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Evaluation of liver and kidney functions in common carp fishes due to environmental toxicity with acetamiprid pesticide

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***Abstract*---**This study was applied at Wasit University - College of Science - Department of Life Sciences, Iraq. The experiments were done in the laboratories of the department of biology and some private laboratories. As well as the laboratories of the Ministry of Science and Technology - Department of Environment and Water. Blood samples of carp fish were collected during the period of December 2021- May 2022. Two hundred samples were collected from common carp fish and about 150 samples of fish were exposed to the acetamipridpesticide and 50 samples of fish werenot exposed to the pesticide. The objective of this study was to evaluate of the liver and kidney functionsin common carp due to the toxicity with Acetamiprid Pesticide in Tigris River. There are a significant increase was observed in serum Urea and Creatinineat ($P \leq 0.01$) in fish exposed to the pesticide compared with the samples that not exposed to the pesticide. whilein liver enzymes tests, the results showed a significant difference in the level of SGOT enzyme were (216.42 ± 0.63), SGPT enzyme (0.27 ± 101.82) and ALP enzyme (100.99 ± 0.21), while to the totals of the control samples were (75.93 ± 0.71), (30.19 ± 0.26) and (35.93 ± 0.86), respectively. The research conclude that the acetamipridpesticide has a negative effect on the fish, the functions of the fish's body organs and on the various biomarkers.

Keywords---common carp, acetamiprid pesticide, environmental toxicity biochemical tests, liver enzymes.

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Introduction

Recently, Iraq was ranked as one of the ten countries that recorded the lowest in environmental safety (YCELP, 2012). This record was based "mainly" on data-based environmental performance criteria such as environmental pollution with different types of toxic substances and their impact on human health. There are a significant gap appeared in environmental data, particularly the related to exposure to toxic chemicals, water quality, industrial pollutants, soil quality, and gas emissions.Limited information regarding levels and sources of environmental pollutants such as toxic metals and organic compounds prevents the development of control strategies to protect the environment and public health (Ala'din, 2004). Pollution levels in Iraq have reached the high level due to government neglect and no effective strategies to treat water pollution. Recently, pollution in Iraq has been reached high levels and this is due to various factors including wars, factory waste and draining water from homes and hospitals. It has contained many polluting materials (chemicals, pesticides and fertilizers) which are directly thrown into rivers (Agbozu et al., 2007).

Pesticides have been used to control pests, including insects, aquatic weeds, and plant diseases. They are a group of toxic compounds that have a major effect on aquatic life and water quality.Pesticides are useful chemicals. It can protect crop and farm losses form pests. It can help in more efficient food production (Dias et al. 2020).The toxicity of pesticide indicates its toxicity. Some pesticides are extremely toxic, while others are relatively nontoxic.Exposure refers to the length of time the animal has been exposure to the pesticide. A brief exposure to some chemicals may have little effect on the fish, while a longer exposure may cause some effects (Calow, 1998). The pesticide dose refers to the amount of pesticides that the animal is exposed to that pesticide (oral or dermal).A small dose of a more toxic chemical may be more harmful than a large dose of a less toxic chemical. The pesticide dose is measured as the weight of the toxin per unit (kg) of body weight (expressed in mg of pesticide/kg body weight) or as the concentration of the toxin in the water or food supply (usually expressed in ppm or ppb) (Beard, 2009). Pesticides are easy to use, and cost-effective. However, the pesticide benefits are not without consequences. Thus, pesticides must be used very carefully to protect human, animal health and environment. The pesticides disadvantages is their toxicity to humans, animals and plants, and the persistence of some of these chemicals in the environment for a longer period (Stenersen, 2004).

Materials and Methods

Sample collected

The samples for this study were collected with the help of a local fisherman in the work area by two fishing methods:

- Cast nets are circular nets made locally, with a diameter of two meters and a side of 1.5 cm.
- Gill nets are fishing nets of different ages and lengths (2-3-4-5-6 cm).

The type of these fish was detected according to Coad (2010) and knowledge of scientific names based on the website (2018). The fish were kept in a glass tank containing water from the same place that sample was taken to keep the fish alive. Then, 10 mm of blood was drawn from the caudal vein of carp fish using plastic syringes. Then, 3 ml of blood was placed in a tube containing an anticoagulant EDTA for the purpose of blood testing (red blood cell count and hemoglobin concentration measurement). This is according to the (1973, Daislly, Blaxhall) method. Then, the remaining blood in the Gell tubes was evaluated. After that (3 ml) was placed in tubes and (4 ml) in other tubes and were identified with numbers and symbols for the purpose of identifying them when conducting laboratory tests.

The Biochemical and enzymatic tests as well as measuring the concentration of the pesticide in the blood. All samples were placed in a plastic ice box and transported to the laboratory as soon as possible. To separate the serum from the rest of the blood components using a centrifuge at (3000 rpm) for 10 mins, then put the serum into Eppendorf test tubes using micropipettes. The remaining blood serum was preserved in numbered Eppendorf tubes and kept in deep freezing (-40 °C) according to (Chng, Yang, 2003). The Cobias CIII (Roche Company, Germany) equipment was used in the experiment. It has been used for isolated the serum and I select 200 µl of it, then put it in a special tube for the equipment. Then, open the equipment and give an order where it gives a code to the sample and it specify the required test and more than one test can be selected at the same time. After that, press (start), then the duration of giving the result will be displayed on the screen. For example, creatine needs 9 minutes, urea needs 5 minutes, and liver enzymes need 12 minutes …. etc. After the end of the specified period for the examination, the equipment reports the result and prints it automatically.

Statistical analysis

The SAS (2018) program has been used to estimate the effect of various factors on different indicators in the study parameters. The T-test has been adopted to find the significant differences between the means of the variables. The correlation coefficient and CI between the different variables were also estimated and calculated to know the effect of the pesticide on the biomarkers.

Results and Discussion

Estimation of Serum Urea and Creatinine

The results have been showed that the effect of the acetamiprid pesticide on urea and creatinine for fish that exposed to this pesticide compared with fish that not exposed to this pesticide. In the crap sample, the results of urea showed a significant decrease in the value (31.64 ± 0.10) that affected by the pesticide, while the value in the control samples was (15.02 ± 0.10). All of these data were significantly different at ($P \leq 0.01$) with the control treatment (15.02 ±0.10). The analysis of variance shows highly significant effects for each exposure period at ($P \leq 0.01$) (Table 1).A previous study indicated that acetamipridpesticide were significantlydifferent at (P<0.01) on the level of urea in the blood compared to the control.

There was a significant difference in the average levels of urea and creatinine in the treated samples compared to the control. Some studies have been found that creatinine levels did not show a significant increase in creatinine level at a high dose relative to the control (Khare et al., 2019; Ayyasamy and Tamilselvan, 2022). The changes in creatinine level of plasma reflect changes in glomerular filtration in relation to kidney function.Measurement of serum creatinine is one of the most common methods that used to assess kidney function. It was observed that samples treated with a high dose of the pesticide showed a significant differences in creatinine levels, with no change in urea levels.

This could be explaining that the liver is related to the stimulation of red blood cell regeneration in the bone marrow. This stimulation may result in uncontrolled dividing cells and may lead to an increased frequency of micronuclei in the bone marrow. It is also possible that this pesticide has genetically toxic effects on bone marrow cells (Jin et al., 2010; Ayyasamy and Tamilselvan, 2022).Creatinine is filtered in the blood mainly by the kidneys (glomerular filtration and proximal tubular secretion). If there is a deficiency in the filtration of the kidneys, blood levels rise. Therefore, creatinine levels in blood and urine can be used to calculate creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR).However, in severe renal dysfunction, filtration is clinically significant as it is a measure of renal function (Khare et al., 2019).Creatinine test has been recorded the mean value (1.508 ±0.01) in the common carp sample, while the value in the control samples was (0.446 ±0.02). All these data were significantly different at (P≤0.01), which higher than the control samples (0.446 ± 0.02).

Table 1
Effect of acetamiprid pesticide on urea and creatine tests of exposed fish and control

Group	Mean ± SE	
	Urea (g/dl)	Creatinine (g/dl)
Patients (No=150)	31.64 ±0.10	1.508 ±0.01
Control (No=50)	15.02 ±0.10	0.446 ±0.02
T-test	0.374 **	0.0445 **
P-value	0.0001	0.0001
O.R. (C.I.)	2.29 (1.33-4.67)	1.26 (0.85-2.611)
** (P≤0.01).		

** Indicates high significant differences at (P≤0.01).

Estimation of Liver enzymes

Table (2) has been showed that the residues of acetamiprid on the liver enzyme indices of exposed fish compared with that of unexposed fish since the liver is the main metabolic center, where most of the vital substances are metabolized and detoxified. Therefore, it is one of the body organs that face a major attack of an unwanted chemical invasion. Thus, liver function is likely to be disrupted under these conditions (Umamaheswari et al., 2019), GOT and GPT are two key enzymes that are a sensitive measure for assessing hepatocyte damage and some liver diseases (Ramesh et al., 2018).

Increased of aminotransferases in the blood may reflect myocardial and hepatic toxicity. It is led to a wide release of enzymes in the blood (Ramesh et al., 2014). The study of the SGOT enzyme, the present results showed a significant differences in the value (216.42 ± 0.63) that affected by the pesticide, while the value in the control samples was (75.93 ± 0.71).The main hepatic cellular component affected by ambient toxins and pesticides is the cell membrane, whereby pesticides either increase the membrane permeability, which leads to increased filtration of enzymes, or reduce permeability, forcing enzymes to accumulate in cells. Cellular damage is another reason for decreased enzyme synthesis in living organisms (Umamaheswari et al., 2019).

The SGPT is found mostly in the liver, with smaller amounts in the kidneys, heart and muscle. The SGPT is a more specific indicator of hepatitis than SGOT. This may also be elevated in diseases affecting other organs, such as the heart or muscles in myocardial infarction, as well as in acute pancreatitis, acute and acute hemolytic anemia and kidney disease (Yassin and Al-Shanti, 2016). The results of the SGPT test have beenreported a significant differences (101.82 ±0.27) in the common carp sample that affected by the pesticide, while the value in the control samples was (30.19 ±0.26). All these data were significantly at ($P \leq 0.01$) higher than the control samples, which recorded (30.19 ±0.26). A study was conducted on the hepatic responses of carp fish against water pollution. They found that the increase and decrease in various biochemical parameters and enzymes in the liver of the studied fish samples compared to control showing the harmful effect of water pollution on fish health (Yousafzai and Shakoori, 2011). A previous study was foundthat the effect of heavy metal accumulation on fish enzyme activity, and it was found that the increase in GOT and GPT activities is associated with liver cell damage (Ibrahim and Mahmoud, 2005).

Acetamiprid pesticide is completely absorbed into the tissues. It is found in high concentration in the liver and kidneys. It is eliminated through the urine (Mondal et al., 2014). Acetamiprid has been found to cause oxidative damage to the liver and kidneys, indicated by markers of injury including cholesterol levels, alanineaminotransferase and aspartate aminotransferase enzymes. All of these may result from liver injury. In addition, acetamiprid was more toxic to the liver according to histopathological tests (Karaca et al., 2019).The acetamiprid leads to increase in the levels of hepatic marker enzymes such as (AST), (ALT) and (ALP), in addition to a severe and toxic effect on the liver according to histopathologicaltests. Histopathological changes have occurred in fatty liver cells. Both liver and kidney tissues showed an increase in the amount of collagenous fibers and immune reactivity of fatty acid synthase (Toghan et al., 2022).

Many environmental stressors affect the liver, causing muscle damage, metabolic disturbances, and possibly death. Various cellular levels (histopathological, morphological, physiological, biochemical, cellular, molecular, exposure, sensitivity, and effective biomarkers) can be used to monitor pollution indicators

(Abdul Lateef et al., 2021). The results have been showed a significant increase in ALP in the mean value (100.99 ±0.21), while the value in the control samples was (35.93 ±0.86). All these data were significantly ($P \le 0.01$) higher than the control samples (35.93 ±0.86). The analysis of variance shows highly significant effects for each exposure period ($P \le 0.01$).

Table 2
The effect of the acetamiprid pesticide on liver enzymes for exposed fish and the control

Group	Mean ± SE		
	AST (g/dl)	ALT (g/dl)	ALP (g/dl)
Patients (No=150)	216.42 ±0.63	101.82 ±0.27	100.99 ±0.21
Control (No=50)	75.93 ±0.71	30.19 ±0.26	35.93 ±0.86
T-test	2.294 **	0.975 **	1.226 **
P-value	0.0001	0.0001	0.0001
O.R. (C.I.)	3.074 (106-5.88)	1.86 (0.86-3.53)	3.47 (1.09-4.67)
** ($P \le 0.01$).			

** Indicates high significant differences at ($P \le 0.01$).

Alkaline phosphatase (ALP) is a very important biomarker of cellular responses to cell toxicity (Lohner et al., 2001). Changes in ALP levels in the organs, blood, and tissues of fish were determined when exposed to pesticides. In addition, ALP has a necessary function in the transfer of metabolites across the cell membrane and the carboxylic acid (TCA) cycle for energy production (Das et al., 2004). On the other hand, an increase in ALP levels in tissues can be viewed as an indicator of tissue damage for energy production (Atli et al., 2006). The previous study showed similar results with (Vasanth et al., 2012).

Conclusion

There are a significant increase was observed in urea and creatine at ($P \le 0.01$) that exposed to the pesticide compared with the samples that not exposed to the pesticide. The research conclude that the acetamiprid pesticide has a negative effect on the fish, on the functions of the fish's body organs and on various biomarkers.

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