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PYRAZOSULFURON-ETHYL INDUCED ALTERATION IN HAEMATOLOGY AND BLOOD BIOCHEMISTRY OF *OREOCHROMIS MOSSAMBICUS:* SUB-ACUTE STUDY

ABSTRACT
Aquatic ecosystems are the ultimate descend of natural and anthropogenic inputs of contaminants into the environment. Many of the environmental pollutant especially pesticides enter into aquatic ecosystem through agricultural run off and ultimately affects the different non target aquatic animals like bivalves, crustaceans, molluscs, prawn and fish, which are of great economics important to humans. Herbicides are a known class of chemicals used to treat weeds, one of the known chemical Pyrazosulfuron-ethyl (PE) is a group of sulfonylurea which is widely used in today's world. Among the sulfonylurea herbicides, pyrazosulfuron ethyl received the most attention because there is limited information available on the fate of pyrazosulfuron ethyl in the environment. Blood hematological and serum biochemistry parameters are often used to assess the health status and as stress indicator in fish. The present study was carried out the impact of sublethal toxicity of herbicide pyrazosulfuron ethyl on haematological and biochemical parameter on freshwater fish *Oreochromis mossambicus.* The fishes were treated with PE During the sublethal treatment (7d,14d) study estimation of hemoglobin(Hb),hematocrit(Hct),erythrocyte count(RBC), mean cellular volume(MCV),mean cellular hemoglobin(MCH), mean cellular hemoglobin concentration (MCHC),and total leucocytes count(WBC) Where as the biochemical profile plasma glucose, total serum protein, albumin, globulin, urea, creatinine, blood urea nitrogen measured. In the present study, the herbicide PE caused the alterations on haematological as well as biochemical parameter of *O.mossambicus* and these alterations can be used as non specific biomarkers in pesticide contaminated aquatic ecosystem.

KEYWORDS
Pyrazosulfuron ethyl, Haemetology, Blood Biochemistry, Sub acute

INTRODUCTION

Injudicious and indiscriminate use of agrochemicals such as fertilizers, pesticides, insecticides and fungicides to boost crop production with the sole aim of getting more yield, water bodies like ponds, lakes, river and low lying water areas are continuously getting polluted [1]. Normally these pesticides reach the aquatic environment through surface runoff, sediment transport from treated soil and direct application as a spray to water bodies to control the inhabiting pests [2]. Thus, Aquatic ecosystems are the ultimate descend of natural and anthropogenic inputs of contaminants into the environment. The agrochemicals used in agriculture are posing a great threat to aquatic fauna especially to fishes, which constitute one of the major sources of protein rich food for mankind.
Herbicides are the most commonly used pesticides, and are the most often detected in surface waters [3]. Numerous commercial formulations containing different herbicides (glyphosate, paraquat, sulfonolurea etc) have become popular around the world due to their effective action and low toxicity to mammals [4, 5, 6]. however; they have proved to be harmful to the environment. Sulfonylurea herbicides are an important class of herbicides used worldwide for controlling weeds in all major agronomic crops. Among sulfonylurea products, pyrazosulfuron-ethyl herbicide is widely used for selective post-emergence control of annual and perennial grasses and broad-leaved weeds in cereals. PE is widely used in rice crops in India [7]. There is limited information available on the fate of PE in the environment.

Blood is a liquid vital fluid and important index for health, environmental effect and growth and reproduction cycle. Haematological analyses has been routinely used in determining the physiological state of animals and known to be affected by different environmental factors, it is used as a guide in the diagnosis of many diseases and in evaluating the responses to therapy in both animals and human [8]. Hematology is used as an index of fish health status in a number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia etc. [9, 10] Hence it use is growing and becoming very important for toxicological research. Shah and Altindag (2004) noted that studies on fish blood gives the possibility of knowing physiological conditions within the fish long before there is an outward manifestation of pathological/disease condition because under stressful condition as well as environmental imbalances some parameters in the fish blood changes in response to reflect the change, the present study therefore examine changes in hematological parameters on sub acute exposure of PE after 7th and 14th day. Nutritional value of fish depends on their biochemical composition, which is affected by the agrochemicals [12]. Alterations in biochemical components as response to environmental stress are authenticated by many authors. Biochemical analysis can provide the valuable information for monitoring the health conditions of fishes. There is vast amount of scientific information available on different herbicides' toxicity on different fish's global level for paraquat [13]on Benny fish *Mesopotamichthy* and by [14]on the haematological parameters of the African catfish, *Clarias gariepinus.* Effect of Glyphosate herbicide has been also been reported on the serum growth hormone (GH) levels and muscle proteins in Nile Tilapia (*Oreochromis Niloticus*) by [15] Behavioral alteration of the common carp (*cyprinus carpio*) has been documented by [16]. However the effects of PE on fresh water Tilapia fish *O. mossambicus* are not well understood. There is a paucity of information about the effects of PE on the hematological and biochemical parameters of *O. mossambicus.* The objective of the present study was to determine the sub-acute toxicity of PE and its effects on haematogical and biochemical parameters of *O. mossambicus* in order to enrich the present knowledge on herbicide toxicity and to show the toxic effects of the herbicide.

MATERIALS AND METHODS

Experimental design:

The specimens of freshwater fish, *O. mossambicus* of similar size in length (12 ± 2 cm) and weight (25 ± 1.9 g) were brought to the laboratory from a local pond of Baroda district and were stocked in well aerated tanks containing chlorine free water for 10 days. Temperature, pH, and dissolved oxygen of the water were maintained at 27 ± 2° C, 7.1 ± 0.5, and 3.9 ± 0.02 mg/L, respectively. If in any batch, mortality exceeded 5% during acclimatization, that entire batch of fish was discarded. They were fed with commercial fish pallets. 30% Water was renewed every day to provide freshwater, rich in oxygen. Ten well-acclimatized fish were transferred from the stock to each experimental tank containing 40 L of water exposed to different concentrations of PE. A control group was also maintained in the same condition for the basic test. The LC_{50} values in the respective time intervals were calculated using software by transforming mortalities (percentage values) into a probit scale [17].

Experimental Procedure

On basis of LC_{50} value sub acute study dose $LC_{50}/10$ was chosen for hematological and biochemical studies. The experimental regime was maintained in the laboratory for14 days. A control group was also maintained. The experiment was performed semi statically with a group of 10 fish in two experimental aquaria, one control aquaria. Hematological and biochemical examinations of the experimental as well as the control fish were carried out at 7th and 14th days of exposure. All the groups were kept under continuous observation during the experimental period. Commercially food pallets were given to fish's once in day during the experiment Ad libitum. Behavior of the test fishes was observed during the experiment period. After the completion of the exposure fish were caught very gently using a small dip net, one at a time with least disturbance. They were slowly released in the tough containing 1% clove oil to make it immobile, and then after each fish was held and wrapped with a clean, dry towel and the posterior half of its body were blotted with a clean coarse filter paper. The total body weight was noted.

Haematological and biochemical estimation of fish: The caudal peduncle of the fish was severed with a single stroke from a heavy, sharp seizure. After discarding the first drop of blood, the freely oozing blood was collected using separate heparinized disposable syringe. The blood was then transferred to the ependrof containg anticoagulant, thoroughly mixed using a thin, blunt glass rod, during the process of collection itself. The blood was stored in -4°C prior to hematological and biochemical estimations. An alteration in the hematology and biochemical profile was performed using NIHON KOHDEN Automated Hematology Analyzer (Celtics α, Japan). The difference between the control and the PE exposed fishes was determined by Multivariate Test (MANOVA). If there was significant difference, Tukey's Post Hoc was employed to recognize difference in the alteration. The significant level of the tests was set at 5% ($p<0.05$).

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RESULTS AND DISCUSSION

The alterations in the hematology and biochemical parameters are presented in Table 1 and in Fig 1 and 2. The results of this investigation are as presented in table 1 indicating wilk's lambda test. Levene's test of Equality of error variances and dunnett's test respectively. The positive value in the post hoc tests indicates increases in the activity of the parameter studied and vice versa. The exposure of fish to PE caused clear significant decrease ($p<0.05$) in hematocrit and hemoglobin levels (Fig 1) after 7th and 14th days of exposure periods, respectively, in comparison with control. The results also revealed that after PE exposure, other hematological indices including MCV, MCH experienced considerable decrease (Fig 1). Blood forms a unique compartment between external and internal environments and any agent including toxic substances that causes stress and can alter blood composition either directly or indirectly by altering osmotic and ion regulation. Blood parameters are considered good physiological indicators of the whole body conditions and therefore can be used in diagnosing the structural and functional status of fish exposed to toxicants [18, 19]. The exposure of *O.mossambicus* to sub acute concentrations of PE resulted into a significant and progressive decrease in the PCV with time i.e on day 7th and 14th respectively compared to the control. A decrease in the erythrocyte count or in the percent of haematocrit indicates the worsening of an organism state and developing anaemia. In the light of the present study, PE exposure led to anemia, as indicated by the significant decrease in Hb and PCV values leading to anemia and as a response might have led to a fall in the red blood cell count, hemoglobin concentration and haematocrit volume. Hypoxia, anemia, and hyperthermia are related stresses causing an osmotic imbalance and decreased capacity of the RBC to carry sufficient oxygen unless otherwise compensated by erythropoiesis or suitable physiological adjustments. The anemic condition in fish results from an unusually low number of red blood cells or too little hemoglobin in the red blood cells. According to [20]the pesticide induced anemia in fish may be due to the inhibitory effect of the toxic substance on the enzyme system responsible for the synthesis of haemoglobin. It may also be due to impaired intestinal absorption of iron, as suggested by [1] Furthermore, this is an indication of disruptive effects of PE on erythropoietic tissues as well as cells viability [21]. Moreover, there are reports that dilution of blood in an organism is an indication of suppressed osmoregulation [22, 23, 24] Similar kind of actions has previously considered by other researchers for herbicides Paraquat [13] Roundup [25. 26], for Atrazine [27] and some of the pesticides such as Imidachloprid. The MCV, MCH and MCHC also decreased considerably compared to the control. This is in agreement with the work of [28] following a short-term exposure of tench (*Tinca tinca*) to agrochemical metal. These alterations were attributed to direct responses of structural damage to RBC membranes resulting in haemolysis and impairment in haemoglobin synthesis, stress related release of RBCs from the spleen and hypoxia, induced by exposure to agrochemical [28].

Biochemical analysis can provide valuable information for monitoring the health conditions of fishes. Biochemical changes depend on the fish species age, the cycle of sexual maturity and health condition. Moreover, analyses of serum biochemical constituents' levels have shown useful information in detection and diagnosis of metabolic disturbances and diseases in fishes ([29]. Determination of glucose concentration in blood serum is widely used as an indication of stress response. Generally, glucose is continuously required as an energy source by all body cells and must be maintained at adequate levels in the plasma. In many fish species, the blood glucose level has the tendency to increase due to experimental stress. In the present study the significant time dependent increase in glucose may be considered to be manifestation of stress induced by PE herbicide. Blood glucose is caused by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stresses. A variety of stressors stimulate the adrenal tissue resulting in increased level of circulating glucocorticoids and catecholamines. Both of these groups of hormones produce hyperglycemia. It is generally thought that, under conditions of stress, hyperglycemia may provide additional energy during times of high metabolic need such as "fight and flight" response [10].

Concerning the results of total protein, albumin, globulin and A/G ratio proteins 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. Also proteins play an important role in the metabolism and regulation of water balance [30]. Proteins play a vital role in physiology of living organisms. All biological activities are regulated by enzymes and hormones, which are also proteins. Assessment of protein content can be considered as

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a diagnostic tool to determine the physiological phases of the cells [31]. Results revealed total hypoproteinaemia, hypoglobulinaemia, hypoalbuminaemia and increased A/G ratio in exposed fish during sub acute exposures .The survival ability of animals exposed to stress mainly depends on their protein synthesis potential. The decrease in protein content was probably due to reduced/perturbation of microsomal protein synthesis suggested as suggested by many workers. The degradation of protein suggests the increase in proteolytic activity and possible utilization on their products for metabolic purpose and cause damage. The depletion of protein fraction in serum in present study may have been due to their degradation and possible utilization of degraded products for metabolic purposes [32] The quantity of protein is dependent on the rate of protein synthesis or on rate of its degradation. Decreased protein level may be attributed to stress mediated immobilization of these compounds to fulfil an increased element for energy by the toxicant.(Seth and Saxena 2003). Serum albumin measures as considerable diagnostic value in laboratory animals because it relates general nutritional status, the integrity of the vascular system and liver function. Albumins in fish organism participate in plastic metabolism and perform transport functions of substances necessary for life activities. In our study Protein, albumin and globulin were decreased significantly. [24] also observed same results in Sevin exposed to *Clarias battrachus.*

Stress results in an increase in cortisol levels in fish stimulating both glycogenesis and gluconeogenesis, as well as an increase in protein catabolism and ammonia production. Ammonia is toxic to all vertebrates. It can be converted to the less toxic urea, but this is a metabolically expensive process which is found only in terrestrial vertebrates. Teleost fishes are primarily amminotelic but their blood contains significant amount of urea and indeed in some teleosts it may account for 20 % or more of the total nitrogen excreted. Occurrence of uremia was reported by many workers [33, 34] Hence, freshwater fish excrete ammonia along with a small quantity of urea as they use urea as an osmotic filter. [35] Furthermore, renal disorders also are known to elevate serum urea values, which are parallel with an significant increase in the BUN, suggesting renal disturbances. The Lake Magadi tilapia excretes all nitrogenous waste as urea produced via the ornithine urea cycle .Many fish embryos have an active ornithine urea cycle and convert ammonia to urea to avoid ammonia toxicity during the early stages of development .Thus urea formation is used by several fish species during development or under certain environmental conditions, such as air exposure or alkaline water pH, to avoid problems of ammonia accumulation and toxicity [36].

Creatinine is derived mainly from the catabolism of creatine found in muscle tissue and its catabolism to creatinine occurs at a steady rate. Severe kidney damage will lead to increased creatinine levels. In the present study serum Creatinine showed insignificant decrease in experimental group in comparison to control animal suggesting that there occurs an alteration in glomeruli filtration rate. Excretion occurs through a combination of glomerular filtration (70 to 80%) and tubular secretion [37, 38]. The alteration in the levels of serum creatinine may, therefore, be due to a combination of these two factors. [39, 40]. Our results are in agreement with the results of [41] who reported that the level was significantly unaffected in O. niloticus exposed to sublethal concentration of atrazine. However, they, disagree with that of [42, 43] This difference in results may be due to the difference in fish species and the nature of the pesticide used.

BUN levels should be viewed by clinicians as a potential indicator of disrupted nitrogen excretion. Elevated blood urea nitrogen level in teleost may serve as a clinical indication of respiratory and excretory compromise due to respiratory epithelial cell hypertrophy and hyperplasia [44]. Thus the elevated blood urea in the present study can be expalined as a protection of fish against oxyhaemoglobin oxidation in the blood [45].Furthermore, as there is no significan change in the Creatinine level it is possible that the increased level of blood urea nitrogen is trying to overcome the respiratory stress induced by the herbicide.
To conclude, the PE exposure of *O.Mossambicus* at sub-acute concentration caused alterations to hematological and biochemical indices, all of which resulted in stress to the organism. The herbicide therefore can be classified as toxic for fish. It also points to the fact that the haematological parameters are the most sensitive parameters in monitoring the toxicity.

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Table 1: Haemetological and blood biochemical Parameter in *O.mossambicus* affected by sub acute exposure of PE:

Variables	Control vs Treated (7 d)	Control vs Treated (14 d)
	MD± SE	MD± SE
Haematological parameter		
Hb	-0.413 ± 0.197	-0.866±0.197***
RBC	-0.056±0.022	-0.106±0.022**
PCV	-1.166±0.224**	-2.133±0.224***
MCV	-5.266±1.614*	-8.733±1.614**
MCH	-3.166±1.298	-8.350±1.298**
WBC	-6090±5.140***	-9070.0±5.140***
Biochemical parameter		
Glucose	-73.666±1.981**	-53.333±1.981***
Protein	-5.833±0.538**	-4.600±0.538***
albumin	-3.833±0.243**	-3.366±0.243***
globulin	-3.366±0.372**	-3.200±0.372***
Urea	12.766±0.787**	28.800±0.787***
BUN	6.000±0.41096**	13.000±0.410***
Creatinine	-0.6000±0.176	-0.300±0.176
Ratio	18.566±0.568**	18.833±.568***

MD± SE Mean difference ± Standard error
The mean difference in significant at 0.05 level
*indicates mean difference is significant at 0.05 level.
**indicates mean difference is significant at 0.001 level.
*** indicates mean difference is significant at 0.0001 level.

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Fig 1: Changes in Haemetological Parameter of PE treated fish *O.mossambicus*. values are mean ± SE of five individual observation

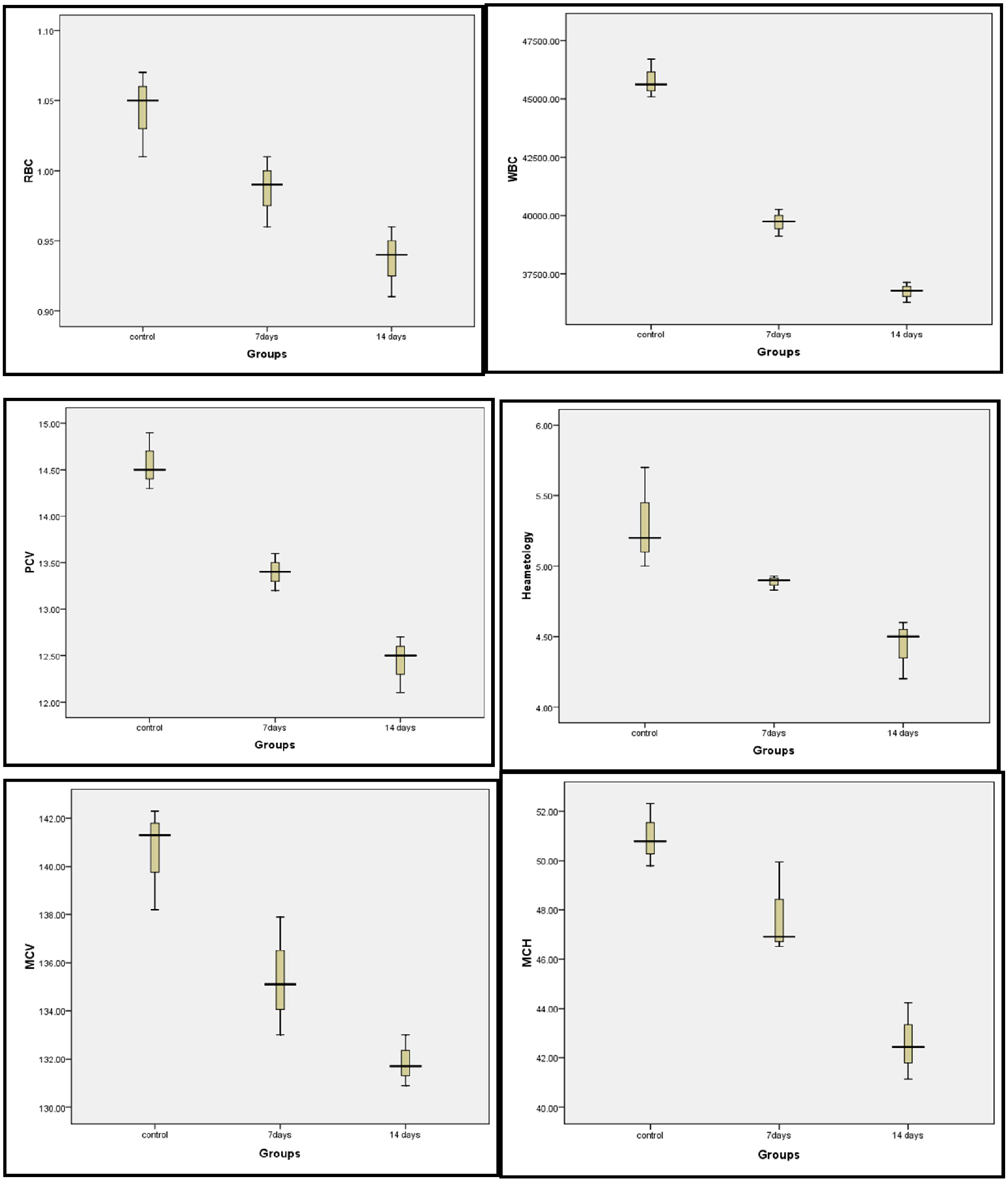

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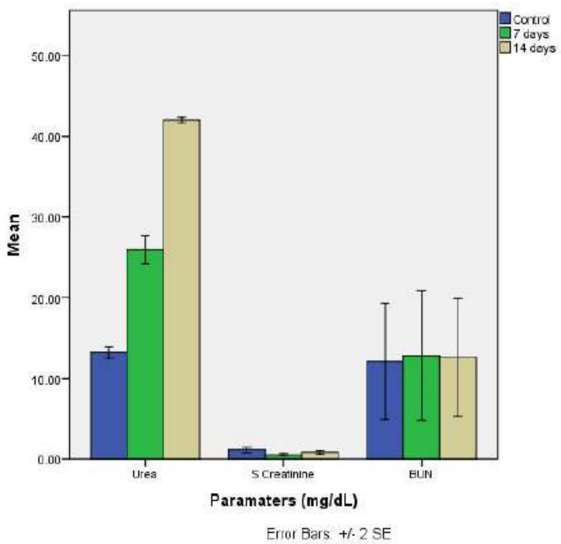
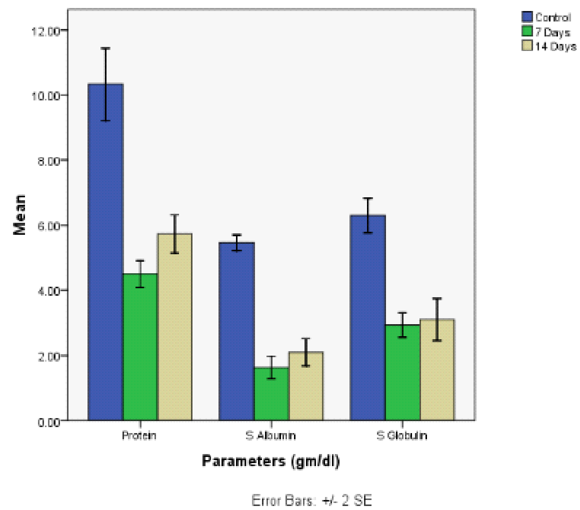
Fig.2 Changes in Blood Urea, Creatinine, BUN, and Total Serum Protein of PE treated fish *O.mossambicus*





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