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Hematology and serum biochemical changes in response to change in saline concentration in fresh water fish *Notopterus notopterus*

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ABSTRACT

The present investigation aim was to study the hematological and serum biochemical changes under increased saline medium in the fresh water fish *Notopterus notopterus*. The fish was able to thrive well in higher well in higher saline medium up to 0.16%. The exposure to saline medium was for a period of one month. After the termination of exposure to saline medium, the fish blood was collected and proceeded for hematological and serum biochemical studies. The result indicates that there was no change in the blood glucose, urea, where as there was an increase in the blood protein, triglycerides and cholesterol along with hemoglobin, RBC, WBC, creatinine, enzymes like SGPT, SGOT, alkalinephosphatase. The blood electrolytes like sodium, potassium and calcium also increased in experimental fishes.

***Keywords*:** Saline medium; Fresh Water fish; *Notopterus notopterus*; hematology

1. INTRODUCTION

Fishes are subjected to stress conditions very often in the natural environment and have developed physiological and biochemical adaptations to cope with that or minimize or

eliminate the deleterious effects, which are called, stress response. The stress response is divided into primary, secondary and tertiary responses (Goos and Consten, 2002; Ham *et al.*, Davis 2004). Stress hormones (catecholamines and corticosteroids) are considered as primary responses and physiological changes are considered as secondary responses. The change in the somatic growth and population is considered as tertiary responses. Stress disturbs the fine internal balance, homeostasis and has further detrimental effects on behavior, growth, reproduction, immune function and disease tolerance. (Chen *et al. 2004*).

Chen *et al.* (2004) describes that evaluation of hematological parameters has provided a tool for facilitating fish health management and Lerman (2004) and Koeypudsa (2007) states that blood chemistry parameters are used as important indicators of physiological stress responses in fish. Catecholamine, cortisol, haematocrite, hemoglobin, glucose, lactate, amino acid and liver glycogen are classical stress indicators (Guerriero 2002, Morales *et al.*, 2005, Koeypudsa *et al.*, 2006). Peterson and Meador study on effects of salinity on freshwater fishes in coastal plain drainages in the southeastern U.S. are summarized their study as follows:

- Some studies suggest that most freshwater fishes cannot reproduce in salinities greater than about 3 to 4%. However, few studies have addressed this issue, and our understanding of this critically important aspect of freshwater fish life history is limited.
- Distributional surveys, salinity tolerance and preference test, and behavioral studies indicate that many freshwater fish species can withstand extended exposure to salinities <9%. However, age and acclimation history can influence this generalized pattern.
- Inability of freshwater fish to survive chronic exposure to salinities greater than about 9% is attributed to the lack of efficient bronchial and renal mechanisms. Osmotic stress is often accompanied by changes in Na^+, K^+, ATP as activity, carbon dioxide levels, and acid-base balance.
- Salinity cannot be viewed as a solitary factor influencing freshwater fish; a number of other factors in saline environments contribute to observed responses in fresh water fish species and communities. These other factors include habitat complexity, extreme physicochemical conditions characteristic of the environment at the interface between fresh and salt water, and biologic interactions, such as competition and predator-prey relations.
- In low-salinity habitats, as salinity increases to the point where the blood becomes isotonic, growth may be reduced in some species (relative to growth in fresh water). However, the effect of salinity on growth of fresh water fishes is not always negative, and the methodology used to determine growth must be standardized.

The effect of stress resulting from aquaculture practices on fish and methods of minimizing such effects have received considerable attention through the years (Barton and Iwama, 1991; Mazik *et al*., 1991; Cech *et al.*, 1996). The stress induced by common practices such as handling, crowding, transport, or poor water conditions can increase the incidence of diseases and mortality and salinity fluctuations undoubtedly impose stress on the physiology of the fish which can modify their structure and structure and is therefore an important factor affecting the economics of aquaculture (Tsuzuki *et al*., 2001; Usha, 2011). The evaluation of biochemical parameters has provided a tool for facilitating fish health management (Chen *et al.* 2004). Finally Petrso and Meador concluded that a new research focus is needed to further refine conceptual model of the ecological role of freshwater fishes in saline environments.

The object of their study was to evaluate the secondary stress response of *Notopterus notopterus* as the studies related to this fish on haematological study is scant. Hence the present study is undertaken.

2. MATERIAL AND METHODS

Fresh water fish *Notopterus notopterus* caught 30 fishes by a local fisher man from Bheema river (16°24'36"N, 770°17'6"E) near Gulbarga and brought to the laboratory. They were kept in 30L fiberglass tanks containing 30 liter aerated water. The fish were allowed to acclimate for a period of one week before start of the experiment. Among 30 fishes, 15 fishes were gradually and continuously exposed to saline medium starting from 1gm up to 50gm and final saline medium contains 50 g/30 liters of water and kept for 30 days. Equal number of fish was kept as control. After the termination of the experiment exposing to saline medium for 30 days, the fish blood was collected from the caudal region with the aid of 2-3cm disposable plastic syringes and a 21 gauge disposable hypodermic needle and transferred to plastic tubers. The sample was then mixed gently and thoroughly and blood samples were transferred to laboratory for testing the serum glucose, serum protein, serum triglycerides and serum cholesterol.

Serum glucose was measured by GOD-POD, End point Assay and Kinetic Assay method, Serum protein was measured by using the modified Biuret method, GPO/PAP method (SR Kit) for the determination of triglycerides, (HOD/PAP method for the determination of cholesterol in blood serum. Hemoglobin was measured using the standard cyanmethemoglobin method described by Baker and Silverton (1976). Haematocrite value was determined by standard Wintrobe method, and expressed in percentage. Blood sample were loaded in Wintrobe tubes and spun in a centrifuge at 3000 rpm for 5 min and measured. Creatinine was determined by modified Jaffe's method Kinetic test without deproteinisation according to the Jaffe's method. Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) activity was assayed following modified International Federation for Clinical Chemistry (IFCC) method using commercial kit. Serum alkaline phosphatase activity was determined by kinetic assay (IFCC) method using commercial kit. Sodium and Potassium are determined by colorimetric method. Calcium was determined by Modified Arsenazo method. RBC and WBC was enumerated in an improved Neubauer Haemocytometer method described by Dacie and Lewis (1984).

2. 1. Calculation

Salinity was found to be 0.16% or 1666.66 ppm, by following standard formula.
ppm = mass solute (mg) / volume solution (L), ppm = 50×1000/30 = 1666.66 ppm, conversion of ppm to %., % = 1666.66×100/1000000 = 0.16%.

3. RESULTS

The fresh water fish *N. notopterus* was exposed to 0.16% saline medium and on exposure for 30 days no mortality occurred and found that the fish can tolerate higher concentration of salinity even in the environment. The fish were comfortable with proper

movement coming to the surface for gulping air. The results obtained have been presented in the Table 1.

There is no change in the serum glucose. The serum protein in control fish was 6.23 gm/dl, while it was in experimental fish 8.43 (Fish exposed to 0.16% salinity). There was 2.20 gm/dl increased in fish exposure to salinity. The serum triglycerides in control fish were 308.25 mg/dl, in experimental fish it was 366.2 mg/dl. There was considerable increase in the serum triglycerides in experimental fishes when compare to control fishes. The plasma cholesterol of control fish was 232.75 mg/dl. The experimental fish serum cholesterol was found to be 450.5 mg/dl. The serum cholesterol has been increased two fold in the experimental fishes.

Table 1. Showing changes in the hematological and serum biochemical parameters of the fresh water fish, *Notopterus notopterus* exposed to chronic saline medium.

S. No	Parameters	Control Group			Experimental group		
		Mean	SD	SE	Mean	SD	SE
1	Ser. Glucose (mg/dl)	72.16	±0.9	0.4	72.83	±0.7	0.3
2	Serum Protein (gm/dl)	6.23	±0.6	0.30	8.43	±0.7	0.3
3	Serum Triglycerides (mg/dl)	308.25	±1.1	0.5	366.2	±0.6	0.3
4	Serum Cholesterol (mg/dl)	232.75	±1.9	0.8	450.5	±1.8	0.8
5	Blood urea (%)	80.1	±0.9	0.4	80.17	±0.7	0.3
6	Haematocrite (%)	41.83	±1.1	0.5	42.66	±1.5	0.6
7	Creatinine(mg/dl)	0.78	±0.05	0.025	0.89	±0.7	0.3
8	SGPT (U/L)	29.8	±0.51	0.23	44.66	±0.5	0.2
9	SGOT (U/L)	60.61	±0.55	0.24	102.15	±0.5	0.24
10	Alkaline phosphates (IU/L)	90.13	±0.68	0.30	102.16	±0.16	0.07
11	Sodium (mmol/l)	77.56	±0.58	0.26	100.41	±0.66	0.29
12	Potassium(mmol/l)	13.56	±0.90	0.40	17.0	±0.70	0.31
13	Calcium(mg/dl)	07.65	±0.28	0.12	10.35	±0.83	0.37
14	RBC (millions/cc.mm)	04.2	±0.01	0.006	04.49	±0.01	0.006
15	WBC (millions/cc.mm)	4001	±1.50	0.67	6002.16	±2.22	0.99
16	Hemoglobin (%)	14.2	±0.17	±0.07	14.71	±0.13	±0.05

[All values are expressed as means ±SD, N = 6)

4. DISCUSSION

The concentration of total protein is used as a basic index for health status of fish (Mustafa et al., 2009) and it is an important non specific immune parameter (Magnadotti *et al.*, 2006). In the present study there is an increase in the serum protein in experimental fishes. The control fish serum protein was 6.23 g/dl and (experimental fish) fishes exposed to 0.16% was 8.43 gm/dl. According to Rakovac *et al*, (2005), increased concentrations of total protein can be caused by structural liver alterations reducing aminotransferase activity impaired control of fluid balance. Serum Kuck *et al., (*2012), also reported that there was increase in total protein as salinity increases. Their result was, at 50% (Sw) Salinity there total protein was 50.4±5.3, at 100% (Sw) salinity the total protein was 40.0±7.9150% (Sw), at 150% salinity the total protein was 35.6±8.9, at 200% (SW) salinity the total protein was 35.7±6.0. On the contrary Mohammad Reza et al., (2012) reported that serum protein levels decreased with increase in salinity. In their study serum protein was 48.33±6.51 at 12 ppt salinity, 53.00±4.00 mg dl^{-1} at 6 ppt and 64.00±4.58 mg dl^{-1} at 0 ppt. Marcel et al., (2009) reported that, in cells of stressed organisms there is an increase I n the production of heat shock protein or stress protein which are required to assist the folding of nascent polypeptide chains, act as a molecular chaperone and mediated the repair and degradation of altered or denatured protein to maintain homeostasis. Protein are the most important and abundant macromolecules in living beings, which play a vital role in architecture and physiology of the cell and in cellular metabolism. Proteins also play an important role in the metabolism and regulation of water balance. (Heath *et al.*, 1995). All biological activities are regulated by enzymes and hormones, which are also proteins. Assessment of protein content can be considered as a diagnostic tool to determine the physiological phases of the cells. (Kapila *et al*., 1991). The survival ability of animals exposed to stress mainly depends on their protein synthesis potential (Padma *et al.*, 2012). The decrease in protein content was probably due to reduced/perturbation of microsomal protein synthesis. The degradation of protein suggests the increase in proteolytic activity and possible utilization on their product for metabolic purpose and cause damage (Padma *et al.* 2012). The quantity of protein is dependent on the rate of protein synthesis or on the rate of its degradation (Catharios *et al.*, 2004).

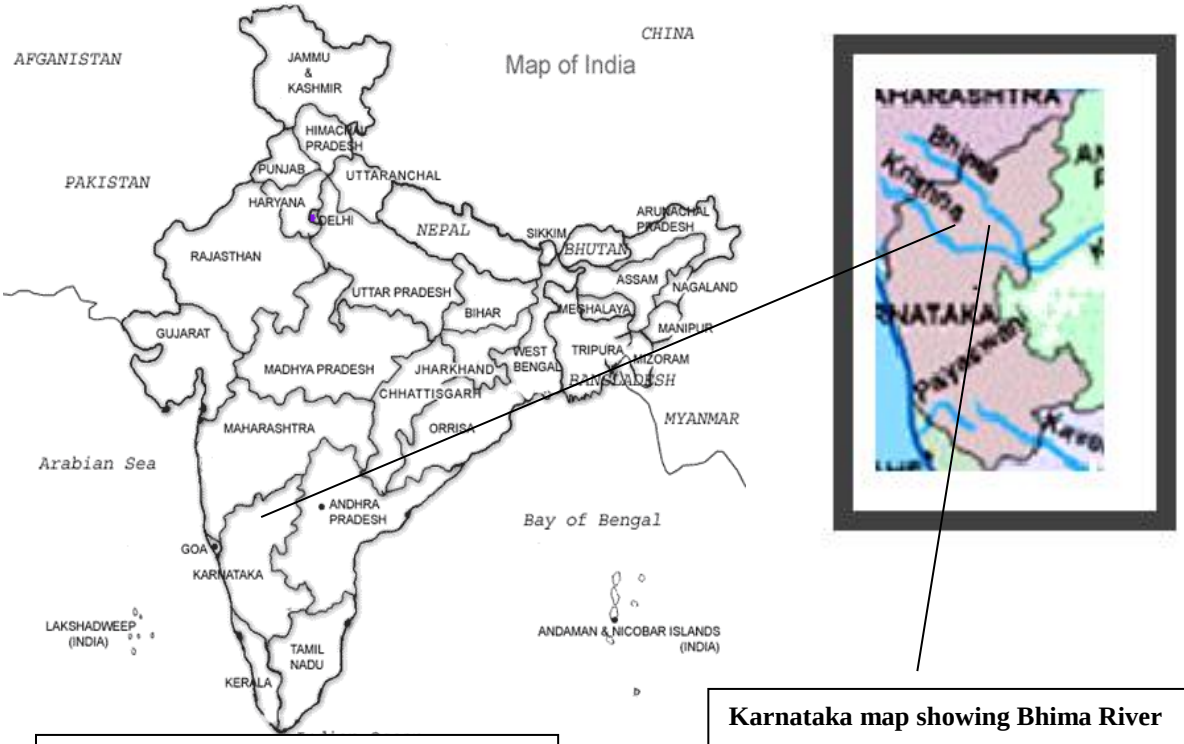
In the present investigation there was no change in the glucose level of fishes exposed to salinity (0.16%). Gelis Tarihi (2004) reported that, there was no change in the glucose level of tilapia, *Saratherodon melanotheron* exposed to 9 ppt salinity, but glucose level was relatively high in fish exposed to 18 ppt for 72 hours and the glycogen level in liver tissue significantly reduced ($P < 0.05$), this shows that higher salinity results in high rate of glycogenolysis activity to meet high energy demand and that resulted in reduced glycogen levels in liver. From the study of Gelis Tarihi (2004) it is clear that as salinity increases glucose level increases, and fishes have the ability to withstand some saline stress, this is revealed in the fish exposed to 9 ppt salinity, it was similar to those of controlled fishes. The same result was also observed in our study in the fish *Notopterus notopterus*. There was no change in the blood glucose level at 0.16% salinity. Hyperglycemia is an expected result of stress or exhaustive exercise in fishes (Barton and Iwama 1991; Hrubec *et al*., 1997). Blood glucose levels may elevate immediately but catecholamines which facilitate gluconeogenesis (Baton and Iwama, 1991). It is known that the degree of hyperglycemia may change depending on the type of stress and the sampling times (Rotlland *et al*., 1997).

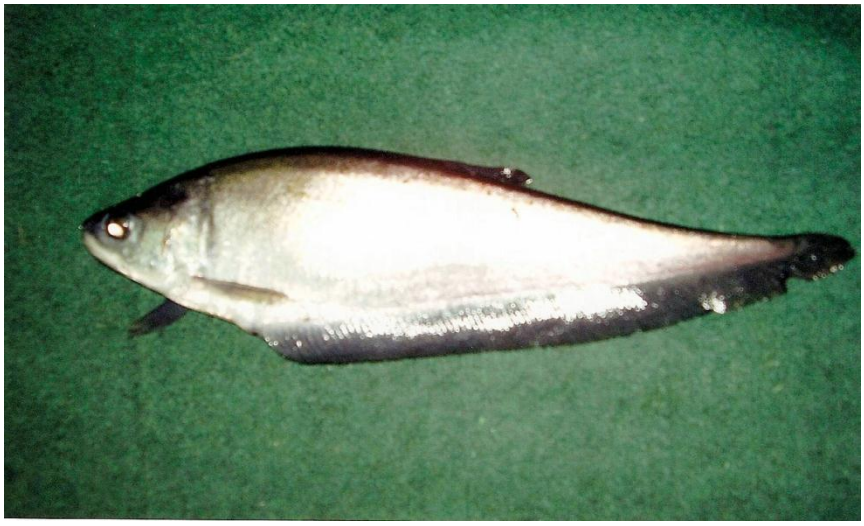
In the fish *Notopterus notopterus*, serum triglycerides level increase in experimental fishes by 57.95 mg/dl. In experimental fish the triglycerides level was 366.2 mg/dl and in control fishes 308.25 mg/dl. Arjon *et al.* (2009) also reported that serum triglycerides increased in *Solea senegalensis,* to the saline condition that is 208 mM at *15 ppt* and 10.7 mM at 39 ppt. the result of Serma *et al.*(2012) was completely reversed when compare that serum triglycerides significantly decreased in parallel with increase in salinity. The result of their report was 2.7 mM at 8 ppt and 1.1 mM at 24 ppt. And the same result was also reported by Mohammad Reza *et al.* (2012) in gold fish. In their study also the serum triglyceride level decreases with increase in salinity. At 0 ppt the serum triglyceride level was 306.67 mg dl^{-1} and at 6 ppt it was 294 $mmdl^{-1}$ and at 12 ppt it was 257.67 $mgdl^{-1}$. However, in our study the triglycerides increased on exposure to saline medium and this may be because of increased in metabolic rate and demand for lipid accumulation. Cholesterol is a necessary compound of the structure of the cell membrane. In fishes exposed to 0.16% salinity (*Notopterus notopterus*) was 450.5 mg/dl. It is twofold more than that of control fishes. The same result is also for Yamawaki *et al.* (1986) and Rancho *et al*, *(*1997). According to them increased concentrations of cholesterol in serum can be a result of damages to liver or kidney. Elevated levels of cholesterol indicate disorders of lipid and lipoprotein metabolism, especially liver disease, (Kavadias *et al.* 2004); it is known that the serum biochemistry can be influenced by many biotic and abiotic factors such as water temperature, seasonal pattern, food, age and sex of the fish (Annino *etal.*, 1976). In the present investigation the serum cholesterol level was two fold increases in the saline exposed fish compare to control fishes. Therefore a high concentration of blood cholesterol may suggest the dietary lipid imbalance (Wedemeyer *et al.*, 1990). This lipid imbalance in *N. notopterus* may be because of saline stress that leads to the imbalance of lipid metabolism.

It has been observed that blood parameters such as haematocrite, hemoglobin and RBC cells are related to environmental factors such as water temperature and salinity. Effect of salinity changes on hematological parameters of the tiger shark, *Pangasius hypothalamus* has been observed and reported that hemoglobin increases, RBC, WBC count reduces and it was suggested that reduction of RBC and WBC due to sudden changes in saline medium (Usha, 2011). In the present study both hemoglobin (Hb) and haematocrite (Hct) increases with increasing salinity. Plaut (1998) states that, the Hct and RBC increased with increasing salinity levels up to 8% salinity afterwards, they subsequently decreased as the salinity increased further. Similar results were observed in the fish, *N. notopterus*.

The study done by Martinez Alvarez *et al*. (2002) also supports the Plaut (1998) observation. Thus at the beginning of exposure to a hyperosmotic environment, the fish would lose water passively and thereby undergo increases in the concentration of Hb and HCT. Afterwards, the compensatory increase in water ingestion would provide transitory dilution of the blood parameters. Finally these would return to initial values as a result of the rest of the osmoregulatory mechanisms, which act to re-establish the extracellular volume (Mortinez Alvarez *et al*. 2002).

According to Jones and Randall (1978) increase in Hb and Hct concentration as a result of increased muscle activity and the concomitant movement of water from plasma to muscle. According to Yamamoto *et al.* (1980), Wells and Weber (1990) increase in Hb and Hct is because of induction of splenetic contractions and the subsequent mobilization of stored erythrocytes.





***Notopterus notopterus* fish collected from Bhīma River**

Figure 1. Showing Map of Bhīma River and Notopterus fish.

(The Bhīma River is a major river in South India. It flows southeast for 861 kilometers through Maharashtra, Karnataka, and Telangana States, before entering the Krishna River, Basin area: 70,614 km² , Length: 861 km, Source: Temple, Country: India, Mouth: Krishna River-This information collected from Wikipedia, map is also downloaded from Wiki images and edited according to the need.)

Effect of salinity on hematological parameters of *Pangassius hypothalamus* was studied by Usha (2011), the RBC count in normal fish was 5.2 million/cc.mm and the fish in salinity was 3.5 million/cc.mm, WBC in normal fish was 6.7 million/cumm and in the test fish it was 4.9 million/cumm, the reduction in the RBC and WBC count were due to the sudden change from fresh water to saline water. In *N. notopterus* the WBC and RBC count is increased on exposure in comparison to control fishes. Our results are completely deviated from the results of R. Usha (2011), may be because of *N. notopterus* is more saline resistant than *Pangassius hypothalamus* thus indicating that with increasing RBC, WBC, Hct and Hb concentration, the fishes may be trying to cope up with the changing salinity condition of the water. Kamal sarma *et al.*, studied alkaline phosphatase (ALP) activities in muscle and liver after exposing saline water *Clarius batrachus*, in their study the ALP value decreased with increased salinity that is in 0% salinity the ALP activity in muscle and liver are 2.900±0.37 and 16.773±0.68 respectively, this data indicates that there is a considerable decrease in the ALP activity from 0% to 8% salinity. From the above result it is obvious that ALP concentration decreases with increase in the salinity. However, in our study the ALP value in control fish it is 90 Iu/L and experimental fish is 102 iu/l that is ALP value is higher than in control fishes. According to Madhuban and Kaviraj (2009) decrease of alkaline phosphates activity might result in altered –cell membrane, decrease in glycogen level and Inhibitory effect on cell growth and proliferation.

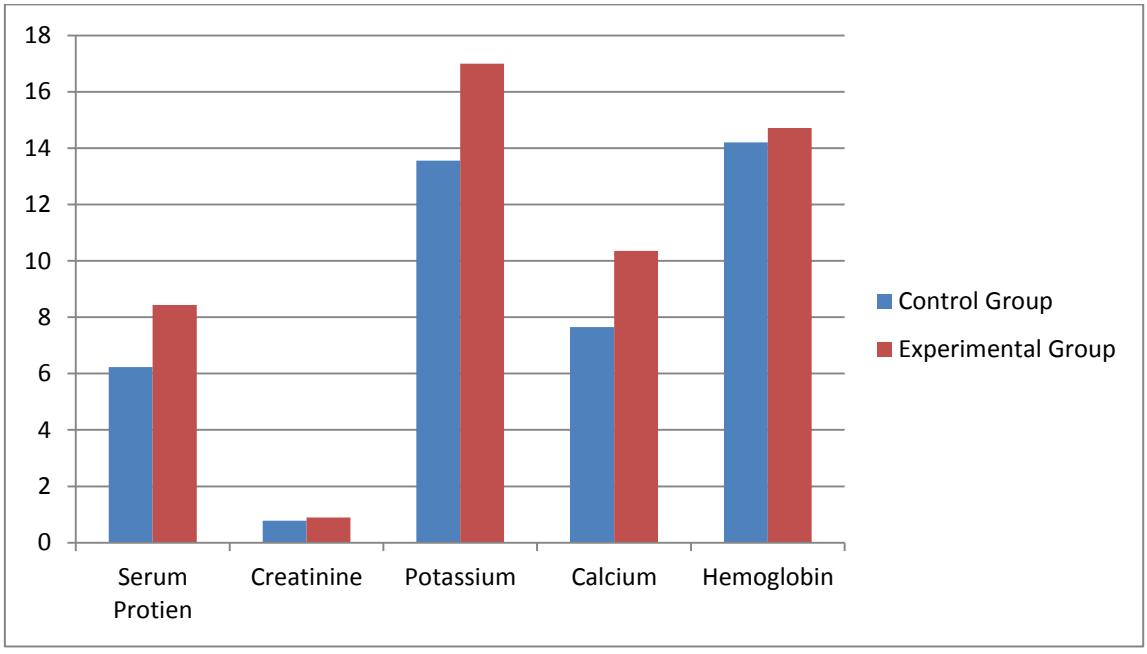


Figure 2. Showing blood biochemical parameters in control and experimental fish.

The ALP value of our study indicates that (more in experimental fishes compared to control) fishes are trying to adopt in the changing environmental conditions that is increased salinity from 0% to 0.16% so that they can maintain its normal –cell membrane transport, glycogen level, cell growth and cell proliferation as in the case of *Oreochrmi*s because of saline [Rao, 2006]. In the fish, *N. notopterus*, the concentration of sodium, potassium, and

calcium increased on exposure to saline water. The similar results were also reported by Chreck (1990), Gelis Tarihi (2004). The stress response of *N. notopterus* to increased salinity changes at this level (electrolyte level) can reflect loss of homeostasis and demonstrate a compensatory response due to the disruptions of homeostasis. According to Schreck (1990) stress leads to a hydro mineral imbalance (Mazeaud *et al.*, 1997) and rectification of the stress related osmotic dysfunction places an energetic load on the fish. In addition electrolytes serve as a general measure of osmoregulatory dysfunction (Robertson *et al.*, 1987). In our study there is an increase in the concentrations of creatinine, SGPT; SGOT in experimental fishes indicates the effect of saline medium on kidney. In the fish, tilapia, *Oreochromis niloticus* (Gelis Tarihi, 2004) exposed to 18 ppt salinity for 72 hours were relatively high creatinine, SGPT, SGOT when compared to control. Similarly, the plasma glucose level of the fish exposed to 9 ppt saline water was similar to control.

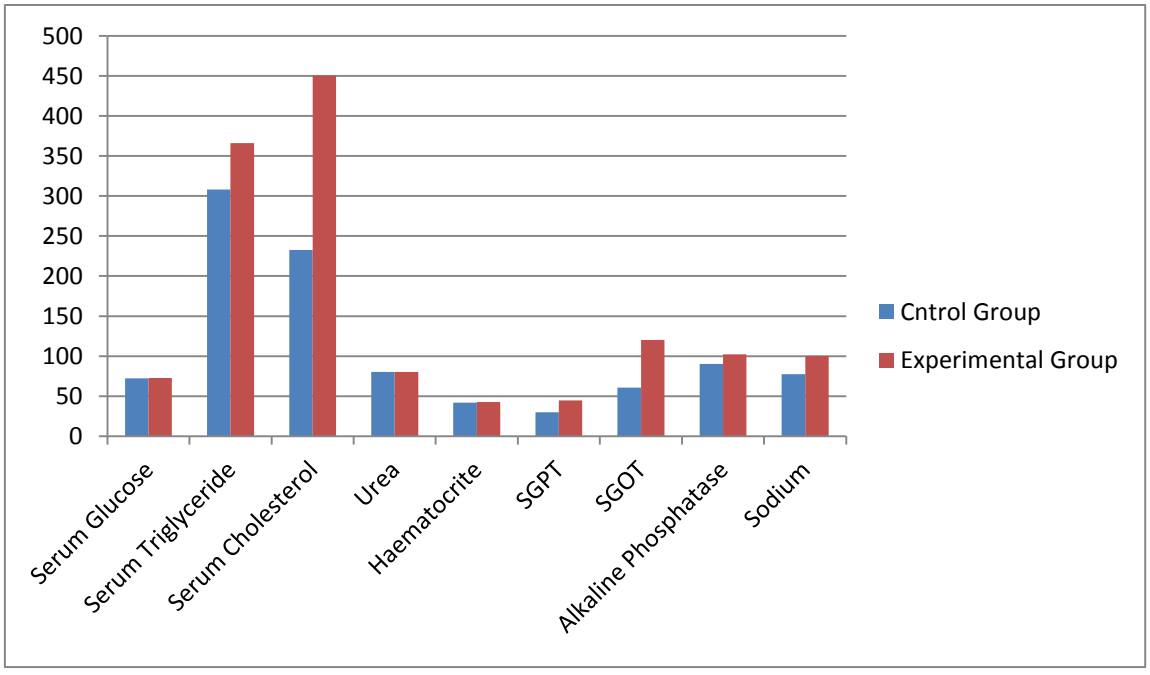


Figure 3. Showing blood biochemical parameters in control and experimental fish.

5. CONCLUSION

Fresh water fish *Notopterus notopterus* shows response to increased saline medium by increasing the level of blood Protein, triglycerides, cholesterol, hemoglobin, RBC, WBC, Enzymes (SGOT and SGPT) and inorganic salts such as Sodium, Potassium, Calcium. These changes are because to cope with changing environment so that it can withstand the stress to thrive well in the changed water saline concentration. Considering the parameters measured in this study N.notopterus appeared to exhibit a stress response to increased saline concentration of water, nevertheless, the magnitude of stress response was related to salinity level. The stress may affect negatively on growth, behavior, endocrine system, and reproduction.

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