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Research Article

BIOCHEMICAL ALTERATION OF TRICLOSAN AND THE PRODUCTIVE ROLE OF GARLIC EXTRACT AND VITAMIN C IN ZEBRAFISH (*BRANCHYDANIO RERIO)*

D. Nagarajan* and T. Ramesh Kumar

Department of Zoology, Annamalai University, Annamalai Nagar, Chidhambaram-608002, Tamil Nadu, India

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ABSTRACT

Zebrafish has been a prominent model vertebrate in a variety of biological discipline. Sustainable information gathered from developmental and genetic research, together with mear completion of the zebrafish genome project, has placed zebrafish in an attractive position for use as a toxicological model. There is a clear potential for *Branchydanio rerio* to provide valuable new sights into chemical toxicity, drug discovery and human diseases using recent advances in forward and reverse genetic techniques. The present study was carried out the impact on exposure of sub lethal concentration of triclosan induced biochemical parameters. This experiment was carried out from 7th day to 28th days of the exposure of triclosan, triclosan with garlic extract, triclosan with vitamin C, garlic extract, vitamin C and maintained with control. Triclosan of 0.32g, 1ml of garlic extract and 1g of vitamin C were used in this study. The result proved that garlic extract and vitamin C were played a productive role in treated with triclosan of zebrafish and also enhances the biochemical and enzymological parameters, and it act as detoxifying agent of triclosan. This study shows the changes in biochemical parameters of blood, which has been recovering by garlic extract and vitamin C against triclosan exposed zebrafish.

Keywords: Zebrafish, Triclosan, Vitamin C, Biochemical, Garlic extract.

INTRODUCTION

Environmental pollution, apart from all the pollution, water pollution is a serious issue in all the developing countries. It is not only affect the survival and reproduction of aquatic organisms but also adversely impacts human health through bio concentration. Sensitivity to different contaminants make the zebrafish as an ideal model organism for environmental monitoring. The international organization for standardization (ISO) first published the zebrafish toxicity test in 1984. There after multiple countries promulgated their own toxicity test standards by using zebrafish according to ISO 7346-1996 such as the British Standard BS/EN/180 7346-1998, The German standard DIN/EN/180 7346-1998 and the Chinise standard GB/T 13267-91. In brief the pollutants detected *in vivo* studies the zebrafish are widely used. There are numerous advantages for the use of zebrafish as a toxicological model species (Spits Bergan and Kent, 2003, Terooka *et al.,* 2003a) as well as for other description. In the early (1990s) less than 100 zebrafish related publications were annually submitted. This rose to ~1,000 at the turn of the century and now average around 3,500 per year.

Among the emerging class of environmental pollutants of (PPCPS) pharmaceuticals and personal care products which are used not only for human and veterinary medicine but also for the promotion of growth in livestock and aquaculture species (Daughton and Ternes, 1999). Triclosan (TCS; 2, 4, 4-trichloro-2'-hydroxydiphenyl ether) is one of the main known antibacterial agents and is added to a wide range of consumer products (eg; toothpaste, soaps, deodorant, textiles, shoes and cosmetics). It has been marketed for over 30 years, and its use has increased over time. The triclosan has been shown to be biodegradable and photounstable and it continuous to breakdown following its release into the aquatic environment. The usage amount of triclosan was more than 3 million tons per year and it was

*Corresponding Author: D. Nagarajan, Research Scholar, Department of Zoology, Annamalai University, Annamalai Nagar, Chidhambaram-608002, Tamil Nadu, India, Email: l.d.premnagu@gmail.com

more than 3.5 million tons per year in Europe (Haldane and Paul, 2005). TCS is used extensively from more than 30 years. Fishes as bioindicators, pollutants effects are very sensitive to changes in their environment and play major role in assessing potential risk associated with contaminations of new chemicals in aquatic environment (Lakra and Nagpure, 2009).

The garlic (*Allium sativum L.*) as a cultured plant, suggesting that its uses as a spice and medicine (Hahn, 1996; Groppo *et al.*, 2002). Vitamlin C (ascorpic acid) is a naturally occurring antioxidant, which is an essential nutrient for the biosysnthesis of collagen, L-carnitine and the conversion of dopamine to norepinephrine (Li and Schellhorn, 2007). Vitamin - C exhibits a powerful scavenging property against free radicals and activated oxygen species. As an electron donor, vitamin C protects by neutralizing reactive oxygen species (ROS) and decreasing oxidative damages (Wilson, 1993). Furthermore; it act as a metabolic antioxidant, detoxifying numerous peroxide metabolites, thus protecting cell membranes and other intracellular components and process that are sensitive to oxidation (Masumoto *et al.*, 1991; Sandel *et al.*, 1988). Vitamin C also exhibits anti-inflammatory effects, prevents endothelial dysfunction and apoptosis, and reduces the risk of cardiovascular diseases (Ozkanlar and Akcay, 2002; El-Ashker *et al.*, 2015). Vitamin C has been demonstrated to play an important role in the functioning of the immune system (Blazer, 1992). Several researchers have investigated the use of different methodologies to make leads to deliver water-soluble substances to marine suspension feeders (Buchal and Langdon, 1998; Nordgreen *et al.*, 2009; Onal and Langdon, 2004). The use of more stable forms of vitamin C is also a crucial requirement in this field.

MATERIALS AND METHODS

Experimental design

The studies were done with 350 adult individuals from the same brood of a zebrafish "*Branchydanio rerio"* Hamilton-Buchnan (Cyprinidae) with 2.5 to 3.5 cm total length and 0.17g mean weight. The fish were maintained for acclimation for two weeks in 2000L aquarium, in filtered and de-chlorinated tap water, at the temperature of 25ºC (±1ºC), P^H 7 (±0.2) and a photoperiod of 10 hours light /14 darkness. The animals were fed twice a day, at the beginning at the end of the light period with commercial tubifex worm feed. After acclimation 100 fish were used for the determination of the LC_{50}. (TCS concentration that is lethal for 50% of the population) and also individual for tests with a sub-lethal dose, all of them maintained at the same environmental condition during acclimation. The experimental setup was maintained with six Groups.

Group I : Control

Group II : Triclosan, (TCS)

Group III : Triclosan + Garlic Extract

Group IV : Triclosan + Vitamin C

Group V : Garlic Extract (GE)

Group VI : Vitamin C (Vit C)

0.32g of triclosan, 1ml of garlic extract and 1g of vitamin C were added to the experimental setup, the garlic and vitamin C are used as recurring agent of triclosan treated zebrafish. The experiment was done in 7^{th} day and 28^{th} day of triclosan exposure.

Collection of blood

Blood sample were collected from control and triclosan treated groups by cardiac puncture. Plastic disposal syringe with 26 gauge needle which was already moisture with heparin was used. The collected blood was expelled into separate heparinised plastic vials and kept immediately on ice. The blood sample was centrifuged within 10 minutes at 0.5g (Eppendorph Centrifuge 5403), the serum is at the top layer of the tube. With a pipette, aspirate the serum, making sure to get the serum while keeping both layers well divided and stable. Transfer the serum into a new microtube and it is ready to used in biochemical analysis (Gabriele *et al.*, 2012).

Biochemical parameters

Sodium, potassium, calcium concentrations measured by Flame Photometer. The serum total protein and albumin concentration estimated by Biuret and Dumas method (Varley Harold, 1980), glucose by glucose oxidase method, cholesterol by cholesterol dehydrogenase/ peroxidase method, blood urea nitrogen (BUN) by glutamate dehydrogenase method, creatinine by Jaffe's kinetic method, uric acid by Enzymatic Photometric test by IFCC method, total lipid level in the fish measured by the method of (Folch *et al.*, 1957). Albumin by bromocresal green method uric acid by carbonate method.

Statistical analysis

The basic statistics, means, standard errors and ranges of the measured parameters were estimated. The patterns of variation due to triclosan, garlic extract and vitamin C doses and their combinations were studied by the data of 7 and 28 days sub-lethal toxicity test was statistically subjected to one-way ANOVA analysis by using SPSS version 17.0 Duncan's multiple range tests was carried out for post hoc comparison of mean. A significance level of $P<0.05$ was used.

RESULTS

The sodium, potassium and calcium level in the 7 and 28 days period is given in (Table 1 and 2). Triclosan reflected high significant decrease in sodium, potassium and calcium level at both period (P<0.05). The normal glucose level of zebrafish *Brachydanio rerio* (Ham) in the 7 and 28 days periods is given in (Table 1 and 2). Triclosan main effect was significantly increase in the both periods (P<0.05). The main effect of garlic extract and vitamin C in the two periods was (P<0.05) highly significant. Dietary supplementation with vitamin C and/or garlic extract improved TCS-GE group compare with control group. The total protein level of zebrafish *Brachydanio rerio* (Ham) in the 7 and 28 days periods is given in (Table 1and 2). Triclosan reflected higher significant decrease in total protein level at both periods (P<0.05). The main effect of garlic extract and vitamin C in the two periods was not significant. Highly significant interaction effect between TCS-GE-Vitamin C was recorded in 28 days period. The protein level decreased in the triclosan exposed fish compare to control group. The total lipid level of zebrafish *Brachydanio rerio* (Ham) in the 7 and 28 days periods is given in (Table 1 and 2). Triclosan reflected high significant decrease in the both periods (P<0.05). The main effect of garlic extract and vitamin C in the two periods was (P<0.05) significant. Vitamin C and its interaction with garlic didn't show significant interaction between TCS-GE and TCS-Vitamin C was recorded in the two periods.

Dietary supplementation with vitamin C and/or garlic extract alleviate the level of total lipids compare that of control and TCS treated group (P<0.05) especially in the 28 days period. The albumin level of zebrafish *Brachydanio rerio* (Ham) in the 7 and 28 days periods is given in (Table 1 and 2). Triclosan reflected high significant decrease in the both periods (P<0.05). The main effect of garlic extract or vitamin C in the two periods was not (P<0.05) significant. Highly significant interaction effect between TCS-GE-Vitamin C was recorded in the 7 and 28 days periods. The creatinine level of zebrafish *Brachydanio rerio* (Ham) in the 7 and 28 days periods is given in (Table 1 and 2). Triclosan reflected high significant decrease in the both periods (P<0.05).

The main effect of TCS-Vitamin C in the two period was significant (P<0.05) compare to control group. The uric acid level of zebrafish *Brachydanio rerio* (Ham) in the 7 and 28 days periods is given in (Table 1and 2). Triclosan main effect was highly significantly decrease in the both periods (P<0.05). The main effect of vitamin C in the two periods was (P<0.05) significant. Vitamin C did not show significant effect compare to control. The blood urea nitrogen level of zebrafish *Brachydanio rerio* (Ham) in the 7 and 28 days periods is given in (Table 1 and 2). Triclosan reflected high significant decrease in the both periods (P<0.05). The main effect of garlic extract was (P<0.05) significant in both period compare to control group. The cholesterol level of zebrafish *Brachydanio rerio* (Ham) in the 7 and 28 days periods is given in (Table 1and 2). Triclosan main effect was significantly decrease in the both periods (P<0.05). The main effect of garlic extract and vitamin C in the two periods was (P<0.05) significant. Supplementation with vitamin C and/or garlic extract improved the GE and Vitamin C alone in the both compare to control group.

Table 1. Biochemical parameters of zebrafish *Brachydanio rerio* (Ham) exposed to triclosan (0.32 mg/L), Vitamin C (Vit C), Garlic extract (GE) and their combinations for 7 days.

Biochemical Parameter	Treatment					
	Control (I)	Exposure (II)	TCS+Garlic Extract (GE) (III)	TCS+Vit C (IV)	Garlic Extract Alone(GE) (V)	Vitamin C Alone (VI)
Sodium (mg/dl)	10.43 ± 1.82^{a}	12.54 ± 1.32^{b}	12.75 ± 0.78^{ab}	11.27 ± 0.52^{c}	10.48 ± 0.41^{d}	10.98 ± 0.71^{a}
Potassium(mg/dl)	4.21 ± 1.88^{bc}	4.43 ± 1.81^{a}	4.00 ± 2.00^{c}	4.60 ± 0.84^{ab}	4.46 ± 1.01^{abc}	4.73 ± 0.65^{bc}
Calcium(mg/dl)	19.80 ± 2.97^{a}	10.17 ± 2.6^{bc}	12.98 ± 1.17^{c}	15.48 ± 1.28^{ab}	17.61 ± 1.97^{a}	18.98 ± 0.58^{ab}
Glucose(mg/dl)	25.08 ± 1.75^{a}	41.61 ± 3.64^{c}	46.98 ± 5.46^{cd}	38.58 ± 2.88^{abc}	$27.31\pm0.^{61ab}$	26.90 ± 0.53^{ab}
Total protein(mg/dl)	5.43 ± 0.05^{de}	4.57 ± 0.45^{bcd}	4.59 ± 0.06^{a}	4.27 ± 0.11^{bcd}	5.13 ± 0.16^{cd}	6.41 ± 0.01^{ef}
Total lipid(mg/dl)	8.32 ± 0.13^{d}	7.08 ± 0.07^{cd}	4.59 ± 0.06^{a}	8.19 ± 0.12^{d}	7.10 ± 0.66^{cd}	8.37 ± 0.27^{d}
Albumin(mg/dl)	0.68 ± 0.06^{bc}	0.41 ± 0.06^{ab}	0.37 ± 0.06^{a}	0.23 ± 0.12^{ab}	0.30 ± 0.12^{c}	0.31 ± 0.02^{d}
Creatinine(mg/dl)	0.493 ± 0.05^{ab}	0.363 ± 0.05^{b}	0.515 ± 0.05^{ab}	0.525 ± 0.05^{ab}	0.415 ± 0.05^{b}	0.425 ± 0.05^{ab}
Uric acid(mg/dl)	3.68 ± 0.230^{a}	3.82 ± 0.230^{a}	2.13 ± 0.240^{bc}	2.25 ± 0.240^{bc}	2.81 ± 0.250^{b}	3.81 ± 0.280^{bc}
BUN(mg/dl)	8.53 ± 1.830^{ab}	9.16 ± 1.830^{ab}	10.93 ± 1.830^{ab}	8.16 ± 1.830^{ab}	7.81 ± 1.830^{ab}	8.13 ± 1.830^{abc}
Chloesterol(mg/dl)	140.35 ± 0.88^{a}	119.53 ± 1.8^{b}	121.45 ± 0.79^{b}	114.96 ± 0.91^{c}	118.46 ± 0.79^{ab}	120.48 ± 0.89^{c}

The data are presented as means ± S.E. Different letters indicate significance at p<0.05.

Table 2. Biochemical parameters of zebrafish *Brachydanio rerio* (Ham) exposed to triclosan (0.32 mg/L), Vitamin C (Vit C), Garlic extract (GE) and their combinations for 28 days.

Biochemical Parameter	Treatment					
	Control (I)	Exposure (II)	TCS+Garlic Extract (GE) (III)	TCS+Vit C (IV)	Garlic Extract Alone(GE) (V)	Vitamin C Alone (VI)
Sodium(mg/dl)	9.78±0.02^{a}	8.57±0.02^{ab}	10.73±0.75^{a}	7.87±0.05^{c}	7.89±0.05^{d}	10.98±0.05^{ab}
Potassium(mg/dl)	2.02±0.05^{b}	1.43±0.81^{ab}	4.12±0.24^{a}	4.90±0.98^{a}	3.89±0.32^{a}	3.90±0.05^{a}
Calcium(mg/dl)	19.81±2.98^{a}	8.18±1.27^{b}	11.89±1.71^{ab}	16.48±1.18^{c}	18.84±1.27^{bc}	19.27±1.78^{a}
Glucose(mg/dl)	26.9±1.05^{a}	58.76±.67^{c}	30.25±0.79^{a}	37.95±1.34^{ab}	27.95±0.96^{a}	30.43±1.17^{a}
Total protein(mg/dl)	6.06±0.13^{e}	4.25±0.15^{bc}	5.85±0.10^{de}	5.30±0.20^{cde}	7.26±0.40^{e}	7.62±0.43^{e}
Total lipid(mg/dl)	8.53±0.25^{d}	6.07±0.12^{abc}	8.42±0.42^{d}	5.03±0.04^{ab}	8.65±0.04^{d}	9.27±0.44^{d}
Albumin(mg/dl)	0.82±0.12^{de}	0.67±0.12^{d}	0.56±0.02^{a}	0.42±0.12^{a}	0.36±0.12^{a}	0.31±0.12^{a}
Creatinine(mg/dl)	0.617±0.05^{a}	0.542±0.05^{a}	0.619±0.05^{a}	0.593±0.05^{a}	0.527±0.05^{a}	0.598±0.05^{bc}
Uric acid(mg/dl)	2.24±0.230^{bc}	2.85±0.240^{b}	1.76±0.230^{c}	1.190±0.230^{c}	1.86±0.290^{b}	2.78±0.283^{bc}
BUN(mg/dl)	9.66±1.830^{ab}	6.23±1.830^{b}	10.83±1.830^{ab}	12.50±1.830^{ab}	10.81±1.201^{c}	12.48±2.81^{bc}
Cholesterol(mg/dl)	98.32±0.98^{a}	83.52±0.81^{ab}	81.45±0.78^{b}	92.96±0.98^{c}	102.54±0.87^{b}	112.58±0.75^{c}

The data are presented as Means ± S.E. Different letters indicate significance at $p<0.05$.

DISCUSSION

In the present study the productive effect of garlic extract and vitamin C against triclosan induced toxicity in blood of zebrafish and the result has been shows the modification in biochemical parameters in all the treated groups and the control. The biochemical parameters of treated and control fish were recorded in (Table 1 and 2). The garlic extract and vitamin C act as a productive agent against triclosan in zebrafish. Das and Mukherjee (2002) reported that exposure to sub-lethal concentration of cypermethrin alters the biochemical and enzymes level and exerts stress on the fish. Garlic is an important medicinal herb extensively cultivated in many countries and has played an important dietary function as well as medicinal role for centuries. Vitamin C is cheap, mean while shows promising result regarding the growth and survival in addition to enhancing the disease resistance in Nile tilapia reported by Ibrahem *et al.* (2010). Garlic alone can provide us with over two hundred unusual chemicals that have the capability of protecting the human body from a wide variety of diseases. The sulfur containing compounds found in garlic afford the human body with protection by stimulating the production of certain beneficial enzymes (Mansell and Reckless, 1991). Ascorbic acid is an essential micronutrient for fishes. Vitamin C is essential for fish growth, reproduction and health (Dabrowski *et al.*, 1990; Soliman *et al.*, 1986; Yamamoto *et al.*, 1985; Xie *et al.*, 2006), adverse stress, minimize toxicity by water contaminants and exerts an immunomodulatory effect (Li and Lovell, 1985; Tewary and Patra, 2008; Eo and Lee, 2008). In the recovery phase was a treatment dependent, those components are used for recovering purpose that act as a detoxifying agent of triclosan. Moreover the supplementation of garlic extract and vitamin C have induced a significant changes in the biochemical parameters of zebrafish exposed to triclosan.

CONCLUSION

Result of our study shows biochemical effect in zebrafish under sublethal exposure to triclosan. These biochemical investigation can be used to study the mode of action of triclosan and the productive role of garlic and vitamin C. Thus biochemical alterations in zebrafish are considered as biomarkers to access the health status of the fishes as well as aquatic bodies polluted with triclosan. Thus environmental protection is the major requirement of the society.

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