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Toxic Effects of Parathion Pesticide on Haematological and Biochemical Parameters in Freshwater: Fish *Barbus Luteus* (H)

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ABSTRACT

The primary objective of this study was to elucidate the haematological and biochemical alterations induced by the toxic effects of parathion pesticide under controlled laboratory conditions. Significant declines were observed in Red Blood Cell (RBC) count Haemoglobin (HB) and Haematocrit (HTC) levels as compared to control. Conversely, Mean Corpuscular Haemoglobin (MCH) pg, Corpuscular Haemoglobin Concentration (MCHC) exhibited substantial increases. An appreciable elevation in Mean Corpuscular Volume (MCV) reaching 175 fl, 186 fl, and 280 fl, was noted, in contrast to the control value of 165 fl. Biochemical parameters, including protein, glycogen, and cholesterol, demonstrated significant decreases. Concurrently, an elevated trend was observed in blood glucose levels (31.05, 42.2, and 51.03 mg/dl), compared to the control group (27.51) mg/dl, with these increases corresponding to the concentration and duration of exposure. The findings of this study underscore the value of haematological and biochemical indicators as reliable measures to detect the impacts of chemical pollutants, such as pesticides, on organism health.

1. INTRODUCTION

Parathion, or Dimethyl-(4-nitrophenoxy)-Sulfanylidene-5-phosphane, is a highly toxic organophophorous insecticide primarily employed for the control of agricultural pests. This pesticide is characterised by its short-term, high toxicity exposure, typically through inhalation, leading to skin, respiratory, and nervous system injuries [1, 2]. Prolonged exposure, termed "long-term effects," often results in decreased cholinesterase enzyme levels, fainting, and headaches. Furthermore, the Environmental Protection Agency (EPA) has classified parathion as a carcinogen [3].

The aquatic environment is frequently subject to pollution resulting from human activities, most notably through direct discharge, such as factory waste, or indirect routes, such as runoff from agricultural lands where pesticides are applied to eliminate pests [4, 5]. Misapplication of pesticides by farmers often results in only half of the applied amount reaching the target pests, while the remainder contributes to environmental pollution. These pollutants, often transported by storms and rainwater, pose significant risks to major water bodies [4].

Pesticides' detrimental effects on non-target organisms have been well-documented [6, 7]. Fish, being integral components of the food web, provide a valuable metric for assessing environmental impacts of pollutants. They serve as effective bioindicators owing to their ability to reflect the level of harmful substances in water and provide early warnings of effluent toxicity [8].

The introduction of pesticides and other chemical and organic pollutants into water bodies alters their quality and reduces their capacity to support life, particularly during periods of reproduction or migration. Hence, fish are commonly used for water quality assessments, especially in the presence of a multitude of known and unknown pollutants. Haematological and biochemical tests on laboratory animals offer precise and rapid determinations [9-11].

Acute concentrations of pesticides' survival and metabolic effects have been the focus of numerous studies. These studies often analyse changes in the blood parameters of fish exposed to waterborne pollutants and pesticides over time. Changes in haematological processes act as a negative reflex and an indicator of the fish's exposure to rapid stresses [12]. Moreover, alterations in protein, glycogen, lipids, and enzymes in fish tissues or blood are considered crucial indicators of fish health [13, 14]. Chronic effects, such as changes in tissues and enzymatic structure, result from the accumulation of pesticides, particularly in highly sensitive fish species [15, 16].

Thus, the current study investigates the effects of the organophophorous pesticide Methyl parathion (O, O-dimethyl O-4-nitrophenyl phosphorothioate) on haematological and biochemical variables during a 15-day period of long-term exposure to sub-lethal concentrations of the pesticide.

2. MATERIALS AND METHODS

2.1 Collection samples

Barabus Luteus were collected from the Shatt-Arab bank north of Basra governorate, then transferred to the laboratory for one week to acclimatise them to appropriate laboratory conditions such as temperature and ventilation before being used for experiments. The fish were placed in a plastic tang tank with a capacity of 250 litters. The fish were 10 ± 3 gm in weight and 12 ± 3 in length. The fish are acclimated to laboratory conditions at temperatures between 26 ± 28 for a period of one week only. The acclimatization tanks were provided with aeration devices to follows the fishes to settle into laboratory conditions have no signs of stress. After a week, they are distributed to the experiment ponds in five replications, each of which contains five samples.

2.2 Experimental design

After the acclimation period, the concentrations of Parathion pesticide were prepared at 0.25, 0.5, and 0.75 mg/L. The concentrations of pesticides in this experiment are designed depending on the mitigation equation (C1V1 = C2V2) and the percentage of the active ingredient installed on a container of parathion pesticides, which is 50%. These concentrations were chosen according to the value of the lethal concentrations (LC50 = 0.955 mg/L) as the experimental fish were exposed to five replicates containing five samples of fish for a period of 15 days for exposure to sub lethal concentrations of parathion. The fish are also exposed to sub-lethal concentrations for a period of semi-chronic exposure estimated at 15 days in order to clarify the effects on blood ratios and chemical measurements after the end of the period. The measurements are taken from the experimental fish.

Blood samples were taken by drawing blood with a special pipette in order to calculate blood parameters (RBC, HB, HCT, MCV, MCH, and MCHCH). Estimation of RBC using a hemocytometer or Neubauer chamber, followed by blood drawn from the caudal area of the fish and diluted by RBC fluid of formalin-citrate, then placed in a cytometer with a slide cover using the Blaxhall and Daisley [17] method. Estimate of haemoglobin by the cyanmethemoglobin method using Drabkins' reagent [18].

2.3 Biochemical samples

Its including:

Estimation of glycogen

10 mg of liver were taken for 15 samples from the experimental replicates of the fish *Barabus Luteus* exposed to sub lethal concentrations of parathion, the tissues were thoroughly crushed in 10 ml of trichloroacetic acid. The solution was placed in a centrifuge for 20 minutes at a speed of 3500 rpm. 2 ml of filtrate was taken, and 6 ml of acid was added to it (H_2SO_4 concentrate). The test tube was kept in water for 6 minutes after the formation of the colour. We measured its optical density busing a spectrophotometer with a wavelength of 515 nm. The glycogen level is calculated by Srinivasan and Krishnaswamy [19].

Estimation of cholesterol

A certain amount of tissue liver weight by an exact weight. After crushing the tissue with a known 10 ml amount of acetone and leaving the solution in the test tube for 2-3 hours, the cholesterol is extracted. The tube is placed in the centrifuge for 5 minutes. The filtrate is transferred to another test tube and evaporated to complete drying by water bath with a temperature of 100℃ to complete dryness in a water bath, the extract of tissue cholesterol is dissolved in 5 ml of chloroform, and 2 ml of anhydrous acetate mixture 0.1 ml of H_2SO_4 concentrate is added to it, and it is left in a dark shade for 8 minutes after the formation of the color. The sample is measured by spectrophotometer, with wave length of 620 nm, the cholesterol value calculated from the stranded. Estimation of protein: the tissues (10 mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 minutes, and the clear supernatant was used for the analysis of total proteins. Total protein concentration was estimated by the method of Lowry [20].

Estimation of glucose

Blood samples are taken using a needle syringe, and the blood is transferred to a glass tube containing heparin, which is placed in the centrifuge at 400 rpm for 15 minutes at 4℃, after which the plasma is separated for glucose according to the method described by Haider and Rauf [21].

3. RESULTS AND DISCUSSION

The results of haematological and biochemical analyses are analysed using one-way ANOVA to determine significant differences in blood and chemical parameter values compared to the control. The SPSS programme used the mean S.D. of all statistical measurements the probability level ($P<0.05$). The results demonstrate the differences in blood parameters under sub lethal concentrations of parathion pesticides.

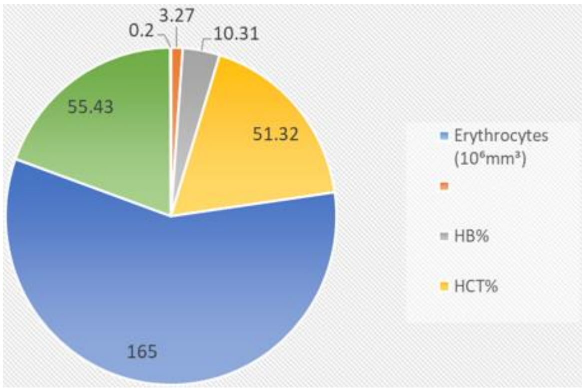

Figure 1. Influence of parathion at 0.25, 0.5, and 0.75 mg/L after 15 days of exposure on haematological variables in *Barbus Luteus* fish

Table 1. Effect of parathion on hematological variables of *Barbus Luteus* fish at 0.25, 0.5, 0.75 mg/L after 15 days of exposure

Parameters	Control	0.25mg/L	0.5mg/L	0.75mg/L
Erythrocytes (10^6mm^3)	3.27±2.2	2.57±0.41	2.16±0.12	1.11±0.52
HB %	10.31±1.7	8.2±0.8	6.61±1.03	3.01±0.02
HCT %	51.32±3.05	30.01±2.2	14.31±1.3	7.05±0.14
MCV (fl)	165±2.7	175±4.13	186.2±5.0.1	280±6.31
MCH (pg.)	55.43±3.2	42.15±2.17	39.17±1.03	27.2±0.04
MCHC %	0.20±0.35	0.27±0,22	0.38±0.51	0.44±0.02

Regarding Erythrocytes, the number of R.B.C. count in the control group was 3.27 (106 mm^3), whereas erythrocyte count decreased (2.57, 2.16, 1.11 106 mm^3) due to concentrations of 0.25, 0.5, and 0.75 mg/L, respectively, compared to an increase in concentrations of parathion pesticide after 15 days of exposure, as shown in Table 1 and Figure 1.

Furthermore, the average haemoglobin concentration was recorded in the control (10.31%), but a significant reduction due to parathion pesticide application resulted in values (8.2, 6.61, and 3.01%) after 15 days of exposure, as shown in Table 1.

On the other side, the average values of HTC were recorded in the control samples, its numerical value (51.32). In the treated samples exposed to sub lethal concentrations for a period of 15 days, a significant decrease was found in all samples, each according to the concentration exposed to it. Table 1 shows the values (30.01, 14.31, and 7.05%).

The sub lethal effects of parathion pesticide concentrations (0.25, 0.5, and 0.75 mg/L) on the average volume of recorded cells (M.C.V.) showed a significant increase with the increase in pesticide concentrations, as its levels reached (175, 186, and 280 fl) compared with the control (165 fl), as for the mean measurement of red cell haemoglobin (M.C.H.), where the count showed a significant decrease as its rates increased. Also increased are the levels of 0.27, 0.38, and 0.44% compared to the control (0.20%).

Hematological indicators are considered one of the important physiological factors in determining the extent of susceptibility to pollutants in fish and other organisms [22]. The rest indicators are impacted by the violation of the blood and standard indicators. Therefore, the emphasis on the safety of red blood cells, haemoglobin values, and the increased of anaemia by decrease the R.B.C count and HB values have a significant impact on the safety of the circulatory system, and during exposure to pollutants like pesticide pollutants, the extent of damage increases with concentration and period. After 15 days of exposure to sub lethal concentrations of the organophophorous pesticide Parathion, we estimated the haematological and biochemical parameters of the freshwater fish *Barabus Luteus*. Based on the above results, it was found in this study that there was a significant decrease in the values of blood cells and haemoglobin values compared to the control, and this was confirmed by Khan et al. [23] in their study on fish (*Labeo rohita*) exposed to the pesticide Cypermethrin and also indicated by Nagaraju et al. [24].

Many scientists looked into what happened to the fish's blood and cells when they were exposed to water pollutants like pesticides. For example, Ghayyur et al. [25] indicated during their study on blood variables, decreases in RBC, HB, and HCT values were found. In another study, Ghafari Farsani et al. [26] noted that there are differences in the blood profile of Hetropoheutes fossiillis due to exposure to pesticides and industrial irregularities. Other studies by Satheeshkumar et al. [27] and Alwan et al. [28] reported a drop in the blood profile. Many environmental stresses that fish face cause changes in blood parameters, so there was a significant decrease ($P < 0.05$) in packed cell volume (PCV), red blood cells (RBC), and haemoglobin (HB) in the current study, which is similar to the study by Adewumi et al. [29] on blood parameters of fish (Clarias ariepinus) exposed to pesticide (Chlorpyrifos). It was also shown from the tables that the hematocrit values started to decrease. This result is consistent with what was observed by Amaeze et al. [30]. RBCs are a type of blood cell that are abundant due to the function of hemoglobin.

They are the main means of carrying oxygen to tissues of the body, and this is how the environment and its accessories affect blood variables, including red blood cells, which leads to a decrease in number and in turn leads to a decrease in haemoglobin and causes anaemia. This is what is observed in many studies such as El-Bouhy et al. [31], as the red blood cells' cytoplasm is rich in haemoglobin, which contains an iron molecule that binds it to oxygen. It can transport oxygen. Here, chemical pollutants like pesticides reduce the number of red blood cells to make them unable to carry oxygen to the tissues of the body, which is obvious by Alishahi et al. [32], from the reduction in the oxygen consumption of the fish. At the time of exposure, and this could be due to shrinkage of the respiratory epithelium or possibly due to mucus accumulation on the gills.

Al-Otaibi et al. [33] discovered a significant increase in the number of hematocrit cells. The hematocrit values also showed that they began to decline as concentrations increased. This result is consistent with what was noticed by Maurya et al. [34] when recording the HCT values of fish (Heteropneustes fossilis) during exposure to toxic concentrations of pesticide. However, the current study recorded a significant increase in the average red cell size (MCV) when long-term exposure to parathion pesticides at the three concentrations (pesticides from industrial waste water) occurred. To say that the type of anaemia for the above concentrations is large for the current study and that the causes of anaemia can also be determined by red blood cell size values, it is described as anaemia caused by iron deficiency [35].

Under the chronic effects of Parathion, the average red blood cell size of the *Barabus Luteus* fish increases significantly. The average value of MCH was used to identify the type of anaemia in terms of the abundance of haemoglobin pigment; when it is high, it is called hyperchronic anaemia. And when it is low-term hypochromic anemia, there is a significant decrease ($P < 0.05$) in the value of MCH red cells exposed to sub-lethal concentrations of parathion organophophorous pesticide, where the presenters agreed that the pesticide effect caused hyperchronic anemia. This indicated that the pesticide Seven caused a significant decrease in the values of MCH corpuscular haemoglobin [36]. Also, this decrease is the result of the damage caused by pesticides; as a result of estimating the values of MCHCH, a significant reduction was observed, and this is similar to what was found in other studies.

The biochemical changes were studied because it is the most important function in achieving the biochemical activations in the body, whether in humans or animals, including fish.

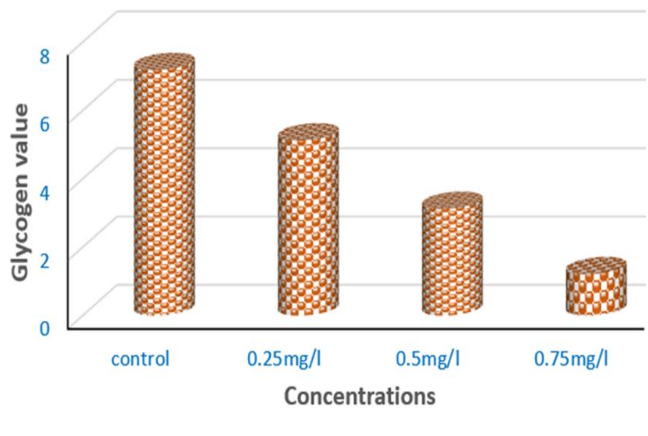

Figure 2. Effect of sub-lethal concentrations pesticide on glycogen content in the liver freshwater fish *Barabus Luteus* after 15 days of exposure

The result showed a significant decrease in liver glycogen level observed during exposure to sub lethal concentrations of parathion pesticide (0.25, 0.5, and 0.75 mg/L), the values were as follows: 5.12, 3.17, and 1.21%, as shown in Figure 2, compared with control (7.18%).

The results confirmed that there was a significant decrease in the level of liver protein according to the concentration values (18.05, 15.17, and 11.05%), where the values indicated a significant reduction compared to what was recorded by the control (22.33%), as shown in Figure 3.

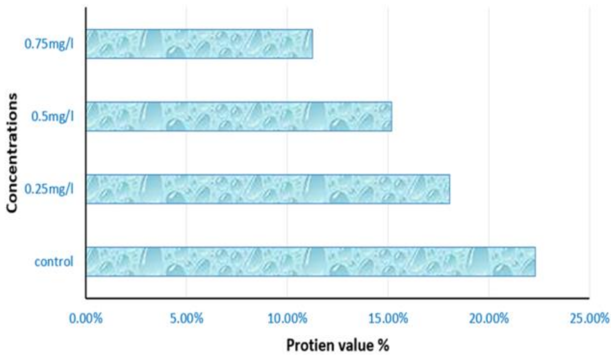

Figure 3. Effect of sub-lethal concentrations pesticides on protein content in the liver of freshwater fish *Barabus Luteus* after 15 days of exposure

According to the current study estimation, the glucose value in the blood increased with a significant increase in pesticide concentration (31.05, 42.2, and 51.03 mg/dL) compared to the group control (27.51 mg/dL), as shown in Figure 4.

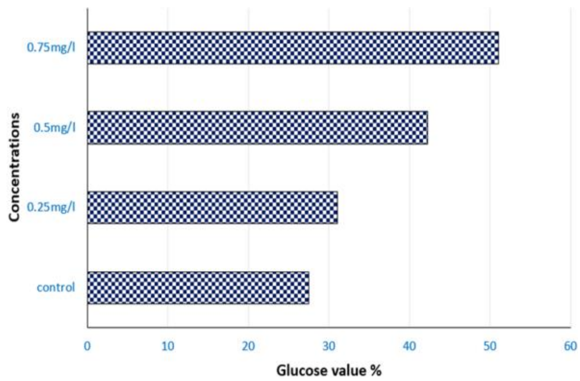

Figure 4. Effect of sub-lethal concentrations pesticide on glucose content in the blood of freshwater fish *Barbus Luteus* after 15 days of exposure

Figure 5. Effect of sub-lethal concentrations pesticides on cholesterol content in the blood of freshwater fish *Barabus Luteus* after 15 days of exposure

Significant decreases were observed in cholesterol values in the liver of fish (8.96, 4.32, and 3.19%) in comparison with the control (12.78%), as shown in Figure 5.

Pollutants are negative factors affecting the aquatic environment, so they cause a lot of stress on the organism's tissues, and biochemical processes such as protein, glucose, glycogen, and cholesterol respond [37].

According to the data presented above, the present investigation discovered a statistically significant drop in protein levels in *Barabus Luteus* fish after subjecting them to sub-lethal quantities of parathion insecticide for a period of 15 days. Accordingly, the decrease was proportional to the amount of concentration, which is in line with what Tasneem and Yasmeen [38] found to be the case. Pesticides influence the metabolic pathways of glycogen levels in the liver of *Barabus Luteus* fish through their effect on degrading enzymes such as lactate dehydrogenase. The proteolysis of protein into amino acids in order to use them in metabolism processes may be the reason for the reduction. Therefore, pesticides make it more difficult for these enzymes to do their jobs, which in turn decreases the liver's capacity to store energy. According to Prakash [39], a decrease in glycogen leads to hypoxia as a result of the low energy required to start breathing. As a result, the body turns to anaerobic respiration in order to speed up the processes of glycogenesis.

As for the blood glucose level tests, the results of the present study showed that they varied depending on the concentrations and the exposure period. An increase in glucose values was observed when compared to the control, which is similar to what Somaiah et al. [40] discovered for an increase in the concentration of glucose in the blood due to the surrounding conditions that intake the fish responded to the toxic pollutants.

Kamalaveni et al. [41] indicated during their study found increased activity of glucose-6-phosphate dehydrogenase in various tissues of the Cyprinus carp carpio fish with confirmed the decrease in succinat ehydrogenase and increase in glycogen was observed in *Labeo rohita* on exposure to Malathion. Therefore, a reduction in energy requirements is due to the need to resist the effects of environmental hazards that cause a change in the biochemistry of fish. The results of the present study varied a decrease in cholesterol values in the liver of fish exposed to three sub-lethal concentrations, this indicated the reason for lowering cholesterol and inhibiting its effectiveness. Amin and Hashem [42] showed that the reason for the reduction of cholesterol in the liver is to take advantage of fats by substituting them for glucose and glycogen as a source of energy. All results are in agreement with the findings of many studies to achieve biochemical responses to various fish under the influence of organophosphate pesticides Baldissera et al. [43].

4. CONCLUSIONS

Under laboratory conditions, the current study aims to investigate the haematological and biochemical changes in response to the toxic effects of parathion pesticide. The present study found significant differences in some haematological and biochemical parameters when exposed to sub lethal of parathion concentrations for 15 days. These changes in the vital values that are related to the metabolic activity of the animal are indicators of environmental pollution and the life of living organisms such as fish, which is an important component of food safety in human nutrition and health.

With the growth of the (M.C.H) concentrations and the

(M.C.H.C) concentrations, several blood parameters indication reveals substantial declines in blood indicators, R.B.C., HB, and HTC when compared with control. In comparison to the control, the current results demonstrate a considerable rise with high concentrations (M.C.V.) reaching 175 fl, 186 fl, and 280 fl (165 fl). The outcomes also showed that some biochemical variables, including protein, glycogen, and cholesterol, had significantly decreased. As concentrations and exposure times rose, the results also showed a substantial rise in blood glucose levels (31.05, 42.2, and 51.03) mg/dl in comparison to the group control (27.51) mg/dl. One of the best indications of exposure to the toxicity of chemical pollutants, including pesticides, is thought to be haematological and biochemical indicators.

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