International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (1): 759-762
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Histopathological Changes of Zebrafish (*Branchydanio Rerio*) Brain with Treated Triclosan and Recovered by Using Garlic Extract and Vitamin C

D. Nagarajan, S. Sridevi and T. Ramesh Kumar
Department of Zoology, Annamalai University, Annamalai Nagar, Chidhambaram, Tamil Nadu

Received: 11 Oct 2018 / Accepted: 9 Nov 2018 / Published online: 1 Jan 2019
Corresponding Author Email: l.d.premnagu@gmail.com

Abstract
Triclosan is a synthetic, broad spectrum of biocide and act as a antimicrobial agent. Triclosan has been extensively used along the years in a diversity of tropical application. The present study was carried out to study the histopathology of brain in zebra fish *Brachydanio rerio* (Ham) which are exposed to triclosan (0.32mg/l) for 28 days. The histopathological changes of brain were observed microscopically showed increasing degree of damage in the tissue correlation with the concentration of triclosan and the control groups exhibited a normal architecture. The histopathological studies have been conducted to establish the causal relationships between contaminant and exposure and other various biological responses. The present investigations have also been proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in the laboratory experiment such analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the brain.
Keywords
Zebrafish, Vitamin C, Histopathological Changes

*****

INTRODUCTION
Pollution refers to the contamination of the environment with harmful and undesirable wastes. Pollutants have now become a part with in the conventional crimes. water pollutants affect marine ecosystem, wild life health and humans. The answer to solving pollution is to make changes in our daily habits and pay more attention to the types of product we consume. Water pollution has been extensively documented as a contributor to health problems in human and marine animal ecosystem. Triclosan is added to a wide range of consumer products to offer long lasting protection against bacteria, moulds and yeast. The majority of its usage is associated with household and personal care product. These uses result in the substance being released to the aquatic environment. In this context the zebra fish (*Brachydanio rerio)* has come to attention recently as a genetically tractable. Vertebrate model system as early as the 1930s, the zebra fish was being used as s classical development and embryological model. Fish as bio- indicators of pollutants effect are very sensitive to the changes in the environment and play significant role in assessing

potential risk associated with contaminations of new chemicals in aquatic environment. The sub- lethal toxicity of pesticides decreases plankton abundance and water quality in fish ponds. Moreover, pesticides have been noticed to interfere with fish health and reproduction. Triclosan has a tremendous effect on physiology that brings about characteristics of alterations in different functions of the vital organs such as kidney, liver, brain, heart, lungs and tissues like muscles, skin, bone etc. (Graham Scott and Sloman, 2004).) Histopathology deals with the study of pathological changes induced in the microscopic structure of the body tissue. Any peculiar type of alteration of cells may indicate the presence of the disease or the effect of toxic substance. The monitorization of histological changes of fish brain is a highly sensitive and accurate way to assess the effect of xenobiotic compounds inthe field and experimental studies. In fishes, it is observed that, the external organs are affected due to toxic chemicals, causing loss of equilibrium, increase in opercular movements and for irregular vertical movements, finally leading to death. This may be attributed to the significant damage to the internal organs. Histopathological studies thus give us useful data concerning tissue change prior to external manifestation (Ananth and Mathivanan, 2014). This study was under taken to investigate the effect of different sub- lethal triclosan concentration on histological aspect of brain of zebra fish *Brachydanio rerio.* Therefore, in the present study an attempt was made to investigate the effect of triclosan (0.32 mg/l) 7 and 28 days on the histoarchitecture of the brain zebra fish *Brachydanio rerio.*

MATERIAL AND METHODS

The fish zebrafish, *Brachydanio rerio (Ham)* having a mean weight of 23 to 25gm and length 4 to 6 cm were collected the trough was sterilized with 0.1% $KMNO_4$ solution and then kept for acclimatization for a period of nine days. They were feed by commercial feed regularly. The triclosan was used in this study and stock solutions were prepared LC_{50} values were (0.32 mg/l) taken as sub- lethal concentrations for this study. One hundred fish were selected and divided into 4 groups of each 100 in the experimental setup. The experiment was carried out for a period of 28 days. The water was replaced daily for the treatment. So as to maintain the constant concentration, triclosan was appropriately diluted and mixed with aquarium water just before use the experiment. Fish from control group were maintained in pesticide free water. The brain was dissected out and fixed in Bowin's fixative, dehydrate using ethyl alcohol, cleared in xylene and embedded in paraffin wax (58 to 60 ºC). Sections of 6mm thickness were obtained, stained in haematoxylin and eosin were mounted in DPX (Dextrene plasticizing xylene). Then the damages were identified and analysed based on the microscopic studies.

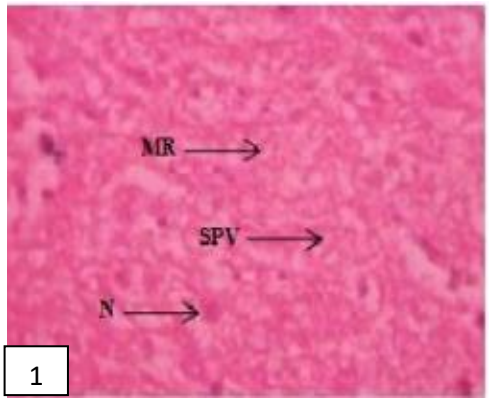
Experimental setup was maintained with four Groups.

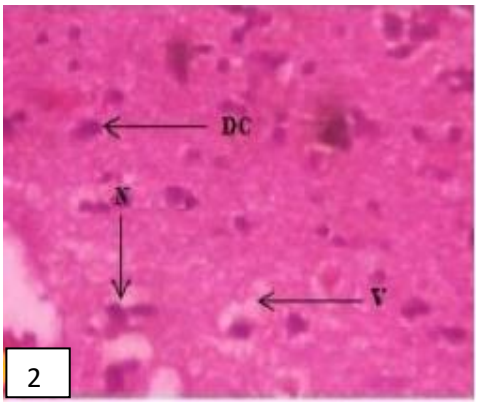
Group I: Control
Group II: Triclosan, (TCS)
Group III: Triclosan + Garlic Extract
Group IV: Triclosan + Vitamin C

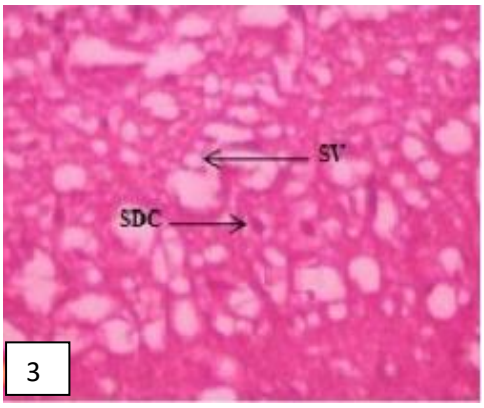
RESULT

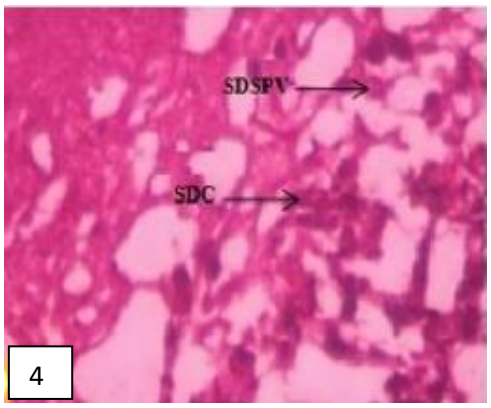
The triclosan exposure of zebrafish at the concentration of 0.32 mglL of exposures at induced marked pathological changes in the brain of exposed fish. The brain also shows damage for 28 days treatment with sub lethal concentration of triclosan in which the nucleus and molecular region (N and MR) were detached from each other and proliferation, stratum periventricular region was detached with shortening gaps, stratum periventricular detachment and necrosis in the neurons of the DGC, SDC, SDGC and SDSPV while it showed vacuolization around mononuclear proliferation. The degenerative changes (DC) in neurons, vacuolation and neurons were seen in granular cells. The fish exposed to sublethal concentration of triclosan, the outer layer stratum periventricular space (SPV) is extensively degenerated and detached from the stratum plexiformet fibrosum externum formation of vaculoses seen in (SPV) and agglutination of neurons is noted. Deformed and clumped cells are seen in the SEP. SDGC Splitting, necrosis and extensive vacuolization in the SDGC region were observed. The degeneration of granular cells, slight vacularization, slight degenerative of granular cells of styratum periventricular are also seen. During the recovery period the garlic extract, and vitamin C treatment reduced the degenerative changes such as molecular region such as stratum periventricular space, vaculation, necrosis, degeneration of granular cells, slight vaculation, slight degenerative of granular cells and slight disintegrated of stratum periventricular space. The above-mentioned changes were seen in figure 1,2,3 and 4.

Fig. 1: Control, Fig. 2: Triclosan with Garlic Extract, Fig. 3: Triclosan with Vitamin C, fig. 4 : Garlic Extract with Vitamin C









N – Nucleus, **MR** - Molecular region, **DC** - Degenerative changes, **V** – Vacuolation, **N** – Necrosis, **DGC** - Degeneration of granular cells, **SDC** - Slight degeneration changes, **SV** - Slight vacuolation, **SDGC** - Slight degenerative of granular cells, **SDSPV** - Slight disintegrated of stratum perventricular

DISCUSSION

In all vertebrates, the brain exhibits three primary vesicles *i.e.,* fore brain, mid brain and hind brain. The mid brain of fish *Branchydanio rerio* forms a relatively large structure and divided into the dorsal optic tectum and the ventral tegmentum. Both tectum and tegmentum of the mid brain are connected with the cerebellum. The tectum is divided into optic lobes by longitudinal furrows which are larger in teleosts than elasmobranch. Well-developed optic lobes were observed in *Branchydanio rerio* in the present study which coincides with the observation in *Channa punctatus* (Abha Mishra and Yogita Devi, 2013). The mid brain is connected reciprocally with the other parts of the brain. The optictectum is laminated and forms superior border of the third ventricle and tegumentum lying above hypothalamus forms the interior border of the third ventricle in *Branchydanio rerio* as the observations made in *Channa punctatus* by Zhang *et al.* (2008) and in *Sebasticus marmoratus* by Abha mirsha and Yogita devi (2013), exposed to arsenic trioxide in *Ctenopharygodon idella* by Ananth (2014). In the present studies shows the exposure of triclosan caused degenerative changes in brain tissue of zebrafish. Similar reason is suggested by Kamal Sarma *et al.* (2010). Das and Mukherjee (2000) reported that hexachlorocyclohexane was neurotoxic and induced vacuolation of brain parenchyma and moderate swelling of pyramidal cells of the cerebrum and opined that vacuolation may have been due to glycolysis leading to microsomal and mitochondrial dysfunctions. Loss of nissel substances and glial cell reaction, with evidence of glial nodule formation in places, were proof of the neurotoxic nature of the chemical.

Santhakumar *et al.* (2000) studied the pathological effects of monocrotophos on the brain by exposing the fish *Anabas testudineus* to sublethal concentrations for 21 days and reported that the pesticides produced rupture of cortex, atrophy of molecular and granular layer, necrosis of neurofibrillar region, vascular dilation, nuclear pyknosis, fibrosis, vacuolation, cerebral oedema and interzonal detachment. The histopathological changes were found to be dose dependent. The vacuolation, dilation of blood capillary, fibrosis, agglutination of neurons and loss of definite demarcation between layers were observed on optic tectum of *Channa punctatus* by Karuppasamy (2000). The above findings were also in support to the observation of Bhattacharya and Mukherjee (1978), in the optic tectum of *Clarias batrachus* and *Channa punctatus*; Joshi and Dubey (1984), *Oxygaster bucarila* to industrial effluents. Altinok and Capkin (2007) reported increasing methiocarb concentrations on fish rainbow trout (*Oncorhynchus mykiss*) caused telangiectasis and necrosis between the molecular and granular layers of the cerebellum where Purkinje cells are located. Similar findings were also observed by Dalela *et al.,* (1979). Trickle bank (2001) found the same result when damselfish, Parma microlepis, was exposed to aldrin and dieldrin. Histological changes in brain due to zinc toxicity to *Labeo rohita* exposed to 5 mg/L showed swelling of pyramidal cells with binucleated nuclei and at 10 mg/L exposure severe necrosis of neuronal cells of cerebrum was observed by Loganathan *et al.* (2006), indicating loss of nissel substances mild vacuolar changes with empty spaces appeared due to increased concentration and duration. The results of the present study conclude, indicate that sublethal concentration of triclosan may have a direct effect on brain and its tissues. As brain plays an important function in governing the whole-body function, any pathological lesion may affect the brain metabolism, resulting in serious consequences in the growth and reproduction of an organism.

REFERENCES

Abha Mishra and Yogita Devi (2013) Histopathological alterations in the brain (optic tectum) of the freshwater teleost, *Channa punctatus* in response to acute and subchronic exposure to the pesticide Chlorpyrifos, Journal homepage wwwelsevierde/acthis

Altinok I, Capkin E (2007) Histopathology of rainbow trout exposed to sublethal concentrations of Methiocarb or Endosulfan Toxicologic Pathology 35: 405–410

Ananth S, Mathivanan V (2014) Histopathological changes in gill and kidney of *Ctenopharyngodon idella* exposed to arsenic trioxide International Journal of Current Research in Chemistry and Pharmaceutical Sciences (pISSN: 2348-5213; eISSN: 2348-5221)

Bhattacharya S, Mukherjee S (1978) Histopathology of the brain (optic tectum) of teleosts exposed to Industrial Pollutants Indian Biologists 10(1):31-36

Dalela, RC, Bhatnagar, MC,Tyagi, AK Verma, SR (1979) Histological damage of gills in *Channa gachua* after acute and subacute exposure to endosulfan and rogor. Mikroscopie 35: 301–307

Das BK, Mukherjee SC (2000) A histopathological study of carp (*Labeo rohita*) exposed to hexachlorocyclohexane. Vet Arhiv 70(4):169–180

Graham R. Scott and Katherine. A. Sloman (2004). The effects of environmental pollutants on complex fish behavior integrating behavioural and physiological indicators of toxicity. Elsevier journal of Aquatic toxicology. Vol: 68 Pp.no: 369-392.

Joshi, R K and Dubey, G K (1984) Histopathological lesions in the brain (optic teetum) of a teleost, *Oxygaster bucaila* exposed to industrial effluent Proc Sem Effect of Pesti Aquat Fauna the Academy of Environment Biology, India Muzaffarnagar; p 45-49

Kamal srma ., N. Ravisanker S. dam roy g. grinson and R.C. Srivastava (2010) . prospects of fish culture in the broad bed and furrow system in Ansmn .J. Envir and Ecology 28(1): 86-90.

Karuppasamy, (2000) Tissue histopathology of *Channa punctatus* (Bloch) under phenyl mercuric acetate toxicity. Bull Pure and Appl Sci,Vol 19A(2):109-116

Loganathan k, B. Velmurugan, J. Hongray Howrelia, M. Selvanayagam and Bharat Bhusan Patnaik (2006) Zinc induced histological changes in brain and liver of Labeo rohita (Ham.) journal of environmental biology 27(1) 107-110

Santhakumar M, Balaji M, Ramudu K (2000) Histopathological lesions in the brain of a teleost Anabas testudineus exposed to monocrotophos. J Environ Biol 21(4): 297-299

Tricklebank KA (2001) Histological alterations in fish from Sydney reefs: possible biomarkers for environmental effects Biomarker 6: 26-32

Zhang J, Zuo Rong C, Chen Y, Wang C (2008) Tributyltin exposure causes brain damage in *Sebastiscus marmoratus Chemosphere* 73: 337–43