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IMPACT OF SIMAZINE AND CHELATE PROPERTIES OF *SOLANAM XANTHOPIUM* IS THE FRESHWATER FISH *CIRRHINUS MRIGALA* FOR BLOOD BIOCHEMICAL CHANGES THE PERIOD OF 120 HOURS

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ABSTRACT

Haematological parameters have been recognized as valuable tools for monitoring fish health. Haematological and serum biochemical parameters were studied and compared different feeding behaviour of teleost fishes. *Cirrhinus mrigala* were carried out in order to find out a normal range of blood parameters which would serve as baseline data for assessment of the health status of the fish as well as reference point for future comparative surveys. Blood parameters such as red blood cell count (RBC) and white blood cells count (WBC), haemoglobin, haematocrit, mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), mean cell haemoglobin, glucose, protein, cholesterol and urea were estimated from teleost fishes of different trophic level. Statistical analysis revealed that differences in haematological parameters between marine fish were significant. The result revealed that haematological RBC/WBC ratio; MCV and MCHC were significantly correlated study the impact of Simazine and Chelate properties of *Solanam xanthopium* is the freshwater fish *C. mrigala* for blood biochemical changes the period of 120 hours. Blood serum biochemical parameters can be used for confirming the maturity and monitoring any changes in the quality of waters and related soils.

Keywords: Simazine, Chelate properties, *Solanam xanthopium*, *Cirrhinus mrigala*.

INTRODUCTION

The heavy metal and pesticide contamination of aquatic system has attracted and attention of researchers to all over the world and has increased in the last decades due to extensive use of them in agricultural, chemical and industrial processes that are becoming threats to living organisms (Dutta and Dalal, 2008).

Environmental contamination by pesticides has been documented in both biotic and abiotic components. The random use of different pesticides often causes lot of damage on non-target organism (Adhikari *et. al.*, 2003). Organophosphate pesticides constitute a large proportion of the total synthetic chemicals employed for the control of pests in the field of agriculture, veterinary practices and public health. The pollution of environment due to use of pesticides has become an increasing problem over the last century with the development of industry, agriculture and increase in population (Bernard and Grazyna, 1999). The organophosphorous compounds are widely used because of their rapid biodegradability and non persistent nature. Recently studies have proved that extremely low quantities of pesticides which enter the aquatic environment can affect productivity of organisms to kill eggs and larvae. The contaminations affect all group of organisms in aquatic ecosystem like invertebrate (Meenambal *et al*., 2012).

Simazine can be found in trace amount or at higher concentrations in soil and air. In mammals, simazine can accumulate in body fat, skin, liver, kidney, adrenal glands, ovaries, lung, blood and heart (Sumpter, 1997). The main target for simazine is the central nervous system. Symptoms of simazine toxicity in laboratory animals include pawing, burrowing, salivation, tremors, writhing,

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and seizures. In humans, high dose of simazine result simazine exerts its neurotoxic effect through voltage-dependent sodium channels and integral protein (Borges *et al.,* 2007). Indiscriminate and extensive use of insecticides to protect crops possesses a serious threat to humans and the surrounding environment. Almost all pesticides are volatile in nature when applied to crops. These pesticides can be circulated into different ecosystems by different agents after entering into the environment have a deleterious effect on fish and subsequently to man (Chandran *et al.*, 2005). According to Weichselbaum *et al.*, (1946), Al-Attar (2006) most pesticides may enter into the food channels and cause physiological damage. Among all pesticides, the organophosphates (OP) are widely used to control pests because of their rapid effectiveness and easy biodegradation (Monteiro, 2005).

Fish stocks suffer from natural mortality and high fishing pressure at the end of the dry season-contamination of water by pesticides either directly or indirectly can lead to sift kills, reduced fish productivity of devoted concentration of undesirable chemical in edible dish tissue which can be affect the health of humans eating these fishes Al-Attar (2006) .

Herbicides are widely used for the control of water plants, which may impede the flow of water during the summer, when sudden heavy rain can cause flooding. While the direct effect of herbicides addition is the loss of macrophytes, non-target organisms such as fish may also be affected through loss of habit and food supply (Vasantharaja *et al.,* 2012).Worldwide herbicide usage has increased dramatically during the past two decades, coinciding with changes in farming pesticides and increasingly intensive agriculture (Tamizhazhagan and Pugazhendy, 2016). As a consequence, residuals amount of herbicides and their metabolites have been found in drinking water and food (Vettrivel and Pugazhendy, 2013; Tamizhazhagan and Pugazhendy, 2016). Chemical pesticides have contributed greatly to the increase of yields in agriculture by controlling pest and disease and also towards clacking the insect-borne disease such as malaria, dengue, encephalitis, filariasis, etc. in human health sector (Reddy and Philip, 1994).

The acute toxicity test indicates the relative species sensitivity and lethal concentration which can be used as a basis for long term tests to establish the requirements necessary for the well being of aquatic life. In fishes, biochemical changes are induces by the pollutants before they acquire cellular and systemic malfunction. It is inferred from several investigation that biochemical parameters could be effectively used to detect the effect of pollutants (Vettrivel and Pugazhendy, 2013). Exposure to pesticides results in acute and chronic health problems. Pesticides being used in agricultural tracts are realised into the environment and come into human contact directly or indirectly.

Aerobic organism superoxide anion radical, (O_2) hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) as a result of oxidative metabolism. OH can initiate lipid peroxidation in tissues. The sensitivity of the cell is attenuated by antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxide (GPx), catalase, glutathione reductase (GR). The antioxidant enzymes maintain a relatively low rate of the reactive and harmful OH. Oxidative stress occurs as a result of the effect of xenobiotics causing the disturbances in the antioxidant enzymes system. Glutathione-S-transferase (GST) is a group of multifunctional enzymes involved in biotransformation and detoxification of xenobitoics (Karan, 1998). Highly reactive electrophilic components can be removed they covalently bid to tissue nucleophilic compounds which would lead to toxic effects.

Paradoxically, they are highly vulnerable to toxic chemicals because firstly, their large surface area facilitates greater toxicant interaction and absorption and secondly, their detoxification system is not as robust as that of liver (Tamizhazhagan *et a*l.,2016)Acid phosphatise on the other hand is a phosphatise which frees attached phosphate groups from other molecules during digestion. It is a lysosomal, hydrolytic enzyme with an acid pH optimum. It takes part in the dissolution of dead cells and as such serve as a good indicator of stress condition in the biological system (Viran *et al.,* 2003). The present study was aimed to investigate the impact of simazine and chelate properties of *Solanam xanthopium* on serum biochemical parameters of teleost fishes, *Cirrhinus mrigala* for period of 120 hours.

MATERIALS AND METHODS

Collection and maintanence of the experimental animal

The fresh water fish *C. mrigala* were collected from the fish farm located in Pinnaloor at Navarathna form, Cuddalore district. The fish were brought to the laboratory and transferred to the rectangular cement tanks (100 × 175) of 500 liters capacity containing chlorine free aerated well water, fishes of the same size and weight were used irrespective of their sex for the experiments.

The fishes were exposed to sublethal concentration for treated and control period of 120 hours. A control group was maintained with identical environment. The toxicant water as well as normal water was renewed every day. The fish were sacrificed both experimental and control group on 120 hours.

Toxicity studies

Acute toxicity tests conducted to measure the impact of toxicity studies, the renewal technique of acute static test was adopted in which fish were periodically exposed to the concentrations of the same composition, usually once in every 24 hours by transferring the animals from one test chamber to another.

Estimation of serum protein

Protein in the serum was determined after trichloroacetic acid precipitation by the method (Lowry *et al.*, 1951). The CONH group in the alkaline medium gave a blue colour which was read at 620 nm. 100 mg of BSA was dissolved in 100 ml of water in a standard flask. Small quantities of alkali could be added to make completed dissolution of BSA. 10 ml of stock was diluted to 100 ml/ ml. 0.5 ml of samples preparation was precipitated sample was dissolved in 1.0 ml of 0.1 N NaoH. From this, an aliquot was taken and to this 5.0 ml of alkaline copper reagent was added and allowed to stand at room temperature for 10 min and the blue colour developed was read after 20 min at 620 nm. A standard curve was obtained using BSA and was used to determine the protein level of enzyme activity. The protein levels were expressed as mg/100 ml of serum.

Estimation of serum glucose

Blood glucose was determined by the method of Murrel and Nace, (1988). The blood samples 0.1 m were collected by cardiac puncture using heparinized hypodermic syringe and were immediately deproteinized in 10 ml of 10 percent tungstic acid. The solutions were filtered and the filtrate was used for glucose estimation 0.5 ml of the filtrate and 0.5 ml of dilute tungstic acid was taken in a clean test tube and then 1.0 ml of potassium ferric cyanide solution was added. The test tubes were placed in boiling water bath for 25 seconds and cooled in running tap water, after the content were sufficiently cooled, 1.0 ml of cyanide carbonate solution (buffer solution) was added and the tubes were again placed in boiling water bath for 15 minutes and then quickly cooled to 25-30°C.

Assay of serum aspartate aminotransferase

Serum alanine aminotransferase was assayed by using the diagnostic kit based on the method of Retiman and Frankel, (1957). The buffered substrate was added to 0.1 ml of serum and placed in a water bath at 37°C. To the blank tubes, 0.1 ml distilled water was added instead of serum. Exactly an hour later 2 drops of aniline citrate reagent and 0.5 ml of DNPH reagent were added and kept at room temperature for 20 min. finally 5.0 ml of 0.4N sodium hydroxide was added. A set of pyruvate standards was also treated similarly and read at 520 nm after 10 min.

The result is expressed as IU/L for serum.

IU = amount of enzyme that catalyzes the transformation of 1 micromole of substrate per minute under standard conditions.

Assay of serum alanine aminotransferase

Serum ALT was assayed by using the diagnostic kit based on the method of Retiman and Frankel, (1957). Procedure was the same as that used for the assay of AST except that the incubation time, which was reduced to 30 min (60 min for AST).

The result is expressed as IU/L serum.

IU = amount of enzyme that catalyzes the transformation of 1 micromole of substrate per minute under standard conditions.

Estimation of alkaline phophatase (ALP) Blood serum

Serum alkaline phosphatase was estimation by using the diagnostic kit based on the method of Retiman and Frankel, (1957). ALP catalyses disodium phenyl phosphate into phenol and disodium hydrogen phosphates at pH 10.0 phenol so formed reacts with 4-aminoantiprine in alkaline medium in the present of oxidizing agent potassium ferric cyanide to from a red colored complex whose absorbance is proportional to the enzyme activity. The incubation mixture, contained 1.0 ml of buffered substrate 3.1 ml of deionised water and 0.1 ml of serum was incubated at 37^{0}C. Exactly after 15 min, 2.0 ml of coluor reagent was added to all tubes. The control tubes received the enzyme after the addition of colour reagent. 0.1 ml of standard and 0.1 ml of distilled water (blank) were also treated simultaneously and the colour developed was read at 510 nm. The enzyme activity was expressed as IU/L of serum.

Estimation of acid phophatase (ACP) Blood serum

Serum alkaline phosphatase was estimated by using the diagnostic kit based on Retiman and Frankel method (1957). The incubation mixture contained 1.0 ml of buffered substrate 0.1 ml of deionised water and 0.1 ml of serum was incubated at 37°C exactly after 15 min, 2.0 ml of colour reagent 0.1 ml of standard and 0.1 ml of distilled water (blank) were also treated simultaneously and the colour developed was read at 510 nm. The enzyme activity was expressed as IU/L of serum.

Assay of serum lactate dehydrogenase

Serum lactate dehydrogenase was estimation by using the diagnostic kit based on method of King (1955). 25 µl of serum was added to the incubation mixture containing 675 µl potassium phosphate buffer, 25 µl distilled water. After incubation for 20 min at 25°C the reaction was arrested by adding 25 µl sodium pyruvate. The substrate incubated in

the absence of serum, under the same conditions was used as a reference blank. The decrease in optical density of the test was measured against blank at 340 nm in spectrophotometer at 25°C and the rate of change in extinction was recorded for 6 min and the enzyme activity was calculated by multiplying with a facter 4286. LDH activity is expressed as IU/L serum.

RESULTS

The protein content in blood exhibits remarkable changes from the control levels. When the *C. mrigala* are exposed to group-2, the protein content in blood decreases than in the control (group-1) and the increased percent changes are -16.36, -34.40, -45.95, -54.61 and -66.24 for 24, 48, 72, 96 and 120 hours respectively. When in the fish exposed to (group-3), the percent changes were -6.59, -8.97, -12.62, -15.52 and -17.41 for 24, 48, 72, 96 and 120 hours respectively. In the supplementary feed along with simazine exposed group near to normal the present changes are +11.68, +38.78, +61.67, +86.13 and +144.80 for 24 to 120 hours respectively. In the group-4, increase in the level of protein content is noticed than in group-2 and group-3. The percent changes are +0.19, +0.42, +0.52, +0.75 and +0.91 for120 hours respectively. The recorded protein content in blood parameters for four groups are statistically significant at 1% and 5% levels (Table 1).

The significant changes were induced by the simazine toxicity in haematological parameters of *C. mrigala* in the glucose content in group-2 increases when compared to control (group-1) are +7.56, +11.38,+14.29, +18.71 and +21.12 for 24, 48, 72, 96 and 120 hours. While in the simazine along with supplementary feed (group-3) fish protein content was recovered compared to control group the percent changes are +3.07, +4.44, +7.39, +9.15 and +10.68 for 24 to 120 hours respectively while the treatment of *Solanam xanthopium* in group-3 compare group-2, the recovery effect of *S. xanthopium* has great significant with group-2 the percent changes are -4.17, -6.23, -6.03, -8.05 and -8.62 as the glucose content is higher than in group-1 and compare to group-4. The percent changes are +0.04, +0.05, +0.08, +0.12 and +0.14 for 24 to 120 hours respectively. The levels of glucose content for four groups are statistically significant at 1% and 5% levels (Table 1).

Serum AST activities of *C. mrigala* was increased in response to simazine exposure when compared to control (group-1), the AST levels are increases in group 2 than in group-1. The percent changes are +6.82, +12.26, +18.60, +22.50 and +26.47 for 24, 48, 72, 96 and 120 hours respectively. In the fish exposed to group-3, slight recovers is observed. The percent recoveries are +3.45, +5.38, +7.31, +10.68 and +12.50 for 24, 48, 72, 96 and 120 hours respectively. The exposure of *S. xanthopium* has higher recovery in group-3 when compare to group-2 are -3.15, -6.12, -9.50, -9.65 and -11.04 for 24, 48, 72, 96 and 120 hours. In group-4, the AST level is slightly increased than all the three groups. The percent changes are +0.004, +0.07, +0.09, +0.11 and +0.13 for 24, 48, 72, 96 and 120 hours respectively. The recorded AST levels for four groups are statistically significant at 1% and 5% levels (Table 2).

In the present investigation simazine treated (group-2) fish, ACP content was increased when compared to control (group-1). The percent increase are +14.26, +22.86, +31.63, +34.11 and +37.43 for 24, 48, 72, 96 and 120 hours respectively. While in the simazine along with supplementary feed (group-3) fish ACP content recovered compared to control group the percent changes are +5.98, +8.90, +10.70, +13.86 and +15.33 for 24 to 120 hours respectively. The exposure of *S. xanthopium* has higher recovery in group-3 when compared to group-2. They are -7.24, -11.36, -15.90, -15.90 and -16.08 for 24, 48, 72, 96 and 120 hours. In the supplementary feed alone exposed group 4 showed near towards normal the percent changes are +0.10, +0.10, +0.12, +0.16 and +0.21 for 24 to 120 hours ACP content in all the four groups are statistically significant at 1% and 5% levels (Table 2).

There was a concentration dependent increased in the ALP activity in the blood serum with significant changes in some of the treated group relative to their respective control are +11.41, +20.15, +29.45, +36.70 and +43.74 for 24, 48, 72, 96 and 120 hours respectively. (Group-3). While in the simazine along with supplementary feed *S. xanthopium* (group-3) fish ALP content recovered compared to group 1 the percent changes are +3.73, +5.91, +8.21, +11.42 and +12.34 for 24 to 120 hours respectively. While the treatment of *S. xanthopium* to group-3 compared to group-2, the recovery effect of *S. xanthopium* has great significant with group-2 percent changes are -6.89, -11.84, -16.41, -18.49 and -21.85 for 24 to 120 hours as the ALP content is higher than in group-1 and compared to group-4. The percentage recoveries are +0.05, +0.09, +0.13, +0.16 and +0.21 for 24 to 120 hours respectively. The levels of ACP content for four groups are statistically significant at 1% and 5% levels (Table 2).

Serum LDH activity of *C. mrigala* increased in response to simazine exposure when compared to control (group-1) are +9.63, +15.42, +22.05, +30.06 and +35.39 for 24, 48, 72, 96 and 120 hours respectively. The simazine along with *S. xanthopium* treated fish (group-3) recorded significant recoveries from the effect of simazine (group-1). The percent recoveries are +3.93, +6.88, +8.84, +10.31 and +.11.88 for 24, 48, 72, 96 and 120 hours respectively. In the supplementary feed along with simazine exposed group near towards normal the percent changes are -5.20, -7.40, -10.79, -15.19 and -17.37 for 24 to 120 hours respectively. In the fish exposed to *S. xanthopium* alone (group-4), slightly decrease no changes occur and it is equal to

normal. The percentage changes in *S. xanthopium* (group-4) are +65, +0.96, +1.20, +1.47 and +1.32 for 24 to 120 hours respectively. The LDH content in blood cell counts for groups 2, 3 and 4 are statistically significant at 1% and 5% levels (Table 2).

Table 1. Variations of blood protein (g/100 mL), glucose (mg/dL), aspartate transaminase (IU/L), alanine transaminase (IU/L) activity in the freshwater fish *Cirrhinus mrigala* exposed tosimazine and *Solanam xanthopium* for 120 hours.

Blood	Groups	Hours of exposure				
		24	48	72	96	120
Protein	Group-I Control	6.130 ± 0.046	6.133 ± 0.067	6.135 ± 0.051	6.134 ± 0.048	6.133 ± 0.056
	Group-II simazine	5.127** ± 0.038	4.023** ± 0.030	3.316** ± 0.061	2.784** ± 0.047	2.069** ± 0.048
	% COC	% -16.36	% -34.40	% -45.95	% -54.61	% -66.24
	Group-III simazine + *Solanam xanthopium*	5.726** ± 0.036	5.583** ± 0.047	5.361** ± 0.040	5.182** ± 0.036	5.065** ± 0.050
	% COC	% -6.59	% -8.97	% -12.62	% -15.52	% -17.41
	% COT	% +11.68	% +38.78	% +61.67	% +86.13	% +144.80
	Group-IV *Solanam xanthopium*	6.142^{NS} ± 0.046	6.159^{NS} ± 0.040	6.167^{NS} ± 0.057	6.180^{NS} ± 0.047	6.189^{NS} ± 0.038
	% COC	% +0.19	% +0.42	% +0.52	% +0.75	% +0.91
Glucose	Group-I Control	50.170 ± 0.561	50.175 ± 0.481	50.178 ± 0.808	50.180 ± 0.861	50.182 ± 0.767
	Group-II simazine	53.966* ± 0.786	55.883** ± 0.867	57.347** ± 0.572	59.567** ± 0.886	60.780** ± 0.764
	% COC	% +7.56	% +11.38	% +14.29	% +18.71	% +21.12
	Group-III simazine+ *Solanam xanthopium*	51.713^{NS} ± 0.786	52.401* ± 0.668	53.888* ± 0.621	54.774** ± 0.563	55.543** ± 0.768
	% COC	% +3.07	% +4.44	% +7.39	% +9.15	% +10.68
	% COT	% -4.17	% -6.23	% -6.03	% -8.05	% -8.62
	Group-IV *Solanam xanthopium*	50.185^{NS} ± 0.866	50.199^{NS} ± 0.645	50.220^{NS} ± 0.806	50.239^{NS} ± 0.764	50.254^{NS} ± 0.628
	% COC	% +0.04	% +0.05	% +0.08	% +0.12	% +0.14
AST	Group-I Control	120.430 ± 1.320	120.442 ± 1.080	120.455 ± 0.958	120.464 ± 1.153	120.475 ± 0.971
	Group-II simazine	128.643** ± 0.909	135.205** ± 1.181	142.835** ± 1.242	147.571** ± 1.397	152.366** ± 1.004
	% COC	% +6.82	% +12.26	% +18.60	% +22.50	% +26.47
	Group-III simazine+*Solanam xanthopium*	124.591 ± 0.911	126.925** ± 1.071	129.267** ± 1.276	133.331** ± 1.065	135.536** ± 1.006
	% COC	% +3.45	% +5.38	% +7.31	% +10.68	% +12.50
	% COT	% -3.15	% -6.12	% -9.50	% -9.65	% -11.04
	Group-IV *Solanam xanthopium*	120.475^{NS} ± 1.160	120.526^{NS} ± 1.256	120.567^{NS} ± 0.970	120.595^{NS} ± 1.390	120.636^{NS} ± 1.228
	% COC	% +0.04	% +0.07	% +0.09	% +0.11	% +0.13
ALT	Group-I Control	41.813 ± 0.899	41.818 ± 0.757	41.823 ± 0.784	41.826 ± 0.677	41.829 ± 0.888
	Group-II simazine	47.091** ± 0.778	52.463** ± 0.938	56.711** ± 0.742	59.128** ± 0.797	61.683** ± 0.704
	% COC	% +12.62	% +25.45	% +35.60	% +41.37	% +47.46
	Group-IIIsimazine+ *Solanam xanthopium*	44.573^{NS} ± 0.671	45.646* ± 0.747	46.281* ± 0.834	47.801** ± 0.614	48.655** ± 0.586
	% COC	% +6.60	% +9.15	% +10.66	% +14.28	% +16.32
	% COT	% -5.35	% -12.99	% -18.39	% -19.16	% -21.12
	Group-IV *Solanam xanthopium*	41.826^{NS} ± 0.779	41.840^{NS} ± 0.841	41.858^{NS} ± 0.776	41.871^{NS} ± 0.832	41.884^{NS} ± 0.792
	% COC	% +0.03	% +0.05	% +0.08	% +0.11	% +0.13

Values are mean ± S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels. (+, -) denotes decreased and increased. % COC (change over control); % COT (change over treated).

Table 2. Variations of blood acid phosphatese (IU/L), alkaline phosphatase (IU/L) and lactate dehydrogenase (IU/L) activity in the freshwater fish *Cirrhinus mrigala* exposed tosimazine and *Solanam xanthopium* for 120 hours.

Blood	Groups	Hours of exposure				
		24	48	72	96	120
ACP	Group-I Control	25.373 ± 0.452	25.380 ± 0.542	25.386 ± 0.530	25.390 ± 0.482	25.393 ± 0.578
	Group-II simazine	28.991** ± 0.652	31.181** ± 0.675	33.415** ± 0.895	34.051** ± 0.820	34.898** ± 0.759
	% COC	% +14.26	% +22.86	% +31.63	% +34.11	% +37.43
	Group-III simazine + *Solanam xanthopium*	26.891^{NS} ± 0.664	27.640* ± 0.613	28.103* ± 0.799	28.910** ± 0.668	29.287** ± 0.828
	% COC	% +5.98	% +8.90	% +10.70	% +13.86	% +15.33
	% COT	% -7.24	% -11.36	% -15.90	% -15.90	% -16.08
	Group-IV *Solanam xanthopium*	25.388^{NS} ± 0.685	25.405^{NS} ± 0.781	25.417^{NS} ± 0.432	25.430^{NS} ± 0.512	25.446^{NS} ± 0.750
	% COC	% +0.10	% +0.10	% +0.12	% +0.16	% +0.21
ALP	Group-I Control	21.350 ± 0.674	21.354 ± 0.513	21.356 ± 0.522	21.359 ± 0.696	21.360 ± 0.709
	Group-II simazine	23.786* ± 0.520	25.656** ± 0.819	27.646** ± 0.604	29.198** ± 0.866	30.703** ± 0.764
	% COC	% +11.41	% +20.15	% +29.45	% +36.70	% +43.74
	Group-III simazine+ *Solanam xanthopium*	22.146^{NS} ± 0.584	22.617^{NS} ± 0.639	23.110* ± 0.407	23.798* ± 0.597	23.995* ± 0.524
	% COC	% +3.73	% +5.91	% +8.21	% +11.42	% +12.34
	% COT	% -6.89	% -11.84	% -16.41	% -18.49	% -21.85
	Group-IV *Solanam xanthopium*	21.361^{NS} ± 0.914	21.373^{NS} ± 0.691	21.385^{NS} ± 0.741	21.395^{NS} ± 0.679	21.406^{NS} ± 0.579
	% COC	% +0.05	% +0.09	% +0.13	% +0.16	% +0.21
LDH	Group-I Control	1527 ± 2.844	1556 ± 2.726	1587 ± 3.805	1630 ± 2.801	1667 ± 2.302
	Group-II simazine	1674** ± 2.766	1796** ± 3.801	1937** ± 2.267	2120** ± 3.884	2257** ± 4.056
	% COC	% +9.63	% +15.42	% +22.05	% +30.06	% +35.39
	Group-III simazine+ *Solanam xanthopium*	1587** ± 2.463	1663** ± 2.371	1728** ± 3.080	1798** ± 3.624	1865** ± 4.700
	% COC	% +3.93	% +6.88	% +8.84	% +10.31	% +11.88
	% COT	% -5.20	% -7.40	% -10.79	% -15.19	% -17.37
	Group-IV *Solanam xanthopium*	1537^{NS} ± 3.786	1571^{NS} ± 4.118	1606^{NS} ± 3.978	1654^{NS} ± 2.960	1689^{NS} ± 4.199
	% COC	% +0.65	% +0.96	% +1.20	% +1.47	% +1.32

Values are mean ± S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels. (+, -) denotes decreased and increased. % COC (change over control); % COT (change over treated).

DISCUSSION

The results of the present investigation indicated that the serum glucose content significantly increased as compared with the control when exposed to different concentrations of both the toxicants this suggests that hyperglycemia has set in to help the animal by providing energy substrates to different tissues to cope with the increased energy demand, (Hai *et al.*, 2009).

The increase in blood glucose concentrations is known as a general secondary response to stress of fish to acute toxic

effects and is considered as a reliable indicator of environmental stress. Today environmental pollution has become not only a national but also an international problem (Tamizhazhagan, 2015). Increase in serum glucose levels in fish under stress was reported by (Karan *et al.,* 1998). Hyperglycemic response illustrated in the present study is an indication of a disruption in carbohydrate metabolism, possibly due to enhanced glucose 6-phosphatase activity in liver, elevated breakdown of liver glycogen, or the synthesis of glucose from extra hepatic tissue proteins and amino acids. Pesticides increases the glucose content in blood because of intensive glycogenolysis and the synthesis of glucose from extra hepatic tissue proteins and amino acids. Vettrivel and Pugazhendy (2013) have suggested that the increase in blood glucose by pesticide treatment may indicate disrupted carbohydrate metabolism due to enhanced breakdown of liver glycogen, possibly mediated by increase in adrenocorticotrophic and glucagon hormones and/or reduced insulin activity.

Changes in glucose concentration are most often associated with renal injury. Plasma concentration of glucose is regulated by complex interaction of hormones such as glucagon and cortisol (Vasantharaja *et al.,* 2012). Increased plasma glucose concentration can cause hyperglycemic condition. This was apparent in the present study under toxicity of simazine toxicity rate of hyperglycemia increased during prolonged exposure periods in *C. mrigala.*

Stress is an energy demanding process and the animal mobilizes energy substrates to cope with stress metabolically (Vijayan *et al*., 1997). Glucose is one of the most sensitive indices of the stress state of an organism: its high concentrations in blood indicate that the fish is in stress and it is intensively using energy reserves i.e., glycogen in liver and muscles (Kakoi *et al*., 2007). The stress hormone cortisol has been shown to increase glucose production in fish, by both gluconeogenesis and glycogenolysis, and likely play an important role in the stress associated increase in plasma glucose concentration (Lumsden *et al.,* 1980). Plasma glucose levels, used as stress indicators, increased during the waterborne copper exposure period are significantly correlated with each other (Mukoyama *et al*., 2007). In the present investigation shows that the activities of ALT and AST increased as the concentration of simazine increased in all the organs tested in a dose dependent pattern. Similar result was also reported by Plotka *et al.* (1988) when they exposed *Cyprinus carpio* to diazinon for 96 hrs which produced depressed activities in the enzymes (AST, ALT, ACP and ALP).

A significant increase in the activity of the major enzymes used for this purpose transaminase (AST) indicates stress based tissue impairment (Tozaki *et al.,* 2003). The decrease in total proteins could be attributed in part to the damaging effects of pesticide on liver cells as confirmed by the increase in the activities of serum AST and ALT observed in this study. A decline in serum total protein level was reported in fish *R. quelen* (Borges *et al*., 2007) and *O. niloticus* (Padalino *et al.,* 2014) in response to simazine and copper exposure respectively. All biological activities are regulated by enzymes and hormones, which are also proteins.

In the present study, the activities of two transaminases, AST and ALT, increased significantly with the increase in the time period of confinement. This suggests that confinement stress generates higher free amino acid mobilization, which in turn might have produced glucose to cope up with the stress, via the process of higher gluconeogenesis. Higher activity of AST in the higher packing density at 120 hours and that of ALT at 120 hours also confirm that higher packing densities can be stressful. Similar observation was recorded in tilapia after being exposed to confinement stress (Vijayan *et al*., 1997). Sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) juveniles raised in semi-intensive conditions had higher transaminases activity when compared to those raised in normal densities (Stull and Rodiek *et al.,* 2000).

Marked elevations were noticed in the activity of (AST) and (ALT). The liver is the primary organ of detoxification as well as a major site for detoxification reaction. Therefore, a significant increase in liver enzymes suggests explanations for the presence of the simazine or its toxins in liver. This picture was confirmed histopathologically by the marked vacuolar degeneration of hepatocytes. The changes in enzyme activity with simazine intoxications varies with the deferent concentrations and fish considered. In *Cyprinus carpio,* the levels of ALT and AST were increased. AST and ALT, in conjunction with LDH, have been found to be involved in gluconeogenesis from amino acids and the effects of changes in the activities of the transaminases. Increase in the transaminases is an immune mechanism, which occurs at the initial stages of diseased condition (Chang *et al*., 2005). LDH is a tetrameric enzyme recognized as a potential marker for assessing the toxicity of a chemical. The elevated levels of LDH in the haemolymph might be due to the release of isozymes from the destroyed tissues (Mishra and Shukla, 2003). In this study, after acute exposure to the LDH, AST and ALT levels were significantly higher than those in the control, which agreed with the previous results (Wagemann *et al*., 2014).

CONCLUSION

The present study impact of simazine and chelate properties of *Solanam xanthopium* tratedd fishes aquatic ecosystems can affect aquatic fauna in different ways. Alterations in physico-chemical properties of water, destruction of the delicate balance of the environment, entry into the food chains and physiological damage to the vital tissues of aquatic fauna are the threatening issues of the modern day pesticides. Long term exposure to these products causes countless abnormalities and reduces the life span of aquatic organisms. Blood biochemical alteration occurs and many changes fish

body .Finally, we conclude that simazine is highly toxic to fish, and impose life threatening effect on fish at both lethal and sublethal concentrations. Altered haematological responses can be used as tools in bioassessment to monitor ecotoxicological risks associated with pesticides such as simazine to various fish. It affected entire aquatic food chains.

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