INTRODUCTION

According to Bouck (1951) environmental stress causes a variety of detectable and recognizable changes in the blood of fish. Blaxhall (1972) reported that fish live in intimate contact with their environment, and are therefore, very susceptible to physical and chemical changes which may be reflected in their blood components. The author further stated that, as many poisons are introduced into the environment of fishes, like metals, phenolic derivatives, detergents and pesticides in sublethal level, there is a possibility of some haematological changes. According to Mawdesley-Thomas (1971), scientists are relatively ignorant of fish pathology, fish biochemistry and of the toxic substances being introduced into aquatic environments.

It has been reported by Doudoroff and Kate (1957) that chronic or cumulative intoxication may stunt growth, increase disease susceptibility and decrease reproduction in fish. Harlow and Selye (1937), Dunbar (1969) and Enomato (1969) have observed changes in haematological parameters due to disease in fish. Kawatsu (1969) found that brook trout *Salvelinus fontinalis* suffering from a macrocytic exhibited anemia, resembled human pernicious anemia. A megaloblastic macrocytic anemia was produced in coho salmon fed on folic acid deficient diet (Smith and Halver, 1969).
Crandall and Goodnight (1963) observed that heavy metals produced cardiac damage and blood cell destruction. The larvae of rainbow trout, Salmo gairdneri exposed to organophosphate insecticide dylox, were found to show abnormal behaviour and changes of various tissues and blood cells (Matton and Laham, 1969).

Many workers have emphasized the need for the establishment of normal haematological values in fish with a view to the diagnosis of disease (Hesser, 1960; Snieszko, 1960; Larsen and Snieszko, 1961; Summerfelt, 1967; Blaxhall, 1972) and in connection with pollution and its effects (Mawdesley-Thomas, 1971). Smit and Hattingh (1980) stated that fish biologists have started to realize the importance of haematology in disease aetiology. Changes in the haematological parameters such as packed cell volume (PCV), haemoglobin content, blood electrolytes, and red and white blood cell counts in fish can be induced by incidental factors such as capture and sampling (Bouck and Ball, 1966; Lowe-Jinde and Niimi, 1983), as well as chronic factors such as, exposure to disease and environmental contaminants (Christensen et al., 1977; Johansson-Sjöbeck and Larsson, 1978; Barham et al., 1980).

Pant et al. (1987) suggested that monitoring of blood parameters, both cellular and non-cellular, may have
considerable diagnostic value in assessing early warning signs of pesticide poisoning. It has been already established by Narain and Srivastava (1979), Narain and Nath (1982), and Srivastava and Narain (1982,1985) that environmental contaminants produce haematohistological, leucocytic and hemostatic anomalies, and affect the blood chemistry in the freshwater catfish, *Heteropneustes fossilis*. Ranganatha Koundinya and Rama Murthi (1979) reported that organophosphorous pesticides, sumithion and sevin decreased erythrocyte count and haemoglobin concentration in the blood of *Tilapia mossambica*. According to Blaxhall (1972), and Goss and Wood (1988), estimation of haemoglobin can be used as an index of anemia and fluid volume disturbance. Sastry and Sharma (1981), Singh and Srivastava (1981), Gluth and Hanke (1985), and Ferrando and Moliner (1991) noticed hyperglycemia in *Heteropneustes fossilis*, *Ophiocephalus punctatus* and *Cyprinus carpio* treated with endosulfan, diazinon and lindane, respectively. Similar observations were made during sublethal treatment of *Oreochromis mossambicus* to cadmium (Fu et al., 1990), *Labeo rohita* to copper (Radhakrishnaiah et al., 1992), *Tilapia nilotica* to nickel (Ghazaly, 1992a) and *Lagodon rhomboides* to CCl₄ (Folmar et al., 1993a).
Selye (1950) reported that increased whole blood glucose (hyperglycemia) and plasma cortisol concentration have all been associated with stressful conditions in mammals. Change in each of these blood components has been suggested as useful general indicators of stress in teleosts (Wedemeyer, 1972; Silberfeld, 1974; Donaldson and Dye, 1975; Casillas and Smith, 1977; McLeay, 1977). The increase in the blood glucose level has been used by Wedemeyer (1970), and Nemcsok and Boross (1982) for demonstrating metabolic stress. Elevation in glucose level is a typical response in carps exposed to various pollutants (Hanke et al., 1982; 1983; Gluth and Hanke, 1983). Plasma glucose has been widely monitored to study stress in fish (Mazeaud et al., 1977; Donaldson, 1981; Pickering et al., 1982) and may reflect elevated plasma cortisol (Chan and Woo, 1978).

Pillai and Mane (1984) in Catla catla exposed to fluoride effluent, Sastry and Siddiqui (1983) in Channa punctatus treated with endosulfan toxicity, Kumar and Pant (1981) in Puntius conchonius exposed to zinc and Folmar et al. (1993a) in Lagodon rhomboides treated with CCl₄ observed depletion in the plasma protein levels. Reduction in the protein content of different tissues of fish when exposed to sublethal levels of various toxicants have been well documented by many workers (McLeay and Brown,
According to Alexander and Ingram (1980), the concentration of protein in the serum of fish has been used as an indicator of their general state of health. The symptoms of pesticide toxicity generally involve respiratory distress (Holden, 1973). An increased glycolytic rate and a decrease in oxidative metabolism, protein and RNA synthesis were the prominent biochemical changes observed by Gupta (1986), Rao et al. (1986) and Ghosh (1987) during organophosphorous pesticide poisoning in fishes. Goss and Wood (1988) pointed out that the changes in serum protein levels may lead to fluid volume disturbance.

The foregoing review of literature indicates that not much work is available on the impact of organophosphorous toxicity on the blood chemistry of Cyprinus carpio var. communis. Hence, in the present study, the effect of kitazin toxicity, on some blood components of fish was studied, which may be useful as nonspecific bioindicators of organophosphorous toxicity.
MATERIAL AND METHODS

Blood samples for the estimation of haemoglobin, glucose and protein, both from acute and sublethal treatments, were obtained as outlined in the material and methods section of chapter II of this thesis.

ESTIMATION OF HAEMOGLOBIN

Haemoglobin content of the blood was estimated by Cyanmethemoglobin method using diagnostic reagent kit supplied by J.Mitra and Bros. Pvt. Ltd., New Delhi, India (Cat. No:CR 080500/CR 082000).

Principle

Haemoglobin can undergo several reactions; it binds oxygen and carbon-monoxide to form oxyhemoglobin and carboxyhemoglobin, respectively. Oxidation of the ferrous ion to the ferric form results in the formation of methemoglobin. Methemoglobin binds cyanide ions to form cyanmethemoglobin. Haemoglobin can be measured in any of these forms, but the most satisfactory method of assay, from the viewpoint of accuracy and simplicity involves the conversion of all forms of blood haemoglobin to cyanmethemoglobin.

The principle of the cyanmethemoglobin technique is the use of a single reagent, Drabkins solution, that
reacts with all forms of haemoglobin, except sulphahemoglobin. The latter pigment normally occurs in minute quantities in blood. According to this technique, the total haemoglobin at alkaline pH, is rapidly converted to the cyanoderivative by the initial oxidation of ferrous ions of haemoglobin to ferric state by potassium ferricyanide. Methemoglobin is subsequently converted to cyanmethemoglobin. Absorbance of cyanmethemoglobin is measured at 540 nm at which it is maximum. The colour intensity at this wave length is proportional to the total haemoglobin concentration.

**Reagents**

Reagent 1: Haemoglobin reagent
Reagent 2: Haemoglobin standard reagent (15 g/dl)

**Procedure**

The blood was drawn from control and experimental groups for haemoglobin assay with hypodermic syringe using heparin as an anticoagulant. Then, 0.02 ml of blood was dropped in separate 'Test' tubes containing 5.00 ml of haemoglobin reagent (Reagent-1). Similarly for 'Blank', 0.02 ml of distilled water was added to 5.00 ml of haemoglobin reagent. For 'Standard', the given haemoglobin standard reagent (Reagent-2) was taken as such in a test tube. The contents in all the test tubes were mixed well and incubated
at room temperature (30 ± 5°C) for 10 min. and the optical
density of the Standard (S), Control (C) and Test (T)
against Blank (B) was measured at 540 nm using spectronic-20
(Bausch and Lomb, U.S.A.).

Calculation

\[
g/dl \text{ of haemoglobin in blood} = \frac{\text{O.D. of Test}}{\text{O.D. of Standard}} \times 15
\]

**ESTIMATION OF GLUCOSE**

Plasma glucose was estimated by one step method of
Dubowski (1962) and Hyvarimen and Nikkila (1962) using
diagnostic reagent kit supplied by Beacon Diagnostics Pvt.
Ltd., Navsari, India (Code No : J 14/A).

**Principle**

Glucose reacts with 0-toluidine in presence of hot
acetic acid to give a blue-green colored complex, which is measured colorimetrically.

**Reagents**

Reagent 1 : Glucose reagent
Reagent 2 : Working glucose standard reagent (mg/100 ml)

**Procedure**

To 3.00 ml of glucose reagent (Reagent-1), 0.03 ml
of plasma from control and experimental fish was added
separately. For 'Blank', 0.03 ml of distilled water was added to 3.00 ml of glucose reagent and for 'Standard', 0.03 ml of working glucose standard reagent (Reagent-2) was added to 3.00 ml of glucose reagent. The contents in the test tubes were mixed well and kept in a boiling water bath for 10 min. Then, they were cooled quickly under running tap water to room temperature and the optical density of Standard (S), Control (C) and Test (T) was measured against reagent Blank (B) in spectronic-20 at 630 nm.

Calculation

\[
\text{The concentration of glucose in mg/100 ml =} \ \frac{\text{O.D. of Test}}{\text{O.D. of Standard}} \times 100
\]

ESTIMATION OF PROTEIN

Plasma protein estimation was done according to the method of Lowry et al. (1951).

Principle

The final blue colour of protein is produced by the reaction of carbamyl groups of protein molecules in the sample with alkaline copper and potassium of the reagent. This complex together with tyrosine and tryptophan of the sample gets reduced with phosphomolybdate of the Folin-Phenol reagent.
Reagents

Solution - A

2.00 g of sodium carbonate was dissolved in 100.00 ml of 0.1 N NaOH.

Solution - B

500.00 mg of copper sulphate was dissolved in 100.00 ml of 1% sodium potassium tartarate solution.

Solution - C

50.00 ml of solution-A was mixed with 1.00 ml of solution - B.

Folin-Phenol Reagent

1.00 ml of Folin-Phenol reagent was mixed with 1.00 ml of double distilled water.

Procedure

To 0.1 ml of diluted plasma from the control and experimental animals taken in separate 'Test' tubes, 0.99 ml of distilled water was added. They were treated with 5.00 ml of Solution-C for 10 min. Finally, 0.5 ml of Folin-Phenol reagent was added to the above mixture. Similarly for 'Blank', 1.00 ml of distilled water was mixed with 5.00 ml of Solution-C, kept for 10 min. and 0.5 ml of Folin-Phenol reagent was added. Colour intensity (O.D) of Control (C) and
Test (T) against reagent Blank (B) was read after 15 min. at 720 nm by spectronic-20.

For the preparation of 'Standard', 1.00 mg of bovine serum albumin (Sigma-Chemical Company, U.S.A.) was added to 10.00 ml of 1 N NaOH and made upto 100.00 ml in a standard flask. From this, 1.00 ml of solution was taken and mixed with 5.00 ml of Solution-C, kept for 10 min. and 0.5 ml of Folin-Phenol reagent was added. The optical density of the Standard (S) was read as mentioned above.

Calculation

\[
\frac{\text{O.D. of Unknown}}{\text{O.D. of Known}} \times \text{pg of Standard} = \text{pg of protein in ml of plasma.}
\]

Dilution factor = 1 : 10

STATISTICAL ANALYSIS

The statistical analysis was done individually for each sample and the mean value of five individual observations was taken for each parameter. The standard error for the sample mean was calculated and given in appropriate tables. The significance of sample mean between control and kitazin treated fish was tested by using Student's 't' test (Campbell, 1981) and Duncan's Multiple Range Test (Duncan, 1951). The analytical data together with Tables and Graphs/Bar-diagrams are presented in appropriate places in the text.
RESULTS

The data on the changes in the haemoglobin, glucose and protein levels of fish treated with median lethal concentration of kitazin are presented in Table 11 and Figs. 7 a, b, c. The haemoglobin content decreased to $1.746 \pm 0.056$ g/dl in the experimental fish from $2.845 \pm 0.103$ g/dl in control fish showing a per cent reduction of 38.63. However, the plasma glucose registered 32.26 per cent increase in its level over that of the control values. In the case of plasma protein, the experimental fish registered a 58.85 per cent decline when compared to that of the control values.

Table 12 and Fig. 8 present the results on the haemoglobin content of blood of fish treated with sublethal concentration of kitazin from treatment-I and II. At the end of 5th day, fish from treatment-I exhibited a per cent reduction of 5.79 which increased to 29.27 per cent at the end of 10th day of treatment. However, after 20th day of treatment, the haemoglobin content of fish started recovering giving 53.07, 24.10 and 74.14 per cent increase at the end of 20th, 25th and 30th days of treatment, respectively.
Table 11: Changes in the haemoglobin, plasma glucose and protein levels of *Cyprinus carpio* var. *communis* exposed to 24 h LC$_{50}$ concentration of kitazin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Experimental</th>
<th>Per cent change</th>
<th>Calculated 't'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>2.845 ± 0.103</td>
<td>1.746 ± 0.056</td>
<td>-38.63</td>
<td>8.356*</td>
</tr>
<tr>
<td>Glucose (mg/100 ml)</td>
<td>118.572 ± 2.828</td>
<td>156.818 ± 3.015</td>
<td>+32.26</td>
<td>8.275*</td>
</tr>
<tr>
<td>Protein (µg/ml)</td>
<td>462.500 ± 5.528</td>
<td>190.333 ± 1.776</td>
<td>-58.85</td>
<td>41.950*</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of 5 individual observations

- Denotes per cent decrease over control
+ Denotes per cent increase over control
* Values are significant at 5% level

Degrees of freedom at 8 t 0.05 = 2.306
Fig. 7. Bar-diagram showing changes in haemoglobin, plasma glucose and protein levels of *Cyprinus carpio* var. *communis* exposed to 24 h LC$_{50}$ concentration of kitazin.

a. Haemoglobin

b. Glucose

c. Protein
Fig. 7a

Fig. 7b

Fig. 7c
<table>
<thead>
<tr>
<th>Treatment period (in days)</th>
<th>Control</th>
<th>Treatment-I</th>
<th>Treatment-II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Haemoglobin content (g/dl)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(g/dl)</td>
<td>(g/dl)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>5</td>
<td>2.780 ± 0.086</td>
<td>2.619 ± 0.148</td>
<td>1.791 ± 0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-5.79)</td>
<td>(-35.58)</td>
</tr>
<tr>
<td>10</td>
<td>2.651 ± 0.113</td>
<td>1.875 ± 0.086</td>
<td>1.649 ± 0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-29.27)</td>
<td>(-37.80)</td>
</tr>
<tr>
<td>15</td>
<td>2.328 ± 0.103</td>
<td>2.295 ± 0.092</td>
<td>1.507 ± 0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-1.42)</td>
<td>(-35.27)</td>
</tr>
<tr>
<td>20</td>
<td>2.619 ± 0.148</td>
<td>4.009 ± 0.172</td>
<td>3.453 ± 0.042</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+53.07)</td>
<td>(+31.84)</td>
</tr>
<tr>
<td>25</td>
<td>2.871 ± 0.115</td>
<td>3.563 ± 0.024</td>
<td>3.498 ± 0.037</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+24.10)</td>
<td>(+21.84)</td>
</tr>
<tr>
<td>30</td>
<td>2.896 ± 0.088</td>
<td>5.043 ± 0.116</td>
<td>3.660 ± 0.017</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+74.17)</td>
<td>(+26.28)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of 5 individual observations

Values in parantheses are per cent change over control

- Denotes per cent decrease over control

+ Denotes per cent increase over control
Fig. 8. Bar-diagram showing changes in haemoglobin content of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of kitazin from treatment-I and II.
Fig. 8

Haemoglobin (g/dl)

Control  L — ) Treatment-

Treatment (in days)

6.04 4.04 3.04 2.04 1.04

Fig. 8
The haemoglobin content of the blood of fish from treatment-II showed a marked reduction (ranging from 35 to 37%) in its level upto 15th day of treatment (Table 12 and Fig. 8). The per cent reduction in the haemoglobin content of the experimental fish during the above period was higher than that of the fish from treatment-I. The haemoglobin level recovered giving 31.84, 21.84 and 26.38 per cent increase at the end of 20th, 25th and 30th days of treatment, respectively, showing a lower per cent recovery when compared to that of the fish from treatment-I.

Statistical analysis on the changes in the haemoglobin content of blood of *Cyprinus carpio* exposed to sublethal concentration of kitazin from treatment-I and II by DMRT gave the following results (Table 13).

The haemoglobin content observed after 5th day was found to be on par with each other in control and treatment-I groups, while both of them were significantly different from treatment-II. A similar trend was observed after 15th day also. The haemoglobin content observed after 10th, 20th and 30th day was such that the control, treatment-I and II groups were significantly different from each other. On the other hand, the haemoglobin content observed after 25th day was on par with each other in treatment-I and II, but were significantly different from that of control group.
Table 13: DMRT table for haemoglobin content in the blood of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of kitazin from treatment-I and II

<table>
<thead>
<tr>
<th>Treatment period (in days)</th>
<th>Control</th>
<th>Treatment - I</th>
<th>Treatment - II</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.780</td>
<td>2.619</td>
<td>1.791</td>
<td>2.397 III</td>
</tr>
<tr>
<td>10</td>
<td>2.651</td>
<td>1.875</td>
<td>1.649</td>
<td>2.058 IV</td>
</tr>
<tr>
<td>15</td>
<td>2.328</td>
<td>2.295</td>
<td>1.507</td>
<td>2.043 IV</td>
</tr>
<tr>
<td>20</td>
<td>2.619</td>
<td>4.009</td>
<td>3.453</td>
<td>3.360 II</td>
</tr>
<tr>
<td>25</td>
<td>2.871</td>
<td>3.563</td>
<td>3.498</td>
<td>3.311 II</td>
</tr>
<tr>
<td>30</td>
<td>2.896</td>
<td>5.043</td>
<td>3.660</td>
<td>3.866 I</td>
</tr>
</tbody>
</table>

Mean: 2.691, 3.234, 2.593

Small letters are represented for comparison of row means (Different treatments for a particular period)

Capital letters are represented for comparison of column means (Different periods for a particular treatment)

Roman letters are represented for comparison of period means

Greek letters are represented for comparison of treatment means

Means of similar letters are on par
The haemoglobin content in the control groups was found to be on par with each other after 5th, 10th, 20th, 25th and 30th day of treatment, but they were significantly different from that of 15th day. The haemoglobin content found after different periods in treatment-I was found to be significantly different from each other. The haemoglobin content of fish from treatment-II after 5th, 10th and 15th day was on par with each other, but they were significantly different from the haemoglobin content found after 20th, 25th and 30th day of treatment. The haemoglobin content found at the end of 20th, 25th and 30th day was on par with each other, but they were significantly different from that of 5th, 10th and 15th day of treatment.

Taking the haemoglobin content from different periods irrespective of the treatments, the following were observed. The haemoglobin content after 5th day was found to be significantly different from that of other periods. The haemoglobin content of fish after 10th and 15th day was found to be on par with each other, but they were significantly different from the haemoglobin content of other periods. The haemoglobin content found at the end of 20th day was on par with that of 25th day but significantly differed from 5th, 10th, 15th and 30th day.
Considering the haemoglobin content of the blood of fish from different treatments irrespective of the periods, treatment-II group was on par with control group, but both of them were significantly different from the haemoglobin content of the fish from treatment-I. In general, upto 15th day, treatment-II had a pronounced effect on haemoglobin content than treatment-I. But after 15th day of treatment period, treatment-I exhibited more effect on haemoglobin content than treatment-II, which was statistically significant.

Table 14 and Fig. 9 give the data on changes in the plasma glucose level of the fish from treatment-I. After 5th day of treatment, the elevation in the plasma glucose was 29.44 per cent. The increase in the plasma glucose level showed a steady and gradual elevation during the subsequent sublethal exposure periods giving 109.52 per cent increase over that of their controls at the end of 30th day of treatment.

The fish from treatment-II also exhibited a marked increase in their plasma glucose level throughout the treatment period giving 46.25 per cent increase after 5th day and 233.97 per cent after 30th day of treatment (Table 14 and Fig. 9). In treatment-II, the elevation in the plasma glucose level of fish was almost two fold when compared with that of the fish from treatment-I.
Table 14: Glucose level in the plasma of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of kitazin from treatment-I and II

<table>
<thead>
<tr>
<th>Treatment period (in days)</th>
<th>Glucose level (mg/100 ml)</th>
<th>Control</th>
<th>Treatment-I</th>
<th>Treatment-II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>125.000 ± 3.685</td>
<td>161.806 ± 1.643</td>
<td>182.813 ± 2.073</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+29.44)</td>
<td>(+46.25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>141.667 ± 2.485</td>
<td>253.125 ± 4.146</td>
<td>311.364 ± 5.379</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+78.68)</td>
<td>(+119.79)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>135.000 ± 3.944</td>
<td>248.333 ± 2.211</td>
<td>273.438 ± 3.698</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+83.95)</td>
<td>(+102.55)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>121.875 ± 2.795</td>
<td>251.563 ± 3.698</td>
<td>364.063 ± 3.698</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+106.41)</td>
<td>(+198.72)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>93.750 ± 3.062</td>
<td>178.125 ± 4.146</td>
<td>359.375 ± 4.146</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+90.00)</td>
<td>(+283.33)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>116.667 ± 3.514</td>
<td>244.444 ± 3.143</td>
<td>389.063 ± 2.073</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+109.52)</td>
<td>(+233.97)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of 5 individual observations

Values in parantheses are per cent change over control

+ Denotes per cent increase over control
Fig. 9. Bar-diagram showing changes in plasma glucose level of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of kitazin from treatment-I and II.
Glucose (mg/100ml)

Treatment-I  Treatment-II
Treatment (in days)

Control

Fig. 9
The DMRT on the plasma glucose level of *Cyprinus carpio* from treatment-I and II is given in Table 15. The plasma glucose levels of fish after 5th day was found to be significantly different from each other in control, treatment-I and II groups. Similar observations were also noted for 10th, 15th, 20th, 25th and 30th day exposures.

In control groups, the plasma glucose level found after 5th and 15th day, 10th and 15th day, and 5th, 20th and 30th day was on par with each other. While they were significantly different in the haemoglobin content from the rest of their counterparts. In treatment-I group, the plasma glucose level of fish was on par with each other after 10th, 15th, 20th and 30th day, while they significantly differed from that of 5th and 25th day of treatment. The plasma glucose level in treatment-II group revealed that it was on par with each other during 20th and 25th day, while they were significantly different from that of 5th, 10th, 15th and 30th day which were significantly different from each other.

Analysis of means for different periods irrespective of the treatments revealed that glucose level of fish was found to be on par for 20th and 30th day, while it was significantly different from that of 5th, 10th, 15th
Table 15: DMRT table for glucose level in the plasma of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of *kitazin* from treatment-I and II

<table>
<thead>
<tr>
<th>Treatment period (in days)</th>
<th>Control</th>
<th>Treatment - I</th>
<th>Treatment - II</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>125.000 (\text{CBC})</td>
<td>161.806 (\text{bC})</td>
<td>182.813 (\text{aE})</td>
<td>156.540 (\text{V})</td>
</tr>
<tr>
<td>10</td>
<td>141.667 (\text{CA})</td>
<td>253.125 (\text{bA})</td>
<td>311.364 (\text{aC})</td>
<td>235.385 (\text{II})</td>
</tr>
<tr>
<td>15</td>
<td>135.000 (\text{CAB})</td>
<td>248.333 (\text{bA})</td>
<td>273.438 (\text{aD})</td>
<td>218.924 (\text{III})</td>
</tr>
<tr>
<td>20</td>
<td>121.875 (\text{CC})</td>
<td>251.563 (\text{bA})</td>
<td>364.063 (\text{aB})</td>
<td>245.834 (\text{I})</td>
</tr>
<tr>
<td>25</td>
<td>93.750 (\text{CD})</td>
<td>178.125 (\text{bB})</td>
<td>359.375 (\text{aB})</td>
<td>210.417 (\text{IV})</td>
</tr>
<tr>
<td>30</td>
<td>116.667 (\text{CC})</td>
<td>244.444 (\text{bA})</td>
<td>389.063 (\text{aA})</td>
<td>250.058 (\text{I})</td>
</tr>
</tbody>
</table>

Mean

---

Small letters are represented for comparison of row means (Different treatments for a particular period)

Capital letters are represented for comparison of column means (Different periods for a particular treatment)

Roman letters are represented for comparison of period means

Greek letters are represented for comparison of treatment means

Means of similar letters are on par
and 25th day. When the glucose level in the plasma of fish was analysed from different treatments irrespective of their periods, treatment-I and II were significantly different from control and also among each other.

The effect of treatment-I and II was significant from control. Whereas, treatment-II had a high stress than that of treatment-I, so that the plasma glucose level was high in treatment-II when compared with the other group.

Plasma protein content of fish from treatment-I registered a decrease in its level throughout the experimental period over that of the control (Table 16 and Fig. 10). The reduction in the protein content was maximum at the end of 15th day of treatment (55.94 per cent) and minimum after 25th day of treatment (12.86 per cent). The decrease in the plasma protein content of fish from treatment-II was more pronounced than that of their counterparts from treatment-I (Table 16 and Fig. 10). The per cent decrease in the plasma protein level varied between a low value of 31.49 after 20th day of treatment and a high of 48.17 after 10th day treatment.

The plasma protein content of Cyprinus carpio when analysed statistically by DMRT showed the following results (Table 17). The plasma protein content of fish from control, treatment-I and II groups was significantly different from
Table 16: Protein content in the plasma of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of kitazin from treatment-I and II

<table>
<thead>
<tr>
<th>Treatment period (in days)</th>
<th>Protein content (µg/ml)</th>
<th>Protein content (µg/ml)</th>
<th>Protein content (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment-I</td>
<td>Treatment-II</td>
</tr>
<tr>
<td>5</td>
<td>462.500 ± 3.727</td>
<td>315.000 ± 4.854</td>
<td>275.833 ± 2.560</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-31.89)</td>
<td>(-40.36)</td>
</tr>
<tr>
<td>10</td>
<td>475.500 ± 7.204</td>
<td>320.000 ± 2.108</td>
<td>244.917 ± 1.507</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-32.28)</td>
<td>(-48.17)</td>
</tr>
<tr>
<td>15</td>
<td>476.667 ± 2.494</td>
<td>210.000 ± 0.943</td>
<td>318.750 ± 0.553</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-55.94)</td>
<td>(-33.13)</td>
</tr>
<tr>
<td>20</td>
<td>460.417 ± 2.764</td>
<td>340.000 ± 2.829</td>
<td>315.417 ± 0.553</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-26.15)</td>
<td>(-31.49)</td>
</tr>
<tr>
<td>25</td>
<td>466.667 ± 2.357</td>
<td>406.667 ± 3.399</td>
<td>313.750 ± 0.986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-12.86)</td>
<td>(-32.77)</td>
</tr>
<tr>
<td>30</td>
<td>464.167 ± 4.460</td>
<td>316.667 ± 4.714</td>
<td>287.083 ± 0.986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-31.78)</td>
<td>(-38.15)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of 5 individual observations
Values in parantheses are per cent change over control
- Denotes per cent decrease over control
Fig. 10. Bar-diagram showing changes in plasma protein content of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of kitazin from treatment-I and II.
Fig. 10

Protein (μg/ml)

Treatment (in days)

500
400
300
200
100
0

Control  Treatment-I  Treatment-II

Fig. 10
Table 17: DMRT table for protein content in the plasma of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of kitazin from treatment-I and II

<table>
<thead>
<tr>
<th>Treatment period (in days)</th>
<th>Control</th>
<th>Treatment - I</th>
<th>Treatment - II</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>462.500 aBC</td>
<td>315.000 bC</td>
<td>275.833 cC</td>
<td>351.111 IV</td>
</tr>
<tr>
<td>10</td>
<td>472.500 aAB</td>
<td>320.000 bC</td>
<td>244.917 cD</td>
<td>345.806 V</td>
</tr>
<tr>
<td>15</td>
<td>476.667 aA</td>
<td>210.000 cD</td>
<td>318.750 bA</td>
<td>335.139 VI</td>
</tr>
<tr>
<td>20</td>
<td>460.417 aC</td>
<td>340.000 bB</td>
<td>315.417 cA</td>
<td>371.945 II</td>
</tr>
<tr>
<td>25</td>
<td>466.667 aABC</td>
<td>406.667 bA</td>
<td>313.750 cA</td>
<td>395.695 I</td>
</tr>
<tr>
<td>30</td>
<td>464.167 aBC</td>
<td>316.667 bC</td>
<td>287.083 cB</td>
<td>355.972 III</td>
</tr>
</tbody>
</table>

Mean 467.153 318.056 292.625

Small letters are represented for comparison of row means (Different treatments for a particular period)

Capital letters are represented for comparison of column means (Different periods for a particular treatment)

Roman letters are represented for comparison of period means

Greek letters are represented for comparison of treatment means

Means of similar letters are on par
each other during all the exposure periods namely, 5th, 10th, 15th, 20th, 25th and 30th day.

When the control group was analysed, the protein content of fish found at the end of 5th and 15th day, 5th and 20th day, 5th and 30th day, and 10th and 20th day was significantly different from each other. While all others were on par with each other. In treatment-I, the protein content of fish from 5th, 10th and 30th day was on par, while they were significantly different from that of 15th, 20th and 25th day values. Likewise, the protein content of 15th, 20th and 30th day also differed significantly from each other. The plasma protein of fish from treatment-II was on par at the end of 15th, 20th and 25th day, while they significantly differed from that of 5th, 10th and 30th day of treatment-II. Also, 5th, 10th and 30th day varied significantly from one another.

Irrespective of treatments, the mean values for different periods were significantly different from each other from 5th day to 30th day. Likewise the mean values of different treatments were significantly different from each other in spite of different exposure periods. From the above results it may be inferred that depletion of protein content was high in treatment-II even though both the treatments significantly differed from control. So it can be concluded that treatment-II has a very high impact on fish.
DISCUSSION

The major vehicle by which insecticides are distributed throughout the body is blood for vertebrates and body fluid-haemolymph for invertebrates (Matsumura, 1985). Since the majority of insecticides are not readily soluble in aqueous solutions, the mechanisms by which the blood carries them have aroused scientific curiosity. Moss and Hathway (1964) found that solubility of teleodrin® and dieldrin in rabbit serum was 4000 times greater than their solubility in water. The above authors further observed that in the blood, pesticides were found mainly in the erythrocytes (particularly in the erythrocyte contents) and plasma and not in the leukocytes, platelets or stroma indicating that they mainly bind with haemoglobin.

Heavy metal toxicity decreased haemoglobin level and hematocrit values in many fish (Larsson, 1975; Christensen et al., 1977; Sjobeck et al., 1984; Gill and Pant, 1985; Garg et al., 1989; Ruparelia et al., 1990; Sen et al., 1992). Studies of Natarajan (1981), Verma et al. (1982), Sastry and Siddiqui (1984), and Andaya and Di Giulio (1987) revealed that both organochlorine and organophosphorous insecticides were found to decrease the haemoglobin content, hematocrit value and other blood parameters in fish
Hypoxia refers to any condition in which there is an inadequate supply of oxygen to the tissues. According to Smith (1980) arterial or anoxic hypoxia is characterized by lower-than-normal $\text{PO}_2$ in arterial blood when the oxygen capacity and rate of blood flow are normal or elevated; in toxic insults this type of hypoxia results from exposure to pulmonary irritants or drugs that depress the respiration. Although the rate of haemoglobin oxidation is greatly increased by exposure to a variety of chemicals, heme group oxidation occurs spontaneously in air; the spontaneous or autoxidation accounts for very low concentrations of haemoglobin found in the normal circulating blood; some chemicals are capable of mediating the oxidation of haemoglobin both in vivo and in vitro (Smith, 1980).

In vitro incubation of mammalian erythrocytes with dichlone (Sikka et al., 1974) and menadione (Mezick et al., 1970) resulted in rapid and extensive oxidation of haemoglobin, leading to formation of methemoglobin and significant increase in osmotic fragility of erythrocytes resulting in cell lysis. Matkovics et al. (1987) observed in Cyprinus carpio a quick decrease in haemoglobin content in response to paraquat toxicity and the authors suggested that it might be presumably through methemoglobin formation and a direct response of $O_2^-$ radical. Bus et al. (1976) and
Barabas et al. (1983) reported that the redox reactions involving paraquat in the presence of oxygen produced superoxide ($O_2^-$) radical, an agent with a strong influence on the structure and function of important cellular molecules supporting the findings of the above workers.

The decrease in the haemoglobin content during acute toxicity and up to 15 days of sublethal treatment in the present study may be due to rapid oxidation of haemoglobin to methemoglobin or release of $O_2^-$ radical brought about by the toxic stress of kitazin. The work of Goldberg and Stein (1976) revealed that hydrogen peroxide formed from the coupled oxidation of oxyhemoglobin stimulated the formation of large amounts of methemoglobin. It is increasingly recognized that xenobiotics capable of undergoing redox cycling can exert toxic effects via the generation of oxygen-free radicals (Freeman and Crapo, 1982; Mason, 1982; Pryor, 1982; Di Guiseppi and Fridovich, 1984), which may reinforce the observations of the present study. However, further work is needed to ascertain the exact mechanism responsible for the decrease in the haemoglobin content of fish.

Several authors have observed an increase in the haemoglobin content in fish when exposed to heavy metals and insecticides (Christensen et al., 1972; Niimi and Lowe-
According to Chitra and RamanaRao (1986), the hyperactivity may be due to hypoxia faced by the fish due to gill damage by the irritants and increased haemoglobin content may be a response to compensate impaired respiratory efficiency. Pant et al. (1987) also noted an increase in the haemoglobin content in the blood of Barbus conchonius and suggested that the hypoxemia may trigger an exodus of erythrocytes from haemopoietic loci in an attempt to compensate for the reduced oxygen carrying capacity of the blood. In the present study also, the recovery in haemoglobin level of experimental fish from treatments - I and II may be a compensatory mechanism in response to hypoxia produced by kitazin toxicity, recalling the observations of the above workers.

Heavy metals, industrial effluents and pesticides produce hyperglycemic and hepatic glycogen depletion in various teleost species (Sakaguchi, 1972; McLeay, 1977; Das et al., 1980; Sastry and Subhadra, 1982; Srivastava, 1982). Silbergeld (1974) reported that hyperglycemic condition may be generally due to nonspecific response to pesticide induced stress. The increased glucose may result from activation of glycogen stores by adrenalin-mediated through stress (Nakano and Tomlinson, 1967).
The organophosphorous insecticides are believed to be nerve poisons and block synaptic transmission in the cholinergic part of the nervous system (Metcalf, 1971; Reddy and Rao, 1988), which may lead to the excessive accumulation of acetylcholine at nerve cell junctions. A similar observation was made by Brzezinski and Ludwicki (1973), who reported that organophosphorous insecticides may result in accumulation of acetylcholine at synaptic junctions with a simultaneous increase in the secretion of catecholamines in mammals. Stressful stimuli elicit rapid secretion of both glucocorticoids and catecholamines from the adrenal tissue of fish, leading to hyperglycemia (Singh and Srivastava, 1981).

The hyperglycemia may be a physiological response to meet the critical need of the brain tissue for increased energy in the form of glucose during methylparathion exposure in *Metapenaeus monoceros* (Reddy and Rao, 1991). In the present study, the fish exposed to kitazin, an organophosphorous fungicide has showed a similar physiological response which finds support from the observations of Silbergeld (1974), and Reddy and Rao (1991) who suggested that hyperglycemic condition may be generally due to nonspecific response to pesticide induced stress.
In teleosts, an analogous system to the mammalian PAS (Pituitary Adrenal System), the pituitary – interrenal axis (PIA) exists with the interrenal (adrenal) gland secreting the same types of corticosteroids and with, in the main, the same physiological functions as those in mammals (Bentley, 1976). Physiological changes in teleosts subjected to stressors have shown enough similarities with a mammalian model (General Adaptation Syndrome; Selye, 1936) to suggest that teleostean response to stressors are basically the same.

Whole body protein concentrations are influenced by a variety of environmental factors (Claybrook, 1983). According to Florkin and Scheer (1970), Gilles (1970) and Bayne (1973), under conditions of stress many organisms will mobilize proteins as an energy source via the oxidation of amino acids. Kabeer et al. (1978) reported that pesticide stress in fish resulted in serum protein degradation and the products of which are fed to TCA cycle through aminotransferase system to meet high energy demand. During toxicant-induced stress, protein catabolism increased in oysters exposed to naphthalene toxicity (Riley and Mix, 1981), in freshwater crabs treated with sumithion (Bhagyalakshmi et al., 1983) and in amphipods exposed to pentachlorophenol (Graney and Giesy, 1986).
The decrease in the serum protein content of asphyxiated *Scorpaena porcus* was interpreted by Cordier and Chanel (1958) as a disturbance in the dynamic equilibrium between anabolic and catabolic processes in protein metabolism in which catabolism prevails. Rao et al. (1987) suggested that inhibition of protein synthesis by fluoride in bivalve mollusc, *Indonaia caeruleus* result from the inhibition of initiation of new peptide chains and the dissociation of 80 S ribosome.

Moore et al. (1982) found that lysosomes may be destabilized in animals found in polluted waters and thus hydrolytic enzymes could be released leading to protein breakdown. But according to Dalela et al. (1981) the decrease in the plasma protein levels of pesticide treated *Mystus vittatus* may be due to the excretion of proteins by kidney due to kidney disorder (albuminuria) or impaired protein synthesis as a result of liver disorders. The reduced levels of serum protein has been correlated to either its excessive loss due to nephrosis or to reduced protein synthesis due to liver cirrhosis by Lynch et al. (1969). In the present study, the decrease in the plasma protein levels of fish treated with acute and sublethal level of kitazin may be due to hepatic impairment or nephrosis or protein degradation due to increased
activity of proteolytic enzymes or any fermentation combination of the above factors which may find support from the above authors.

According to Klaassen (1980) that several proteins within the plasma can bind normal physiologic constituents in the body as well as some foreign compounds; most foreign compounds that are bound to plasma proteins are bound to albumin; the fraction of toxicant in the plasma bound to plasma proteins is not immediately available for distribution into the extravascular space or for filtration at the kidney. Petermann (1961) pointed out that toxicants must usually exist in the free state, to react with biological molecules and interfere with biochemical mechanisms. The author further suggested that the total amount of plasma protein available for the binding and transport of toxic substances plays an important role in the toxicological consequences of these compounds. Reduction in the plasma protein levels of fish treated with kitazin may lead to the availability of free state of the pesticide in large quantity in plasma which may interfere with the biochemical mechanisms of fish making it more susceptible to the toxicant.

Simon and Blackman (1967) observed that the effect of pH on toxicity does not depend on pH alone, but also a
function of pK. The total concentration of toxic material necessary to produce the same responses from the organism, rise at the higher pH levels, but the point along the pH scale at which this rise commences in governed by pK. Hence, the effect of pH on toxicity is directly related to the degree of dissociation of toxic substances. Dissociation of toxicants may result in the formation of ions which must act as anion and cations (Simon and Blackman, 1967). Thus there is a competition between cations and hydrogen ions for negative centres, and between anions and hydroxyl ions for positive centres.

Cameron (1979) observed respiratory acidosis (CO₂ excess) in fish which was atypical and implicated some limitation in CO₂ diffusion and (or) catalysed HCO₃⁻ dehydration at the gill. Endogenous (lactic acid; Krishna Murthy et al., 1981) and exogenous factors (H⁺ influx or base efflux, McDonald and Wood, 1981; Ultsch et al., 1981) as well as changes in the relative intensities of the counterion exchanges across the gill (Na⁺/H⁺ or NH₄⁺ and Cl⁻/HCO₃⁻ or OH⁻, Maetz, 1973), might all have contributed to the observed metabolic acidosis in fish; since, an increase in gill permeability to H⁺ has been documented at low ambient pH (McWilliams and Potts, 1978), enhanced branchial H⁺ influx would be the most possible explanation for the observed acidosis (Hobe et al., 1983). Probably, a similar
situation may prevail in the present study also leading to metabolic acidosis, resulting in more pronounced alterations in the haemoglobin, plasma glucose and protein values of fish from treatment- II.