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Case Report

ADVANCES IN BIORESEARCH EFFICACY OF *PISONIA ALBA* AND *SOLANUM XANTHOCARPUM* ON HEMATOLOGICAL AND BLOOD BIOCHEMICAL RESPONSES OF FRESH WATER FISH *LABEO ROHITA* (HAMILTON) EXPOSURE TO SIMAZINE HERBICIDE

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A B S T R A C T

This study that looked at simazine (2-chloro-4,6-bis(ethylamino)-s-triazine), a triazine herbicide that has been used to manage weeds and algae in agriculture and the aquatic environment for a long time. For a long time, haematological tests have been thought to be good indicators of fish health. Haematological and serum biochemical indicators were used to compare different eating activity of teleost fishes. Labeo rohita were tested to obtain a normal range of blood parameters that could be used as a baseline for assessing the fish's health and as a benchmark for future comparisons. Blood parameters such as red blood cell count (RBC) and white blood cell count (WBC), haemoglobin, haematocrit, mean cell haemoglobin concentration (MCHC), mean cell volume (MCV), mean cell haemoglobin, glucose, protein, cholesterol, and urea were estimated from freshwater fish of various trophic levels. A statistical investigation revealed that the haematological features of marine fish differed significantly. When compared to control, haematological profiles demonstrated a significant (P0.01) decrease in leukocyte count at all concentrations. In a study of the exposed to simazine and improvements in bioresearch efficacy of *Pisonia alba* and *Solanam xanthopium* in the freshwater fish *Labeo rohita* for haematological modifications over a 120-hour period, the haematological RBC/WBC ratio, MCV, and MCHC were all substantially associated. Biochemical characteristics of the blood serum To detect the functional condition of fish during acute exposure, blood biochemical measures are used as health indicators. As a result, blood biochemical parameters such as plasma glucose, protein, albumin, and globulin were investigated in order to assess the herbicide's toxic potential. This information can be used to confirm the maturity of waters and related soils, as well as to track any changes in environmental quality.

INTRODUCTION

Simazine ($C_7H_{12}C_lN_5$) is a triazine herbicide with a CAS name and number of 2-chloro-4,6-bis(ethylamino)-1,3,5-triazine and 122-34-9, respectively. Simazine was first registered in Canada in 1963 under the trade names Simadex, Simmaprim, and Princep (Agriculture and Agri-Food Canada 1997). Simazine is a selective herbicide used in raspberries, loganberries, highbush blueberries, apples, pears, grapes, asparagus, and ornamentals to control annual broadleaf and grass weeds. Total weed control in industrial zones, airports, and along shelterbelts and rights-of-way, as well as aquatic weed management in ditches, farm ponds, fish hatcheries, aquaria, and fountains, are examples of noncrop applications. As evidenced by BCFs 100, the bioaccumulation potential is minimal. If the organism is transported to uncontaminated water, the depuration half-life in fish is 7 days, showing that simazine is rapidly eliminated or metabolized (Rodgers 1970 and Niimi 1987).

Various studies have found oxidative anxiety in numerous fish species (Blahov *et al*., 2013; Mela *et al*., 2013), performance differences (Plhalova *et al*., 2012), and biochemical changes after exposure to severe, sublethal, or lethal doses. Atrazine has been linked to negative impacts on productivity (Tillitt *et al*., 2010), resistance reactions (Kreutz *et al*., 2012), and the decontamination system (Kreutz *et al*., 2012). (Fu *et al*., 2013). After being exposed to atrazine, many tissues such as the liver,

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kidney, gills, and other fish organs are impacted (Paulino *et al*., 2012).

Changes in fish performance and pathological function can occur after a high dose of exposure (Solomon *et al*., 2008). Because of its well moment within the soil, it has the potential to mix with water (Waring and Moore, 2004). The total erythrocyte count (RBC), total white blood cell count (WBC), hematocrit (PCV), haemoglobin concentration (Hb), erythrocyte indices (MCV, MCH, MCHC), white blood cell differential count, and the evaluation of stained peripheral blood films are all part of the hemogram evaluation. Campbell, (2004). After erythrocytes, thrombocytes have been described as the most abundant blood cells.

Hematological parameters are strongly linked to an animal's response to its environment, suggesting that the environment in which fish reside may have an impact on their haematological characteristics (Gabriel *et al*., 2004). Atrazine reaches water, according to Hussein *et al*. (1996), due to the immediacy of agronomic nations of aquatic locations, or sometimes because to improper treatment in certain situations. Fish has been severely harmed as a result of present environmental issues since it is directly exposed to ecological pollution, demonstrating the natural endowments of ecological effluence in waters. Because it is predicted to remain persistent in the environment, there is a chance that it will end up in the aquatic environment via run-off. As simazine is used as a selective systemic herbicide to suppress annual grasses and broad-leaved weeds, it is released into the environment directly (Tomlin 1997). Simazine is a non-volatile compound that is moderately soluble in water (6.2 mg/L at 25 °C) (Wilson and Wilson 2010).

Due to its high mobility in soil, simazine is also predicted to leak into ground water systems. Simazine has been discovered to photodegrade in soil but is resistant to abiotic processes in water. Because of its low vapour pressure, volatilization is not considered to be a significant process for simazine (Wauchope *et al*., 1992). In the food web, simazine does not bio accumulate or bio magnify. Although the effects of acute and subchronic exposure of fish to simazine, another s-triazine herbicide, have been well reported, there is a paucity of evidence on simazine's chronic toxicity to Labeo rohita at environmentally relevant quantities.

The high impact of simazine has prompted researchers to investigate its toxicity in aquatic life. Phosphatase that releases attached phosphate groups from other molecules during digestion is known as acid phosphatise. It's a lysosomal hydrolytic enzyme that works best at an acid pH. It aids in the breakdown of dead cells and so serves as a useful indicator of the biological system's stress level (Viran *et al*., 2003). The purpose of this study was to see if simazine had any effect on haematological and blood biochemical responses, as well as the potential of *Pisonia alba* and *Solanum xanthocarpum* to restore serum biochemical parameters in *Labeo rohita* for a period of 120 hours.

MATERIALS AND METHODS

Collection and maintenance of the experimental animal.

Labeo rohita, a freshwater fish, was taken at the VGM fish farm in Kurinjipadi, Cuddalore district. The fish were transported to the lab and transferred to rectangular fibre glass tanks (100 175 cm) with a 500 litre capacity and chlorine free aerated well water fish of the same size and weight were utilised for the studies, regardless of their sex.

Procurement and rearing of experimental fishes

In India's freshwater, *Labeo rohita,* sometimes known as 'rohu,' is widely dispersed. Labeo rohita was obtained from a fish farm in the Cuddalore district's Kurinjipadi. The gathered fish were transferred in half-filled polythene bags with the least amount of disturbance possible. Each bag held about 100 fish, and the water was aerated with pressured air from a cylinder.

Throughout the course of the study, there was no mortality in any of the consignments, indicating that this means of transportation was successful. The fish were brought to the lab and acclimatised in a fibre aquarium for a week before being employed in the experiment. The fungal infection was kept at bay by washing the fish tanks (aquarium) with potassium permanganate solution. The fish were disinfected with a 0.1 percent potassium permanganate solution and kept in well-aerated tap water for three weeks. They were acclimatised to experimental tanks for at least one week prior to experimentation. For the studies, fish measuring 10-12 cm in length and 9-14 gramme in weight were chosen regardless of sex. The fish were fed oil-free groundnut cake on a daily basis. After 2 hours, the unused food was removed, and the water was replaced on a daily basis.

Diseases, stress, physical damage, and mortality were all carefully examined in the test fishes. Individuals who were very sick, abnormal, or dead were discarded. To limit the cumulative influence of animal excreta in the test trough, feeding was stopped two hours before to the start of the trials (Arora *et al.,* 1972). The fish were exposed to sublethal concentrations during a 120-hour treatment and control period. A control group was kept in the same conditions as the experimental group. Every day, the hazardous water and regular water were replaced. On 120 hours, both the experimental and control groups of fish were slaughtered.

Toxicity studies

In acute toxicity tests, the renewal technique of acute static test was used to assess the efficacy of Pisonia alba and Solanum anthocarpum on haematological and blood biochemical responses, as well as recovery ability toxicity studies, in which fish were exposed to the same composition concentrations on a regular basis, usually once every 24 hours, by transferring the animals from one test chamber to another.

Source of Simazine

The herbicide simazine, used for the experiment was sourced from the present study commercial formulations of Macspred Simazine 900DF herbicide is a long acting pre-emergrnt herbicide which kills certain annual board leaf and perennial weeds by absorption through the root system.with the trade name Exports Ltd (India) was purchased from the market. It has a role as a herbicide and an environment contaminant. It is a chloro -1, 3, 5- triazine and a diamino-1,3,5-triazine.

Extraction of Pisonia alba and Solanum xanthocarpum

The leaves of *Pisonia alba and Solanum xanthocarpum* powder is made by using grinder to get coarse powder. Equal amount of powder can be sieved through 40 mesh sieve to get coarse powder of particle size desired. The powder was

exposed to Soxhlet extraction with aqueous and ethanol (95% v/v) at 60oC. Colorless solvent in the siphon tube was collected as the end of extraction. The extracts are concentrated by distillation to 3/4 of its original volume. The extracts concentrated were taken in a china dish and evaporation done on thermostat controlled water bath till thick paste is formed. The extract is dried and stored in a glass bottle in a refrigerator at 4oC. The dried, crude extracts concentrated were labeled as ALPA and ELPA. The yield was 12.8% w/w.

Supplementary feed

Pisonia alba and *Solanam xanthopium* leaves, both disease-free, were collected in and around Cuddalore and Kudikadu, and the plant was identified. The leaves were washed in running tap water for 10 minutes before being dried. Aerial parts (1kg) of *Pisonia alba* and *Solanam xanthopium* were macerated thrice at room temperature and powdered, and an equal amount of rice brane was mixed well with a small amount of water added and a small pellet as feed was prepared.

Design of the an experiment

Group-1 (Untreated control) - For 120 hours, fish were exposed to freshwater.

Group-2 (Simazine) - For 120 hours, fish were exposed to simazine (18 mg/L sublethal dose).

Group-3 (Simazine and *Pisonia alba*, *Solanam xanthopium*) - For 120 hours, fish were subjected to simazine (18 mg/L) and pisonia alba, solanam xanthopium (2.0g+2.0g).

Group-4 (*Pisonia alba* and *Solanam xanthopium*) - For 120 hours, fish were exposed to *Pisonia alba* and *Solanam xanthopium* alone (2.0g+2.0g).

Healthy Labeo rohita were obtained from a freshwater farm in the Cuddalore district's VGM fish farm Kurinjipadi. They were acclimatised for a maximum of 15 days in a laboratory setting. The experimental experiments were conducted on fish measuring 4.5 to 6.0 cm in length and weighed 5 to 6 g each. For 120 hours, Labeo rohita fingerlings were exposed to a sublethal dosage of simazine (18 mg/l). The fish in the sublethal and control groups were slaughtered so that biochemical and blood parameters could be determined. Blood samples were taken from both the control and treated groups' candal veins of live fish. According to McKnight (1966), a part (1 ml) was mixed thoroughly in a clean dry vial containing EDTA anticoagulant (1.5 mg/ml) to test RBC, WBC, haemoglobin, and haematocrit, among other things.

Neubauer's haemocytometer was used to count red blood corpuscles (RBC) and white blood corpuscles (WBC) utilising Hayem's and Tuerk's solutions as diluting fluids, respectively. Wintrope's method was used to determine haematocrit values, and for haemoglobin (Hb) estimation, a blood sample was treated with N/10 HCl, and the colour of the acid haematin was matched to the specified standards using Sahli's haemoglobinometer. Following conventional formulas, mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated (Dacie and Lewis, 1991).

$$MCV = \frac{PCV/1000 \text{ ml blood}}{RBC \text{ in millions } / \text{ mm}^3} = \text{fl}$$

$$MCH = \frac{Hb \text{ in g}/1000 \text{ ml blood}}{RBC \text{ in millions } / \text{ mm}^3} = \text{pg}$$

The data obtained from the control and experiment were subjected to statistical analysis by student‘t’ test.

Albumin and globulin level in serum

The concentration of albumin was determined by a commercially available kit (Siemens Ltd., Gujarat, India) and the absorbance was measured at 628 nm on a spectrophotometer (UV–VIS Systronics, 118). Globulin level was obtained by subtracting the albumin concentration from the total proteins.

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, (1958). 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 25-30ºC

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 37ºC. 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.

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 370C. 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.

Table 1 Variations of RBC ($\times 10^6/mm^3$), WBC ($\times 10^3/mm^3$), Hb (g/L) and PCV (%),MCV (fL), MCH (pg) and MCHC (%) values in the freshwater fish *Labeo rohita* exposed to simazine followed by the supplementary feed of *Pisonia alba* and *Solanam xanthopium* exposed to 120 hours

Blood	Groups	Hours of exposure				
		24	48	72	96	120
RBC ($\times 10^6/mm^3$)	Group-I Control	2.248 ± 0.008	2.250 ± 0.006	2.246 ± 0.005	2.240 ± 0.008	2.238 ± 0.006
	Group-II Simazine	1.076** ± 0.006	0.929** ± 0.005	0.824** ± 0.006	0.835** ± 0.004	0.771** ± 0.007
	% COC	% -12.97	% -25.75	% -33.94	% -40.73	% -45.81
	Group-III Simazine+ *Pisonia alba and Solanam xanthopium*	1.185** ± 0.004	1.157** ± 0.008	1.124** ± 0.007	1.079** ± 0.005	1.044 ± 0.006
	% COC	% -4.25	% -7.36	% -9.87	% -13.06	% -15.75
	% COT	% +11.05	% +25.79	% +35.46	% +47.68	% +56.45
	Group-IV *Pisonia alba and Solanam xanthopium*	1.256^{NS} ± 0.005	1.261^{NS} ± 0.006	1.262^{NS} ± 0.005	1.263^{NS} ± 0.004	1.267^{NS} ± 0.006
	% COC	% +0.56	% +0.81	% +1.29	% +1.84	% +2.27
WBC ($\times 10^3/mm^3$)	Group-I Control	1.266 ± 0.025	1.268 ± 0.032	1.260 ± 0.027	1.262 ± 0.031	1.263 ± 0.039
	Group-II Simazine	1.704** ± 0.028	1.922** ± 0.029	2.164** ± 0.031	2.282** ± 0.028	2.319** ± 0.031
	% COC	% +35.59	% +52.70	% +71.67	% +80.66	% +83.53
	Group-III simazine+ *Pisonia alba and Solanam xanthopium*	1.372* ± 0.028	1.422* ± 0.029	1.449** ± 0.031	1.496** ± 0.028	1.514** ± 0.031
	% COC	% +9.08	% +12.96	% +15.79	% +18.46	% +19.95
	% COT	% -18.55	% -24.03	% -31.55	% -33.43	% -32.64
	Group-IV *Pisonia alba and Solanam xanthopium*	1.261^{NS} ± 0.036	1.264^{NS} ± 0.025	1.268^{NS} ± 0.031	1.276^{NS} ± 0.038	1.281^{NS} ± 0.037
	% COC	% +0.33	% +0.57	% +0.72	% +1.04	% +1.36
Hb (g/L)	Group-I Control	2.345 ± 0.035	2.348 ± 0.047	2.352 ± 0.058	2.355 ± 0.031	2.357 ± 0.038
	Group-II Simazine	2.937** ± 0.038	2.659** ± 0.040	2.492** ± 0.031	2.281** ± 0.047	2.019** ± 0.033
	% COC	% -14.68	% -20.88	% -25.67	% -32.04	% -39.87
	Group-III Simazine+ *Pisonia alba and Solanam xanthopium*	3.163* ± 0.036	3.056** ± 0.040	3.039** ± 0.058	2.982** ± 0.049	2.910 ± 0.058
	% COC	% -5.44	% -8.72	% -9.38	% -11.12	% -13.31
	% COT	% +7.74	% +15.32	% +22.10	% +30.78	% +44.21
	Group-IV *Pisonia alba and Solanam xanthopium*	3.353^{NS} ± 0.049	3.365^{NS} ± 0.051	3.377^{NS} ± 0.046	3.385^{NS} ± 0.052	3.399^{NS} ± 0.065
	% COC	% +0.24	% +0.52	% +0.74	% +0.88	% +1.24
PCV (%)	Group-I Control	27.938 ± 0.531	27.943 ± 0.431	27.945 ± 0.573	27.947 ± 0.428	27.946 ± 0.633
	Group-II Simazine	26.870** ± 0.446	24.370** ± 0.548	22.261** ± 0.633	22.045** ± 0.424	18.298** ± 0.588
	% COC	% -10.60	% -19.25	% -26.55	% -30.75	% -33.33
	Group-III Simazine+*Solanam xanthopium*	27.503^{NS} ± 0.397	26.685* ± 0.456	26.083* ± 0.584	25.611** ± 0.428	25.141** ± 0.532
	% COC	% -4.96	% -7.80	% -9.89	% -11.52	% -13.14
	% COT	% +6.32	% +14.19	% +22.69	% +27.78	% +30.29
	Group-IV *Pisonia alba and Solanam xanthopium*	28.951^{NS} ± 0.427	28.962^{NS} ± 0.630	28.978^{NS} ± 0.477	28.991^{NS} ± 0.548	28.998^{NS} ± 0.473
	% COC	% +0.06	% +0.07	% +0.11	% +0.16	% +0.19
MCV (fl)	Group-I Control	231.878 ± 1.808	232.096 ± 1.663	232.301 ± 1.272	232.543 ± 1.358	232.712 ± 1.869
	Group-II Simazine	243.209** ± 1.971	251.828** ± 2.061	258.330** ± 1.988	272.717** ± 2.393	287.603** ± 1.389
	% COC	% +4.87	% +8.51	% +11.22	% +16.27	% +22.89
	Group-III Simazine+ *Pisonia alba* and *Solanam xanthopium*	234.148^{NS} ± 1.610	235.743^{NS} ± 1.630	237.264* ± 1.236	238.575* ± 1.471	240.041 ± 1.055
	% COC	% +0.98	% +1.58	% +2.14	% +2.59	% +3.15
	% COT	% -3.73	% -6.35	% -8.53	% -12.52	% -16.56
	Group-IV *Pisonia alba* and *Solanam xanthopium*	234.326^{NS} ± 2.877	230.870^{NS} ± 1.855	230.110^{NS} ± 1.973	229.830^{NS} ± 2.067	229.066^{NS} ± 1.826
	% COC	% +1.05	% -0.55	% -0.96	% -1.19	% -1.58
MCH (pg)	Group-I Control	26.502 ± 0.495	26.680 ± 0.567	26.802 ± 0.405	26.966 ± 0.395	27.030 ± 0.484
	Group-II Simazine	28.030* ± 0.315	28.845* ± 0.478	29.567** ± 0.411	30.770** ± 0.378	31.076** ± 0.514
	% COC	% +5.75	% +8.12	% +10.33	% +14.12	% +14.98
	Group-III Simazine+ *Pisonia alba* and *Solanam xanthopium*	27.068^{NS} ± 0.495	27.790^{NS} ± 0.339	28.061^{NS} ± 0.427	28.660* ± 0.430	28.908 ± 0.365
	% COC	% +2.13	% +4.16	% +4.70	% +6.16	% +6.90
	% COT	% -3.42	% -3.65	% -5.12	% -6.85	% -6.94
	Group-IV *Pisonia alba* and *Solanam xanthopium*	26.580^{NS} ± 0.443	25.803^{NS} ± 0.503	25.959^{NS} ± 0.428	26.161^{NS} ± 0.569	26.218^{NS} ± 0.505
	% COC	% +0.29	% +0.46	% +0.58	% +0.61	% +0.69
MCHC (%)	Group-I Control	12.562 ± 0.066	12.570 ± 0.075	12.585 ± 0.067	12.592 ± 0.073	12.599 ± 0.059
	Group-II Simazine	12.240* ± 0.054	12.135** ± 0.049	12.016** ± 0.066	10.870** ± 0.050	10.450** ± 0.070
	% COC	% -2.78	% -3.76	% -4.91	% -6.23	% -9.91
	Group-III Simazine+ *Pisonia alba* and *Solanam xanthopium*	12.486^{NS} ± 0.046	12.420^{NS} ± 0.059	12.367* ± 0.048	12.360* ± 0.053	12.315 ± 0.050
	% COC	% -0.61	% -1.30	% -1.88	% -2.00	% -2.45
	% COT	% +2.19	% +2.56	% +8.79	% +8.79	% +8.83
	Group-IV *Pisonia alba* and *Solanam xanthopium*	12.585^{NS} ± 0.067	12.613^{NS} ± 0.071	12.648^{NS} ± 0.052	12.670^{NS} ± 0.072	12.693^{NS} ± 0.061
	% COC	% +0.20	% +0.37	% +0.54	% +0.67	% +0.81

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).

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 37ºC 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 (1965). 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 25ºC 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 25ºC 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.

Statistical Analysis

The results of the present study were statistically evaluated using the student t-test (Milton and Tsokos,1983) to compare means of treatment for the various biochemical parameters studies data against their control ones, and the results were declared significant at the (P<0.05), (P<0.01) level.

RESULT

Fish behavior

Both the control and exposed fish fed normally during the investigation. There were no indicators of respiratory distress, such as rapid breathing, gill opercular motions, or floating near the water's surface. During the experiment, there were no deaths.

Table 2 Variations of blood protein (g/100 mL), glucose (mg/dL), Albumin (g/100 mL), Globulin (g/100 mL) and Cholesterol (g/100 mL), in the freshwater fish *Labeo rohita* exposed to simazine followed by the supplementary feed of *Pisonia alba* and *Solanam xanthopium* exposed to 120 hours

Blood	Groups	Hours of exposure				
		24	48	72	96	120
Protein (g/100 mL)	Group-I Control	7.130 ± 0.046	7.133 ± 0.067	7.135 ± 0.051	7.134 ± 0.048	7.133 ± 0.056
	Group-II Simazine	6.127** ± 0.038	5.023** ± 0.030	4.316** ± 0.061	3.784** ± 0.047	2.069** ± 0.048
	% COC	% -15.36	% -32.40	% -44.95	% -53.61	% -65.24
	Group-III Simazine+ *Pisonia alba* and *Solanam xanthopium*	8.728** ± 0.036	8.584** ± 0.047	8.362** ± 0.040	8.184** ± 0.036	8.064** ± 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 *Pisonia alba* and *Solanam xanthopium*	5.142^{NS} ± 0.046	5.159^{NS} ± 0.040	5.167^{NS} ± 0.057	5.180^{NS} ± 0.047	5.189^{NS} ± 0.038
	% COC	% +0.18	% +0.41	% +0.52	% +0.76	% +0.91
Glucose (mg/dL)	Group-I Control	51.170 ± 0.561	51.175 ± 0.481	51.178 ± 0.808	51.180 ± 0.861	51.182 ± 0.767
	Group-II Simazine	52.966* ± 0.786	54.883** ± 0.867	56.347** ± 0.572	59.567** ± 0.886	61.780** ± 0.764
	% COC	% +7.56	% +11.38	% +14.29	% +18.71	% +21.12
	Group-III Simazine+ *Pisonia alba* and *Solanam xanthopium*	53.713^{NS} ± 0.786	53.401* ± 0.668	53.888* ± 0.621	55.774** ± 0.563	56.543** ± 0.768
	% COC	% +3.07	% +4.44	% +7.39	% +9.15	% +10.68
	% COT	% -4.19	% -6.24	% -6.06	% -8.08	% -8.63
	Group-IV *Pisonia alba* and *Solanam xanthopium*	50.184^{NS} ± 0.866	50.198^{NS} ± 0.645	50.221^{NS} ± 0.806	50.237^{NS} ± 0.764	50.255^{NS} ± 0.628
	% COC	% +0.04	% +0.05	% +0.08	% +0.12	% +0.14
Albumin (g/100 mL)	Group-I Control	120.431 ± 1.322	120.442 ± 1.081	120.455 ± 0.957	120.464 ± 1.154	120.475 ± 0.972
	Group-II Simazine	127.643** ± 0.909	134.205** ± 1.181	143.835** ± 1.242	147.571** ± 1.397	153.366** ± 1.004
	% COC	% +6.82	% +12.26	% +18.60	% +22.50	% +26.47
	Group-III Simazine+ *Pisonia alba* and *Solanam xanthopium*	126.591 ± 0.911	126.925** ± 1.071	127.267** ± 1.276	134.331** ± 1.065	136.536** ± 1.006
	% COC	% +3.45	% +5.38	% +7.31	% +10.68	% +12.50
	% COT	% -3.14	% -6.13	% -9.51	% -9.66	% -11.05
	Group-IV *Pisonia alba and Solanam xanthopium*	121.475^{NS} ± 1.160	121.526^{NS} ± 1.256	121.567^{NS} ± 0.970	121.595^{NS} ± 1.390	121.636^{NS} ± 1.228
	% COC	% +0.04	% +0.07	% +0.09	% +0.11	% +0.13
Globulin (g/100 mL)	Group-I Control	42.813 ± 0.899	42.818 ± 0.757	42.823 ± 0.784	42.826 ± 0.677	42.829 ± 0.888
	Group-II Simazine	46.091** ± 0.778	52.463** ± 0.938	55.711** ± 0.742	58.128** ± 0.797	62.683** ± 0.704
	% COC	% +12.62	% +25.45	% +35.60	% +41.37	% +47.46
	Group-III Simazine+ *Pisonia alba* and *Solanam xanthopium*	45.573^{NS} ± 0.671	45.646* ± 0.747	48.281* ± 0.834	47.801** ± 0.614	49.655** ± 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 *Pisonia alba* and *Solanam xanthopium*	41.828^{NS} ± 0.779	41.842^{NS} ± 0.841	41.857^{NS} ± 0.776	41.874^{NS} ± 0.832	41.888^{NS} ± 0.792
	% COC	% +0.03	% +0.05	% +0.08	% +0.11	% +0.13
Cholesterol (g/100 mL)	Group-I Control	51.282 ± 0.027	51.261 ± 0.036	51.253 ± 0.037	51.246 ± 0.038	51.238 ± 0.042
	Group-II Simazine	55.966* ± 0.786	58.883** ± 0.867	62.347** ± 0.572	64.567** ± 0.886	68.780** ± 0.764
	% COC	% +7.57	% +11.48	% +14.19	% +18.74	% +21.22
	Group-III Simazine+ *Pisonia alba* and *Solanam xanthopium*	53.714^{NS} ± 0.786	54.401* ± 0.669	58.888* ± 0.621	59.774** ± 0.563	57.543** ± 0.767
	% COC	% +3.07	% +4.44	% +7.39	% +9.15	% +10.68
	% COT	% -4.17	% -6.23	% -6.03	% -8.05	% -8.62
	Group-IV *Pisonia alba* and *Solanam xanthopium*	52.186^{NS} ± 0.866	52.199^{NS} ± 0.645	52.220^{NS} ± 0.806	52.239^{NS} ± 0.764	52.254^{NS} ± 0.628
	% COC	% +0.04	% +0.05	% +0.08	% +0.12	% +0.14

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).

Hematological profile after acute exposure to simazine

Table 2 shows the results of haematological profiling. In comparison to controls, all groups exposed to simazine demonstrated a substantial (p 0.01) drop in leukocyte count. All of the groups had similar Hb, PCV, MCH, MCV, MCHC, and Leukogram values.

Biochemical blood plasma profiles after acute exposure to simazine

Table 1 shows the results of haematological and blood biochemical profiling. When compared to controls, blood biochemical profiles of fish Labeo rohita exposed to simazine at an ambient dose of 18 mg/l (group I) exhibited significantly (p 0.01) greater activity of plasma glucose, protein, albumin, and globulin. The biochemical profiles of groups two and three revealed considerably increased plasma blood biochemical activity (p 0.01) and (p 0.05) than the controls. When compared to controls, total protein (p 0.05), albumin (p 0.05), globulin activity (P 0.01), and cholesterol activity (p 0.05) were significantly higher in group four of the fish *Labeo rohita.*

The quantitative changes of haematological and blood biochemical parameters such as RBC, WBC, Hb, haematocrit, MCH, MCV, plasma glucose, protein, albumin globulin, and cholesterol have been observed in the fish Labeo rohita fish in control and sublethal concentrations of simazine (18 mg/l) exposed after 24, 48, 72, 96, and 120 hours and are listed in (Table 1). The suppression of erythropoiesis or the loss of red cells can both result in a decrease in RBC content. The considerable drop in RBC counts during the sublethal study was attributed to anaemia and haemolysis produced by simazine poisoning in the current investigation.

The investigation of haematological features in fish has become a significant tool for gaining a better understanding of normal and diseased processes, as well as their toxicological consequences. In comparison to controls, simazine at dosages of 18 mg/l, 1, 2, and 4 mg/l was linked with a lower leukocyte count. In fish, leukocytes are engaged in the control of immune function as well as a protective response to stress. Anemia was found in Labeo rohita after exposure to simazine in the current study, as evidenced by a substantial drop in RBC count, Hb percent, and Hct (Table 1). The Number of white blood cells of the experimental fish Labeo rohita increases rapidly following exposure to simazine toxicity for 24, 48, 72, 96, and 120 hours (Table 1). Throughout the trial, the level of MCV and MCH increased, possibly indicating a condition of macrocytic anaemia in the atrazine-exposed fish. This organ receives the greatest amount of postbranchial blood in fish. Renal lesions may be considered to be useful indicators of environmental pollution because triazine uptake via the gill appears to be of major importance.

Total protein, albumin, and cholesterol levels have all changed as a result of simazine exposure. Lipid metabolism is linked to cholesterol levels in the blood. Simazine harms the liver, lowering the amount of esterified cholesterol available (Table 2). Proteins are essential for living creatures' physiology. Enzymes and hormones, which are both proteins, govern all biological activity. The sublethal reaction of simazine may be linked to protein metabolism, which is the process of converting blood and structural proteins to energy in order to satisfy the increased energy demand during stressful situations.

For the period of 24 to 120 hours, Labeo rohita treated to sublethal concentrations of simazine (group II) showed a considerable decrease in protein, albumin, and globulin. Albumin levels are shown to be lower in fish exposed to simazine, which can be explained on a functional basis. Albumin is a protein reserve and transport protein with a length to junctions osmotic regulation of blood volume (Table 2).

In the current study, the activities of ALT and AST increased in a dose-dependent way as the concentrations of simazine increased in all of the organs evaluated. When compared to the control (group-I), serum AST activity of Labeo rohita increased in response to simazine exposure. AST levels are higher in group 2 than in group I. At 1% and 5% levels, the observed AST levels for four groups are statistically significant (Fig. 1).

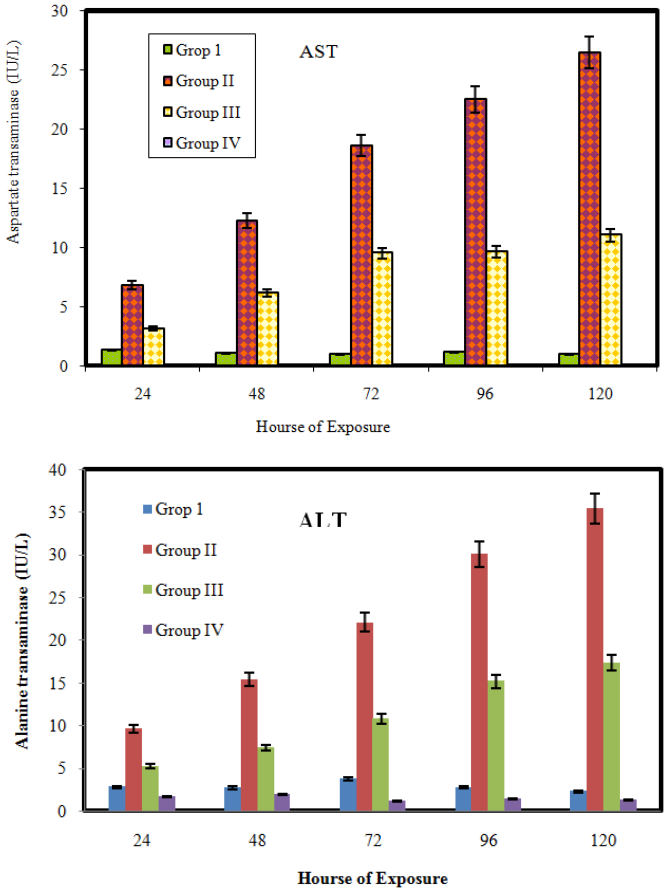

Fig 1 Variations of blood Aspartate transaminase (IU/L), Alanine transaminase (IU/L) activity in the freshwater fish *Labeo rohita* exposed to simazineollowed by the supplementary feed of *Pisonia alba* and *Solanam xanthopium* exposed to 120 hours

ACP content was found to be significantly higher in simazine-treated (group-II) fish than in control fish in this study (group-I). At 1% and 5% levels, the ACP content in each of the four groups is statistically significant (Fig. 2). The decrease in total proteins could be attributable in part to the pesticide's harmful effects on liver cells, as seen by the increase in serum AST and ALT activity obtained in this study. Results suggests that confinement stress causes increased free amino acid mobilisation, which, in turn, may have resulted in increased gluconeogenesis to cope with the stress. The activities of (AST) and (ALT) were found to be significantly elevated (ALT). The liver is both a main detoxification organ and a key detoxification reaction site. As a result, a considerable increase

in liver enzymes could indicate the presence of simazine or its toxins in the liver.

The significant vacuolar degeneration of hepatocytes validated this picture histopathologically. The effects of simazine intoxication on enzyme activity vary depending on the concentrations used and the fish involved. The levels of ALT and AST in *Labeo rohota* were raised. AST and ALT, along with LDH, have been discovered to have a role in gluconeogenesis from amino acids and the consequences of transaminase activity variations. At 1% and 5% levels, the values of ACP content for four groups are statistically significant (Table 4). When compared to control, the serum LDH activity of Labeo rohita increased in response to simazine exposure (group-I). At 1% and 5% levels, the LDH content in blood cell counts for groups II, III, and IV is statistically significant (Fig 2). LDH is a tetrameric enzyme that has been identified as a potential marker for determining a chemical's toxicity.

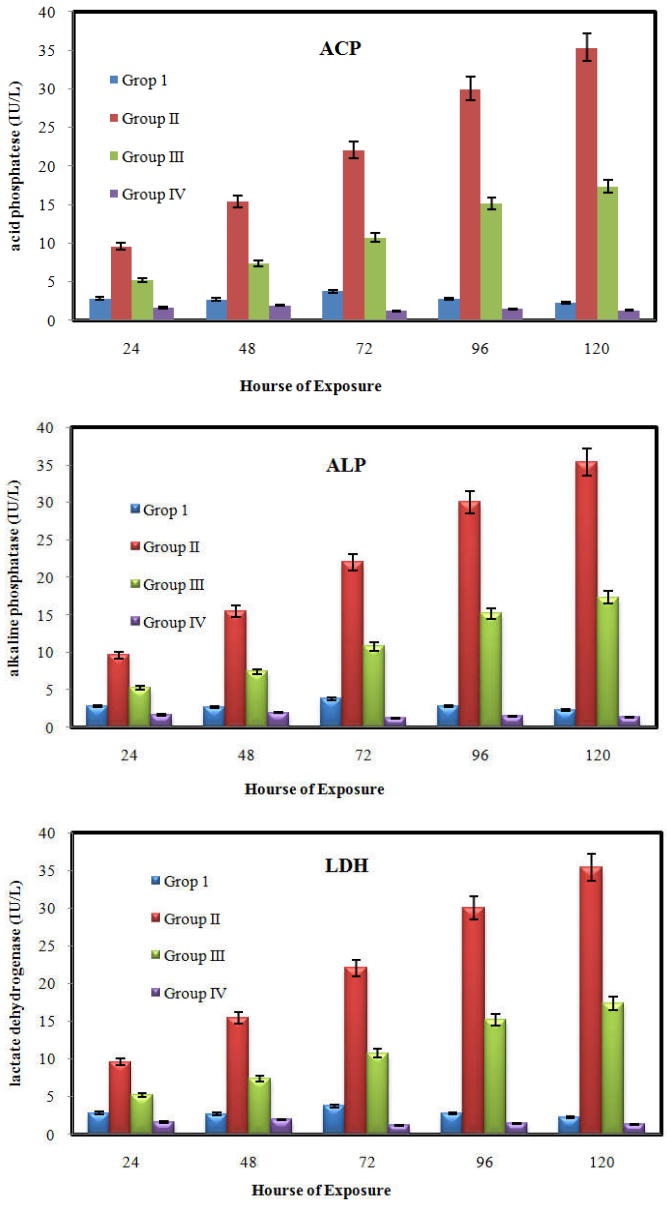

Fig 2 Variations of blood acid phosphatese (IU/L), alkaline phosphatase (IU/L) and lactate dehydrogenase (IU/L) activity in the freshwater fish *Labeo rohita* exposed to simazine followed by the supplementary feed of *Pisonia alba* and *Solanam xanthopium* exposed to 120 hours

DISCUSSION

Acute or chronic stress can alter the blood parameters of teleost fish. Bonga, Wendelaar (1997). Haematological alterations are frequently the first detectable and quantifiable responses to environmental change (Hawkins and Mawdesley, 2006). Changes in lymphoperisis and/or altered lymphocyte release from lymphoid tissues cause a decrease in leukocyte count (Das *et al*., 2004). The decrease in leukocyte count in this study implies that the fish were stressed as a result of simazine exposure, which could have resulted in hypoxia and kidney injury. Oropesa *et al*. (2009), on the other hand, found no influence on the haematological profiles of freshwater fish exposed to 45 mg L1 simazine.

In *Nile tilapia* and catfish (*Chrysichthyes auratus*) treated to 3 and 6 mg/l of atrazine, Hussein *et al*., (1996) found a decrease in % RBC, Hb, and PCV. The considerable drop in Hb concentration could potentially be attributable to an increase in the rate at which Hb is destroyed or a decrease in the rate at which Hb is synthesised (Reddy and Bashamohideen, 1989). The WBC was the most sensitive to environmental changes, while lymphocytes were the most important leucotyes (Karuppasamy and Subathra, 2005). This rise in WBC could be attributable to an increase in leucocyte population, indicating that the fish's immune system is working to protect them from infections when under simazine stress.

The increase in MCV and MCH values with a decrease in MCHC could be attributable to variations in the medium caused by hazardous chemicals. MCV and MCH levels in the blood of cadmium-exposed Oreochromis were found to be significantly higher by Ruperelia *et al.,* (1992).

Pesticides containing triazine have a direct influence on the anatomy and function of the kidneys in freshwater fish (Velisek *et al*., 2008, 2009b). The tubules in the caudal kidney of carp exposed to simazine for a long time were destroyed in our experiment. The kidney is vital for maintaining a stable internal environment in respect of water and salt, excretion, and, to a lesser extent, xenobiotic metabolism (Ortiz *et al*., 2003). Haemolysis and erythrocyte shrinkage, which were implicated in this study, could also be produced by dilution of the plasma volume, which could contribute to a fall in serum protein concentration to some extent (Das *et al*., 2004). Because of the hypoactivity caused by simazine toxicity, this easily available protein reserve fraction may be used up, resulting in a decrease in amount, nucleus shrinkage, and albumin vacuolization production (Hiran, 1996). Plotka *et al*. (1988) observed a similar finding when they exposed Cyprinus carpio to diazinon for 120 hours, which resulted in decreased enzyme activity (AST, ALT, ACP and ALP).

Stress-related tissue damage is indicated by a large rise in the activity of the key enzymes utilised for this purpose, transaminase (AST) (Tozaki *et al*., 2003). Higher AST activity and ALT activity at higher packing densities at 120 hours confirm that higher packing densities can be stressful. After being subjected to confinement stress, tilapia made a similar observation (Vijayan *et al*., 1997). An increase in transaminases is an immunological response that occurs in the early stages of illness (Chang *et al.,* 2005). The ALP activity in the blood serum rose in a concentration-dependent manner, with significant differences in several of the treated groups

compared to their respective controls. The release of isozymes from the damaged tissues could explain the higher levels of LDH in the haemolymph (Mishra and Shukla, 2003). AST and ALT levels were considerably greater in this study following acute exposure to LDH than in the control group, which confirmed earlier findings (Wagemann *et al.,* 2014).

CONCLUSION

The effects of Pisonia alba and Solanum xanthocarpum on haematological and blood biochemical responses, as well as the ability of freshwater fish Labeo rohita to recover after exposure to simazine herbicide, were investigated in this study. Aquatic habitats can affect aquatic animals in a variety of ways. Modern pesticides are problematic because they affect the physico-chemical properties of water, disrupt the ecosystem's delicate balance, enter food chains, and inflict physiological damage to aquatic fauna's essential tissues. Prolonged exposure to these toxins causes a bevvy of malformations and shortens the lifespan of aquatic species. Biochemical changes in the blood cause several alterations in the fish body. Finally, we conclude that simazine is highly toxic to fish and poses a life-threatening threat at both lethal and sublethal concentrations. Alterations in haematological responses can be used in bioassessment to determine ecotoxicological risks associated with pesticides such as simazine to certain fish. It had repercussions across the aquatic food chain.

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