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Triclosan Induced Alterations of Biochemical Parameters in Zebra Fish *Brachydaniorerio* (Ham.) and their Productive Role by Dietary Supplementation of Garlic Extract and L-Ascorbic acid (Vitamin C)

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ABSTRACT

The pesticides have different adverse impacts on different life stages of fish species with attempts to use dietary antioxidants to counteract their effects. Triclosan (TCS) is commonly used as antimicrobial agent that is incorporated into dish soap, detergent, toothpaste, mouthwash, shampoo, etc., in addition to innumerable other personal care and household products. Hence the current study was carried out to investigated the potential protective effects of garlic extract and L-Ascorbic acid (Vitamin C) and their combination versus triclosan-induced changes in zebra fish *Brachydaniorerio* (Hamilton). The median lethal concentration of triclosan for 96h is (0.32mg/L). The species were exposed to sub lethal concentration of triclosan (0.32mg/L) for 7 and 28 days. Triclosan significantly ($P<0.05$) increasesthe lipid peroxidation, supperoxide dismutase, enzyme catalase, and increases the serum glucose level. Addition of garlic extract and/or Vitamin C to the diet of triclosan-treated fish showed the increase level of the serum glucose, serum total protein, total lipid, creatinine, sodium, potassium, calcium, Blood urea nitrogen (BUN), uric acid, albumin and cholesterol , LPO, SOD, CAT, levels in comparison to control fish. On the other hand, triclosan significantly ($P<0.05$) led to negative effect in serum total protein, total lipid, creatinine, sodium, potassium, calcium, Blood urea nitrogen (BUN), uric acid, albumin and cholesterol levels.However, the fish fed with garlic extract and/or L-Ascorbic acid (vitamin C) in diets neutralized the toxic effect of triclosan. The result suggests that, supplemented garlic extract and/or vitamin C can be effectively used to neutralize the toxic effect of triclosan on zebra fish *Brachydaniorerio* (Ham).

Keywords: Triclosan, biochemical parameters, garlic extract, vitamin C, zebra fish .

Introduction

The aquatic ecosystems have known to receive a wide spectrum of pollutants, which may be introduced to it directly or indirectly. Pesticides are extensively used to control the pest population in intensive agricultural production and fish farms, which could be in the form of insects, weeds, molluscs including harmful bacteria and viruses. These pesticides can reach natural waters either via transfer of the chemicals from soil or directly by spraying against target organisms[1] and affects non-target organisms such as fish and prawn which are of great economics importance to humans[2].The uptake of chemicals by aquatic organisms may occur from the water, sediments, suspended particulate matter and from food.

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)-phenol) is a widely used broad-spectrum bactericide. Triclosan can effectively prevent bacterial lipid biosynthesis by blocking enzyme enoyl-acyl carrier protein reductase[3]. Due to its effectiveness and thermal stability, triclosan is widely incorporated into numerous consumer products, including soaps, toothpastes, disinfectants, cosmetics, and detergents [4]. Disposal and usage of the triclosan-containing products result in continuous release of triclosan from these products into wastewater. It was estimated that triclosan-containing products account for~ 96 % of triclosan in wastewater [5].Triclosan and its metabolites are not only detected in rivers, lakes, soils, and sediments, but also in human urine, blood, and breast milk samples [6]. The molecular structure of triclosan has shown in Figure-1.

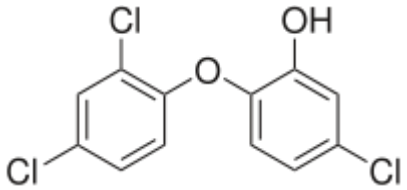

Figure-1:-Molecular structure of Triclosan.

Garlic is probably one of the earliest known medicinal plants [7]. Its bulbs (cloves) had been used as a cure-all

in ancient Egypt and are mentioned in the Ebers papyrus, one of the earliest treaties on medicinal plants. Garlic contains sulfur containing compounds. Alliin is converted to the anti-microbial active alliin when the bulb is cut or bruised. Ajoene which is secondary degradation product of alliin, is presumably the most active compound responsible for the anti-thrombotic activity of garlic [8]. Garlic has also been shown to have a productive nature against gastrointestinal neoplasias, against blood clots (anti-platelet action) due to part to the compounds alliin and ajoene, which have fibrinolytic activity.

Antioxidants are known as potential scavengers of ROS, so they protect biological membranes from oxidants. However, the balance between free radicals and synthesis of antioxidant defense scan be broken by chemicals. Vitamin C is a water-soluble antioxidant found in the cytosol and extracellular fluid that can interact directly with free radicals, thus preventing oxidative and DNA damage [9] . Vitamin C is a powerful antioxidant andfree radical scavenger[10]. Furthermore, results showa strong relationship between low vitamin C levels and cardiovascular diseases[11].

Most fish species cannot synthesize vitamin C, and have to depend on external sources to meet their needs [12]. The vitamin C requirement for normal growth and survival is quite low [13]; however, a higher level is required to improve the stress resistance of fish [14].

Fishes are used as an excellent indicator of aquatic pollution due to their high sensitivity to environmental contaminants which may affect certain physiological and biochemical processes [15]. Indeed, fish blood is widely used in toxicological research and environmental monitoring as a promising indicator of physiological and pathological changes [16].

Several studies have been carried out on the effect of different pesticides on biochemical aspects of fish and other aquatic animals, but very few on its productive role of garlic and vitamin C. Especially, there is lack of data on this topic in relation to zebra fish *Brachydaniorerio* (Hamilton) species. It is necessary to obtain scientific information about the effect of triclosan on zebra fish improve risk-assessment studies and its productive with the help of garlic and Vitamin C. The main objective of this study was to assess the effect of sub-lethal concentration of triclosan exposure period on biochemical parameters of zebra fish *Brachydaniorerio* (Ham) and its productive role of two natural products garlic extract and Vitamin C.

Materials and Methods

Collection of fish

The healthy, zebra fish *Brachydaniorerio* (Hamilton) were purchased from the friend's aquarium, Kolathur, Chennai, having mean weight 3 to 5 g and length 4 to 6cm. Fishes were immediately transported to the fish laboratory in the Department of Zoology, Faculty of Science, Annamalai University. The experimental fishes were reared in glass tanks (100 L capacity) and acclimatized for one month before being used in the experimental study. They were given the treatment of 0.1%$KMNO_4$ solution for bacterial contamination. Then they were fed with tubifex warms regularly.

LC_{50} value of Triclosan (TCS)

Technical grade triclosan $C_{12}H_7Cl_3O_2$ [5-chloro-2-(2,4-dichlorophenoxy)phenol]from [The I.L.E.co., Chennai. India]. The LC_{50} value of triclosan was determined in the laboratory. Three hundred fishes were randomly distributed into six aquarium tanks (100L) filled with different concentration of triclosan (0.15, 0.20, 0.25, 0.30, 0.35mg/L). The mortality was recorded for 96h. The LC_{50} of triclosan calculated with the help of probit analysis using SPSS software. The 96 hr concentration (0.32mg/L) of calculated LC_{50} was selected.

Sublethal study

LC_{50} values (0.32mg/L) were taken as sub lethal study. Sixty fishes were selected and divided into six groups for the experiments. The triclosan was used in this study and stock solutions were prepared. The experiment was carried out for a period of 7 and 28 days.

Group-I: Control fish

Group-II: Triclosan exposure (0.32mg/L)

Group-III: Triclosan exposure (0.32mg/L) + Garlic extract (1ml)

Group-IV: Triclosan exposure (0.32mg/L) + L-Ascorbic acid (Vitamin C-1g)

Group-V: Garlic extract alone (1g)

Group-VI: L-Ascorbic acid alone (Vitamin C 1g)

Garlic extract (GE)

An aqueous extract of whole crude garlic was prepared as follows: freshly peeled cloves of garlic (*A. sativum*, purchased from local market) were sliced into small pieces and ground in a clean mortar using a mortar pestle to produce a fine paste. The working solution was then prepared by dissolving 5 g of the paste in 100 ml of distilled water, where 1 ml of the extract contains 50 mg of crude garlic. Fresh garlic extract was dissolved in the aquarium tank daily.

L-Ascorbic acid (vitamin C)

A pure form of L-(+) ascorbic acid was supplied as pure crystals (I.L.E.Co.,) Kattangulathur, Chennai. A freshly prepared aqueous solution of L-ascorbic acid (1g) was dissolved in to aquarium tank daily throughout the experiments.

Biochemical parameters

Sodium, potassium, calcium concentrations measured by flame photometer. The serum total protein and albumin concentration estimated by Biuret and Dumas method[17],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 [18].

Enzyme assay

At the end of experiment (7 and 28 days), fishes were sacrificed for the collection of liver to carry out the enzyme assay. The activity of SOD, catalase (CAT) was determined spectrophotometrically at 480nm by the epinephrine method by [19], Lipid peroxidation was measured according to the method of [20]. 10(w/v) tissue homogenate from liver was used (this homogenate contained 1% v/v dimethyl sulfoxide to prevent further oxidation). 0.2 ml aliquots of tissue homogenate were added to 0.2 ml sodium dodecyl sulfate solution (8.1%w/v) and 1.5 ml acetic acid solution (20% v/v). The mixture was made up to 4.0 ml with distilled water and heated to 95 ºC for 1 h. The samples were cooled, centrifuged at 2000 rpm for 10 min. and measured at 532 nm using spectrophotometer (Ultrospec 3100 pro, Biochrom Ltd). The results were expressed as nmolmalondialdehyde formation per g tissue.

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

Treatment / Biochemical parameter	Control (I)	Exposure (II)	TCS+Garlic extract (GE) (III)	TCS+Vit C (IV)	Garlic extract alone(GE) (V)	Vit 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±0.^{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}

Table-1: Values of biochemical parameters of zebra fish *Brachydaniorerio* (Ham) exposed to triclosan (0.32mg/L), vitamin C (Vit C), garlic extract (GE) and their combinations for 7 days. The data are presented as Means ± S.E. Different letters indicate significance at $p<0.05$

Treatment / Biochemical parameter	Control (I)	Exposure (II)	TCS+Garlic extract(GE) (III)	TCS+VitC (IV)	Garlic extract alone(GE) (V)	Vit 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}

Table-2: Values of biochemical parameters of zebra fish *Brachydaniorerio* (Ham) exposed to triclosan (0.32mg/L), vitamin C (Vit C), garlic extract (GE) and their combinations for 28 days. The data are presented as Means ± S.E. Different letters indicate significance at $p<0.05$

Antioxidant enzymes	Control (I)	Exposure (II)	TCS+Garlic extract(GE) (III)	TCS+VitC (IV)	Garlic extract alone(GE) (V)	Vit C alone (VI)
LPO(nmol/mg) tissue	1.39±0.59^{ab}	4.20±0.33^{d}	6.47±0.21^{e}	2.61±0.18^{bc}	1.49±0.19^{ab}	1.13±0.11^{a}
SOD(nmol/mg)	255.54±1.92^{d}	365.54±1.68^{b}	378.29±2.54^{c}	359.29±2.54^{a}	374.28±2.12^{a}	368.21±1.63^{c}
CAT(nmol/mg)	65.35±0.91^{c}	75.66±0.87^{b}	89.87±1.33^{a}	89.34±1.22^{a}	81.32±1.27^{a}	90.12±2.18^{a}

Table-3: Values of Antioxidant enzymes of zebra fish *Brachydaniorerio* (Ham) exposed to triclosan (0.32mg/L), vitamin C (Vit C), garlic extract (GE) and their combinations for 7 days The data are presented as Means ± S.E. Different letters indicate significance at p<0.05

Antioxidant enzymes	Control (I)	Exposure (II)	TCS+Garlic extract(GE) (III)	TCS+VitC (IV)	Garlic extract alone(GE) (V)	Vit C alone (VI)
LPO(nmol/mg)	2.77±0.06^{a}	6.73±0.35^{bcd}	8.05±0.29^{b}	7.71±0.49^{bc}	6.30±0.44^{bc}	6.83±0.05^{bcd}
SOD(nmol/mg)	395.78±1.18^{d}	444.65±1.48^{c}	450.38±2.24^{a}	446.65±2.14^{b}	426.25±2.15^{b}	415.27±2.15^{a}
CAT(nmol/mg)	75.33±0.97^{d}	85.65±0.97^{b}	99.48±1.32^{a}	99.77±1.32^{a}	81.22±1.27^{a}	82.72±1.35^{a}

Table-4: Values of Antioxidant enzymes of zebra fish *Brachydaniorerio* (Ham) exposed to triclosan(0.32mg/L), vitamin C (Vit C), garlic extract (GE) and their combinations for 28 days The data are presented as Means ± S.E. Different letters indicate significance at p<0.05

Biochemical parameters

Sodium, potassium, calcium levels.

The sodium, potassium, calcium levels in the 7 and 28 days periods is given in table 1 and 2. Triclosan reflected high significant decrease in sodium, potassium, calcium levels at both periods (P<0.05). The main effect of garlic extract and Vit C in the two periods was decreased. In garlic and Vit C alone is highly significant compare to control group.

Glucose level

The normal glucose level of zebra fish *Brachydaniorerio* (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 Vit C in the two periods was (P<0.05) highly significant. Dietary supplementations with Vit C and/or garlic extract improved TCS-GE group compare with control group.

Total protein level

The total protein level of zebra fish *Brachydaniorerio*(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 Vit C In the two periods was not significant. Highly significant interaction effect between TCS-GE-Vit C was recorded in 28 days period. The protein level decreased in the triclosan exposed fish compare to control group.

Total lipid level

The total lipid level of zebra fish *Brachydaniorerio* (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 Vit C in the two periods was (P<0.05) significant. Vit C and its interaction with garlic didn't show significant interaction between TCS-GE and TCS-Vit C was recorded in the two periods. Dietary supplementation with Vit 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.

Albumin level

The albumin level of zebra fish *Brachydaniorerio* (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 Vit C in the two periods not was (P<0.05) significant. Highly significant interaction effect between TCS-GE-Vit C was recorded in the 7 and 28 days periods.

Creatinine level

The Creatinine level of zebra fish *Brachydaniorerio* (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-Vit C in the two period was significant (P<0.05) compare to control group.

Uric acid level

The uric acid level of zebra fish *Brachydaniorerio* (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 Vit C in the two periods was (P<0.05) significant. Vit C did not show significant effect compare to control.

Blood urea nitrogen level (BUN)

The blood urea nitrogen level of zebra fish *Brachydaniorerio* (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.

Cholesterol level

The cholesterol level of zebra fish *Brachydaniorerio* (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 Vit C in the two periods was (P<0.05) significant. Supplementation with Vit C and/or garlic extract improved the GE and VIT C alone in the both compare to control group.

Enzyme assay

Lipid peroxidation (LPO)

The liver lipid peroxidation values of zebra fish *Brachydaniorerio* (Ham) in the 7 and 28 days periods is

given in table 3 and 4. Triclosan main effect of TCS-GE-Vit C and their interaction were highly significant in the both periods (P<0.05). LPO in the level of TCS exposer is decreased. Supplementation with Vit C and/or garlic extract improved especially in the first period.

Superoxide dismutase (SOD)

The antioxidant enzymes activity in the liver of zebra fish *Brachydaniorerio* (Ham) in the 7 and 28 days periods is given in table 3 and 4. Triclosan main effect was significantly increase in the both periods (P<0.05). The supplementation of garlic extract and Vit C showed in the two periods was (P<0.05) significant compare to control group.

Enzyme catalase (CAT)

The antioxidant enzymes activity in the liver of zebra fish *Brachydaniorerio* (Ham) in the 7 and 28 days periods is given in table 3 and 4. Triclosan main effect was significantly increase in the liver homogenate tissue (P<0.05). Supplementation of garlic extract and Vit C showed in the two periods was (P<0.05) significant compare to control group.

Discussion

The results of this study provide further data on long-term exposure to triclosan for consideration in risk assessment. The findings contribute to knowledge of the toxic potential of triclosan to zebra fish *Brachydaniorerio* (Ham).

In order to make an accurate assessment of the hazards that a contaminant may pose in a natural system, behavioral indices selected for monitoring must reflect the organism's behavior in the field [21]. On the other hand, [22] reported accelerated respiration and loss of movement coordination in rainbow trout and carp following acute poisoning with metribuzin. These characteristics have also been reported in *Oreochromisniloticus*, and *Chrysichthyesauratus*[23] and by Saglio and Trijasse[24]in *Carassiusauratus*following acute poisoning with atrazine. Movement imbalance in freshwater fish (*Labeorohita*, *Mystusvittatus*, and *Cirrhinusmrigala*) acutely exposed to simazine and cyanazine has been reported by [25]. [26]reported respiratory distress such as rapid ventilation, increased rate of gill cover movements, or floating at the surface of water in common carp after exposure to simazine. The exposure of zebrafish, *Brachydaniorerio*, to atrazine caused changes in behavior, such as significant preference for habitat with a dark substratum, even at concentrations lower than 6µg1^{-1} [27]. Our results differ from these, as, supplementation of garlic extract and vitamin C improves the biochemical parameters of zebra fish *Brachydaniorerio*. A similar result was observed[28].

Sodium potassium, calcium levels indicate the operation of a variety of homeostatic mechanisms in the body [29].Calcium levels higher than values reported [30]. Garlic extract and/or vitamin C were found to be valid in counteracting stress-induced changes in Sodium, potassium, calcium level. In the present study, triclosan-induced hyperglycemia was revealed in zebra fish *Brachydaniorerio*. Similar findings were observed by [31] and [32] after exposure to different doses of pesticides. The source of such hyperglycemia seems to be due to the liver glycogenolysis, resulting from the increased plasma catecholamines and corticosteroid hormones [33]as well as amino acids through the activation of gluconeogenesis process [34]. Garlic extract and/or vitamin C reduced triclosan-induced hyperglycemia revealed in *Brachydaniorerio*. Similar results were recorded by [28]for *O. niloticus* stressed by cadmium. [35] reported a reduced blood glucose level after stress in Atlantic halibut (*Hippoglossushippoglossus* L.) fed vitamin E supplemented diets.

In the present study, decrease in serum total protein level (hypoproteinemia) was recorded in triclosan exposed *B. rerio*. Similar findings were recorded by [36,37,28,31,32,38]and [39]after exposure to different doses of pesticides and heavy metals. Such hypoproteinemia may be due to direct effect of the utilization of body protein as an energy supply to meet the increasing physiological demands to overcome the stress in the polluted medium [40]hypoproteinemia may also be attributed to several pathological processes including plasma dissolution, renal damage and elimination in the urine, decreased liver protein synthesis, alteration in hepatic blood flow and / or hemorrhage into the peritoneal cavity and intestine [41]. [42] observed an increase in the stressed total protein level in male rat fed Tomato-juice as supplemented diets. [43]recorded an increase in total protein level after Diazinon-induced and Nitrate-induced stress in male rats fed vitamin E as supplemented diets.

In the present study, decrease in serum total lipids level was recorded in triclosan-treated *B.rerio*. Similar findings were observed by [44]and 32after exposure to different doses of pesticides and heavy metals. Such induced decrease in serum total lipids level may be due to the increase in secretion of catecholamines and corticosteroids with enhanced metabolic rate and in turn reduced metabolic reserves[44]. [45]found that vitamin E as a diet supplementation improved total lipids level in muscle and liver tissues of rats. The present results have clearly demonstrated the ability of sub lethal dose of triclosan to induce oxidative stress in fish liver as evidenced by increased thiobarbituric acid reactive substance.

The albumin has been reported to be an osmoregulator of blood volume, an easily available protein reserve and a transport protein [46]. [47]described that hyperactivity caused by deltamethrin may lead to the utilization of this easily accessible protein reserve-fraction containing albumin, resulting in a decreased quantity. Another possible reason for the lowered amount of albumin may be a decreased albumin synthesis in the hepatocytes[48]. In the present study,

similar findings have been noticed when exposed with triclosan. Improvement in albumin content was observed with the increased supplementation of garlic and /or vitamin C compared to treated group.

Biomarkers of renal functions include Blood urea nitrogen (BUN), creatinine and uric acid. The elevated level of creatinine is an indicative of kidney dysfunction. In broilers, uric acid (UA), and not urea, is the main end product of nitrogen [49]. The elevation of uric acid occurs following significant disease in the kidneys [50]. The present study, have been noticed that, the improvement in BUN, creatinine,uric acid content was observed with the increased supplementation of garlic and /or vitamin C compared to treated group.

[51]reported that cholesterol concentrations increase as the fish size increased. A high blood urea concentration recorded in *M. cephalus* is likely to be a sign of stress associated with the increase in the cortisol level [52]. In the present study, similar findings have been noticed when exposed with triclosan. Improvement in cholesterol content was observed with the increased supplementation of garlic and /or vitamin C compared to treated group.

The rise in lipid peroxidation level in liver means a modification in the physical characteristics of cell membrane[53]since lipid peroxidation leads to hydrolysis of phospholipids into hydroperoxy fatty acids [54]. In the present study, the level of lipid peroxidation was decreased in liver of triclosan-exposed fishes fed diets supplemented with vitamin C and/or garlic extract especially in the first period. Similar results were recorded by [28] in concern with cadmium-exposed *O. niloticus*. The mechanism for protection of garlic involves scavenging potentially toxic and mutagenic electrophiles and free radicals and modification of phase-II enzymes and the phase-I profile, which enhances detoxification pathways. The allyl group of garlic constituents enhances the level of glutathione-S-transferase (GST) thereby accelerating the detoxification of mutagens and carcinogens [55].

SOD activity in blood serum and liver tissue homogenates showed significant increase in fish groups source fed on diets contained garlic compared to the control group. This result is similar to results showed the use of garlic in fish farming enhanced the activity of non-specific defense systems in *O. niloticus*[56], also its powder increase the antioxidant capacity in hamsters [57]. Garlicoil and its component enhanced SOD activity in liver [58]. Garlic extract exerts antioxidant action by scavenging reactive oxygen species, enhancing the cellular antioxidant enzymes SOD in the cells [59]. On the other hand, effect of garlic oil on SOD activities was decreasedin tissue homogenates of liver and kidney aftergarlic treatment [60].

CAT activity in serum and in liver tissue homogenates also showed significant increase in fish groups fed on diets contained garlic compared to control group. This result is similar to results showed that, the use of garlic in fish farming enhancing the activity of non-specific defense systems in *O. niloticus*[56]. Garlic oil and its component enhanced CAT in the cells [59].On the other hand, this study demonstrates that supplementation of garlic extract and /or L-ascorbic acid (vitamin C) had the ability to reduce the biochemical effect of triclosan.

Conclusion

To summarize the above findings, biochemical parameters clearly indicated the triclosan induced impairment of metabolism as fish were observed to be under severe metabolic stress. The evaluated parameters like biochemical alterations and variations in different enzyme activities.These alterations were reversed to a great extent with supplementation of garlic extract and/or vitamin C in their diet. Taking this into account, fishes can be protected against any possible pesticide toxicity using natural antioxidants such as garlic extract and vitamin C by incorporating it at higher level.

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