuti, I., 2015. Detection of urinary 8-hydroxydeoxy-guanosine (8-OHdG) levels as a biomarker of oxidative DNA damage among home industry workers exposed to chromium. Procedia Environ. Sci. 23, 290–296.
Shalini, B., Sharma, J., 2015. Beneficial effects of *Emblica officinalis* on fluoride-induced toxicity on brain biochemical indexes and learning-memory in rats. Toxicol. Int. 22 (1), 35.
Shi, X., Zhuang, P., Zhang, L., Feng, G., Chen, L., Liu, J., Qu, L., Wang, R., 2009. The bioaccumulation of fluoride ion (F−) in Siberian sturgeon (*Acipenser baerii*) under laboratory conditions. Chemosphere 75 (3), 376–380.
Singh, R., Hussain, M.A., Kumar, J., Kumar, M., Kumari, U., Mazumder, S., 2017. Chronic fluoride exposure exacerbates headkidney pathology and causes immune commotion in *Clarias gariepinus*. Aquat. Toxicol. 192, 30–39. https://doi.org/10.1016/j.aquatox.2017.09.006.
Skórka-Majewicz, M., Goschorska, M., Żwierełło, W., Baranowska-Bosiacka, I., Styburski, D., Kapczuk, P., Gutowska, I., 2020. Effect of fluoride on endocrine tissues and their secretory functions–review. Chemosphere 260, 127565.
Surachetpong, W., Roy, S.R.K., Nicholson, P., 2020. Tilapia lake virus: the story so far. J. Fish Dis. 43 (10), 1115–1132.
Suvarna, K.S., Layton, C., Bancroft, J.D., 2018. Bancroft's Theory and Practice of Histological Techniques E-Book. Elsevier Health Sciences.
Tu, W., Zhang, Q., Liu, Y., Han, L., Wang, Q., Chen, P., Zhang, S., Wang, A., Zhou, X., 2018. Fluoride induces apoptosis via inhibiting SIRT1 activity to activate mitochondrial p53 pathway in human neuroblastoma SH-SY5Y cells. Toxicol. Appl. Pharmacol. 347, 60–69.
Uddin, M., Alim, M., Islam, S., Rashid, H., Shahjahan, M., 2018. Temperature changes alter the acute toxicity responses of cypermethrin in zebrafish. Progress. Agric. 29 (1), 64–70.
van de Vis, H., Kolarevic, J., Stien, L.H., Kristiansen, T.S., Gerritzen, M., van de Braak, K., Abbink, W., Sæther, B.-S., Noble, C., 2020. Welfare of farmed fish in different production systems and operations. Welfare Fish 323–361.
Vorhees, C.V., Williams, M.T., Hawkey, A.B., Levin, E.D., 2021. Translating neurobehavioral toxicity across species from zebrafish to rats to humans: implications for risk assessment. Front. Toxicol. 3, 629229.
Wang, X., Xi, Y., Zeng, X., Zhao, H., Cao, J., Jiang, W., 2018. Effects of chlorogenic acid against aluminium neurotoxicity in ICR mice through chelation and antioxidant actions. J. Funct. Foods 40, 365–376.
Wauquier, F., Boutin-Wittrant, L., Viret, A., Guilhaudis, L., Oulyadi, H., Bourafai-Aziez, A., Charpentier, G., Rousselot, G., Cassin, E., Descamps, S., 2021. Metabolic and anti-inflammatory protective properties of human enriched serum following artichoke leaf extract absorption: results from an innovative ex vivo clinical trial. Nutrients 13 (8), 2653.
Wu, G., Xu, B., Lu, L., 2006. Comment on fluorine pollution and prevention of Shilongba hydro-electric power station. J. Kunm. Univer. Sci. Technol. (Sci. Technol.) 31, 55–58.
Yang, M., Ji, L., Zhang, X., Fan, Y., Ren, Z., 2017. The relationship between behavior responses and brain acetylcholinesterase (ache) activity of zebrafish (*danio rerio*) in cadmium stress. Int. J. Fish. Sci. Res. 1, 1002.
Yoshitomi, B., Nagano, I., 2012. Effect of dietary fluoride derived from Antarctic krill (*Euphausia superba*) meal on growth of yellowtail (*Seriola quinqueradiata*). Chemosphere 86 (9), 891–897.
Zhan, X.A., Wang, M., Xu, Z.R., Li, W.F., Li, J.X., Biology, 2006. Effects of fluoride on hepatic antioxidant system and transcription of Cu/Zn SOD gene in young pigs. J. Trace Elements Med. 20 (2), 83–87.