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Diazinon induced changes in blood biochemistry of *Channa striatus*

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ABSTRACT

The acute effect of diazinon on the Channa striatus (Bloch) was assessed by comparing the biochemical blood plasma profiles of a control group and a group exposed to the effect of the pesticide DiazintolR. The fishes were exposed to sublethal concentration of diazinon and control for 96 h. Fish of particular size (22-24 cm) and weight (25-30 g) range were used irrespective of their sex for the experiments. The test solutions were renewed every 24 hr to maintain the optimum dissolved oxygen level. To evaluate the acute toxicity studies the static renewal toxicity test were conducted according to the methods recommended by American Public Health Association (1960). In the present investigation the median lethal concentration (LC50) of diazinon for 24, 48, 72 and 96 hr were analyzed. The toxicant concentration used in the present series of tests were approximately the wide range of concentrations viz., Control, LC10, LC20, LC30, LC40, LC50, LC60, LC70, LC80, LC90, LC99 aqueous solutions were prepared. Each group twenty one fishes were introduced with three replications. The blood samples from the challenged fishes were taken after every 20, 40 and 60 minute in fishes exposed to diazinon. The challenged fishes showed a significant rise in A:G ratio, creatinine, urea, uric acid, glucose, cortisol, bilrubin and cholesterol. However, opposite trend was observed in protein, albumin, globulin, liver glycogen, serum lipid and serum phosphorous.

1. Introduction

Pesticide use is known to cause serious environmental problems, especially in the dry season, because during this period the dilution capacity of the water systems is low, thus increasing the risk of high concentrations of toxic chemicals. Moreover, the dry season is often the critical period for many animals, especially fish and birds. Fish stocks suffer from natural mortality and high fishing pressure at the end of the dry season. Contamination of water by pesticides either directly or indirectly can lead to fish kills, reduced fish productivity or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans eating these fishes (Adedeji et al., 2009). Diazinon is a widely used toxicant in a number of organophosphorous pesticides (Robert and Hutson, 1998). Although the aquatic environment is not the target one for the use of such pesticides the results of a number of monitoring studies have showed the presence of diazinon and its metabolite, diazoxon, in surface waters (De Vlaming et al., 2000).

The toxicity of a chemical is totally dependent on the concentration of the chemical in the aqua systems and organisms or even the concentration at the target receptor in the organism. Only the bioavailable fraction of the pesticide can be accumulated by aquatic organisms and thereby reach the specific target receptors (Hamelink and Spacie, 1977). The actual amount of the pesticide that enters the organism, is therefore of critical importance and determines the biological ramifications. In environmental toxicology, environmental concentration is often used as indices for knowing the actual amount of a chemical entering an organism. After bioaccumulation, the pesticides change the metabolism courses which can well be defined by the changes in biochemical values in the blood of the target animal.

Biochemical characteristics of blood are among the important indices of the status of internal environment of the fish (Edsall, 1999). Changes in the biochemical blood profile mirror changes in metabolism and biochemical processes of the organism, resulting from the effects of various pollutants, and they make it possible to study the mechanisms of the effects of these substances. The major biochemical response to the effect of diazinon in fishes is the inhibition of enzymes. Hamm et al. (1998) also observed changes in carbohydrate metabolism in the eel, Anguilla anguilla. The glycogen contents in the liver and muscular tissue was significantly decreased while glucose and lactate concentrations in the blood were significantly increased.

Biochemical composition of blood gives an idea about the nature of change in the external environment influencing the internal milieu of the fish. In order to evaluate the effect of diazinon [0,0-diethyl-0-(2-isopropyl-6-methylpyrimidin-4yl) phosphorothioate] on common carp (Cyprinus carpio L.), Luskova et al. (2002) assessed the biochemical blood plasma profiles of a control group and a group exposed to the effect of the pesticide Basudin 600 EW (containing 600 g.l-1 diazinon as the toxic substance). The authors observed that the total protein and lactate concentration were significantly lower ($p < 0.05$) in the experimental group, compared with the control group. On the contrary, glucose concentration in the plasma of the experimental group was significantly higher ($p < 0.01$) than in that of the control group.

Diazinon toxicity was evaluated through acute and chronic exposures of fish to 1/2 and 1/10 the calculated LC50 for 1w and 6 w respectively by Safinaz et al. (2009). Results showed a significant increase in cortisol, glucose, urea, uric acid, creatinine and serum Ca++ levels. Furthermore, diazinon exposures were associated with a decrease in total protein concentration in serum with a reduction in both albumin and globulin. 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 (Mommsen and Walsh, 1992). Also proteins play an important role in the metabolism and regulation of water balance (Heath, 1995). Results revealed total hypoproteinaemia, hypoglobulinaemia, hypoalbuminaemia and increased A/G ratio in exposed fish during short and long term of exposures, in comparison with the control group, supported by Khalaf – Allah (1999) and Hanna and Sahar (2007). The decrease of total protein levels might be due to the increase of cortisol which suppresses the immunoglobulin (Wedemeyer, 1996; Reddy and Leatherland, 1998). Furthermore, under stress conditions, the protein consumed by fishes is not stored in the body tissue (Baskaran and Palanichamy, 1990) and hence the exposed fish meet their extra energy requirements from body proteins, which are mobilized to produce glucose, which is made available for fishes by the process of gluconeogenesis (Vasanthi et al., 1990).

Creatinine, urea and uric acid revealed a significant increase in diazinon exposed fish during the short and long term exposure compared to the control group. This can be explained by a disorder of the glomerular filtration rate (G.F.R.) (Hernandez and Couslon, 1967). The decrease in the rate of excretion of urea nitrogen, produces an increase in the concentration of BUN in plasma as described by Coles (1986), Haggag (2004) and Radwan and El–Said (2006). Blood glucose is considered as indicator for stress response in fish. The result of serum glucose showed a significant increase in all diazinon exposed fish groups, as supported by Haggag (2004) and Sweilum (2006). The hyperglycaemic condition induced by pesticides might be explained in part by inhibition of cholinesterase at neuro effector sites in the adrenal medulla, leading to hypersecretion of adrenaline and cortisol which stimulates the break down of glycogen to glucose (glycogenolysis) (Gupta,1974). Also, in this respect, hyperglaecemia can be viewed as a physiological response of the fish to meet the critical need for energy under toxic stress. This need may be met by increased break down of liver and muscle glycogen as described by Ferrando and Andreu-Moliner (1991). The elevated glucose level may be due to enhancement of the break down of liver glycogen due to reduced insulin activity. The findings agree with those of Wedemeyer (1996) and Reddy and Leatherland (1998).

In order to investigate the toxic effect of diazinon pesticide on the health status of C. gariepinus after its release from the fish body and to detect its public health significance on the human consumers, a clearance experiment has been carried out for 14 days during which different previously mentioned biochemical and residual parameters have been measured at 7th day and 14th day. Regarding the results of total protein, albumin, globulin and A/G ratio after the recovery period, the total protein, albumin and globulin values increased but the values of A/G ratio decreased at the 7th day and started to increase again at the 14th day of clearance. These results support those of Abou – Zeid et al. (1996) but disagreed with those of Sancho et al. (1998) who mentioned that levels of proteins did not approach the normal values. This may be attributed to time of clearance (192 h.) and different fish species, (Anguilla anguilla). A similar pattern of urea, uric acid and creatinine values had been detected during the clearance period. Results disagreed with Radwan and El – Said (2006), which may be due to variance in both fish species and concentration of pesticide used.

The results of Notopterus notopterus by Manoj et al. (2014) exposed to lethal concentration (0.1 ppm) of endrin revealed significant ($P < 0.05$) increase in Bilirubin (mg/dL) Na+ and K+. The results also revealed a decrease in plasma protein (g/dL), Albumin (g/dL), Globulin (g/dL), Glucose (mg/dL), as well as lipid profile, which included analysis of Cholesterol (g/dL), Triglycerides, HDL (mg/dL), LDL (mg/dL), and VLDL (mg/dL). A significant ($P<0.05$) decrease in serum ionic substances like Ca, Mg, Chloride (mEq/L) and phosphorus were also observed. The study revealed the peculiar impact of the test pesticide on the health condition of the fish in controlled condition. This could give an idea about the total stress on the fish in natural waters. Present study aims to evaluate the acute effect of diazinon on plasma profile of the Channa striatus following the exposure to different subsets of LC50 of diazinon.

2. Materials and Methods

Collection and maintenance of fish:

Fish of particular size (22-24 cm) and weight (25-30 g) range were used irrespective of their sex for the experiments. Only healthy individuals collected from the same body of water were employed in all tests. The test solutions were renewed every 24 hr to maintain the optimum dissolved oxygen level. While conducting the experiments, care was taken not to deviate from the modified main principles of bioassay techniques outlined by Sprague (1973) and recommended by APHA (1989). Fish were fed with 2% of body weight fish feed once a day. Before attempting, the fish divided into groups were kept in 1 x 1.2m (wide-deep) fiber-glass tanks one week for adaptation. Aeration was allowed during the trial. The quality parameters of water were measured as pH=7.3-7.5, Temperature=27 ± 1 º C, DO: 7.2-7.4 ppm, salinity=0.42-0.49 ppt, alkalinity=260 mg/L as $CaCO_3$, hardness=323 mg /L $CaCO_3$.

Acute Toxicity:

To evaluate the acute toxicity studies the static renewal toxicity test were conducted according to the methods recommended by American Public Health Association (1960). In the present investigation the median lethal concentration (LC_{50}) of diazinon for 24, 48, 72 and 96 hr were analyzed. The screening test was conducted to avoid delay and to save time and effort. The objective of this test is to obtain approximate indication of the concentration of a substance likely to be hazardous to the test fish and fishes in general in their natural environment (Alabaster and Lloyd, 1982). The toxicant concentration used in the present series of tests were approximately the wide range of concentrations viz., Control, LC_{10}, LC_{20}, LC_{30}, LC_{40}, LC_{50}, LC_{60}, LC_{70}, LC_{80}, LC_{90}, LC_{99} aqueous solutions were prepared. In each group twenty one fishes were introduced with three replications. The tests were conducted in the plastic troughs. The troughs were cleaned well and dried before conducting experiments. Then the tests were conducted by allowing twenty one fishes of Channa striatus in each plastic trough containing 10 liters of water with particular concentration of the diazinon. The screening tests was continued to assess the concentration at which all fishes survived for 24 hr and likewise the concentration at which most of the fishes died simultaneously (Bansal *et al.,* 1980)

Biochemical Analysis:

Blood samples were quickly collected from tail blood vessel by heparinized syringes and immediately stored on ice as described by Schmitt *et al.* (1999) and 2 ml was decanted in heparin after which the plasma was separated by centrifugation and thus separated into plasma, bufy coat and packed erythrocytes. were centrifuged immediately at 2750*g* and the plasma promptly removed and frozen at –10°C. For each age group, 20 samples (composite or individual) were analyzed. The plasma was then decanted into labeled Ependorf tube with the aid of Pasteur pipette and stored at -20°C prior to

subsequent analysis, which was conducted within 48 h of sample collection. The plasma obtained after exposing the fish to 24.61 ppm of diazinon was analyzed to evaluate the effects of diazinon on the plasma. After sampling, the blood was centrifuged for 15 min at 400 g. The total protein (TP), Blood urea nitrogen (BUN), glucose (GLU), creatinine were determined using the automatic analyser COBAS MIRA (Hoffmann, La Roche, Co. Switzerland) and using optimized tests of Boehringer Mannheim GmBH by means of spectrophotometer Varian DMS 200. Globulin was calculated by subtracting albumin value from total protein value. Uric acid was estimated by the method of Brown (1945). Glycogen was determined by the method of Seifter *et.al.* (1950). Cortisol and cholesterol was estimated through spectrophotometery. Bilrubin concentration was determined by using Malloy and Evelyn method. Estimation of total lipids was estimated by the method of Floch *et al*. (1957). Serum phosphorus was estimated by following the method suggested by Gomari (1942).

Statistical analysis:

The variations through the medium of (±SEM) between groups were evaluated using Independent Samples-t test. 95% confidence limits were considered important. SPSS 15.0 software was used for data analysis.

3. Results

The biochemical values are important indicators of stress due to environmental pollution, indicating the catabolic end products, which mark the level of stress after the level of the components cross the threshold. The trends of change (R1 and R2) in biochemical values of Channa striatus after exposure to various concentrations of Diazinon are presented in table 1 and 2. Plasma proteins, also termed serum proteins or blood proteins, are proteins present in blood plasma. They serve many different functions, including transport of lipids, hormones, vitamins and minerals in the circulatory system and the regulation of acellular activity and functioning of the immune system. A low total protein level can suggest a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly. Low levels may be seen in severe malnutrition and with conditions that cause inflammation. The mean±SD value of total protein expressed in g/dL, was 5.17±0.38 during R1 (*N* = 21), which showed a decrease after the exposure, to 3.93±0.05 (*V* = 0.002; *p* >0.1). In R2, total protein was 5.45±0.05, which reduced to 3.99±0.10 (*V* = 0.011; *p* >0.1). Significant (P<0.05) differences were observed among the mean, with *F crit* value of 2.85 (*df* = 21) and variance of 220.71 for 96 h LC_{50}.

Serum albumin, often referred to simply as blood albumin, is an albumin (a type of globular protein) found in blood. Serum albumin accounts for 55% of blood proteins, and is a major contributor in maintaining the osmotic pressure of plasma to assist in the transport of lipids and steroid hormones. Albumin is one of the most extensively studied endogenous proteins which are used in the diagnostic technologies. During the present research period, the mean±SD value of albumin expressed in g/dL, was 2.03±0.05 during R1 (N = 21), which decreased to 1.77±0.02 (V = 0.006; p <0.01). In R2, albumin was 1.99±0.01, which showed a decrease to 1.67±0.02 (V = 0.004; p <0.01). Significant (P<0.05) differences were observed among the mean, with F crit value of 2.85 (df = 21) and variance of 269.82 for 96 h LC50.

The globulins are a family of globular proteins that have higher molecular weights than albumins and are insoluble in pure water but soluble in dilute salt solutions. Some globulins are produced in the liver, while others are made by the immune system. Globulins make up 38% of blood proteins and transport ions, hormones, and lipids assisting in immune function. The mean±SD value of globulin expressed in g/dL, was 3.13±0.07 during R1 (*N* = 21), which showed a decrease to 2.17±0.06 (*V* = 0.003; *p* >0.1). In R2, globulin was 3.24±0.02, which showed a decrease to 2.16±0.18. Significant (P<0.05) differences were observed among the mean, with *F crit* value of 2.85 (*df* = 21) and variance of 260.60 for 96 h LC_{50}.

A:G ratio is best indicator of the relative changes in albumin and globulin. Normally, there is a little more albumin than globulins, giving a normal A/G ratio of slightly over 1. Because uneasy states affect the relative amounts of albumin and globulin, the A/G ratio may provide a clue as to the cause of the change in protein levels. A high A/G ratio suggests underproduction of immunoglobulins. The mean±SD value of A:G ratio was 0.67±0.02 during R1 (*N* = 21), which showed an increase after the exposure with a mean±SD of 0.82±0.04 (*V* = 0.001; *p* >0.1). In R2, A:G ratio was 0.64±0.02, which showed an increase to 0.85±0.03 (*V* = 0.009; *p* <0.01). Significant (P<0.05) differences were observed among the mean, with *F crit* value of 2.85 (*df* = 21) and variance of 292.34 for 96 h LC_{50}.

Creatinine is a chemical waste molecule that is generated from muscle metabolism. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. Creatinine has been found to be a fairly reliable indicator of kidney function. Elevated creatinine level signifies impaired kidney function. As the kidneys become impaired for any reason, the creatinine level in the blood rises due to poor clearance of creatinine by the kidneys. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys. The mean±SD value of creatinine expressed in mg/dL, was 0.27±0.01 during R1 (*N* = 21), which showed an increase to 0.50±0.04, post exposure (*V* = 0.002; *p* <0.01). In R2, creatinine was 0.29±0.01, which showed an increase to 0.55±0.02 (*V* = 0.004; *p* >0.1). Significant (P<0.05) differences were observed among the mean, with *F crit* value of 2.85 (*df* = 21) and variance of 300.13 for 96 h LC_{50}.

The blood urea nitrogen (BUN) test reveals important information about the status of kidneys and liver. Generally, a high blood urea nitrogen level means that the kidneys aren't working well. The elevated urea nitrogen can also be due to dehydration, resulting from not drinking enough fluids or for other reasons including the malfunctioning of kidney and liver on exposure to chemicals/pesticides. The mean±SD value of urea expressed in mg/dL, was 13.57±0.14 during R1 (*N* = 21), which showed an increase to 14.77±0.12, post exposure (*V* = 0.01; *p* <0.01). In R2, urea was 13.57±0.22, which showed an increase to 14.85±0.07 (*V* = 0.05; *p* <0.01). Significant (P<0.05) differences were observed among the mean, with *F crit* value of 2.85 (*df* = 21) and variance of 52.33 for 96 h LC_{50}.

A uric acid blood test, also known as a serum uric acid measurement, determines how much uric acid is present in the blood. The test can help determine how well a fish body produces and removes uric acid. Most uric acid is dissolved in the blood, filtered through the kidneys, and expelled from the body. Sometimes, the body produces too much uric acid or doesn't filter out enough of it. Higher than normal uric acid levels in the blood is called hyperuricemia and can be caused by the over-production of uric acid in the body or the inability

of the kidneys to adequately remove enough uric acid from the body. The mean±SD value of uric acid expressed in mg/dL, was 2.03±0.01 during R1 (*N* = 21), which showed an increase to 2.87±0.03 (*V* = 0.001; *p* <0.01). In R2, uric acid was 2.11±0.05, which showed an increase to 3.00±0.21 (*V* = 0.044; *p* <0.01). Significant (P<0.05) differences were observed among the mean, with *F crit* value of 2.85 (*df* = 21) and variance of 244.87 for 96 h LC_{50}.

A high fasting blood glucose level can be an indicator of underlying health problems. Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy. In suboptimum or stressful conditions (internal or external) the chromaffin cells release catecholamine hormones, adrenaline and noradrenaline toward blood circulation. Those stress hormones in conjunction with cortisol mobilize and elevate glucose production in fish through glucogenesis and glycogenolysis pathways to cope with the energy demand produced by the stressor for the "fight and flight" reaction. Glucose is then released (from liver and muscle) toward blood circulation and enters into cells through the insulin action. The mean±SD value of glucose expressed in mg/dL, was 62.63±1.33 during R1 (*N* = 21), which showed an increase to 109.03±5.73, post exposure (*V* = 32.89; *p* >0.1). In R2, glucose was 60.78±1.12, which showed an increase to 102.3±2.72 (*V* = 1.65; *p* >0.1). Significant (P<0.05) differences were observed among the mean, with *F crit* value of 2.85 (*df* = 21) and variance of 2987.65 for 96 h LC_{50}.

Cortisol is the principal glucocorticoid secreted by the interrenal tissue (steroidogenic cells) located in the head-kidney of teleost fish. This hormone is released by the activation of the hypothalamus-pituitary-interrenal axis (HPI axis). When an organism undergoes stress conditions, the hypothalamus releases corticotropin-releasing factor (CRF) toward blood circulation. This polypeptide further stimulates secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland which finally activates the release of cortisol by the interrenal tissue. The mean±SD value of cortisol expressed in µg/dL, was 8.27±0.06 during R1 (*N* = 21), which showed an increase after the exposure with a mean±SD of 11.93±0.33 (*V* = 0.108; *p* <0.01). In R2, cortisol was 8.40±0.10, showed an increase to 11.86±0.07 (*V* = 0.005; *p* <0.01). Significant (P<0.05) differences were observed among the mean, with *F crit* value of 2.85 (*df* = 21) and variance of 86.33 for 96 h LC_{50}.

Glycogen represents the principal storage form of carbohydrate in the mammalian body, mainly in liver and muscles. It is a branched homoglycan. Glycogen is primarily synthesized in liver and muscle tissue where it can constitute up to 10% of the weight of liver and 1-2% of the weight of muscle tissue. While muscle glycogen is generally utilized locally, liver glycogen serves as an important buffer to regulate blood glucose levels. Glycogen metabolism is dysregulated in the times of stress, leading to higher glycogen concentration. The mean±SD value of liver glycogen expressed in mg/g, was 8.183±0.05 during R1 (*N* = 21), which showed a decrease after the exposure with a mean±SD of 7.012±0.10 (*V* = 0.010; *p* <0.01). In R2, liver glycogen was 8.09±0.035, which showed a decrease to 6.99±0.035, post exposure (*V* = 0.001; *p* >0.1). Significant (P<0.05) differences were observed among the mean, with *F crit* value of 2.85 (*df* = 21) and variance of 161.78 for 96 h LC_{50}.

The bilirubin blood test measures the level of bilirubin in the blood. Bilirubin is a yellowish pigment found in bile, a fluid made by the liver. A small amount of older red blood cells are replaced by new blood cells every day. Bilirubin is left after these older blood cells are removed. The liver helps break down bilirubin so that it can be removed from the body. The liver damage in fishes may be the reason for increased bilirubin level after exposure to pesticides/insecticides. The mean±SD value of serum bilirubin expressed in µmol/L, was 5.78±0.32 during R1 (*N* = 21), which showed an increase to 7.22±0.59, post exposure (*V* = 0.92; *p* <0.01). In R2, serum bilirubin was 5.81±0.32, which showed an increase to 7.34±0.59, post exposure (*V* = 0.92; *p* <0.01). Significant (P<0.05) differences were observed among the mean, with *F crit* value of 2.85 (*df* = 21) and variance of 158.06 for 96 h LC_{50}.

The concentration of total cholesterol and total lipids in serum and tissues of fish have been reported to be moderately sensitive to environmental pollutant but the direction of change in these parameters seems to be dependent on many factors, such as the types of contaminant, the concentration, mode of its action, duration of exposure and fish species. The mean±SD value of serum cholesterol, expressed in mg/dL, was 129.0±2.50 during R1 (*N* = 21), which showed an increase to 139.5±2.63 (*V* = 212.7; *p* >0.1). In R2, serum cholesterol was 132.0±2.50 (*N* = 21), which showed an increase to 141.5±2.63 (*V* = 198.3; *p* <0.01). Significant (P<0.05) differences were observed among the mean, with *F crit* value of 2.85 (*df* = 21) and variance of 6498 for 96 h LC_{50}.

Lipids are an important source of nutrition that provides a significant amount of energy and structural components for reproductive growth. During exposure of fish to various toxic aquatic pollutants, there is a marked reduction in serum lipid content. It might be due to the reduction in absorption of carbohydrate and protein, resulting in the depletion of energy during toxic stress, which leads to the degradation of lipid to combat the required energy. As the level of the protein and carbohydrate absorption decreases the lipid level also decreases due to lipid metabolism to meet the required energy during the stress condition. During the present research period, the mean±SD value of serum lipid expressed in mg/dL, was 6.7±0.13 during R1 (*N* = 21), which showed a decrease to 1.90±0.05 (*V* = 11.0; *p* <0.01). In R2, serum lipid was 6.9±0.13, which showed a decrease to 2.02±0.05 (*V* = 10.98; *p* <0.01). Significant (P<0.05) differences were observed among the mean, with *F crit* value of 2.85 (*df* = 21) and variance of 266.805 for 96 h LC_{50}.

The inorganic phosphate acts as a major cytoplasmic buffer and is the basis of energy exchange. Both hypo and hyperphosphatemia have been recorded in teleosts exposed to various insecticides/pesticides. During the present research period, the mean±SD value of serum phosphorus expressed in mg/dL, was 13.65±0.92 during R1 (*N* = 21), which showed a decrease after the exposure with a mean±SD of 9.33±0.56 (*V* = 8.29; *p* >0.1). In R2, serum phosphorus was 13.82±0.59, which showed a decrease after the exposure with a mean±SD of 9.49±0.79 (*V* = 7.98; *p*

>0.1). Significant (P<0.05) differences were observed among the mean, with *F crit* value of 2.85 (*df* = 21) and variance of 266.81 for 96 h LC_{50}.

4. Discussion

Biochemical characteristics of blood are among the important indices of the status of internal environment of the fish (Edsall, 1999). Changes in the biochemical blood profile mirror changes in metabolism and biochemical processes of the organism, resulting from the effects of various pollutants, and they make it possible to study the mechanisms of the effects of these substances. The major biochemical response to the effect of diazinon in fishes is the inhibition of enzymes. Hamm *et al.* (1998) observed changes in carbohydrate metabolism in the eel, *Anguilla anguilla*. They also reported glycogen contents in the liver and muscular tissue was significantly decreased while glucose and lactate concentrations in the blood were significantly increased after exposure to diazinon.

In order to evaluate the effect of diazinon on biochemical blood profile of common carp (*Cyprinus carpio* L.), Luskova *et al*. (2002) assessed by comparing the control group and a group exposed to the effect of the pesticide Basudin 600 EW (containing 600 $g.l^{-1}$ diazinon as the toxic substance). The authors reported significantly lower (p<0.05) total protein and lactate concentration in the experimental group, compared with the control group. On the contrary, glucose concentration in the plasma of the experimental group was significantly higher (p < 0.01) than in that of the control group. Our findings reported significant (p<0.05) decrease in total protein, albumin, globulin, A:G ratio, as documented by the above authors.

Banaee *et al*. (2008) determined the chronic toxicity of Diazinon and its effects on some hematological parameters and biochemical blood plasma profiles of common carp, *Cyprinus carpio*. The authors reported that the experimental groups showed no significant difference (p < 0.05) in total protein (p< 0.05) and significantly higher (p<0.05) values of plasma glucose, which is in agreement with our findings. In yet another trial, a significant increase in cortisol, glucose, urea, uric acid, creatinine and serum Ca^{++} levels were recorded by Safinaz *et al*. (2009), who investigated the clinical and biochemical alterations associated with diazinon toxicity in *Clarias gariepinus*. Authors reported decrease in total protein concentration in serum with a reduction in both albumin and globulin.

The acute effect of diazinon on the African catfish (*Clarias gariepinus)* was assessed by comparing the biochemical blood plasma profiles of a control group and a group exposed to the effect of the pesticide Diazintol^{R} (162 mg/ml of diazinon as the active substance) by Adedeji (2010). Their results showed a significant decrease (p < 0.05) in the total protein, albumin globulin and lactate concentration in the experimental group compared with the control group. Glucose concentration in the plasma of the experimental group was significantly higher (p < 0.05) than that of the control group. A significantly higher (p < 0.05) concentration of plasma sodium and potassium was observed in the experimental group and a significantly lower (p < 0.05) concentration of plasma calcium and phosphorus, compared with those in the control group.

Diazinon-induced changes in the transaminase activities of *Clarias gariepinus*, were assessed by Inyang *et al*. (2010) who exposed the fish to varying sub-lethal concentrations of diazinon (1.0, 2.5, 5.0, 7.5 and 10.0 mg/L) in semi-static bioassays for 30 days. The authors reported that the levels of total protein in plasma and muscle were significantly lower (p<0.05) in all test concentrations in comparison with the control, in addition to liver, kidney and gills which showed decreased values with increased concentration of diazinon. On the other hand, the common carp (*Cyprinus carpio*) was exposed to sub lethal concentration of diazinon by Zubair (2011). Fishes exposed to sub-lethal concentrations (0.5, 1.0 and 2.0 mg/l) showed increase in plasma glucose level, glutamate-oxaloacetate transminase (PGOT) and plasma glutamate pyruvate transminase (PGPT) activities. Protein level in plasma and glycogen in liver and muscles were found to decrease in treated fish with diazinon during their study.

In order to assess the influence of organo-phosphorus (OP) Diazinon pesticide on adult tilapia fish (*Oreochromis niloticus*) in a semi static renewal bioassay for 30 days, Soyingbe *et al*. (2012) exposed fish to varying sub-lethal concentrations of diazinon (1.0, 2.5, 5.0, 7.5 and 10.0 mg/l) for 30 days and compared with control (untreated). The authors reported that the levels of total protein in plasma was significantly lower (P<0.05) in all test concentrations in comparison with the control; but no concentration-dependent changes among the various concentration of diazinon was observed. Conversely, protein concentrations in muscle, liver, gills and kidney decreased with increased concentration of diazinon (P<0.05).

In yet another trial, Indian carp (*Cirrhinus mrigala*) exposed to two sub-lethal concentrations (0.815 mg/L and 1.63 mg/L) of diazinon for 30 days showed a significant decrease in plasma levels of total protein, albumin, and globulin as documented by Haider and Rauf (2014), and a significant increase in plasma glucose levels when compared with controlled fish (P<0.05). The findings of the above authors lend support to our observations on the effect of diazinon on biochemical alterations in *Channa striatus*. A marked decrease total protein ($y = -1.28\ln(x) + 5.975$), albumin ($y = -0.26\ln(x) + 2.152$), globulin ($y = -0.72\ln(x) + 3.549$), liver glycogen ($y = -0.99\ln(x) + 8.681$), serum lipid ($y = -2.84\ln(x) + 7.136$) and serum phosphorus ($y = -2.50\ln(x) + 14.13$) was reported during the present study. While as post exposure increase in creatinine ($y = 0.192\ln(x) + 0.185$), urea ($y = 0.783\ln(x) + 13.21$), uric acid ($y = 0.876\ln(x) + 1.503$), glucose ($y = 35.84\ln(x) + 44.62$), cortisol ($y = 2.115\ln(x) + 7.650$), bilirubin ($y = 1.220\ln(x) + 5.109$), and cholesterol ($y = 11.3\ln(x) + 123.0$) was recorded during the present investigations.

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Table 1: Values for first replica of mean biochemical parameters of *Channa striatus* (Bloch) affected by sublethal exposure of Diazinon

Indices	Units	Groups	N	Means	SD	Variance	Probability	Sig. 1	Sig. 2	95% Confidence Interval	
										Lower Bound	Upper Bound
Total Protein	g/dl	Control	10	5.17	0.38	0.001	0.99	1.62E-15	1.75E-14	1.42	5.62
		Treated	21	3.93	0.05	0.002					
Albumin	g/dL	Control	10	2.03	0.05	0.002	0.93	3.28E-15	3.18E-15	0.5	0.75
		Treated	21	1.77	0.02	0.006					
Globulin	g/dl	Control	10	3.13	0.07	0.005	0.98	1.07E-15	1.12E-15	0.33	0.8
		Treated	21	2.17	0.06	0.003					
A/G Ratio	--	Control	10	0.67	0.02	0.006	0.86	8.0E-16	7.56E-15	0.25	1.6
		Treated	21	0.82	0.04	0.001					
Creatinine	mg/dl	Control	10	0.27	0.01	0.002	0.94	1.15E-15	1.51E-14	0.31	3.0
		Treated	21	0.50	0.04	0.002					
Urea	mg/dl	Control	10	13.57	0.14	0.01	0.95	3.9E-16	3.85E-14	0.85	3.13
		Treated	21	14.77	0.12	0.01					
Uric Acid	mg/dl	Control	10	2.03	0.01	0.001	0.99	5.8E-18	6.3E-14	3.5	4.6
		Treated	21	2.87	0.03	0.001					
Glucose	mg/dl	Control	10	62.63	1.33	1.78	0.98	9.3E-16	8.5E-15	4.29	4.56
		Treated	21	109.03	5.73	32.89					
Cortisol	µg/dl	Control	10	8.27	0.06	0.003	0.99	1.9E-17	1.85E-16	5.5	33.5
		Treated	21	11.93	0.33	0.108					
Liver Glycogen	mg/g	Control	10	8.183	0.05	0.002	0.98	5.9E-15	1.2E-15	1.84	8.07
		Treated	21	7.012	0.10	0.010					
Kidney Glycogen	mg/g	Control	10	2.62	0.42	0.19	0.99	4.2E-14	3.7E-16	1.5	1.8
		Treated	21	2.01	0.36	0.11					
Bilirubin	µmol/L	Control	10	5.78	0.32	1.03	0.81	2.07E-7	1.90E-5	5.33	5.88
		Treated	21	7.22	0.59	0.92					
Serum Cholesterol	mg/dL	Control	10	129.0	2.50	250.2	0.99	3.19E-08	2.72E-7	0.48	0.63
		Treated	21	139.5	2.63	212.7					
Serum Lipid	mg/dL	Control	10	6.7	0.13	11.60	0.99	5.20E-7	4.83E-8	1.59	2.32
		Treated	21	1.90	0.05	11.00					
Serum phosphorous	mg/dL	Control	10	13.65	0.92	9.33	0.97	1.22E-05	2.56E-06	12.0	13.68
		Treated	21	9.33	0.56	8.29					

Table 2: **Values for second replica of mean biochemical parameters of *Channa striatus* (Bloch) affected by sublethal exposure of Diazinon**

Indices	Units	Groups	N	Means	SD	Variance	Probability	Sig. 1	Sig. 2	95% Confidence Interval	
										Lower Bound	Upper Bound
Total Protein	g/dL	Control	10	5.45	0.05	0.002	0.98	1.5E-15	1.5E-15	2.1	7.4
		Treated	21	3.99	0.10	0.011					
Albumin	g/dL	Control	10	1.99	0.01	0.04	0.99	4.1E-15	2.9E-15	2.0	2.31
		Treated	21	1.67	0.02	0.004					
Globulin	g/dl	Control	10	3.24	0.02	0.006	0.98	2.4E-16	6.9E-15	7.2	25.4
		Treated	21	2.16	0.18	0.032					
A/G Ratio	--	Control	10	0.64	0.02	0.004	0.95	2.6E-17	6.0E-16	0.11	1.5
		Treated	21	0.85	0.03	0.009					
Creatinine	mg/dl	Control	10	0.29	0.01	0.002	0.98	1.0E-15	3.9E-15	0.16	1.33
		Treated	21	0.55	0.02	0.004					
Urea	mg/dl	Control	10	13.57	0.22	0.04	0.93	5.8E-15	1.2E-15	0.34	10.2
		Treated	21	14.85	0.07	0.05					
Uric Acid	mg/dl	Control	10	2.11	0.05	0.003	0.93	3.0E-15	3.7E-15	3.8	5.05
		Treated	21	3.00	0.21	0.044					
Glucose	mg/dl	Control	10	60.78	1.12	1.06	0.99	2.8E-15	2.5E-14	1.55	5.6
		Treated	21	102.3	2.72	1.65					
Cortisol	µg/dl	Control	10	8.40	0.10	0.01	0.99	4.3E-15	2.2E-15	0.75	5.56
		Treated	21	11.86	0.07	0.005					
Liver Glycogen	mg/g	Control	10	8.09	0.035	0.001	0.99	4.6E-15	1.9E-15	1.0	1.11
		Treated	21	6.99	0.035	0.001					
Kidney Glycogen	mg/g	Control	10	2.58	0.58	0.03	0.94	3.9E-12	1.6E-14	1.2	1.3
		Treated	21	1.98	0.16	0.12					
Bilirubin	µmol/L	Control	10	5.81	0.32	1.03	0.89	3.05E-8	2.01E-7	4.39	5.09
		Treated	21	7.34	0.59	0.92					
Serum Cholesterol	mg/dL	Control	10	132.0	2.50	241.7	0.98	5.26E-07	4.89E-8	5.28	4.39
		Treated	21	141.5	2.63	198.3					
Serum Lipid	mg/dL	Control	10	6.9	0.13	11.52	0.98	3.19E-8	4.52E-9	0.48	0.63
		Treated	21	2.02	0.05	10.98					
Serum phosphorous	mg/dL	Control	10	13.82	0.59	8.57	0.86	3.29E-06	4.65E-07	11.56	12.89
		Treated	21	9.49	0.79	7.98					