Influence Of Karate On The Activity Of Enzymes Of The Anti-Oxidizing System Of Rat Liver Protection And Ways Of Their Correction

[1] Parida Mirkhamidova – Professor of the Department of Botany, Tashkent State Pedagogical University named after Nizami,
[2] Dilfuza Tuychieva – Associate Professor of the Department of Zoology and Biochemistry, Andijan State University,
[1] Dilnoza Bobokhonova – Lecturer of the Department of Botany, Tashkent State Pedagogical University named after Nizami,
[2] Mashkhura Parpieva – Lecturer at the Department of Zoology and Biochemistry, Andijan State University
1 – Tashkent State Pedagogical University named after Nizami
2 – Andijan State University
Tashkent, Republic of Uzbekistan, tds.bio@mail.ru

***Abstract:** As a result of research, it has been established that synthetic karate pyrethroid causes functional changes in antioxidant enzymes in microsomes and mitochondria. An important aspect of our study was the comparison of the taxation rate when animals were poisoned with a karate pesticide and when animals were protected from poisoning with an antioxidant of plant origin. The results obtained indicate that the plant antioxidant factor (PAF) has protective properties and accelerates the restoration of enzyme activity.*

***Keywords:** pyrethroid karate, plant antioxidant factor (PAF), liver, mitochondria, microsome, enzyme, free radical lipid peroxidation, glutathione, superoxide dismutase (SOD), glutathione reductase (GR), catalase.*

1. INTRODUCTION

The source of pollution of the biosphere is getting more and more every day. The use of pesticides in the near future is likely to expand due to the need for food for the rapidly growing world population, as pesticides provide 20% of the total increase in crop production. However, even now, local concentrations of chemicals in the human environment are approaching significant values [2, 11, 21, 22, 23, 24].
In this regard, it becomes relevant to search for ways of prevention and protection measures against the effects of pesticides.
Entering the body in various ways, they constantly accumulate in the tissues of humans and animals, exerting a toxic effect on them [2, 11, 12, 21]. Detoxification processes in the body are carried out by all organs, but mainly by the liver. Liver damage can cause severe metabolic, immune response, detoxification, and antimicrobial defenses. Among cellular organelles, mitochondria and microsomes are the main targets of these compounds.
It is known that the emergence and development of a number of pathological conditions in humans and animals is accompanied by the activation of lipid peroxidation of cell membranes [5].

It has been established that a significant number of xenobiotics has a toxic effect through lesions of the lipid phase of cell membranes, involving lipids in free radical peroxidation [12].

It is known that chemical modification of bio-membranes by toxic products of lipid peroxidation disrupts protein-lipid interactions, inactivates membrane-bound enzyme complexes, increases membrane permeability, which leads to profound changes in metabolism and, as a result, to its degradation [1, 3].

In the cell, the protection of membrane lipids from peroxidation is carried out by the enzymatic-antioxidant system. One of the protective enzymes is superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, which detoxify the primary and intermediate products of lipid peroxidation and glutathione transferase, glycosidase, formaldehyde dehydrogenase, which detoxify by-products of peroxidation and other carbonyl compounds. They have a certain specialization both in relation to specific types of radicals and peroxides and in the loci of occurrence of reactive oxygen species [8].

The study of the activity of enzymes of the antioxidant system during intoxication and liver damage is important for elucidating pathological mechanisms and adaptive changes, for developing methods for correcting disorders [13].

From this position, the Department of Botany of TSPU named after Nizami has been conducting research on the toxic effect of pyrethroitakarate for several years. The issues of correlating the consequences of poisoning by means of the application obtained in the laboratory of the department are being studied. Plant antioxidant factor (PAF).

Karate is a synthetic pyrethroid, consisting of a chlorofluoroorganic compound, which is widely used in agriculture.

In this regard, it is necessary to search for and study antioxidant drugs capable of responding to the toxic effect of pesticides by reducing the damaging effect on the structural and functional state of antioxidant enzymes and thereby exerting a protective effect. Based on this, we studied the effect of karate pyrethroid on the activities of superoxide dismutase, glutathione reductase, catalase in mitochondria and microsomes of rat liver and the change in these parameters under the action of a plant antioxidant factor introduced 30 minutes after inoculation.

The objects of the study were white male Wistar rats weighing about 100 grams. Karate rats were injected orally in the form of an aqueous suspension through a probe at a dose of 1/10 LD50 once, the 2nd group of animals after inoculation with karate, after 30 minutes was injected with an alcoholic 5% extract of the plant antioxidant factor (PAF) in an amount of 1 ml for four days. After karate inoculation and PAF administration, all rats were kept on a common diet. On the 1st, 5th, 10th, 20, 30, 40, 50th days, the control and experimental rats were slaughtered for experiments.

The method for determining the activity of superoxide dismutase in isolated hepatocytes is based on the ability of the enzyme superoxide dismutase to inhibit the reduction of nitrotetrozolium blue in an alkaline medium [15].

The method for determining the activity of glutathione reductase is based on the fact that glutathione reductase, with the participation of reduced forms of pyridine nucleotides, converts the oxidized form of glutathione into the reduced one. The enzyme activity is calculated from the increase in the amount of reduced glutathione in the incubation medium (17).

The method for determining the activity of catalase is based on the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts (23).

Protein was determined by the Lowry method.

It is known that the enzyme superoxide dismutase is an essential component of the antioxidant system. Superoxide dismutase (SOD) catalyzes dismutation of superoxide

radicals and thereby prevents the pathogenic action of reactive oxygen species [17]. The enzyme superoxide dismutase converts the oxygen superoxide anion into the less reactive hydrogen peroxide H_2O_2. The formation of oxygen superoxide anion is of great biological importance. It is a highly reactive compound that, due to its high hydrophilicity, cannot leave the cell, and accumulates in the cytoplasm [7]. Our results show that, in control rats, the activity of the SOD enzyme in mitochondria was 1.30 U / mg protein, in microsam 1.21 U / mg protein.

In experimental rats inoculated with a pesticide on the 5th day after poisoning, there is a sharp increase in SOD activity, which was 84% in mitochondria, 57% in microsomes, more than normal. (fig.1, 2)

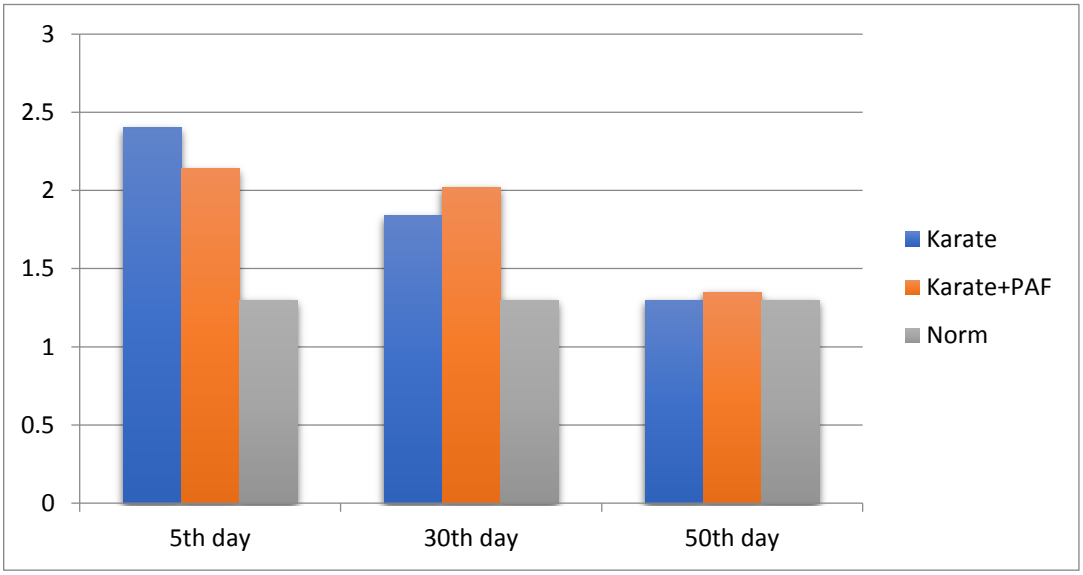

Figure 1. The activity of the enzyme superoxide dismutase in mitochondria (unit / mg protein). Activity equal to 1.21 U / mg protein is taken as 100%.

The observed increase in the activity of the SOD enzyme can be considered as a protective, adaptive reaction of a multicellular organism against lipid peroxidation, which disrupts the structure and function of cell membranes.

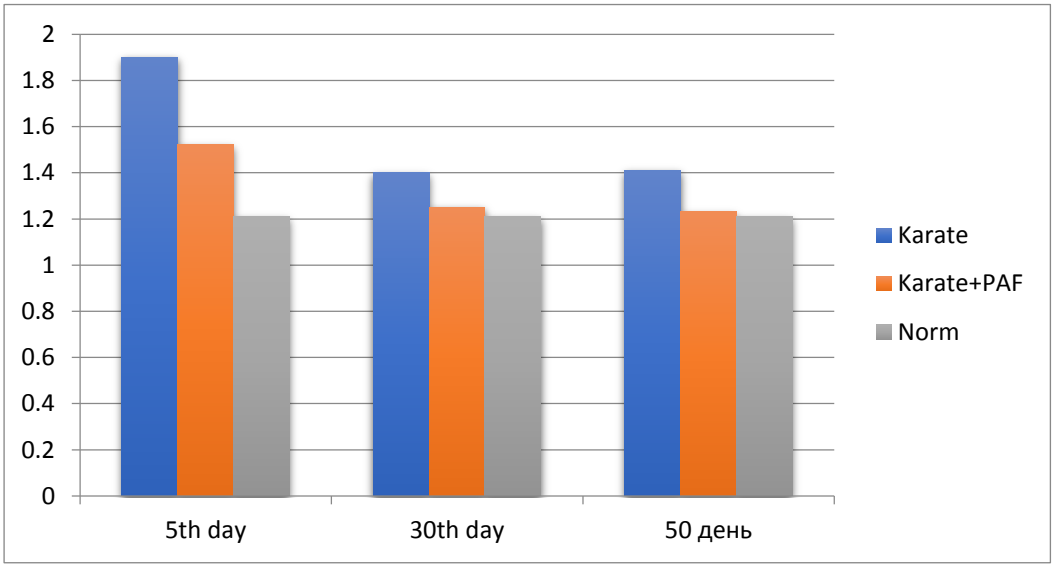

Figure 2. Superoxide dismutase enzyme activity in microsomes (unit / mg protein). Activity equal to 1.21 U / mg protein is taken as 100%.

In rats protected by an antioxidant, the enzyme activity in mitochondria was 41%, and in microsomes it was 25% higher than normal. (fig.1, 2)
Studies on the 30th day after intoxication showed that the activity of SOD in mitochondria is 64%, in microsomes it is 32% higher than normal. In the group protected by the antioxidant PAF, the SOD activity indicator is lower than in the 1st experimental group, and, accordingly, it is higher by 25% in mitochondria, by 3% in microsomes from the control. [25]
On the 50th day after intoxication, the enzyme activity in mitochondria was 35%, and in microsomes it was 24% higher than the norm. In rats that received an antioxidant of plant origin, the SOD activity indicator on the 50th day was practically reduced to normal, in mitochondria 3%, in microsomes 1%.
Thus, the activation of SOD, reaching a maximum on the 5th day in the mitochondria and microsomes of the rat liver.
Studies show that the plant antioxidant factor studied by us exhibits anti-toxic effects; therefore, in rats that received PAF after intoxication, superoxide dismutase activity in mitochondria and microsomes is impaired to a lesser extent.
It is known that glutathione reductase is directly involved in the process of detoxification of many toxic compounds, preserving the sulfhydryl groups of proteins, and also as a coenzyme of glycolysis and a number of other enzymes. Since free cysteine is present only in small amounts, glutathione is the most abundant sulfhydryl compound in cells and its function is to maintain active conformation of many enzymes [21].
Also, glutathione reductase is responsible for maintaining normal levels of reduced glutathione in liver cells, which is most likely compensatory in nature. To find out the degree of participation of glutathione reductase in the detoxification process, its activity was determined in the mitochondria and microsomes of the liver of intact rats. The glutathione reductase activity of mitochondria, according to the results of our studies, is 78.9 nmol NADPH / min / mg protein, and in microsomes - 59.6 nmol NADPH / min / mg protein, which was taken as 100% of the enzyme activity (Fig. 3, 4)
The results of our studies show that the activity of glutathione reductase in the mitochondria of the liver of rats on the 5th day after karate poisoning exceeded the activity of glutathione reductase in the mitochondria of control rats by 246%. With the introduction of PAF, it increased by only 110% (Fig. 3). On days 30-50 after inoculation, this indicator, in comparison with the 5th day, tended to decrease and fell to 105% and 104%. With the use of PAF, by 30-50 days, the activity decreased to 63% and 5%, respectively. This indicates that, under the action of PAF, the activity of glutathione reductase in the mitochondria of rat liver cells is compared with the norm (Fig. 3).

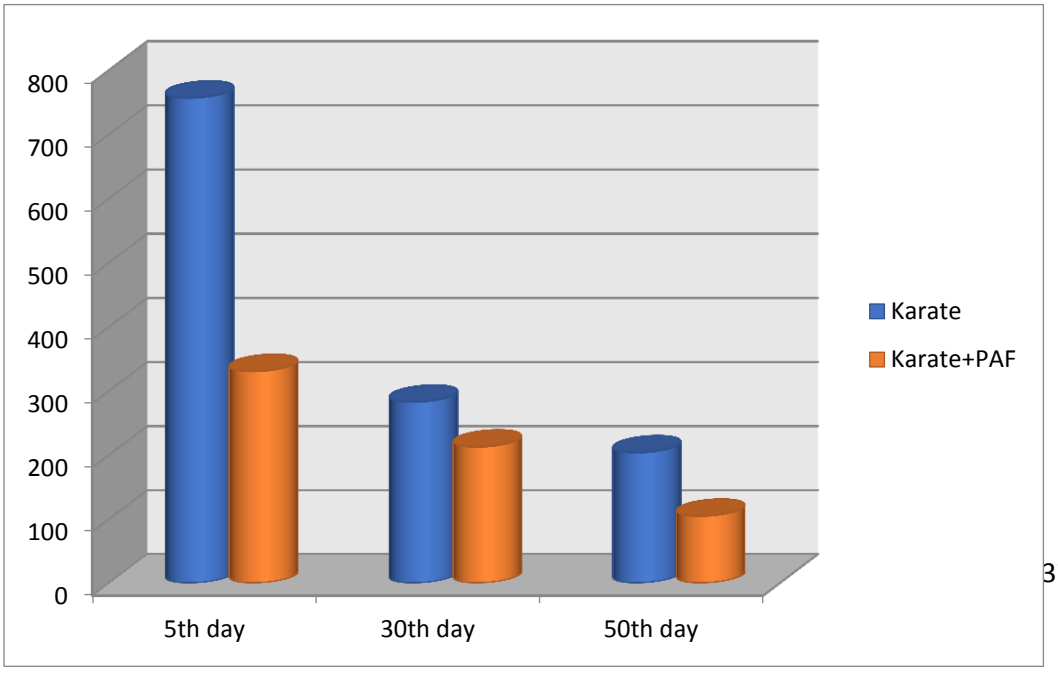

Figure 3. Glutathione reductase activity of mitochondria isolated from rat liver (100% was taken as glutathione reductase activity of rat liver mitochondria in control; glutathione reductase activity of mitochondria is 78.9 nmol NADPH / min / mg protein of rat liver mitochondria).

An approximate picture is also observed in the microsomes of rat liver cells. On the 5th day, the enzyme activity was overestimated by 290% in the poisoned karate microsomes, and in the protected PAF - by 163% of the control, the difference was almost 2 times (Fig. 4).

On the 30th day, the indicator of enzyme activity after inoculation and with the use of PAF, drops to 130% and 29%, respectively.

By the 50th day, glutathione reductase activity decreases in microsomes affected by karate to 130%, and in those protected by PAF - to 17%. (fig. 4).

From the above, it can be seen that the greatest activity of glutathione reductase is observed in microsomes of rat liver cells on the 5th day after priming, and in microsomes protected by PAF, a decrease is observed by the 50th day, to the normal value.

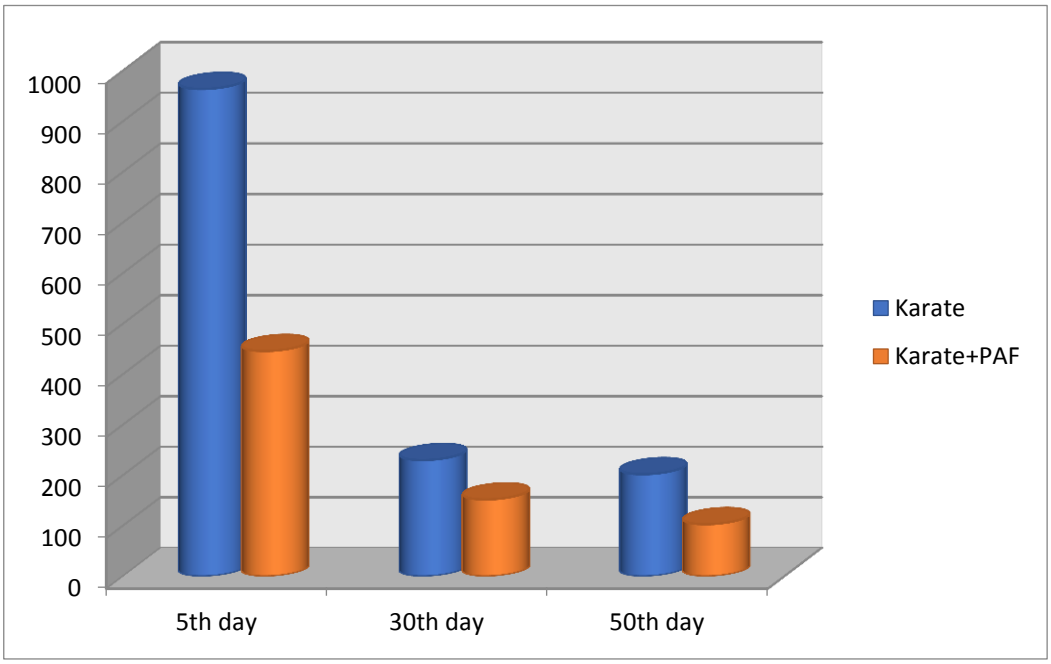

Figure 4. Glutathione reductase activity of microsomes isolated from rat liver (the glutathione reductase activity of rat liver microsomes in control was taken as 100%; glutathione reductase activity of microsomes is 59.6 nmol NADPH / min / mg protein of rat liver microsomes).

The action of the PAF once again confirms our assumptions that the metabolism, disturbed by the action of karate, normalizes much faster than those poisoned, and on the 50th day the activity of the enzyme is normalized.

The results of our experiments show that in the mitochondria and microsomes of the liver of animals after karate poisoning, the activity of glutathione reductase increases, and they also indicate a high sensitivity of the enzyme to the karate pesticide, which is involved in providing a protective antitoxic function.

An increase in glutathione reductase activity in mitochondria and microsomes of liver cells in rats in pathology indicates that the action of the pesticide in the liver increases the level of reduced glutathione, as it is responsible for its normal level.

Thus, the introduction of PAF to animals after karate seeding leads to the restoration of the activity of glutathione reductase, an enzyme of the antioxidant defense system, which in turn

indicates the normalization of free radical processes in liver cells that are unusual for the body in normal conditions.

Catalase is widespread in humans and animals, and the largest amounts of the enzyme are found in erythrocytes, liver and kidneys. The processes associated with the detoxification of certain toxic substances are reactions associated with the formation of hydrogen peroxide. Catalase is an enzyme that is invariably present in aerobic cells. They function very quickly and break down H_2O_2, water and molecular oxygen. Catalase thereby prevents the pathogenic effects of the activity of oxygen species. In this regard, it was interesting for us to study the effect of karate on the activity of antioxidant catalase.

The results obtained for determining the activity of catalase show that the catalog activity sharply decreases on the 5th day of karate poisoning. The activity of catalase is 55.8% of that of the control.

The studies were carried out for 50 days. The results of studying the activity of catalase in the liver are presented in Fig. 5.

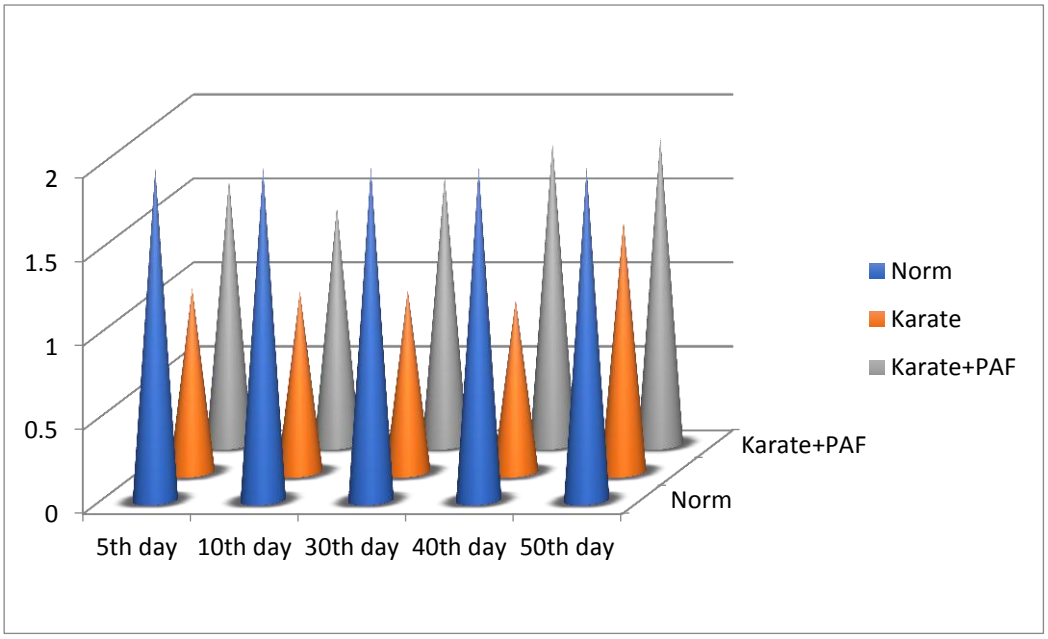

Figure 5. Specific activity of catalase in rat liver.
The activity is taken as 100%, equal to 1.98 μat / l per 1 mg of protein.

Figure 5 shows that catalase activity in rats poisoned by karate sharply decreases by the 5th day. On the 40th and 50th days of the experiment, the indicators of catalase activity are 65.5 and 75.7%, respectively, of the activity of the control sample. With the introduction of the antioxidant PAF to poisoned rats, the catalase activity for the first 5 days drops to 20% and is gradually restored at all periods of the experiment. On the 40th and 50th days in this group of animals, the catalase activity is restored by 91 and 94%, respectively.

From the above research results, it follows that karate leads to a decrease in the activity of the enzyme of the antioxidant system - catalase in the liver. Changes in catalase activity are observed in numerous studies during intoxication of animals with xenobiotics [1,12,18]

Thus, our proposed plant antioxidant factor (PAF) in the affected hepatocytes, apparently, promotes the acceleration of liver detoxification and the normalization of the altered processes.

2. CONCLUSION

Pesticides cause significant changes in the structure and metabolism of tissues, cells and subcellular structures, being nonspecific structural and metabolic poisons.
It is known that the emergence and development of a number of pathological conditions in humans and animals is accompanied by the activation of lipid peroxidation of cell membranes [10].
We have shown that karate has a toxic effect, affecting the lipids of cell membranes, involving them in free-radical peroxidation, which is one of the reasons for the violation of membrane functions. Used PAF as an antioxidant has a positive effect, significantly reducing lipid peroxidation (LPO) in poisoned rats [4].
Our results show that the LPO process is activated in poisoned animals, and its intensity is interrelated with the state of the antioxidant defense system, in particular, the enzymes SOD, glutathione reductase, and catalase.
Our results show that the study of the protection of SOD and glutathione reductase enzymes during pesticide intoxication, the enzyme activity, which reaches its maximum on the 5th day, is higher than the norm. Subsequently, a slight decrease is observed, however, the level of enzyme activity does not compare with the norm on the 50th day.
An important aspect of our study was to compare the degree of toxicity when animals are poisoned with the karate pesticide, and when these animals are protected from poisoning with an antioxidant of plant origin (PAF). The results obtained indicate that PAF has protective properties and accelerates the processes of LPO and the activity of the enzymes SOD, glutathione reductase, catalase in animals protected by PAF, play biochemical changes that occur in the cell in response to the introduction of PAF. The recovery process in animals that received PAF is activated already on the 5th day.
It has been shown that one of the possible biochemical mechanisms of PAF protection is the ability to reduce lipid oxidation disorders. We have shown that the introduction of PAF during intoxication with a pesticide sharply reduces the accumulation of lipid peroxidation [4,24].
It has been established that the enzymes of the antioxidant system glutathione reductase and superoxide dismutase in the mitochondria and microsomes of rat liver cells are sensitive to the action of pesticides, that is, several times higher than the control values for karate poisoning.
The results obtained for determining the activity of catalase show that catalase activity sharply decreases on the 5th day of karate poisoning, the activity is 55.5% of the activity from the control, and by the 5th day, 75% of the activity is detected, which is 24% lower than the norm.
The introduction of PAF, in order to protect the body of rats from the toxic effects of pyrethroid karate, leads to the restoration of catalase activity. In this case, the restoration of catalase activity reaches 92% of the control value.
The results obtained indicate that PAF prevents toxic effects and promotes the utilization of toxic substances in the body, has protective properties and accelerates the recovery process.

3. REFERENCES

[1] Akbarhodzhaeva Kh.N. Effects of hepatotropic xenobiotics on the structure of immunocompetent organs and the development of lipids for their correction: // abstract of thesis for Candidate of Science in Biology, T.2005,;
[2] Actual problems of hygiene, toxicology and occupational pathology. / Materials of the scientific-practical conference of young scientists and specialists with international

participation (Electronic edition). // Ed. Academician of the RAS V.I. Rakitsky, - Mytishchi, FB UN FNTSG named after F.F. Erisman of Rospotrebnadzor, 2019, p. 271.
[3] Alimbabayeva N.T., Mirkhamidova P., Isabekova M.A., Zikiryaev A., Faizullaev S.S. The effect of residual amounts of karate on the activity of mitochondrial enzymes of hepatocytes. // Uzbek Biological Journal, 2005, No. 4, p. 15-19.
[4] Alimbabaeva N.T., Kholitova R.A., Mirkhamidova P., Tutundzhan A.A., Zikiryaev A., Faizullaev S.S. The effect of karate on lipid peroxidation in mitochondria and microsomes of the rat liver. // Uzbek Biological Journal, 2005, No. 6, p. 34-37.
[5] Archakov A.I. Microsomal oxidation.//Moscow, - Science, - 1975.
[6] Beloedova S.N. Comprehensive toxicological and hygienic regulation of the use of a new herbicide based on chlorimun-ethylimazethapyr. // Diss. Cand. Biol. Sciences., Mytischi., 2009.
[7] Berezov T.T., Korovkin B.F. Biological chemistry // Medicine, M. - 1998
[8] Burlakova E.B. The role of lipids in the process of information transfer in the cell // Biochemistry of lipids and their role in metabolism. Moscow. - 1981
[9] Vlasova S.N., Shibunina E.I., Berislegina I.A. The activity of glutathione-dependent erythrocyte enzymes in chronic liver diseases. // Laboratory work, 1990, No. 8, p. 18-22.
[10] Vladimirov Y.A., Archakov A.I. LPO in biological membranes // Moscow, Mir, 1972
[11] Hygiene, toxicology and occupational pathology: traditions and modernity. / Materials of the All-Russian scientific and practical conference with international participation, dedicated to the 125th anniversary of the founding of the F.F. Erisman FNTSG // Ed. Doctor of medical sciences, prof. A.Y. Popova, Academician of the RAS V.I. Rakitsky., - M., ed. Trading corporation "Dashkov and Co", 2016
[12] Kagan Y.S., Panshina T.N., Sasinovich L.M. Biochemical effects of the toxic action of synthetic pyrethroids. // Hygiene and sanitation. M., Medicine, 1986, No. 1, p. 7-9.
[13] Kardischenko A.N. Medical laboratory diagnostics. SPb.: M: Intermedica, - 1997, - p. 21.
[14] Koraluk M.A., Ivanova L., Mayorova I.G. Method for determining the activity of catalase. // Lab.delo, 1988, No. 1, p. 16.
[15] Makarevich O. P., Golikov P. P. The activity of blood superoxide dismutase in the acute period of various diseases. // Laboratory work, 1983, No. 6, p. 24.
[16] Mirkhamidova P., Mirakhmedov A.K., Sagatova G.A., Isakova A.V., Khamidov D.Kh. The effect of butifos on the structure and function of the liver of rat embryos. // Uzbek Biological Journal, Tashkent, 1981, No. 5, p. 52-56.
[17] Putilina F.E.// Methods of biochemical research. / - Leningrad, -1989, p. 181-183.
[18] Saydalieva L.T., Mirkhamidova P., Alimbabayeva N., Mirkhamidova N.G. Correlation of the PAF effect of the pesticide karate on the activity of cytochrome P-450 in the liver of rat embryos. // Bulletin of the Academy of Sciences of the Republic of Uzbekistan, Nukus, "Ilm" - 2014, No. 1 (234), p. 76-78.
[19] Handbook on pesticides (toxicological and hygienic characteristics) / / Issue No. 2, ed. Academician of the Russian Academy of Sciences, ISBN№978-5-903413-37-9, published in 2015
[20] White A., Fundamentals of Biochemistry, // M., Mir, 1981, Vol. 1, p. 358.
[21] Tuychiyeva D., Mirkhamidova P., Babakhanova D., Parpieva M., Alimova R. Influence of pesticides on the activity of some enzymes of rat liver and ways of their correction // Norwegian Journal of development of the International Science, No. 45/2020, VOL. 2, p. 8.
[22] Cutcamp Z. K., Koch R.B., Desaioh D. // Insectiside made of action. Acad.Press. 1982., p. 45.

[23] Joeschke H., Yores Y.I., Coderbaum A.J., Hinson J.A., Pessayre D., Zemasters J.J./ Mechanismes of hepatotoxicity // Toxicol. Science. 2002. No. 65., p. 166-176.
[24] Mirkhamidova P. Babakhanova D.B., Mukhamedov U.J, Alimova R.A. Determination of biologically active substances flavonoides in fruit trees with healing properties // European Science Reviev. 2020. No. 5-6, p. 93-96.
[25] T.S.Xudoyberdiev. B.R.Boltaboev. B.A.Razzakov. M.Sh.Kholdarov. "To The Fertilizer Knife Determination Of Resistance". // Asian Journal of Multidimensional Research (AJMR) // 9 (8), 65-71, 2020. https://www.indianjournals.com/ijor.aspx?target=ijor:ajmr&volume=9&issue=8&article=011