

Study of Oxidative Stress Induced by Okadaic Acid and Its Effect Brain Antioxidant System in Zebra Fish (*Danio Rerio*)

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ABSTRACT

Alzheimer's disease (AD) is a neurological illness associated with aging. To learn more about AD mechanisms and aid in medication discovery, transgenic and pharmacological AD models are researched in great detail. Nevertheless, they are time-consuming and somewhat expensive, which makes it more difficult to find promising anti-AD treatments. Here, we created a novel model of AD in adult zebrafish by co-treating them for 72 hours with okadaic acid (OKA) at varying concentrations. Following treatment we studied oxidative stress using Superoxide dismutase (SOD), Glutathione Peroxidase assay (GPX), Acetylcholinesterase (AChE) activity and DNA fragmentation. We found the brain weight of the treated groups was found to be reduced along with AChE activity. The activity of glutathione reductase (GR) and SOD activity got increased with OKA concentrations and also we did observe prominent DNA shearing with increasing concentrations of OKA. Based on the findings, we may infer that OKA adversely affects antioxidant biochemical pathways linked to the generation of reactive oxygen species in the zebra fish that were studied.

Keywords: Zebra fish, Antioxidants, Okadaic acid, Superoxide dismutase, DNA fragmentation.

INTRODUCTION

Alzheimer's disease (AD) is a complex neurological condition that makes it difficult to design therapeutic interventions that work. Animal models that faithfully mimic the pathophysiology of AD are necessary for comprehending disease mechanisms and investigating possible therapies (Passeri E, 2022). Industrial and residential effluents from anthropogenic activities have contaminated freshwater and marine environments (Dalton et al., 2015). They have increased the occurrence of toxic algal blooms, which are a result of phytoplankton colonies in aquatic habitats fluorescing out of control and creating toxins that can make people sick from eating seafood, primarily mollusks and crustaceans (Tubaro and Hungerford, 2007). Currently, nine groups have been created based on the chemical characteristics of these poisons and how they affect the organism that has been intoxicated.

Okadaic acid (OA) is a prominent liposoluble toxin that is widely distributed in shellfish and mostly causes human diarrheal shellfish poisoning. Its risks are numerous and include neurotoxicity, cytotoxicity, embryotoxicity, and even genotoxicity. However, there isn't any hard evidence that it stunts the growth of human progeny (Figueroa D, 2020). On the Pacific coast of Japan, okadaic acid was found mostly in the black sponge *Halichondria okadai* (Prego-Faraldo et al., 2013). When consumed by people, it typically builds up in the hepatopancreas of mussels and oysters, resulting in diarrhoea syndrome. It is poisonous as a dinophysistoxin because it stimulates the phosphorylation of proteins that regulate sodium-regulated intestinal secretion. According to Ikehara et al. (2010), it is lipophilic and functions as a strong inhibitor of protein phosphatases (PP) 1 and 2 A, causing cells to accumulate hyperphosphorylated proteins and changing a number of metabolic processes. According to Duracova et al. (2018), one of the most well-known toxic consequences is the disruption of cell structure, which results in the loss of cell shape in culture and can be used to identify the presence of the

toxin. While OA is primarily recognized for its ability to cause diarrhea, other research suggests that OA causes the phosphorylated microtubule associated protein tau to accumulate. This accumulation occurs simultaneously with abrupt alterations in the stability of the neuronal cytoskeleton, which ultimately leads to the death of cortical neurons (Valdiglesias et al., 2013). It is therefore hypothesized that OA would encourage neurite disintegration, which would enlarge cerebellar granule cell bodies. In addition, it has the ability to induce DNA fragmentation, which is a feature of apoptosis, condense chromatin, and rearrange the cytoskeleton (Munday, 2013).

Fish and other top trophic level marine creatures can build up OA-group poisons in their tissues. However, in addition to fish's considerable mobility, passive interaction settings can lead to very low levels of OA-group toxin accumulation, such as the process of dilution and dispersion in the aquatic environment (Souid, G., 2018). On the other hand, not much information is known about how exposure affects fish larvae early in their development or about their biochemical reactions. To lessen damage from exposure to xenobiotics that cause oxidative stress, aquatic organisms—including fish—have developed a physiological antioxidant system that includes antioxidant enzymes like glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Prieto, A.I., 2007). Through increased reactive oxygen species (ROS) and lipoperoxidation, OA has been demonstrated to experimentally generate oxidative stress in aquatic species, including bivalves (Figueroa, D., 2020). Overproduction of ROS can lead to intracellular damage that impacts proteins and DNA in addition to membrane lipids. However, the dosage and mode of exposure to the relevant xenobiotics or toxins will primarily determine how antioxidant enzyme activity is altered (Pavagadhi, S., 2012).

In this contribution, we investigated the theory that the OA group toxins generated could cause zebra fish to exhibit decreased antioxidant defense system enzyme activity, favoring oxidative damage and morphological abnormalities. Using zebra fish as a model, the aim of this study was to ascertain the toxicity and biochemical response of the antioxidant defense system to acute exposure to OA.

METHODOLOGY

We purchased zebra fish (*Danio rerio*) with appropriate aeration from a nearby pet store. The animal was kept in a circulating system with filters to aerate the system and maintain the water quality necessary for a healthy aquatic habitat with an ideal temperature of 25–28°C, all while taking care to ensure that it was not disturbed. Fish used for experiments were zebras, kept apart in various aquarium tanks. In the age groups of 4-5 months, adult male Zebra fish weighing between 0.3 and 0.5gm of body weight were utilized.

Prior to experimental initiation, fish underwent two weeks of acclimatization in aerated glass aquaria maintained at 25–28 °C with a 14:10 hour light-dark cycle, following standard husbandry guidelines [37]. Water quality parameters- pH (7.0 ± 0.2), dissolved oxygen concentration, and ammonia levels- were monitored regularly. Fish received standardized commercial zebrafish diet twice daily. All experimental procedures received approval from the Institutional Animal Ethics Committee at Maharani Lakshmi Ammanni College for Women, Bengaluru (**Approval No. mLAC/CCSCA/2024/002**), and were conducted according to Guidelines of CCSEA for experimentation on fishes.

Zebra fish growth rate is most rapid during the first three months after hatching, then the growth rate decreases as it attains 18 months. The difference in the growth rate could be due to the higher food intake in the captivity among the species. (Spencer et al., 2007). A mean life span of domesticated Zebra fish is 42 months with the oldest individual surviving for upto 66 months. Instances like spinal curvature, muscle degeneration associated with senescence become apparent in the domesticated and wild Zebra fish after the second year in captivity and most fishes die naturally before these conditions develop. As per the observations of Gerhard et al., 2002; Kishi et al., 2003 it was also found that the type and amount of food the fishes feed on and the fluctuations in the water parameters results with increased mortality rate in Zebra fish.

In order to check the mortality and natality rate, fishes were monitored for a period of 1 month. It was observed that, out of 30 fishes in each tank there was increased mortality in tank 4, it might be due to fungal growth in the tank as led to contamination of water It was also noticed that the amount of food given for the fishes in tank 4

would have hindered the metabolic activities of fishes. The animal was quarantined and acclimatised before grouping for experimental purpose.

Zebra fish were maintained in five distinct tanks and were grouped as one control containing 20 fishes and four experimental treatment groups contain 30 fishes in each tank, induced with OKA (okadaic acid) at varying concentrations. (0.302 μ g/L, 3.022 μ g/L, 30.22 μ g/L and 302.2 μ g/L). The subjects were monitored for about a month, and were then exposed to cold stress. Prior to exposing the zebra fish to cold stress, their weight was measured. After that, they were placed in a 500ml beaker filled with ice cold water and left for two hours. During this time, the fish's movement was reduced and they were in an involuntary condition. Following the treatment, the brain of these fishes was dissected carefully. The tissues of the brain were promptly removed, weighed, frozen in liquid nitrogen, and kept at -80°C until examination. Using a homogenizer, frozen samples were homogenized (1: 20, w/v) in 50mM potassium phosphate buffer (pH 7.0) with 0.5mM EDTA. The homogenate was centrifuged for 30min at 4°C at 12,000rpm/min to extract the supernatant for the analysis of biochemical parameters (Li Z, 2010).

GROUPS	Concentration of induced oka (okadaic acid) in μ g/l	TOTAL NO OF FISHES FISH
CONTROL	nil	20
OKA-1	0.302	30
OKA-2	3.022	30
OKA-3	30.22	30
OKA-4	302.2	30

Table-1 Experimental groups

The dissected brain was subjected to various biochemical assay.

Glutathione Peroxidase assay (GPX): This experiment was carried out on the principle that residual glutathione reacts with 5, 5'-dithiobis 2-nitro benzoic acid (DTNB) to generate a molecule that absorbs at a maximum wavelength of 450nm. By catalyzing the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) for the consumption of NADPH, the catalytic activity of glutathione peroxidase and glutathione reductase was measured spectrophotometrically at 450nm. The nmol of NADPH consumed per minute per milligram of protein was used to express the particular activities (Marta Bartoskova, 2014). About 1.5ml of sodium phosphate buffer (100mM, pH 7.0) was added to the tubes labelled Tank. About 0.2ml of reduced glutathione (4mM) was added and 0.1ml of respective test samples was added. To the tube labelled standard, 1.6ml of buffer and 0.2ml of reduced glutathione was added and mixed. Tube with 2ml of buffer alone served as blank. The control tube consisted of 1.9ml of buffer and 0.1ml of sample from control tank. About 0.2ml of peroxide (2mM) was added to all the tubes except blank and control. All the tubes were incubated at 37°C for 10min. following incubation, 0.5ml of 8% trichloroaceticacid (TCA) was added and after incubation at room temperature for 30min, absorbance was recorded at 450nm. Reduced glutathione was calculated by the formula (OD test –OD control)/OD of std x concentration of standard (400 μ M).

Acetylcholinesterase (AChE) activity: AChE activity was assessed following treatment as described by Luo L et al (2024) but with slight modifications. To a series of test tubes labelled control and test, about 0.1ml of sample was added. Blank tube was added with 0.1ml of Bovine serum albumin (BSA; 0.05% w/v). Following incubation at 30°C for 10mn, reagent AChE (6.25 mg/ml) was added and incubated for 10min. About 0.2ml of sodium acetate (200mM) was added to all the tubes and incubated 15min at 4°C. The contents were then centrifuged at 3000rpm for 3min and the supernatant collected was added with 1.45ml of 5,5'-Dithio-bis(2-Nitrobenzoic Acid) (0.2mM; DTNB) and incubated at 30°C for 15min. after incubation, tubes were added with 0.1ml of Phenylmethylsulfonyl Fluoride Solution (PMSF) and absorbance was recorded at 412nm in a UV spectrophotometer (1800).

Superoxide Dismutase (SOD) Activity: The procedure adopted was that of Beauchamp and Fridovich (1971) but with little modifications. About 100 μ L of sample at varying concentrations, 2.9mL of 50mM Na-phosphate

buffer (pH 7.8), 13mM methionine, 75µM Nitro Blue tetrazolium (NBT), 10µM EDTA, 2µM riboflavin, and 10µM EDTA were all included in the reaction mixture. Gallic acid was used as positive control. The absorbance was measured at 560nm following a 10-minute incubation period at 30°C. SOD activity is represented as units/mg protein, and one unit of enzyme activity is defined as the amount of protein that causes a 50% inhibition of the NBT reduction rate. The inhibition of blue formazan formation was taken as a superoxide quenching activity.

DNA fragmentation: DNA fragmentation method was followed as described by Mottola F *et al* (2022) but with slight modifications. In brief, the brain tissues were added to a microtuge tube and homogenized. To the suspension, 50µl of extraction buffer (Tris (10mM, pH 8.2); EDTA (10mM); NaCl (200mM) and 0.5% SDS along with Proteinase K (200µg/ml) was added and incubated at 50°C for about 3hr. Following incubation, 100µl of ethyl alcohol was added and incubated on ice for 20-30min. The contents are centrifuged for 10min at maximum speed and the supernatant obtained was added with 200µl 70% alcohol. The contents are centrifuged again at maximum speed to pellet the DNA, and the DNA obtained was stored in 20-50µl TE buffer and stored at -20°C until further use. The DNA was run on 0.25% agarose gel to check the quality of the DNA and assess the DNA damage.

RESULTS

Statistical Analysis: Statistical Analysis was done by using GraphPad Prism 8.4, All the data were mean $\pm$ SE of N=8. Paired 't' test and ANOVA was performed and data considered significant at $p < 0.05$

Effect of OKA on brain weight: Following the subchronic Toxicity Test, the weight of the brain was of each fish were recorded. For every tested concentration as well as the control, the brain weight was computed. Among the examined groups, no discernible changes were discovered (Figure 1). The weight of the treated groups was found to be reduced significantly when compared to control (0.04 ± 0.0032 mg). The weight of the fish was found to be 0.01 ± 0.0012 , 0.02 ± 0.0054 , 0.01 ± 0.001 and 0.01 ± 0.001155 respectively at $0.302\mu\text{g/L}$, $3.022\mu\text{g/L}$, $30.22\mu\text{g/L}$ and $302.2\mu\text{g/L}$.

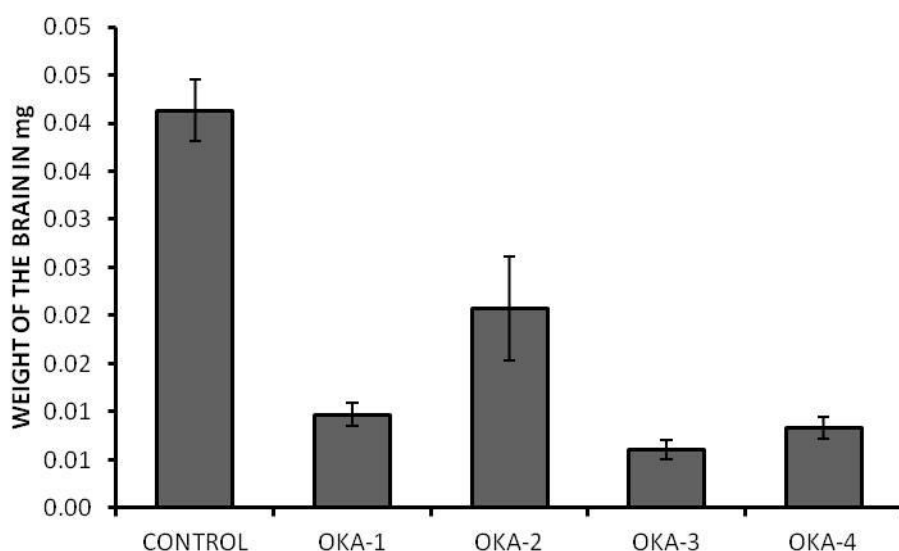


Figure 1: Zebrafish brain weight in subchronic toxicity test. Control: Without treatment. All the values are average of mean $\pm$ SEM. (N=8). Students Paired test was performed to compare the means between groups and considered significant at $P < 0.05$

Glutathione Peroxidase assay (GPX): Zebra Fish treated to all dosages of OKA (0.302, 3.022, 30.22 and 302.2mg/L) showed increased glutathione peroxidase (GPx) activity in comparison to the control group (Figure 2). However, the GPx activity was only considerably higher ($P < 0.01$) in the groups exposed to OKA concentrations than in the control group. When compared to the control group (37.93 ± 1.21 U/L), the activity of glutathione reductase (GR) increased at OKA concentrations was found to be 189.695 ± 2.133 , 151.745 ± 3.21 ,

75.9 ± 1.033 and 113.795 ± 3.242 but the increase did not reach significance (Figure 2). Our results might be because exposure to okadaic acid may have affected the expression levels of genes encoding GPx enzymes. Additionally, modifications in gene expression may have affected the levels and activities of GPx proteins in response to cellular damage, oxidative stress, and protection. (Diego Figueroa, et al., 2020).

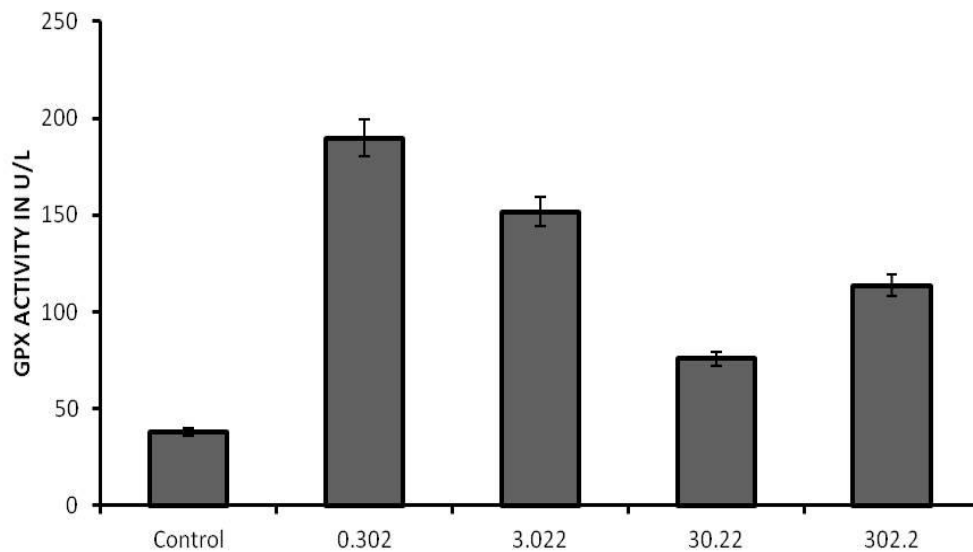


Figure 2: Glutathione peroxidase (GPx) activity in zebrafish exposed to OKA. All the are average values in mean ± SEM) (N=8). Students Paired test was performed to compare the means between groups and considered significant at P < 0.05.

Acetylcholinesterase (AChE) activity: Acetylcholinesterase (AChE), a crucial enzyme that hydrolyzes acetylcholine in the brain and causes cholinergic neuronal dysfunction (Ferreira-Vieira T.H, 2016). In order to verify the stress levels induced we measured the AChE activity of zebra fish after they were treated with varying dosages of OKA. When zebra fish treated with the four chosen combinations of OKA and were compared to the normal control group, we observed that the activity got reduced (Figure 3) significantly when compared to control. When compared to the control group (0.063 ± 0.0011U/L), the activity of AChE reduced at OKA concentrations. The activity was found to be 0.02635 ± 2.133, 0.01411 ± 0.0001, 0.0065 ± 0.00012 and 0.0056 ± 0.0001 but the increase did not reach significance (Figure 3).

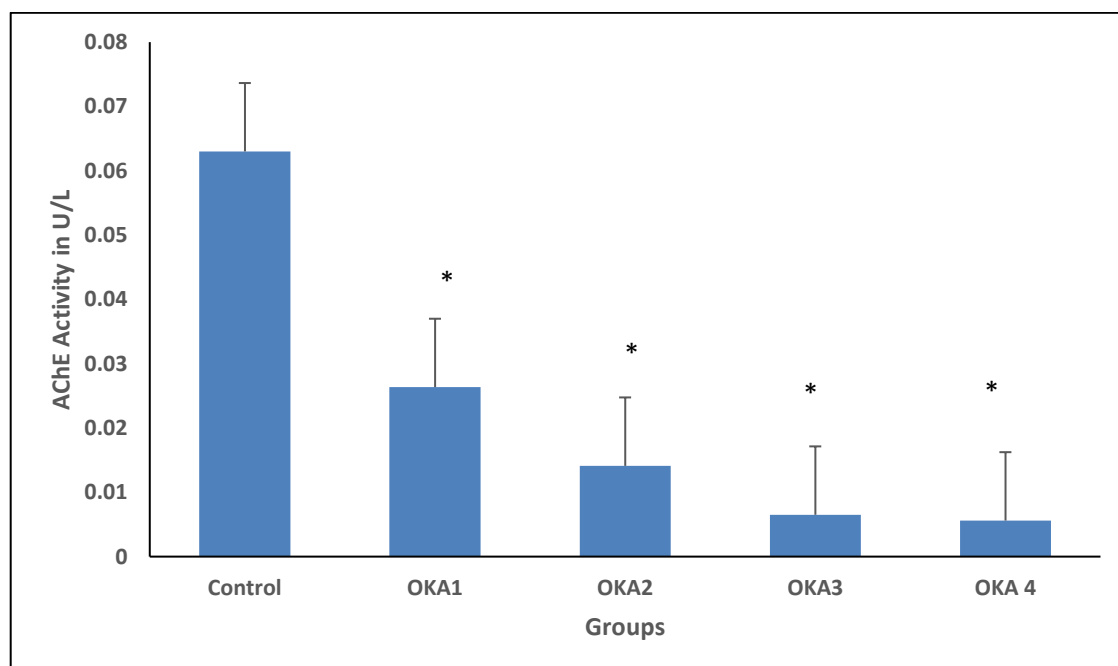


Figure 3: Acetylcholine esterase activity (U/L). Control: Without treatment. All the values are average of mean ± SEM. (N=8). Students Paired test was performed to compare the means between groups and considered significant at P < 0.05

Superoxide Dismutase (Sod) Activity: The effects of prolonged OKA exposure on the SOD antioxidant parameter in zebrafish is depicted in Figure 4. The SOD activity was found to increase to 224.191 from 82.911 (control) at 3.022 μ g/L which is highly abnormal. The concentration of the enzyme activity was found from the standard curve drawn using gallic acid as positive control ($Y=0.0012x + 0.01917$; $R^2 = 0.9965$). This confirms the level of oxidative stress the organism had and we found dose dependant activity ($P<0.05$) till 3.022 μ g/L and thereafter showed no activity ($p<0.05$). On the other hand positive control showed 34.911U/L activity. The SOD activity was found to be 201.353 ± 2.133 and 224.191 ± 0.0001 respectively at 0.302 μ g/L and 3.022 μ g/L. The above graph represents effect of OKA with an increased level Superoxide dismutase (SOD) activity in the OKA-1 and OKA-2 and no activity in OKA-3 and OKA-4. This is due to SOD activity could be affected by okadaic acid-induced changes in protein conformation or post-translational modifications. SOD enzymatic activity can neutralizes superoxide radicals under oxidative stress conditions induced by okadaic acid. Macroscopic evaluation during the exposure period to Okadaic acid showed alterations in zebrafish brain.

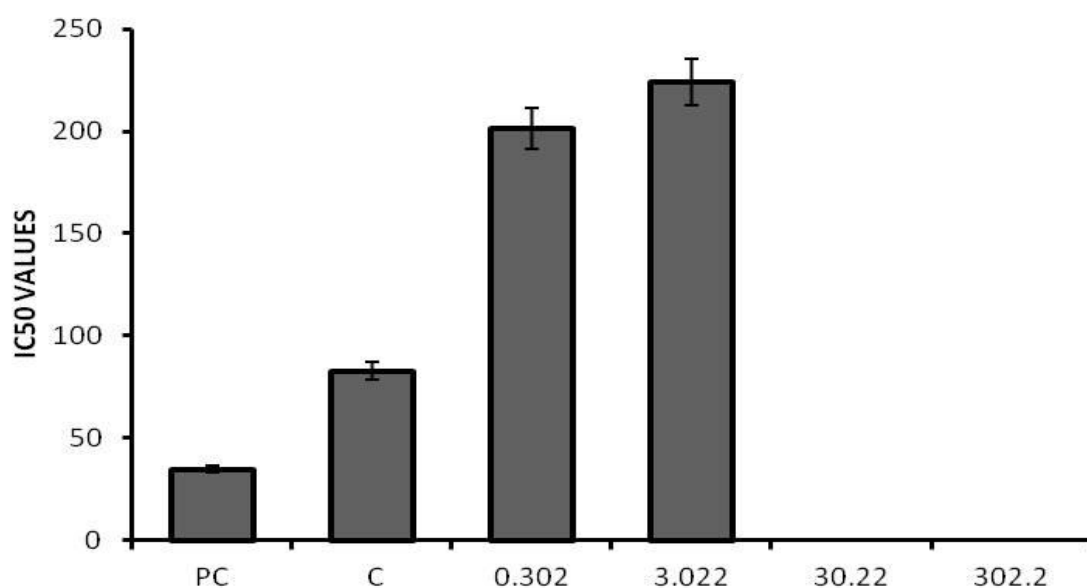


Figure 4: IC50 values from SOD activity. Control: Without treatment. PC: gallic acid. All the values are average of mean $\pm$ SEM. Students Paired test was performed to compare the means between groups and considered significant at $P < 0.05$

DNA fragmentation: Figure 5 displayed representative images of DNA fragmentation analysis on gel electrophoresis. There was clear pattern of DNA damage with increasing concentration of the OKA. The damage was found to be dose dependant ($P<0.05$). The control band was observed clear and distinct. On treatment with OKA at 0.302 μ g/L, the DNA was intact with little damage. When the dosage increased to 3.022 μ g/L and above, the DNA obtained was too low and the shearing of DNA can be seen clearly indicating the damage. The damage was proportional to the concentration.

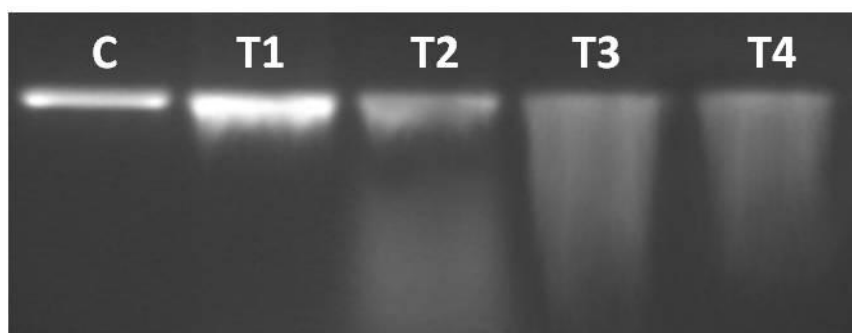


Figure 5: DNA fragmentation assay: C: control; T1: Tank 1; T2: Tank 2; T3: Tank 3; T4: Tank 4. TBE buffer (Tris HCl 11.3gm, Boric acid-5.5gm, EDTA-0.9gm). 0.25% agarose with TBE buffer.

DISCUSSION

Our research revealed the detrimental effects of OKA exposure on a number of zebra fish morphological and biochemical factors. We saw negative impacts on the weight of the brain and antioxidants enzymes. Interestingly, we found that there was a dose-dependent association, with notable impacts being seen as early as 0.302µg/L OKA.

When compared to the control group, the weight of the treated groups was shown to be considerably lower (0.04 ± 0.0032 mg). The fish's weight was measured at 0.302µg/L, 3.022µg/L, 30.22µg/L, and 302.2µg/L, and determined to be 0.01 ± 0.0012 , 0.02 ± 0.0054 , 0.01 ± 0.001 , and 0.01 ± 0.001155 , respectively. Similar works were done by Aisha Siddiqui, et al (2023) wherein they observed discernible neuro degeneration among the treated groups. Our work is also in line with Ankitha Shetty et al (2024) wherein they reported the positive impact of the carica papaya over the brain weight and neurodegeneration caused by OKA.

Primarily employed in developmental biology and molecular genetics, zebrafish have also been recognized as a promising vertebrate model for toxicology and drug discovery, and have been suggested as a model for human disease (Yang et al., 2009). A number of zebrafish antioxidant system components, including as glutathione contents and antioxidant enzyme activity, have been evaluated in relation to oxidative stress in the past (Massarsky et al., 2013). Our study, when compared to the control group, zebra fish treated to all dosages of OKA (0.302, 3.022, 30.22, and 302.2µg/L) had elevated glutathione peroxidase (GPx) activity. Nonetheless, the GPx activity in the groups exposed to OKA doses was only significantly higher ($P < 0.01$) than in the control group. Under oxidative stress circumstances, an increase in GPx activity is also anticipated. According to Jaja-Chimedza et al. (2012), one of the previously cited investigations, GPx activity increased in response to bacterial lipopolysaccharides. Furthermore, varying degrees of oxidative stress can account for a portion of the diversity in antioxidant responses. It's crucial to remember that ROS can stimulate the production of GSH and antioxidant enzymes, boosting the body's antioxidant capacity. On the other hand, elevated ROS levels have the potential to inactivate antioxidant enzymes.

It can be explained by the fact that cells contain oxidative chemicals, which may activate antioxidant enzymes more as a defense strategy (Z. H. Li, 2010). In one experiment, norfloxacin at an ambient concentration of 0.0001 mg L⁻¹ did not result in an increase in GPx activity (M. Bartoskova, 2014). The study conducted on zebrafish exposed to ibuprofen (NSAIDs) at doses of 0.05, 1, 8, and 25 mg L⁻¹ likewise revealed an increase in GPx activity (M. Bartoskova, 2013). We also found that there was a significant decrease in activity in the zebra fish treated with the four selected combinations of OKA when compared to the control group. At OKA doses, AChE activity decreased in comparison to the control group (0.063 ± 0.0011 U/L).

The common neurodegenerative dementia Alzheimer's disease poses treatment problems since amyloid-targeting therapies raise safety concerns. Zebra fish may display OSAT due to a collection of toxins produced, which would reduce the antioxidant-defining system and favour oxidative damage and morphological defects. The purpose of this study was to determine the toxicity and biochemical reaction of AChE and antioxidant enzymes to acute exposure to OKA using zebra fish as a model. The results suggest that in the zebra fish model, OKA negatively impacts antioxidant biochemical pathways connected to the production of reactive oxygen species. Okadaic acid (OA) and its analogues (OA-group toxins) cause considerable toxicity by encouraging excessive generation of reactive oxygen species (ROS), which results in severe oxidative stress, according to research conducted using the zebrafish (*Danio rerio*) model.

CONCLUSION

Our study's findings show that OKA had an adverse effect on zebrafish brain growth. Certain oxidative stress biomarkers are impacted by OKA at the studied concentrations (GPx, AChE and SOD). Upon subchronic exposure to OKA, several antioxidant and biotransformation enzymes become more active in zebrafish. These findings lead us to the conclusion that OKA may be detrimental to various metabolic processes linked to the synthesis of ROS (reactive oxygen species) in aquatic organisms, even at ambient concentrations.

REFERENCES

1. A. Tubaro, J. Hungerford. Toxicology of marine toxins. R.C. Gupta (Ed.), Veterinary Toxicology: Basic and Clinical Principles, Academic Press/ Elsevier, Amsterdam (2007), pp. 52-275
2. Aisha Siddiqui, Syafiq Asnawi Zainal Abidin, Zahoor A. Shah, Iekhsan Othman, Yatinesh Kumari, Behavioural, genomics and proteomic approach to examine Alzheimer's disease in zebrafish, Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, Volume 271, 2023, 109636, ISSN 1532-0456,
3. Ankitha Shetty, Kalyanam Bharathi. Evaluation of Neuroprotective Activity of Carica papaya Seeds Extract in Okadaic Acid Induced Alzheimer's Disease in Zebra Fish Model (Danio rerio). Research Journal of Pharmacology and Pharmacodynamics. 2024;16(2):73-9. doi: 10.52711/2321-5836.2024.00013
4. B.E. Dalton, J. Lu, J. Leips, T.W. Cronin, K.L. Carleton. Variable light environments induce plastic spectral tuning by regional opsin coexpression in the African cichlid fish, Metriaclicha zebra. Mol. Ecol., 24 (16) (2015), pp. 4193-4204
5. Beauchamp, C. and Fridovich, I. (1971) Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. Review. 44, 276-287.
6. Diego Figueroa, Ailen Signore, Oscar Araneda, Héctor R. Contreras, Miguel Concha & Carlos García, Toxicity and differential oxidative stress effects on zebrafish larvae following exposure to toxins from the okadaic acid group. 19 Jul 2020.
7. Ferreira-Vieira T.H., Guimaraes I.M., Silva F.R., Ribeiro F.M. Alzheimer's disease: Targeting the Cholinergic System. Curr Neuropharmacol. 2016;14(1):101–115. doi: 10.2174/1570159x13666150716165726.
8. Figueroa D, Signore A, Araneda O, Contreras HR, Concha M, García C. Toxicity and differential oxidative stress effects on zebrafish larvae following exposure to toxins from the okadaic acid group. J Toxicol Environ Health A. 2020 Aug 17;83(15-16):573-588. doi: 10.1080/15287394.2020.1793046. Epub 2020 Jul 19. PMID: 32686606.
9. Figueroa, D.; Signore, A.; Araneda, O.; Contreras, H.R.; Concha, M.; García, C. Toxicity and differential oxidative stress effects on zebrafish larvae following exposure to toxins from the okadaic acid group. J. Toxicol. Environ. Health A **2020**, 83, 573–588.
10. J. Le Du et al. Embryotoxic effects of dissolved okadaic acid on the development of Longfin yellowtail Seriola rivoliana. (2017) Aquatic Toxicology. Volume 190, September 2017, Pages 210-216
11. Jaja-Chimedza A, Gantar M, Mayer GD, Gibbs PDL, Berry JP. Effects of cyanobacterial lipopolysaccharides from Microcystis on glutathione-based detoxification pathways in the zebrafish (Danio rerio) embryo. Toxins. 2012;4:390–404.
12. Li Z, Zlabeka V, Grabica R, Li P, Machovaa J, Veliseka J, Randaka T. Effects of exposure to sublethal propiconazole on the antioxidant defense system and Na⁺-K⁺-ATPase activity in brain of rainbow trout, Oncorhynchus mykiss. Aquat Toxicol. 2010;98:297–303
13. Luo L, Yan T, Yang L, Zhao M. Aluminum chloride and D-galactose induced a zebrafish model of Alzheimer's disease with cognitive deficits and aging. Comput Struct Biotechnol J. 2024 May 22;23:2230-2239. doi: 10.1016/j.csbj.2024.05.036. PMID: 38827230; PMCID: PMC11140485.
14. Luo L, Yan T, Yang L, Zhao M. Aluminum chloride and D-galactose induced a zebrafish model of Alzheimer's disease with cognitive deficits and aging. Comput Struct Biotechnol J. 2024 May 22;23:2230-2239. doi: 10.1016/j.csbj.2024.05.036. PMID: 38827230; PMCID: PMC11140485.
15. M. Bartoskova, R. Dobsikova, V. Stancova et al., "Evaluation of ibuprofen toxicity for zebrafish (Danio rerio) targeting on selected biomarkers of oxidative stress," Neuroendocrinology Letters, vol. 34, pp. 102–108, 2013.
16. M. Duracova, J. Klimentova, A. Fucikova, J. Dresler. Proteomic methods of detection and quantification of protein toxins. Toxins, 10 (3) (2018), p. 99, 10.3390/toxins10030099
17. M.V. Prego-Faraldo, V. Valdíglesias, J. Méndez, J.M. Eirín-López. Okadaic acid meet and greet: an insight into detection methods, response strategies and genotoxic effects in marine invertebrates. Mar. Drugs, 11 (8) (2013), pp. 2829-2845, 10.3390/md11082829

18. Marta Bartoskova, Radka Dobsikova, Vlasta Stancova, Ondrej Pana, Dana Zivna, Lucie Plhalova, Jana Blahova, and Petr Marsalek. Norfloxacin—Toxicity for Zebrafish (*Danio rerio*) Focused on Oxidative Stress Parameters. Hindawi Publishing Corporation BioMed Research International Volume 2014, Article ID 560235, 6 pages
19. Marta Bartoskova, Radka Dobsikova, Vlasta Stancova, Ondrej Pana, Dana Zivna, Lucie Plhalova, Jana Blahova, and Petr Marsalek. Norfloxacin—Toxicity for Zebrafish (*Danio rerio*) Focused on Oxidative Stress Parameters. Hindawi Publishing Corporation BioMed Research International Volume 2014, Article ID 560235, 6 pages <http://dx.doi.org/10.1155/2014/560235>
20. Massarsky A, Dupuis L, Taylor J, Eisa-Beygi S, Strek L, Trudeau VL, Moon TW. Assessment of nanosilver toxicity during zebrafish (*Danio rerio*) development. *Chemosphere*. 2013;92:59–66.
21. Mottola F, Iovine C, Santonastaso M, Carfora V, Pacifico S, Rocco L. Evaluation of Zebrafish DNA Integrity after Individual and Combined Exposure to TiO₂ Nanoparticles and Lincomycin. *Toxics*. 2022 Mar 8;10(3):132. doi: 10.3390/toxics10030132. PMID: 35324757; PMCID: PMC8954801.
22. Passeri E, Elkhoury K, Morsink M, Broersen K, Linder M, Tamayol A, Malaplate C, Yen FT, Arab-Tehrany E. Alzheimer's Disease: Treatment Strategies and Their Limitations. *Int J Mol Sci*. 2022 Nov 12;23(22):13954. doi: 10.3390/ijms232213954. PMID: 36430432; PMCID: PMC9697769.
23. Pavagadhi, S.; Gong, Z.; Hande, M.P.; Dionysiou, D.D.; de la Cruz, A.A.; Balasubramanian, R. Biochemical response of diverse organs in adult *Danio rerio* (zebrafish) exposed to sub-lethal concentrations of microcystin-LR and microcystin-RR: A balneation study. *Aquat. Toxicol.* **2012**, 109, 1–10.
24. Prieto, A.I.; Pichardo, S.; Jos, A.; Moreno, I.; Camean, A.M. Time-dependent oxidative stress responses after acute exposure to toxic cyanobacterial cells containing microcystins in tilapia fish (*Oreochromis niloticus*) under laboratory conditions. *Aquat. Toxicol.* **2007**, 84, 337–345.
25. R. Munday. Is protein phosphatase inhibition responsible for the toxic effects of okadaic Acid in animals? *Toxins*, 5 (2) (2013), pp. 267–285, 10.3390/toxins5020267
26. Souid, G.; Souayed, N.; Haouas, Z.; Maaroufi, K. Does the phycotoxin okadaic acid cause oxidative stress damages and histological alterations to seabream (*Sparus aurata*)? *Toxicon* **2018**, 144, 55–60.
27. T. Ikehara, S. Imamura, A. Yoshino, T. Yasumoto. PP2A inhibition assay using recombinant enzyme for rapid detection of okadaic acid and its analogs in shellfish *Toxins*, 2 (1) (2010), pp. 195–204, 10.3390/toxins2010195
28. V. Valdiglesias et al. Assessment of okadaic acid effects on cytotoxicity, DNA damage and DNA repair in human cells. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. Volume 689, Issues 1–2, 7 July 2010, Pages 74–79
29. V. Valdiglesias et al. Assessment of okadaic acid effects on cytotoxicity, DNA damage and DNA repair in human cells. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. Volume 689, Issues 1–2, 7 July 2010, Pages 74–79
30. V. Valdiglesias, M.V. PregoFaraldo, E. Pásaro, J. Méndez, B. Laffon Okadaic acid: more than a diarrhetic toxin. *Mar. Drugs*, 11 (11) (2013), pp. 4328–4349, 10.3390/md11114328
31. Yang L, Ho NY, Alshut R, Legradi J, Weiss C, Reischl M, Mikut R, Liebel U, Müller F, Strähle U. Zebrafish embryos as models for embryotoxic and teratological effects of chemicals. *Reprod Toxicol.* 2009;28:245–253.
32. Z. H. Li, P. Li, and T. Randak, “Ecotoxicological effects of short-term exposure to a human pharmaceutical Verapamil in juvenile rainbow trout (*Oncorhynchus mykiss*),” *Comparative Biochemistry and Physiology C*, vol. 152, pp. 385–391, 2010. [25] M. Bartoskova, R. Dobsikova, V. Stancova et al., “Evaluation of ibuprofen toxicity for zebrafish (*Danio rerio*) targeting on selected biomarkers of oxidative stress,” *Neuroendocrinology Letters*, vol. 34, pp. 102–108, 2013.
33. Z. H. Li, P. Li, and T. Randak, “Ecotoxicological effects of short-term exposure to a human pharmaceutical Verapamil in juvenile rainbow trout (*Oncorhynchus mykiss*),” *Comparative Biochemistry and Physiology C*, vol. 152, pp. 385–391, 2010.