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Evaluation of effect to Ivermectin exposure on metabolic enzymes and blood glucose of rohu (*Labeo rohita)* fingerlings

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ABSTRACT

The ivermectin is mostly used an anti-parasitic drug for treatment of Argulosis and other ectoparasite diseases. The present study was aimed to investigate the effect of acute exposure (96 h) of different concentration of ivermectin in the water on metabolism and blood glucose level of rohu (*Labeo rohita*) fingerlings. The graded level of ivermectin such as 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 and 17.5 µg/L in water were selected and a test was conducted in triplicate for each concentration with 10 fishes in each tub of 100 L capacity. At the end of the experimental period of 96 hrs, activity of alkaline phosphatase (ALP) and blood glucose level were significantly decreased ($P < 0.05$) with respect to concentration of ivermectin, while the activities of lactate dehydrogenase (LDH) and transaminase enzymes (alanine amino transferase and aspartate amino transferase) were significantly increased ($P < 0.05$) in exposed groups compared to the control. A negative correlation was recorded between the level of ivermectin concentration in water and blood glucose level. The gross observations noticed were sluggishness, inactivity, dark body color, disorientation and imbalance in swimming.

Keyword: Ivermectin, Rohu, Blood Glucose, Toxicity, Metabolic Enzymes.

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INTRODUCTION

Aquaculture is the fastest growing food production sector with the sustained rise in growth of finfish aquaculture, but past two decades have experienced several problems related to disease prevalence. The disease outbreak that has been estimated by several workers around the world and it accounts for the huge loss to the sector. Parasitic infestation especially ecto-parasites is the major problem in carp culture. Argulus and similar arthropods are very rampant along with some of Monogenian and Digenian trematodes. Argulus so-called fish lice in freshwater and the sea lice in marine cages have been problems of finfish culture [1]. These parasitic infestations lead the poor growth, mortality and subsequent loss of production. The problem has been so pronounced that it has led to the disposal of tons of the pesticide and unregulated use of this drug in the water leading poor growth and mortality in the farm. Among the pharmaceutics used, ivermectin is the most preferred drug for controlling Argulosis in tropical countries especially India.

Ivermectin belongs to the family of avermectins, one of the most recently developed anti-parasitic agents isolated from the actinomycete, Streptomyces avermitilis[2]. Ivermectin (22, 23 Dihydroavermectin B1) was initially used to control sea lice on farmed salmonid in Ireland and possibly Scotland[3]. Ivermectin is a broad-spectrum anti-parasitic drug that effectively controls nematodes and parasitic arthropods in a wide variety of host and has been proposed as an alternative to the organophosphorous pesticides for the controlling sea lice mainly in salmonid aquaculture [3]. But same has not been proven to be safe technique due to slow growth, frequent mortality during the treatment due to unregulated use of ivermectin. Till now no systematic work on its efficacy for treatment, toxicity and pharmacokinetics' has been reported to establish its potential use and safety in Indian carps. There are few data available on the toxicity of ivermectin to fish caused due to the higher dose than the recommended. Therefore, dose-dependent toxicity, become an important aspect of study, particularly its impact on metabolic enzymes and glucose utilization. Hence, considering these the present study was aimed to investigate the toxicity of ivermectin to *Labeo rohita* fingerlings.

MATERIALS AND METHOD

Experimental animals

In the present experiment, rohu (*Labeo rohita)* fingerlings were obtained from the Array Farm Goregaon, Mumbai, India. Two hundred and forty fishes were randomly distributed in 8 distinct experimental groups with 30 fishes in each group. Fishes of uniform size with initial weight

ranging from 5.35 g to 5.75 g were stocked in 24 plastic experimental tubs (rectangular, 300 L) and maintained for 15 days prior to the experiment for acclimatization (10 fish/tub).

Experimental conditions

Round the clock aeration was provided to all the experimental tubs and water temperature was recorded to be in the range of 26.4–28.8 ºC. 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, total hardness (carbonate hardness test kit, Merck, Germany), ammonia (at 635 nm by phenate method), nitrite and nitrate (543 nm wave length APHA, [36]) were recorded during the experimental period. Technical grade (97 %) ivermectin (Malti Enterprises India) was applied in water at different concentration after dissolving in ethanol to dissolve in water.

Determination of toxicity

In this trial, the animals were exposed to a range of concentration in logarithmic scale such 20, 40, 60, 80 and 100 µg/L, first for finding the range of toxicity study. The range of LC_{50} for *Labeo rohita* fingerling (mean wt. 5.4 g) under above mentioned conditions was ascertained to lie below 20 µg/L, since all the fishes died within the 18 hours. Hence for the definitive test, ivermectin concentrations such as 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 and 17.5 µg/L were selected to test. The mortality was recorded at, 24, 48, 72 and 96 hours interval. The maximum ethanol volume used for the dilution of the dosing concentrations was added to control group. The fishes were not fed during the experimental period and dead fishes were removed from tank immediately.

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.

Enzymes of Carbohydrate Metabolism

Lactate dehydrogenase (LDH)

The LDH (E.C. 1.1.1.27) activity was assayed in different tissues by the method of Wroblewski and Ladue [4]. The total 3 ml of the reaction mixture comprised of 2.7ml of 0.1M phosphate buffer

(pH 7.5), 0.1ml of NADH solution (2 mg NADH dissolved in 1ml of phosphate buffer solution), 0.1ml of tissue homogenate and 0.1ml of sodium pyruvate. The reaction was started after addition of substrate sodium pyruvate. The OD was recorded at 340nm at 30 seconds interval. The enzymatic activity was expressed as units/ mg protein/ min at 25^0 C, where 1 unit was equal to Δ0.01OD/ min.

Alkaline phosphatase (ALP)

The assay was done by method of Garen and Levinthal[5] .The assay mixture comprise of the 0.2 ml bicarbonate buffer (0.2 M), 0.1 ml of 0.1 M $MgCl_2$, 0.1 ml tissue homogenate, 0.5 ml of distilled water and the 0.1 ml freshly prepared 0.1 M Para-nitro phenyl phosphate. Incubate the reaction mixture in water bath at 37 degree for 15 minutes and then stop the reaction by adding 1.0ml of 0.1 N NaOH. After incubation take OD at 410nm. The unit is expressed as mole of PNP released/mg protein/min at 37º centigrade.

Transaminase Enzymes

Aspartate amino transferase (AST)

The AST (E.C.2.6.1.1) activity was assayed in different tissue homogenates as described by Wooten[6]. The substrate comprised of 0.2M D, L- aspartic acid and 2mM α-ketoglutarate in 0.05M phosphate buffer (pH 7.4). In the experimental and control tubes, 0.5ml of substrate was added. The reaction was started by adding 0.1ml of tissue homogenate. The assay mixture was incubated at 37^0C for 60 minutes. The reaction was terminated by adding 0.5ml of 1mM 2, 4 dinitrophenyl hydrazine (DNPH). In the control tubes the enzyme source was added after DNPH solution. The tubes were held at room temperature for 20 minutes with occasional shaking. Then 5ml of 0.4ml NaOH solution was added, the contents were thoroughly mixed. After 10 minutes, the OD was recorded at 540nm against blank.

Alanine amino transferase (ALT)

The procedure adopted for ALT (E.C.2.6.1.2) activity was same as for AST activity except the substrate comprised of 0.2 M D, L- alanine instead of aspartic acid.

Blood Glucose

Glucose was estimated by the method of Nelson and Somogyi [7] as described by Oser [8]. Blood sample (0.5 ml) was deproteinised by mixing with 4.75 ml of zinc sulphate followed by addition of 4.75 ml of barium hydroxide. The solution was mixed vigorously and filtered using a filter paper and the filtrate was collected in a dry test tube and 1 ml of alkaline copper sulphate was added to it. The test tubes were placed in a boiling water bath for 20 min. The test tubes were

then cooled to room temperature and 1 ml arseno-molybdate reagent was added. The absorbance was recorded at 540 nm against blank.

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 physio-chemical parameters of water like temperature (^{0}C), dissolved oxygen (mg L^{-1}), pH, carbonate hardness (mg L^{-1}), free carbon dioxide (mg L^{-1}), ammonia (mg L-1), Nitrite N (mg L^{-1}), Nitrate N (mg L^{-1}) were recorded and were observed to be within the optimum range of requirements for the fish. Indian major carps can thrive well at a temperature range of 18-38^{0}C [9], which supports the range of temperature, 26.4^{0}C – 32^{0}C observed during the entire experimental period, to be optimum for *Labeo rohita*. The pH of water in all the experimental groups ranged from 7.2-8.4 and was within the acceptable range (6.5-9.0) [10]. The dissolved oxygen level in different experimental tubs was recorded to be within the range of 6.2 to 8.4 mg L^{-1}, which is within the optimum range 6-7 mg L^{-1} for cyprinids [11]. From the above result, it is assumed that dissolved oxygen was optimum throughout the experimental period, which is due to continuous aeration. In the present study, the carbon dioxide concentration was found to be negligible, and so did not have any adverse effect on the survival of the experimental animals. This may be due to low biomass stocking and daily water exchange during the experimental period.

The carbonate hardness was found to be 236-245 mg L^{-1} during the experimental period**.** Schaperclaus [12] suggested water having a hardness of 250 mg L^{-1} or above as satisfactory for the growth of fish**.** The suggested value of ammonia in water ranges from 0 to 1.0 mg L^{-1} [,8], which is in agreement with the recorded range (0.14-0.27 mg L^{-1}**)** in the present study. Nitrite-N concentration was recorded in the range of 0.001-0.005 mg L^{-1}, which was well within the permissible range for pond aquaculture [13]. In the present study all the water quality parameters were in the normal range so the single factor attributing variation should be the ivermectin concentration.

Table 1: Physiochemical parameters of water during the experimental period of 96 hours for main inlet water taken for toxicity studies.

S. No.	Water parameter	Values ranged
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^{-1}
5	Carbonate hardness of water	236 – 245 mg L^{-1}
6	Ammonia-N	0.14-0.19 mg L^{-1}
7	Nitrite-N	0.003-0.005 mg L^{-1}
8	Nitrate-N	0.04-0.06 mg L^{-1}

Acute toxicity

The cumulative mortality percentage in different treatment groups at different hours of 24, 48, 72 and 96 are given in table 2. LC_{50} values of the ivermectin to *L. rohita* fingerlings (5.4 gm) at 96 hrs. was 7.9 µg /L. There was dose dependent response of mortality with confluence of response at higher dose (table 2). The level of toxicity explains that the *L. rohita* fingerlings are susceptible to ivermectin.

Table 2: Cumulative mortality percentage of Ivermectin exposed L. *rohita* fingerlings (5.4 gm) at different hours. (n=3 for each concentration)

Treatments Ivermectin µg/L	24 hours	48 hours	72 hours	96 hours
0.00	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
2.50	$3.33^{ab} \pm 3.33$	$10.00^{ab} \pm 5.77$	$13.33^{ab} \pm 8.82$	$20.00^{ab} \pm 0.00$
5.00	$13.33^{abc} \pm 3.33$	$26.67^{bc} \pm 6.67$	$26.67^{bc} \pm 6.67$	$26.67^{b} \pm 6.67$
7.50	$20.00^{bc} \pm 5.77$	$30.00^{c} \pm 10.00$	$36.67^{c} \pm 13.33$	$40.00^{bc} \pm 5.28$
10.00	$30.00^{cd} \pm 10.00$	$53.33^{d} \pm 6.67$	$63.33^{d} \pm 3.33$	$63.33^{cd} \pm 3.33$
12.50	$30.00^{cd} \pm 5.77$	$60.00^{d} \pm 5.77$	$60.00^{d} \pm 5.77$	$60.00^{d} \pm 5.77$
15.00	$46.67^{d} \pm 3.33$	$60.00^{d} \pm 0.01$	$66.67^{d} \pm 3.33$	$73.33^{de} \pm 3.33$
17.50	$46.67^{d} \pm 8.82$	$66.67^{d} \pm 3.33$	$76.67^{d} \pm 3.33$	$86.67^{e} \pm 3.33$
p- values	0.001	0.001	0.001	0.001

Metabolic enzymes

Lactate dehydrogenase

Exposure of ivermectin had significant effect ($p<0.05$) on muscle and liver LDH compared to unexposed (control) group (table 3) and activity was significantly increased due to ivermectin exposure. In muscle the LDH activities increased with the exposure level up to the LC_{50}, after which it decreased. However, in liver highest activities recorded at the lowest dose of exposure, which decreased gradually.

Table 3: Activity of LDH and ALP enzymes in liver and muscle tissue of *Labeo rohita* fingerlings exposed to graded level of ivermectin concentration in water.

Concentrations (µg/L)	LDH		ALP	
	Muscle	Liver	Intestine	Liver
0.0	0.93±0.13^{a}	1.30±0.13^{a}	60.45±3.98^{d}	31.29±2.86^{c}
2.5	2.90±0.21^{cd}	6.03±0.58^{c}	24.4±4.13^{c}	21.22±2.99^{b}
5.0	3.20±0.35^{d}	4.76±0.55^{b}	17.46±2.95^{abc}	11.79±2.17^{a}
7.5	2.50±0.12^{c}	4.03±0.48^{b}	16.00±2.13^{abc}	6.83±1.82^{a}
10.0	2.54±0.11^{c}	4.76±0.56^{b}	14.53±2.62^{ab}	8.24±1.12^{a}
12.5	1.75±0.09^{b}	4.39±0.22^{b}	15.43±1.86^{abc}	11.03±2.66^{a}
15.0	2.69±0.10^{cd}	4.12±0.23^{b}	8.23±1.16^{a}	11.71±2.80^{a}
17.5	2.53±0.10^{c}	3.85±0.29^{b}	18.17±3.76^{bc}	11.66±0.92^{a}
P value	0.028	0.014	0.001	0.001

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

LDH[1] (Lactate dehydrogenase): specific activity expressed as Units/ min/ mg protein at 37^{0}C

ALP[2] (Alkaline phosphatase): specific activity expressed as micro mole of Para nitro phenyl phosphate used / min/ mg protein at 37^{0}C

Data expressed as mean ±SE, n=6.

In the present study exposure of ivermectin had significant effect (P<0.05) on muscle and liver LDH compared to unexposed group. The increase in LDH activity may be due to the higher production of lactate, which is the preferred substrate for gluconeogenesis in fish [14, 15]. Such a condition also indicates oxygen limited condition in the cell [16]. This is the condition which prevails in the ivermectin toxicity due to paralytic action and respiratory depression [2]. The higher dose of ivermectin administration leads to reduction in LDH activity. This could be the inability of the body compensatory mechanism to cope with stress and failing of protective mechanism [17].

Alkaline phosphatase

Alkaline phosphatase activity showed the significant (p<0.05) and sustained decrease in intestine as well as in liver of fishes of all exposed group compared to control group. The highest level of activity in intestine was recorded in control group, it decreased to the lowest in dose level 15.0 µg/L. But in liver, trend is biphasic, the highest level of activity was recorded in control group, which decreased to the lowest at LC_{50} dose and later increased (table 3).

ALP is a membrane bound enzyme found at bile pole of hepatocytes and also found in pinocytic vesicle and Golgi complex. It is present on all cell membranes where active transport occurs having the function of hydrolase and transphosphorylase. It is often employed to access the integrity of plasma membrane and metabolic conjugation of drug in liver and excretion in intestine require energy supply. Therefore the alkaline phosphatase activity was significantly

affected by different level of exposure of ivermectin. Alkaline phosphatase activity decreased in all exposed group in intestine as well as the liver ($p<0.05$) compared to control. The highest activity was recorded in intestine and liver of control group and the lowest activity was recorded in group exposed to 15 µg/L. Decrease in ALP activity may be taken as an index of hepatic parenchymal damage and hepatocytic necrosis. According to Okawa inhibition of ALP reflects alteration in protein synthesis and uncoupling of oxidative phosphorylation[18]. The similar decrease in ALP activity of the exposed fish was found in ALP in the liver and kidney of catfish, *Heterpneustes fossilis* after toxication with cadmium [19]. This decrease may be due to the damage and dysfunction of the liver. The decrease in ALP by stressors probably indicates an altered transport of phosphate.

Amino transferase enzymes

Aspartate amino transferase (AST) and alanine amino transferase (ALT) are important aminotransferase enzymes that redistribute amino nitrogen among the amino acids. Exposure of ivermectin had no significant effect ($p<0.05$) on muscle AST activity but had significant ($p<0.05$) increase in AST activity of liver compared to control group and liver AST activity was substantially highest in the highest concentration of ivermectin exposed fish and the least in unexposed group. Exposure of Ivermectin led significant increase ($p<0.05$) in ALT activities of both, muscle and liver compared to control group. The unexposed group had the least ALT activity in both liver and muscle. (Table 4). AST transfers the amino group of aspartic acid to α-keto glutarate and converts to glutamic acid forming oxaloacetate. ALT transfers the amino group from alanine to α-keto glutarate forming glutamic acid and pyruvate. Alanine is the principal amino acid released from muscle tissue during starvation. It is an important substrate for hepatic gluconeogenesis, and alanine transamination is required for the proper maintenance of fasting blood glucose concentrations. Apart from this the amino acids are deaminated or transaminated to produce TCA cycle intermediates, this also helps in energy production in the form of ATP [20].

In Ivermectin toxicity there is GABA (gamma-aminobutyric acid) production in brain, which work through glutamate gated chloride channels leading to hyperpolarization and inhibition of the nervous conduction across the nerve and interneuron synapses [21,22]. GABA is produced from glutamic acid by enzyme glutamate decarboxylase. While in GABA shunt conversion glutamate to 2 oxo glutaric acid is reversible and bidirectional reaction which give GABA production and same time GABA degradation by GABA transaminase to produce succinic semialdehyde (SSA)

and subsequently succinic acid by SSA- Dehydrogenage [23]. But to support the forward reaction of GABA production glutmate is used, which in turn is produced by the aminotransferase enzymes [24]. The these transferase activities are found to be predominantly in liver to promote glutamate production and metabolism via intermediary metabolism and GABA shunt as shown in the figure 3

In the present study, the exposure of ivermectin had not significant effect (P>0.01) on muscle but had significant (p<0.05) increase in AST activity in liver compared to the unexposed (control) group with the highest activity in liver of fishes of the highest ivermectin concentration group (17.5 µg/L). Whereas the unexposed group has least activity in liver and highest in muscle.

Similarly exposure of ivermectin had significant effect (p<0.05) on both muscle and liver alanine transferase enzyme activity under study. The increase in ALT activity in muscle appears to be dose independent compared to the unexposed group. While the value was substantially highest in liver of fishes exposed to the highest concentration (17.5 µg/L) and in muscle the highest activity was found at exposure of 10 µg/L, after that the ALT activity in muscle decreased with increase in level of Ivermectin. This is in agreement that the AST and ALT enzyme activity increase in tune with increasing stress of ivermectin in present experiment, which in turn produce glucose to cope up with energy demand by gluconeogenesis in liver and production of glutamate and subsequently GABA from glutamate. Beside the glutamate It is obvious that glutamate synthesized are used for glutamine production, glutamine synthetase and glutaminase constitute a potential ammonia scavenging cycle, and the two enzymes are commonly present in different cells and this seems to be particularly important in the liver and in the central nervous system. During the time of hypoxia or poor opercular pumping during ivermectin toxicity in fishes when ammonia level raises, it could be a protectant to the brain cell from ammonia toxicity. However stress induced increase in the aminotransferase level is common and similar observations have been reported in *Labeo rohita* [25] and *Cyprinus carpio* [16]. It suggests increased demand for energy causing higher gluconeogenesis in the liver same time increased demand of glutamate for GABA shunt.

Similarly, Jin have reported in nature publication that there is nuclear receptor based suppression of the glucose level after Ivermectin administration, which may be a cause for TCA Cycle and gluconeogenesis activation for brain energy demand and hypoglycemia leading more activity of transferase enzymes in liver [26]. Such changes in enzymes activities resulting from toxicant or contaminant affects in various organs of fish have been reported [27, 28, 29] very commonly. The alterations in fish enzyme activities are aimed at maintaining homeostatic equilibrium in the

presence of these toxicants which are known to disrupt physiological and biochemical processes [30]. Similarly in earlier studies involving 400µg/kg ivermectin in a 14-day course revealed a minimal transient increase in serum and liver aminotransferases [31].

Table 4: Activity of AST and ALT enzymes in liver and muscle tissue of *Labeo rohita* fingerlings exposed to grade level of ivermectin concentration in water.

Concentrations (µg/L)	AST		ALT	
	Muscle	Liver	Muscle	Liver
0.0	17.67±2.38^{b}	18.52±1.20^{a}	8.06±0.+66^{a}	6.50±083^{a}
2.5	18.49±1.55^{b}	32.36±2.01^{bc}	10.76±0.86^{a}	16.08±0.61^{cd}
5.0	16.88±1.89^{b}	33.08±2.58^{bc}	18.44±1.72^{b}	11.99±1.17^{b}
7.5	13.16±1.15^{ab}	29.08±1.26^{b}	15.81±1.37^{b}	11.70±0.54^{b}
10.0	10.16±1.31^{a}	29.88±2.46^{b}	18.84±1.20^{b}	13.77±1.00^{bc}
12.5	13.63±1.03^{ab}	28.62±2.68^{b}	15.84±0.55^{b}	15.04±1.30^{c}
15.0	17.25±3.57^{b}	38.52±2.63^{cd}	17.93±2.98^{b}	15.22±0.78^{c}
17.5	13.19±0.82^{ab}	41.39±2.16^{d}	15.74±0.79^{b}	18.70±1.21^{d}
P value	0.052	0.001	0.003	0.002

Mean values bearing different superscripts under each column vary significantly (P<0.05)

ALT: specific activities expressed as nano moles of sodium pyruvate formed/mg protein/minute at 37^{0}C.

AST specific activities expressed as nano moles of oxaloacetate released/min/mg protein at 37^{0}C

Data expressed as mean ±SE, n = 6.

Blood Glucose

The acute exposure to ivermectin lead significant decrease (P<0.01) in the level of blood glucose than control group. The significant dose dependent decrease in blood glucose has been found among exposed groups. The highest level of blood glucose was found in control group and the lowest blood glucose level in concentration 17.5 µg/L groups (Figure 1).

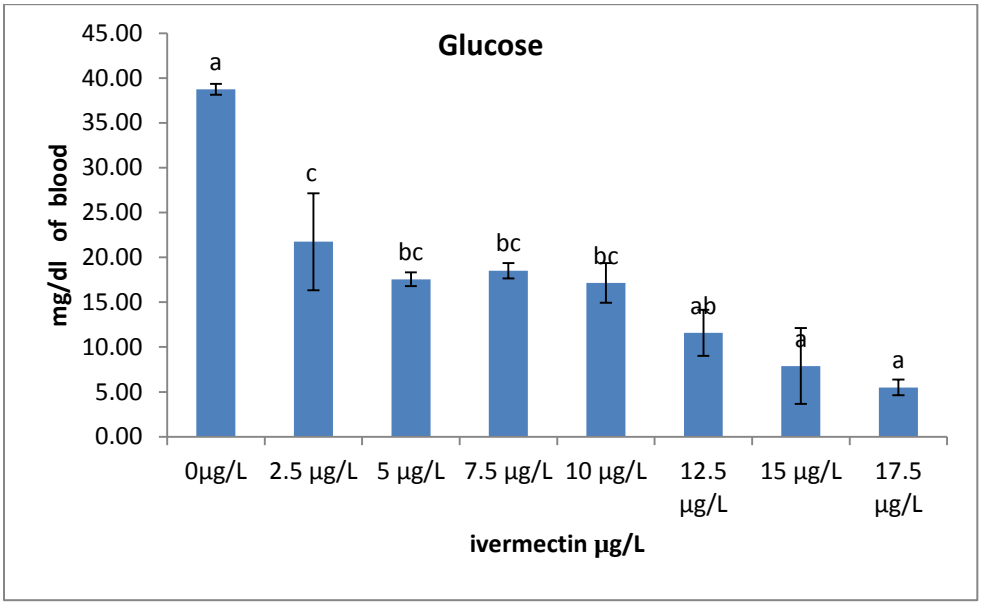

Figure 1: Blood glucose content of *Labeo rohita* fingerlings exposed to different Ivermectin concentrations as experimental groups at the end of 96 hours.

Blood glucose content of rohu fingerlings at the end of experiment was determined and it is expressed in milligram per deciliter of blood, values with different letter differ significantly ($p<0.05$)

Glucose is the major source of energy for living cell and due to stress and glucagon production catabolism of glucose increases. As general adaptor symptoms to cope stress, energy demand and utilization of the glucose by intermediary metabolism and TCA cycle for glutamate production as shown in Figure 2 In The present study, acute exposure to ivermectin led significant dose dependent decrease of among the exposed groups ($P<0.01$) than the unexposed group (control). The 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 show less glucose compared to earlier report in the species [32, 33, 34]. 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 [26]. 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 [35]. 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 [35].

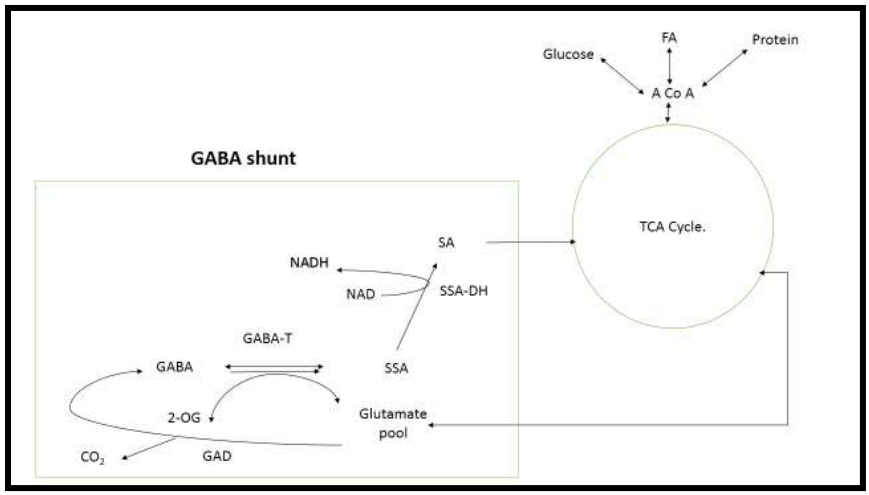

Figure 2: GABA Shunt and intermediary metabolism linking of amino acids and glucose.(GABA ; gama amino butyric acid, GABA-T ;GABA transaminase, 2-OG; 2- Oxo Glutaric Acid, GAD; Glutamate Decarboxylase, SSA; Succinic semi aldehyde, SSA-DH; SSA Dehydrogenase, SA ; Succinic Acid, AcoA; Acetyl coA, FA ; short chain fatty acids

CONCLUSION

In summary the ivermectin can alter the metabolism, precisely the glucose utilization, during bath treatment when used at high concentration in water solution. Moreover, record of decreased ALP activity a may be taken as an index of hepatic damage and hepatocytic necrosis. Inhibition of ALP reflects uncoupling of oxidative phosphorylation system. Overall all these studied parameters of the fish also shows that higher doses of exposure of ivermectin leads harsh and toxic impact.

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