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Effects of intraperitoneal administration of ivermectin on antioxidative enzymes and blood parameters in Rohu (*Labeo rohita)*

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ABSTRACT

Ivermection is widely being use for treatment of ectoprarastic infestation in fishes globally in carp farming. The post treatment mortality of the fish or subsequent slow growth becomes common due to unregulated dose. Therefore, present study was aimed to study the effect of acute sub- lethal ivermectin administration (96 hr) on antioxidative enzymes (catalase, superoxide dismutase, glutathione S-transferase) and hematological parameters like platelets, white blood cell (WBC), red blood cell (RBC), haemoglobin content, blood glucose and Nitro-blue tetrazolium (NBT) of a tropical freshwater fish, rohu (*Labeo rohita)*. The calculated LD_{50} value was found to be 1.81 mg/Kg at 96 h by probit analysis. The three sub lethal doses like 125 125 µg/Kg, 250 µg/Kg , 500 µg/Kg and one control (0.00 µg/Kg) were intraperitoneally administered. After 96 hours it showed significantly altered ($P < 0.05$) activities of anti-oxidative enzymes and hematological profile, in a dose dependent manner. The early gross sign like sluggishness and dark body were recorded during acute toxicity study. The result indicates ivermectin can impact negatively at higher doses to host rohu (*Labeo rohita*)

Keyword: Ivermectin, Intraperitoneal, *L rohita*, Hematological parameters, Anti-oxidative enzymes

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INTRODUCTION

Ectoparasites infestation is the biggest challenge in carp culture and causes huge loss to farmers. It has compelled the farmers to use various pesticides and anti-parasitic drugs. Among the various chemicals and drugs, ivermectin is the most frequently used by the farmers. Ivermectin belongs to a family of avermectins, one of the most recently developed antiparasitic agents isolated from the actinomycete, *Streptomyces avermitilis*. Ivermectin (22,23- dihydroavermectinB1a/22,23-dihydroavermectin B1b) is a broad-spectrum anti-parasitic drug. The known anti- parasitic effect is caused by paralysis of the pharyngeal muscles and paralysis of somatic muscles[1]. The paralyzing effects on the pharyngeal muscles are associated with the interaction of avermectins with glutamate-gated chloride (GluCl) channel receptors. The physiological role of GluCl in the pharynx is to mediate the action of glutamate released from pharyngeal motor neurons. Exogenous glutamate inhibits pharyngeal pumping, which is mimicked by Ivermectin [2]. Paralysis of somatic muscles, on the other hand, is associated with gamma-aminobutyric acid (GABA)-gated chloride channel receptors [1]. The same mechanism is involved in the proposed toxicity or associated altered health and production in fishes.

Argulosis associated alteration in hematological and antioxidant paramaetrs are enviable during severe infestation of Argulus as the fishes get wounded and the open wound becomes a site for secondary infection and osmotic imbalance in fishes, however the same is aggravated by the unregulated use of pesticides and the drugs[3] As the drug induce production of reactive oxygen species (ROS) [4, 5] and ROS may overwhelm cellular defenses and damage proteins, mostly the membranes proteins, lipids, and DNA[6]. The oxidative damage concurrent to damage of hematological parameters leads slow growth and mortality on farm during Argulosis treatment by ivermectin [7, 8].

The fishes like other vertebrate do comprise specific antioxidant enzymes, such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), glutathione peroxidase (GSH-Px; EC 1.11.1.9) and glutathione reductase (GR; EC 1.8.1.7). There is scarce information available pertaining to antioxidative status**,** and haemato-immunomodulatory status of *Labeo rohita* intraperitoneally administered with sub-lethal levels of Ivermectin. Since on farm level of drugs used for treatment of Argulosis in the carp culture system is high. So side effects associated to ivermectin use must be studied. Such studies has been recommended by the earlier authors like Hemaprashant et al., [9] against *Argulus siamensis* infestation in Indian major carp. The studies specially pertaining to effect of ivermectin on metabolic antioxidant and blood parameters of the fish at higher doses have been suggested. Hence, use of this drug should be done based on

systematic assessment of toxicity and physiological changes at sub lethal doses Thus, the aim of this study was to determine the effects of intraperitoneally administered Ivermectin on selected haemato-immunological parameters and oxidative stress in *Labeo rohita*.

MATERIALS AND METHOD

Experimental animals

In the experiment, one hundred and fifty fishes were randomly distributed in 5 distinct experimental groups with 10 fishes in each group. The fish of uniform size with initial weight ranging from 8 g to 20.5 g were stocked in each plastic tub. Experiment was designed for the four drug levels and one control. The intramuscular injection was followed for the 0.1 ml injected volume of ivermectin in propylene glycol. The experiment for study of LD_{50}, was designed after the range finding test with fishes around 8 gm size. *L. rohita* used for the experiment were obtained from the Array Farm Goregaon, Mumbai, India and maintained in the rectangular, plastic 150 L experimental tubs and acclimatized for 15 days prior to the experiment.

Experimental conditions

Round the clock aeration was provided to all the experimental tubs and water temperature was recorded to be in the range of 28.4 ˚C to 32.8 ˚C. The animals were fed ad libitum with experimental diet (30% crude protein) at 3% of their body weight till 48 hr. before the start of the experiment. Water quality parameters viz. dissolved oxygen and temperature (dissolved oxygen and temperature meter, Merck, Germany), pH (digital pH meter, LABINDIA, Mumbai), free carbon dioxide (titrimetric method, [10]; total hardness (carbonate hardness test kit, Merck, Germany), ammonia (at 635 nm by phenate method [10]), nitrite and nitrate (543 nm wave length [10]) were recorded during the experimental period.

Determination of Lethal Dose 50 (LD_{50})

A static non-renewable acute toxicity bioassay was conducted according to standard method (APHA, [10]) to determine LD50 of ivermectin in *Labeo rohita* fingerlings (average weight of 8 g) following exposure of 96 hours. The commercial animal grade ivermectin was purchased from veterinary drug supplier Goregaon, Mumbai, India. Initially, a range finding test was conducted to ascertain the range to be followed in the definitive test. Static non-renewable bioassay was conducted in triplicate for each concentration of ivermectin with 10 animals in each tub. No water exchange and nor feeding were done during the period of experiment. Percentage mortality was recorded at 24, 48, 72 and 96 hours interval. The maximum propyl glycol volume which was used for the dilution of the dosing concentrations was added to control group. The range of LD_{50} for *L.*

rohita fingerling (mean wt. 8 g) under above mentioned conditions was ascertained to lie below 2000 µg/Kg with safe value of 363 µg/Kg. Hence for the sub- lethal toxicity test, ivermectin doses such as 0, 125, 250, and 500 µg/kg body weight were taken. Percentage mortality was recorded at 24, 48, 72 and 96 hours interval. Dead fishes were removed from each tank immediately. The data obtained from the experiment was processed by probit analysis using a computer program, Basic LD50 version 1.1. [11]

Preparation of tissue homogenate

The organs of the fishes from the different groups were dissected carefully, weighed and kept on ice. The iced tissues were than homogenized with chilled sucrose solution (0.25 M) in a glass tube using Teflon coated mechanical tissue homogenizer (MICCRA D-9, Digitronic, Germany) to get 5 % homogenate and centrifuged at 5000 rpm for 20 min at 4 ºC. The supernatant was separated and stored at - 20 ºC for further analysis.

Biochemical assays

Antioxidant enzymes

Catalase

Catalase activity was estimated according to the method of Takahara et al. [12]. To 2.45 ml of phosphate buffer (50mM, pH 7.0), 50 µl of the tissue homogenate was added and the reaction was started by the addition of 1.0 ml of H_2O_2 solution, the decrease in absorbance was measured at 240 nm at 30 sec. intervals for 2 min. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as nmoles of H_2O_2 decomposed per min per mg protein

Superoxide dismutase

The SOD activity was estimated by the method of Misra & Fridovich [13]. The assay is based on the oxidation of epinephrine-adrenochrome transition by the enzyme. The reaction mixture consisted of 50 µl of sample, 1.5 ml phosphate buffer and 0.5 ml epinephrine. The solution were mixed well and immediately read the change in optical density at 480 nm for 2 min in a Shimadzu- UV spectrophotometer. One unit of SOD activity was the amount of protein required to give 50% inhibition of epinephrine auto oxidation.

Glutathione-s-Transferase (GST)

Gluthathione-S-transferase (GST; EC 2.5.1.18) activity was measured by spectrophotometric method of Habing et al. [14], using S-2, 4-dinitrophenyl glutathione (CDNB) as substrate. The method was based on the principle of formation of adduct of CDNB, S-2, 4-dinitrophenyl

glutathione was monitored by measuring the increase in absorbance at 340 nm against blank. The GST activity was expressed as nmol/min/mg protein.

Collection of blood

Each fish was anesthetized with clove oil at 50µl of clove oil per liter of water before taking blood from fish. Blood was withdrawn from *vena caudalis* of rohu using a medical syringe which was previously rinsed with freshly made 2.7% EDTA solution. Blood collected was immediately to test tube already coated with thin layer of EDTA powder (as an anticoagulant) and optimum shacking was applied in order to prevent clotting and hemolysis of blood [15, 38,39].

The blood was kept at low temperature in cooler until analyzed and analyzed within 6 h after collection. Hematocrit value (Hct %) was determined after centrifugation at 15,000 rpm for 3 min in a table centrifuge. Hemoglobin contents (Hb) were determined using more with reliable and accurate cyanomethemoglobin method as with Drabkin's solution [38, 39]. Red Blood Cell count (RBC), white blood cell count (WBC) and differential WBC counts were performed as described in Schaperclaus [16].

Total leucocytes count (WBC), Total erythrocyte count (RBC) and platelets

20 µl of blood was mixed with 3980 µl of WBC, RBC and platelets diluting fluid in a clean glass vial. The mixture was shaken well to suspend the cells uniformly in the solution. Care was taken that here were no air bubbles trapped. The numbers of cells were counted in four big squares under high power (40x) magnification of light microscope and expressed as the number per cubic ml.

Haemoglobin content

The haemoglobin level of blood was analyzed following the cyanmethemoglobin method using Drabkins Fluid (Qualigens). Blood (20 µl) was mixed with 5 ml of Drabkin's working solution. The absorbance was measured using a spectrophotometer at 540 nm. The final concentration was calculated by comparing with the standard cyanmethemoglobin (Qualigens Diagnostics) [38,39] . The hemoglobin concentration was then calculated as described in diagnostic kit.

Nitroblue tetrazolium (NBT) assay

Nitroblue tetrazolium assay was done by the method of Secombe [17] as modified by Stasiack and Bauman [18]. Fifty microliters of blood was placed into the wells of 'U' bottom microliter plates and incubated at 37^{0} C for 1 h to facilitate adhesion of cells. Then the supernatant was removed and the loaded wells were washed three times in PBS. After washing, 50 microliters of 0.2% NBT was added and plate was incubated for further 1 hr. The cells were then fixed with 100% methanol for 2-3 min and again washed thrice with 30% methanol. The plates were then air dried. Sixty microliters 2N potassium hydroxide and 70 microliters dimethyl sulphoxide were added into each

well to dissolve the formazon blue precipitate formed. The OD of the blue colored solution was then read in ELISA reader at 540 nm [39, 40] .

Statistical analysis

The data were statistically analyzed using statistical package of SPSS version 16, in which data were subjected to one-way ANOVA and Duncan's multiple range tests was used to determine the significant differences between the means at 5 % level of significance.

RESULTS AND DISCUSSION

Physio-chemical parameters of water

All the physico-chemical parameters of water were recorded and average values of all the treatments were found within the optimum range. Recorded dissolved oxygen concentration of all the experimental tubs were within the range of 6.2 to 8.4 mg L^{-1} and the water temperature ranged from 28.4 ^{0}C to 32.8 ^{0}C during the experimental period. The pH values were recorded within the range of 7.2 to 8.4 and free carbon dioxide in water was found to be negligible (1.4 to 3.0 mg L^{-1}) and the carbonate hardness was found to be 236 – 245 mg L^{-1}. The total ammonia content of all the experimental tubs were recorded before water exchange. It was found to be in the range of 0.14 to 0.19 mg L^{-1}. The nitrite – N and nitrate-N content were found to be in the range of 0.003 to 0.005 mgL^{-1} and 0.04 to 0.06 mg L^{-1}, respectively.

Table 1: Physio-chemical parameters of water during the experimental period of 96 hours for accessing the effect of intraperitoneal ivermectin administration study.

S. No.	Water parameter	Values range
1	Dissolved oxygen	6.2 to 8.4 mg L^{-1}
2	Temperature	28.4 ^{0}C to 32.8 ^{0}C
3	pH	7.2 to 8.4.
4	Dissolved CO_2	1.4 to 3 mg/l
5	Carbonate hardness of water	236 – 245 mg L^{-1}
6	Ammonia-N(mg/l)	0.14-0.19
7	Nitrite-N(mg/l)	0.003-0.005
8	Nitrate-N(mg/l)	0.04-0.06

LD_{50} Determination

The LD_{50} of the ivermectin to *L. rohita* fingerlings (8 gm) at different hour is given in table no. 2. The value at 24, 48, 72 and 96 hours comes 3.17, 2.31, 1.92 and 1.81 mg/Kg respectively. The safe value of was found to be 0.363 mg /kg with S- value of 1. 83 mg /Kg.

Table 2: LD_{50} value of ivermectin for *L. rohita* (8 gm) at different hours.

Period of Exposure	LD 50 (mg/kg)	95 % confidence interval		S-Value	Safe Level
		Lowe Limit	Upper Limit		
24 hrs	3.17	2.64	4.20		
48 hrs	2.31	1.99	2.65	1. 83	0.363
72 hrs	1.92	1.65	2.18		
96 hrs	1.81	1.41	2.19		

Enzymes of oxidative damage

Catalase and Superoxide dismutase (SOD)

Exposure of ivermectin had significant effect ($p<0.05$) on both gill and liver catalase activity in dose dependent manner compared to unexposed group at the end of experimental period after 96 h (Table 3). The highest activity was found in gill tissue than liver in ivermectin administered groups. However, no significant difference recorded in exposed group of gill tissue. However exposure of ivermectin had no significant effect ($p<0.05$) on liver SOD activity compared to unexposed group. But apparent difference could be seen in gill SOD activity. The highest activities of SOD in both tissues were found in 250 µg/kg group (Table 3).

Table 3: Activity of SOD and Catalase enzymes in liver and gill tissue of *Labeo rohita* at 96 hour administered with graded level of intra-peritoneal ivermectin injection.

Doses	SOD		Catalase	
	Gill	Liver	Gill	Liver
0 µg/kg	50.43 ± 3.32^{b}	74.13 ± 2.10	28.50 ± 2.72^{a}	14.35 ± 1.50^{a}
125 µg/kg	46.95 ± 2.42^{ab}	72.39 ± 3.72	124.12 ± 9.16^{b}	66.84 ± 8.98^{c}
250 µg/kg	43.86 ± 2.72^{ab}	76.61 ± 5.32	121.25 ± 8.34^{b}	68.88 ± 8.81^{c}
500 µg/kg	41.88 ± 1.13^{a}	71.00 ± 3.44	124.65 ± 4.56^{b}	44.25 ± 1.64^{b}
P value	0.001	0.209	0.002	.001

Different superscripts in the same column signify statistical differences ($p<0.05$) (mean ± S.E) (n = 6) Catalase: Units/mg protein) SOD: Super oxide dismutase: Units/mg protein) (n = 6)

Glutathione s- transferase (GST)

The brain, liver and intestine GST activities of fishes after 96 h exposed to different doses of ivermectin are shown in Table no.4. Exposure of ivermectin had significant effect ($P<0.05$) on all tissues in dose-dependent manner compared to unexposed group. Significantly increased trend with increasing dose of ivermectin in both brain and liver were recorded. The highest activities of GST in liver and brain were 1.29±0.06 and 3.73±0.11 respectively at 500µg/kg level group and lowest in control group. Similarly in the intestine, the highest specific activity of GST was also recorded in 500 µg/kg (3.87±0.39) and lowest in control (0.49±0.07).

Table. 4: Activity of GST enzymes in brain, liver and intestine tissue of *Labeo rohita* at 96 hour administered with grade level of intra-peritoneal ivermectin injection.

Doses	GST		
	Brain	Liver	Intestine
0 µg/kg	0.69±0.07^{a}	0.63±0.05^{a}	0.49±0.07^{a}
125 µg/kg	1.60±0.06^{b}	0.78±0.08^{a}	2.61±0.16^{b}
250 µg/kg	3.34±0.38^{c}	1.16±0.03^{b}	2.99±0.41^{b}
500 µg/kg	3.73±0.11^{c}	1.29±0.06^{b}	3.87±0.39^{c}
P value	0.003	0.007	0.001

Different superscripts in the same column signify statistical differences (p<0.05) (mean ± S.E.) (n = 6), GST: Glutathione S- transferase- Units/mg protein.

Haematological parameters

WBC, RBC and platelets

The WBC count (x 10^5 cells/mm^3) of the different ivermectin doses showed significant difference (P<0.05) in all treatment groups compared to control. Significantly higher values were recorded in dose dependent manner against control groups. The highest value was recorded in 250 µg/kg group and lowest in control group. Similarly there was significant difference (P<0.05) in RBC count among control and different treatment groups in a dose-dependent manner after 96 h. Significantly higher value was found in 250 µg/kg (1.77±0.07) group and lowest in control group (1.21±0.05). However, Platelets count in different administered groups showed a significant progressive fall, with highest value in control group and the lowest in group of 500 µg/kg in dose dependent manner (Table 5).

Table 5: Blood parameters of *Labeo rohita* at 96 hour exposed to grade level of ivermectin through intra-peritoneal injection.

Doses	WBC	RBC	Platelets	Hemoglobin	Glucose
0 µg/kg	181.03^{a}±3.4	1.27^{a}±0.06	124.33^{c}±6.49	4.91^{a} ±0.13	52.4^{d}±0.13
125 µg/kg	212.1^{b}±2.3	1.54^{b}±0.10	56.67^{b}±4.10	6.31^{b} ±0.12	17.56^{b}±0.12
250 µg/kg	253^{c}±8.6	1.77^{c}±0.07	36.33^{a}±2.19	7.10^{c} ±0.15	17.14^{b}±0.15
500 µg/kg	226.4^{b}±5.3	1.42^{ab}±0.02	22.67^{a}±2.60	5.84^{b} ±0.23	4.87^{a} ±0.23
P value	0.01	0.01	0.01	0.01	0.01

Values in the same column with different superscript differ significantly (P<0.05);

Platlets, WBC; 10^3 cells/ mm^3, RBC, million cells/ mm^3, Haemoglobin, gm/100 ml of blood; glucose , mg/dl of blood.

Data expressed as Mean ± SE, n = 3

Hemoglobin and blood glucose

The haemoglobin count (x 10^5 cells/mm^3) of the different treatments is given in Table 5. There was significant difference (P<0.05) was found in all treatment groups compared to control.

Significantly higher values were found in all ivermectin administered groups in dose dependent manner. The highest value was recorded in 250 µg/kg (7.10±0.15) group and lowest in control (4.91±0.13) administered group which was significantly different (P>0.05) from all groups. But with administration of ivermectin, blood glucose values were recorded to decrease significantly (P<0.05) compared to control after 96 h exposer in dose dependence manner. Significantly higher glucose level was recorded in control (52.4±0.13) and lowest level in 500 µg/kg (4.87±0.23) groups (Table 5).

Lysozyme and Nitroblue tetrazolium (NBT) assay

Lysozyme activity was significantly increased by ivermectin administration in all treatment group compared with control (P<0.05). Significantly lowest activity was recorded in in control group and the highest in group with dose of 125 µg/Kg. There was significant difference (P<0.05) in NBT activity (Table 6). NBT activity was induced by ivermectin injection, significantly (P<0.05). Among all treatment, 250µg/kg (3.15±0.12) administered group found to have highest value and lowest in (1.15±0.07) group.

Table 6: Serum lysozyme and NBT value of *Labeo rohita* fingerlings exposed to grade level of ivermectin through intraperitonial injection at end of 96 hour

Doses	Lysozyme	NBT
0 µg/kg	8.86 ^{a}±1.90	0.10 ^{a}±0.01
125 µg/kg	29.52 ^{d}±2.47	0.13 ^{a}±0.004
250 µg/kg	16.93 ^{ab}±3.15	0.19 ^{b}±0.01
500 µg/kg	22.20 ^{bc}±2.22	0.25 ^{c}±0.07
P value	0.01	0.01

Values in the same column with different superscript differ significantly (P<0.05);

NBT: Nitrobluetetrazolium, units: optical density at 630 nm

Lysozyme: change in OD/ min/mg protein

Physiochemical parameters of water

The various physio-chemical parameters of water studied during the experimental period are in the recommended optimal range of *L. rohita* [19]. Therefore, the impact of water quality parameter in response to treatment was negligible. This is being reconfirmed by lack of mortality of L. rohita in the control group during 96 h trial.

Acute toxicity and LD_{50}

In the present study, LD_{50} value at 24, 48, 72 and 96 hours comes 3.17, 2.31, 1.92 and 1.81 mg/Kg respectively and the safe value of was found to be 0.363 mg /kg with S- value of 1. 83 mg/Kg. It has been found by earlier studies that the difference between treatment dosage and toxic effects of Ivermectin in Atlantic salmon varies [20]. A commonly recommended treatment dose of Ivermectin

is 0.05 mg/kg, given twice weekly in feed, with no treatments in winter.The toxicity response in animal is determined by factors like weight, age and species of the animal. So, the determined 96 h LD_{50} was to be 0.5 mg/kg [20] with a 96 h LC_{50} set at17 µg/L in Atlantic salmon[21]. This reflects the varying degree of toxicity in different species of fishes.

Symptoms of Ivermectin toxicity indicates neurotoxicity in fishes also as in salmon it triggered loss of appetite, dark skin coloration, lethargy and erratic swimming behavior [20]. These symptoms were also prominent in rohu in present study leading yellow coloration of belly and pelvic portion which was prominent at end of experiment in most of the fishes. This can be attributed due to the elimination of the Ivermectin through intestinal secretion and potential damage of intestinal mucosa. While at the end of experiment loss of scale or loose scale were also noticed in all Ivermectin treated group.

The higher sensitivity to Ivermectin in some fishes like rohu can be attributed to their poor evolutionary adaptation, the poor blood brain barrier ability to restrict the permeation of drug in brain. Further, the high brain concentrations of Ivermectin found in Atlantic salmon, *Salmon salar*, and gilthead sea bream after oral and intraperitoneal administration, respectively [22, 23] point towards a less selective barrier in fish as compared to mammals.

Enzymes of Oxidative Damage

For maintaining normal homeostasis and physiological condition, cells produce reactive oxygen species (ROS) such as H_2O_2 leading to cell damage and cell death. Living organisms are protected from ROS by several defense mechanisms, including antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, etc. Catalase (CAT) activity plays important role in antioxidative defense of the cell by reducing H_2O_2. When the rate of ROS generation exceeds that of their removal, oxidative stress occurs [15].

In the present study**,** exposure of ivermectin had significant effect ($p<0.05$) on both gill and liver catalase activity in dose dependent manner compared to unexposed group at the end of experimental period after 96 h (Table 3). The highest activity was found in gill tissue than liver in ivermectin administered groups. In a similar study done in rabbits infested with *Psoroptes cuniculi*, doramectin caused an increase in CAT activity [24]. In their study, the increased activity of CAT was found in the liver, kidney, heart of rabbit exposed to Ivermectin at 0.02 mg/kg. Similarly, in the present study there was dose dependent increase in CAT activity due to Ivermectin exposure. This indicates excessive, H_2O_2, reactive oxygen species (ROS) and reactive nitrogen species (NOS) free radical production due to ivermectin toxicity [15, 25, 26].

Phagocytosis in fish is the result of phagocytic cells, which include mononuclear phagocytes (monocytes and macrophages) and neutrophils. Phagocytes produce large quantities of superoxide anion (O_2^-) upon stimulation with a variety of agents and pathogen[15]. In the present study SOD activity was not significantly different from the control group in liver. While the values in gill tissues were found to decrease significantly in ivermectin administered group compared to their unexposed counterpart.

In the present study, Ivermectin had significant effect ($P<0.05$) on both liver and brain, intestine GST activity compared to the control group. Exposure of ivermectin had significant effect ($P<0.05$) on all tissues studies (brain, liver and intestine) in dose-dependent manner compared to untreated control group. Significantly increased trend with increasing dose of ivermectin in brain, liver and intestine were recorded. The enzyme activity bears strong correlation (r2= 0. 64 in liver and r2= 0.931 in brain and r2= 0. 89 in intestine) with Ivermectin level, indicating the potentiality of the enzyme activity as biochemical marker for the Ivermectin induced stress and toxicity (fig.1). The same trend has been reported by our earlier publication [15], where immersion dose of ivermectin bears strong correlation with GST enzyme activity (r2= 0.906 in liver and r2= 0.921) in brain. Several other authors have reported, GSTs are a family of enzymes that play a significant role in detoxification of xenobiotics such as insecticides [27]. Increased activity of delta and epsilon class GSTs had been linked to resistance to organophosphates, DDT and pyrethroids [28]. GSTs have also been associated with another macrocyclic lactone resistance in mites, with elevated GST activity observed in abamectin-resistant *Tetranychus urticae* [29]. Additionally, *Caenorhabditis elegans* isolates selected for Ivermectin resistance *in vitro* show increased transcription of GSTs and glutathione conjugate multidrug related protein (MRP) transporters, together with reduced intracellular glutathione, suggesting its ivermectin induced acceleration of drug conjugation and removal [30].

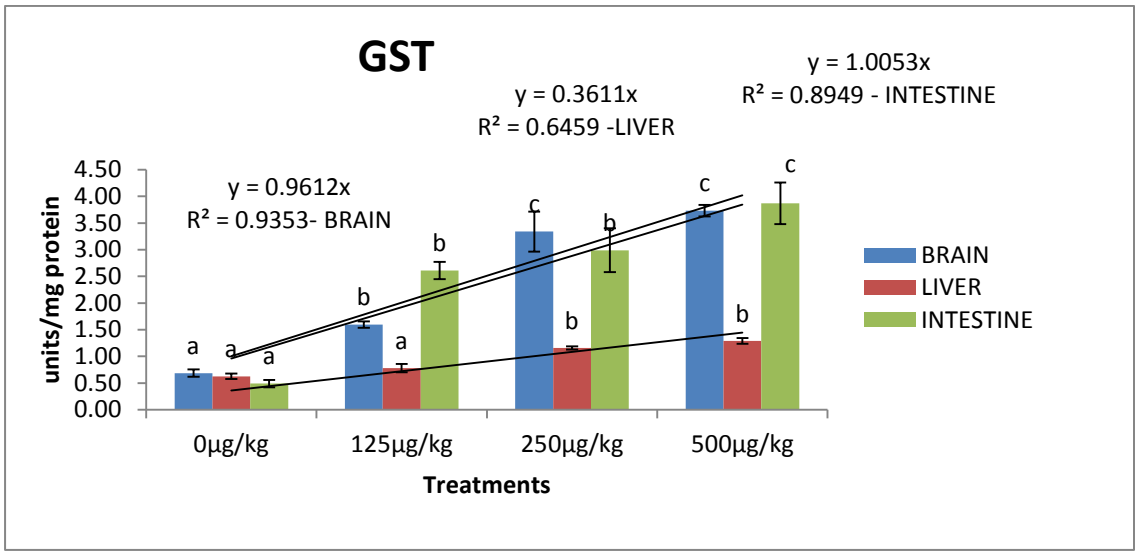

Figure.1: Activity of GST enzymes in brain, liver and intestine tissue of *Labeo rohita* exposed to grade level of ivermectin through intra-peritoneal injection.

Hematological Parameters

In the present study, The WBC and RBC count of the different ivermectin doses showed significant difference (P<0.05) in all treatment groups compared to control. Significantly higher values were recorded in dose dependent manner against control groups. However, Platelets count in different administered groups showed a significant progressive fall, with highest value in control group and the lowest in 500µg/kg in dose dependent manner (Table 5). This could be the body own mechanism to compensate the oxygen deprivation caused due to branchial paralysis and slow opercular pumping. But the same mechanism fails to compensate at the higher toxicity level. Reduced hemoglobin content and elevation in WBC and RBC count was observed in long term toxicity study by Sarma (2003), which is in agreement with Katharios et al. [23]. The platelets count ($x10^3$ cells /mm^3) of the different exposure to different concentration of the Ivermectin showed dose dependent decrease. The dose dependent decrease of the platelets counts could be due to vitamin K deficiency triggered by the Ivermectin. Similar results were reported with the drugs like adbendazole and Ivermectin [31].

There was significant difference haemoglobin content (P<0.05) was found in all treatment groups compared to control. Significantly higher values were found in all ivermectin administered groups in dose dependent manner. But with administration of Ivermectin, blood glucose values were recorded to decrease significantly (P<0.05) compared to control after 96 h exposer in dose dependence manner. The impact can be for adaptive mechanism for coping of decreased branchial

pumping and resulting oxygen deprivation due to paralytic impact of the ivermectin on branchial muscle.

While the glucose decrease has been well explained by ivermectin triggers the inactivation of the animal leading the anabolic inactivation and it might lead low serum glucose. This is clear, as the control or unexposed group also showed less glucose compared to earlier report in the species 32, 33. But dose dependent decrease in the ivermectin exposed group clearly reflect impact of ivermectin in serum glucose reduction. Similar results have already been reported earlier that, the antiparasitic drug ivermectin has been identified as a novel ligand for farsenoid X receptors (FXR) recently [34]. The study reported that ivermectin treatment can reduce serum glucose and cholesterol levels by directly targeting FXR. It was found by them that the crystal structure of ivermectin complexes with the ligand-binding domain of FXR revealing a unique binding mode of ivermectin in the FXR ligand-binding pocket, including the highly dynamic activation factor -2 (AF-2) helix and an expanded ligand-binding pocket. The reduction in serum glucose concentration following the administration of albendazole and its combination with ivermectin has already been reported and explained to be due to inhibition of the uptake and transport of glucose by albendazole [37]. Albendazole has been reported to exert its effect on tubulin polymerization leading to loss of cytoplasmic microtubles and the ability to take up and transport glucose [37].

The serum Lysozyme activity was significantly increased by ivermectin administration in all treatment group compared with control ($P<0.05$). Significantly lowest activity was recorded in in control group and the highest in group with dose of 125 µg/Kg. There was significant difference ($P<0.05$) in NBT activity (Table 6). NBT activity was induced by ivermectin injection, significantly ($P<0.05$).

The finding is similar to the finding by Aklakur et al.[15] where ivermectin immersion in same species lead the increase in the NBT value and in contrast to Siwicki et al. [35] where, exposure to chemotherapeutics caused significant inhibition in the activity of NBT in the blood. In present study the there is significant increase ($P<0.01$), which may be due to stress, leading ROS production or by activation of free radicals [25]. In a similar study on the oxidative burst of eosinophilic granulocytes, the result revealed a dual, dose-dependent modulatory in vitro effect of the investigated anthelmintic drugs on the respiratory burst of eosinophilic effector cells indicating that these compounds may modulate host defense in vivo. Inhibitory effects on the generation of toxic oxygen intermediates were demonstrated for ivermectin at concentrations higher than 200 ng/ml (0.5 micro M). An increased production of the reactive oxygen metabolites was demonstrated at low doses of ivermectin (20-40 ng/ml; corresponding to 0.02-0.04 micro M).[36]

CONCLUSION

In conclusion the higher doses of ivermectin could be toxic in fish. Hematological and antioxidant parameters clearly showed a shift of homeostasis during the application high dose of ivermectin above safe value and reflected significant increase in the level of antioxidant enzyme, indicating adverse impact on antioxidant defense and function of vital tissues and physiology of rohu fingerlings. Moreover, the effect on modulation of hematological parameter of the fish is also indicating that higher doses of exposure of ivermectin lead harsh impact.

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