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Deposit of indoxasacarb as a residue in the organs of pregnant rats and its influence on the activity of transferase enzymes

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ABSTRACT

This article analyzes the residual content of the pesticide indoxacarb in the tissues of certain organs of pregnant rats and their embryos. Also, the influence of safaroflavonoside (SFL) isolated from the plant *Crocus sativus* L. and narsissin flavonoids isolated from the plant *Alhagi canescens* on the content of transferase enzymes and bilirubin in the blood plasma of pregnant rats intoxicated with the pesticide indoxacarb was studied. The pesticide Indoxacarb was administered into the stomach of pregnant rats by the method of per os through a special probe at a dose of LD_{50} 1/10 on days 3, 13, and 17 of pregnancy. On the 20th day of pregnancy, the determination of the amount of residual pesticides in the liver, spleen, brain, and lung tissues of female rats and their embryos was carried out using the LC-MS (China) device. Residual pesticide levels were determined in the liver, spleen, brain, and lung tissues of pregnant rats. A high content of residual pesticide was observed in the liver tissue. In the organs of the embryos of pregnant rats, the presence of the rumen pesticide was also detected. It was found that the residual pesticide content in the liver tissues of pregnant rats and their embryos is higher than in other organs. On the 3rd and 13th days of pregnancy, rats were subjected to single poisoning with the pesticide indoxacarb at a dose of 1/10 LD_{50}, and narcissin and SFL flavonoids were administered orally at a dose of 10 mg kg^{-1} for 7 days from that day. Experimental rats were studied by dividing them into group IV. It was found that flavonoid compounds reduced the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin in pregnant rats poisoned with indoxacarb.

Keywords. Indoxacarb, Pregnant rat, Embryo, cumulation, Liver, Lungs, Spleen, Brain, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Bilirubin, Safaroflavonoside, Narcissin.
Article type: Review Article.

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INTRODUCTION

Currently, more than 1000 pesticides are used in countries around the world to protect agricultural crops. Pesticides are special chemical or biological substances used to protect plants from the effects of insects, weeds, harmful fungi, and other pests, while ensuring their normal growth (Matthews 2015). However, widespread and long-term use of pesticides has led to the accumulation of numerous pesticide residues in food chains, water bodies, and soil, posing various risks to humans. These risks are also directly related to human well-being and ecosystem health (Munir *et al.* 2024). Most pesticides used in agriculture are residual accumulating in the air, soil, and water sourcestransfers directly or indirectly to the organs and tissues of living organisms and can lead to disruption of various biochemical functions in them (Parpieva *et al.* 2023). Studies conducted in the state of Kebbi, Nigeria, have shown that several pesticides accumulate as residues in some organs of farm animals (cows, camels, and goats). That is, pesticides accumulated in the muscle (meat) tissues of these animals - from 0.113 mg kg^{-1} to 2.532 mg kg^{-1} , in liver tissues - from 0.095 mg kg^{-1} to 2.341 mg kg^{-1}, in kidney tissues - from 0.047 mg kg^{-1} to 0.931 mg kg^{-1}, and in heart tissues - from 0.026 mg kg^{-1} to 0.361 mg kg^{-1} (Anthony *et al.* 2022). According to statistics, pesticides worth more than $5.6 billion are used in agriculture annually, which brings great benefits to agricultural productivity (Brown *et al.* 2021). However, the intensive use of these chemicals, in particular their residues in ecosystems, including air, soil, and water sources, has a significant impact on the environment (Megharaj *et al.* 2011). Pesticide pollution emerges as a widespread form of environmental pollution, posing a serious threat to human and animal health. In recent years, the health risk associated with pesticide contamination has attracted significant attention. Reports show that more than 3 million people worldwide suffer annually from pesticide poisoning due to various factors (Dwivedi *et al.* 2014). Low pesticide levels can cause various biochemical changes. Various pesticides negatively affect the formation of various organs during embryonic development. Pesticides can also affect biochemical dysfunctions of biological organs. Changes in liver enzymes such as ALT and AST in the blood plasma of workers poisoned by pesticides have been recorded (Araoud *et al.* 2012). Liver enzymes and nervous system enzymes are studied as dynamic biomarkers for determining hepatotoxicity and neurotoxicity caused by pesticides. As indicators of pesticide action, four enzymes are used, including three liver enzymes, namely ALT, AST, alkaline phosphatase (ALP) and the enzyme of the nervous system, plasma cholinesterase (Mehta Priyanka 2025). Changes in ALT, AST, and IF may indicate disorders of liver physiology. The accumulation of pesticides as residues in liver cells and the neutralization of their toxic effects under the influence of bioactive substances is one of the most urgent tasks. Currently, the effects of many pesticides used in agriculture on organs, tissues, and cells are being studied at the membrane level. However, the accumulation of the pesticide indoxacarb, used in agriculture as an insecticide, as a residue in various organs of pregnant rats, its influence on the activity of transferase enzymes (ALT, and AST), and the correction of disorders using plant flavonoids have not been studied.

MATERIALS AND METHODS

From the Wistar line, outbred' open rats were initially selected for the study, and the semen was inoculated. On the 3^{rd} and 13^{th} days of pregnancy, rats were subjected to single poisoning with the pesticide indoxacarb at a dose of 1/10 LD_{50} . Experimental rats were divided into groups:

3^{rd} day of pregnancy
Group I-Healthy (control)
Group II - Indoxacarb
13^{th} day of pregnancy
Group I-Healthy (control)
Group II - Indoxcarb
17^{th} day of pregnancy
Group I-Healthy (control)
Group II - Indoxcarb

The amount of residual pesticides in various organs (liver, lungs, spleen, and brain) of pregnant rats on days 20-21 of pregnancy was determined using HPLC, LC-MS devices.

Chemical reagents. In LC-MS, a standard solution of Haloxyfop-R-methyl with a concentration of 10 μg mL^{-1} in acetonitrile (HPS Standarts, Germany) was used as a standard. All solvents used in this study were at the HPLC level and were obtained from VWR Chemicals (France). Ultra-pure water was obtained using the Proceers Performa EU15 (China) device.

Pesticide extraction. Organs weighed with an accuracy of 0.0001 g were extracted in a 0.01% solution of formic acid in acetonitrile in an ultrasonic bath at room temperature for 20 minutes. 1.5 mL of the extract was filtered through a 0.22 μm syringe and used for analysis.

Equipment and chromatographic conditions. LC-MS analysis was carried out on the Shimadzu LCMS-2020 (Japan) system, equipped with a CBM-40 system controller, DGU-405 degasser, LC-40D pump, SIL-40 auto-sampler, CTO-40S column furnace, FCV-20AH2 unit. For separation, a Shim-pack Velox C_{18} (150 × 4.6 mm, particle size 5 μm) column with a reversible phase was used at a constant temperature of 30 °C. The mobile phase consisted of a 0.5% aqueous solution of 80% acetonitrile and 20% formic acid. The flow rate was 0.5 mL min^{-1}, and the injection volume was 5 μL. MS detection was carried out on a single quovalldrup mass detector LCMS-2020 equipped with an Electron-Sprey ionizer. To achieve maximum sensitivity values in the positive ion mode of the device, the parameters were optimized as follows: desolvation line temperature (DL) 250 °C, injection gas flow rate 1 L min^{-1}, heating unit temperature 300 °C, drying gas flow rate 15 L min^{-1}, SIM mode 529.00 [(S) - Indoxacarb]. The analysis time was 7 min. The analysis results were analyzed in the LabSolutions V.5.114 program. Nitrogen gas with a purity of 99.5% for drying and nebulation was obtained from the Peak Scientific Genius XE 35 Nitrogen generator.

On the 3rd and 13th days of pregnancy, rats were subjected to single poisoning with the pesticide indoxacarb at a dose of 1/10 LD_{50}, and narcissin and SFL flavonoids were administered orally at a dose of 10 mg kg^{-1} for 7 days from the same day. Experimental rats are grouped as follows:

3rd day of pregnancy	13th day of pregnancy
Group I-Healthy (control)	Group I-Healthy (control)
Group II - Indoxacarb	Group II - Indoxacarb
Group III - Indoxacarb + narcissin	Group III - Indoxacarb + narcissin
Group IV - Indoxacarb + SFL	Group IV - Indoxacarb +SFL

The activity of ALT, AST enzymes and the level of total bilirubin in blood plasma and liver tissue of rats poisoned with the pesticide Indoxcarb were studied on the 20th day of pregnancy after administration of the flavonoids SFL and narcissin. The activity of ALT and AST in the blood serum of experimental animals was determined using the generally accepted method. The principle of the method for determining the activity of these enzymes is based on the formation of pyruvic and oxaloacetic acids as a result of the chain reaction of ketoglutaric, aspartic, and glutamic acids. Under the influence of enzymes that ensure the transition of amino groups of aminotransferases from amino acid to keto acid, pyruvic acid is formed due to the transition reaction of amino groups from amino acid to keto acid. When 2,4-dinethylphenylhydrazine (2,4-DNFH) is added to this mixture, a coloring pyruvic acid hydrosone is formed in an alkaline medium. The resulting color is proportional to the amount of pyruvic acid. ALT catalyzes the transfer of amino groups from alanine to pyruvate and glutamate through 2-oxoglutarate. ALT activity is determined by the rate of decrease in NADH (nicotinamide dinucleotide; Espanola 1987; Approved Recommendation 1986).

Alanine + 2 oxoglutarate ⟶ Pyruvate + Glutamate

Piruvate NADH + H^+ ⟶ Lactate + NAD^+

Normally, ALT in outbred male rats is around 70 ± 1 E L^{-1}, and in female rats – 56 ± 1 E L^{-1}.

Catalyzes the formation of oxalacetate and glutamate from AST aspartate through 2-oxyglutarate amino groups. AST activity is determined by the rate of decrease in NADH. Color intensity was determined by optical density using a spectrophotometer at a wavelength of 340 nm to (Gella 1985).

Aspartate + 2 oxoglutarate ⟶ Oxalate acetate + Glutamate

Oxalate acetate + NADH+H^+ ⟶ Malate + NAD

AST normally is 58 ± 3 E L^{-1} in outbred male rats and about 40 ± 2 E L^{-1} in naked rats.

For biochemical studies, blood from rats was centrifuged at a speed of 3000 rpm for 15 minutes. The levels of ALT, AST, and total bilirubin in the blood of rats were determined using a spectrophotometer. The difference between the values obtained in the *in vivo* experiments o'p was calculated using the t-test bo'. In this case, the values $*p < 0.05$ and $**p < 0.01$ represent statistical significance.

RESULTS

After pesticide residues enter the bloodstream, they can be transferred to various tissues and organs throughout the body. Since most pesticide residues are lipophilic, they tend to accumulate in fatty tissues (Cao *et al.* 2024).

Liver

The liver is the main organ in which pesticide residues are metabolized after entering the body. The enzyme system in the liver, for example, through cytochrome P450 enzymes, can convert pesticide molecules into more easily separable forms, and the resulting metabolites can form oxidation, reduction, or hydrolysis products (Lasch *et al.* 2021). There are studies and literature data on the accumulation of pesticides in different organs in different amounts (Mahmoud *et al.* 2016; Mirkhamidova *et al.* 2021; Mirkhamidova *et al.* 2022). However, the accumulation of pesticides as a residue in the organs of pregnant rats and their embryos has not been sufficiently studied. Therefore, in our research, we set ourselves the goal of studying the residual content of the insecticide indoxocarb, currently the most widely used insecticide in agriculture, in pregnant rats and their embryonic organs (liver, lungs, spleen, and brain). Our initial experiments were conducted on samples of pregnant rat organs. In this case, the residual amount of the pesticide indoxacarb was not found in the chromatogram samples of pregnant rat organs taken for the control group (Table 1, Fig. 1).

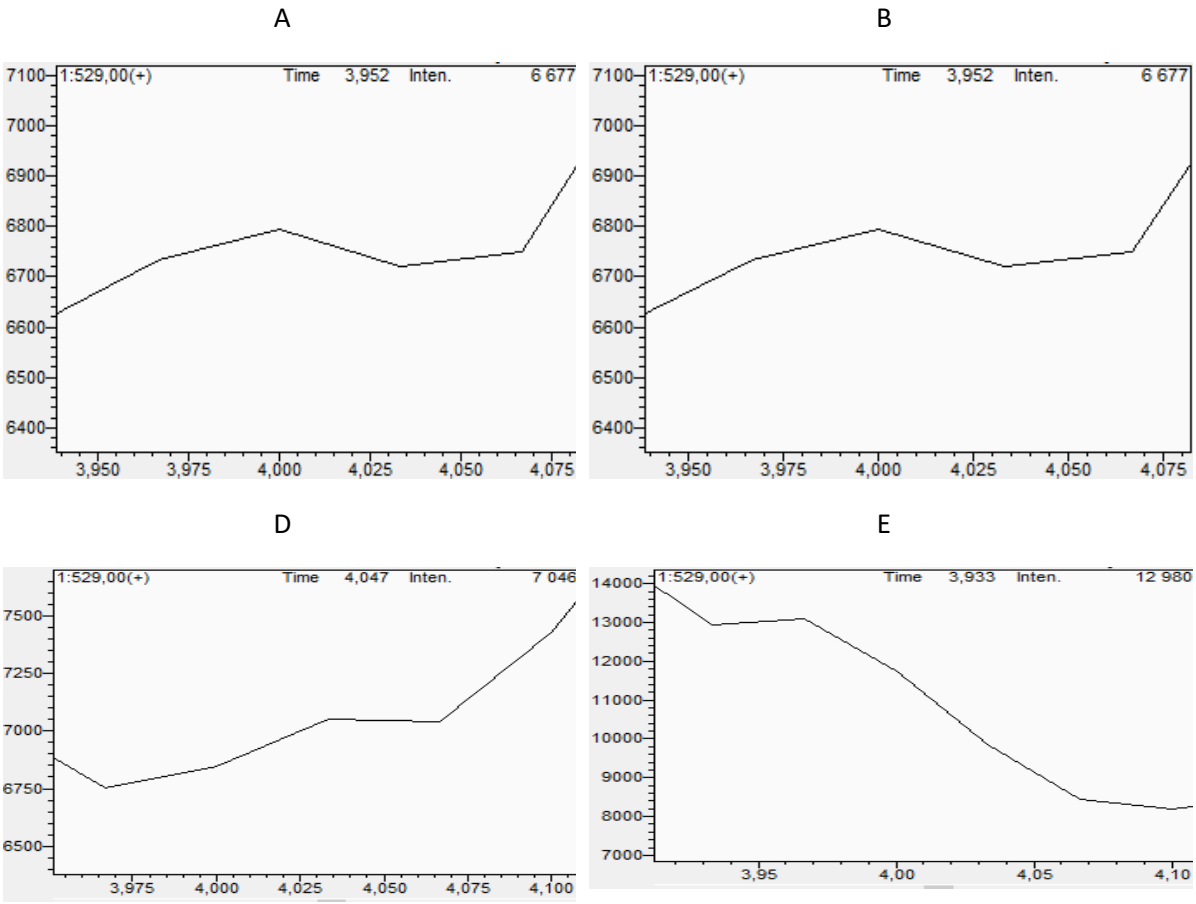


Fig. 1. Chromatograms of pregnant rat organs (liver, lungs, spleen, brain) taken for control. Liver (A), lungs (B), spleen (D), brain (E).

On the 3rd day of pregnancy, in the group of animals poisoned with the pesticide indoxacarb, the residual amount of this pesticide was 12.61437 ± 0.92 mg kg^{-1} in the liver (Table 1, Fig. 2A), 9.35884 ± 0.8 mg kg^{-1} in the lungs (Table 1, Fig. 2B), 4.35887 ± 0.31 mg kg^{-1} in the spleen (Table 1, Fig. 2D), and 11.23101 ± 0.9 mg kg^{-1} in the brain (Table 1, Fig. 2E). In the organs of pregnant rats poisoned with indoxacarb on the 13th day of pregnancy, the pesticide indoxacarb was detected in the liver at a dose of 32.01854 ± 2.2 mg kg^{-1} (Table 1, Fig. 3A), in the lungs at a dose of 11.3512 ± 1.0 mg kg^{-1} (Table 1, Fig. 3B), in the spleen at a dose of 15.06252 ± 1.1 mg kg^{-1} (Table 1, Fig. 3D), in the brain at a dose of 25.62413 ± 1.6 mg kg^{-1} (Table 1, Fig. 3E).

Table 1. Results of chromatographic analysis of residual pesticide levels in the organs (liver, lungs, spleen, and brain) of pregnant rats poisoned with indoxacarb.

№	Day	Liver (mg kg^{-1})	Lung (mg kg^{-1})	Spleen (mg kg^{-1})	Brain (mg kg^{-1})
1.	Healthy	0	0	0	0
2.	3-day	12.61437 ± 0.92	9.358.84 ± 0.8	4.35887 ± 0.31	11.231.01 ± 0.9
3.	13-day	32.0185.4 ± 2.2	11.3512 ± 1.0	15.06.252 ± 1.1	25.62413 ± 1.6
4.	17-day	66.544.46 ± 4.4	13.5132 ± 1.2	21.25212 ± 1.8	30.51392 ± 2.1

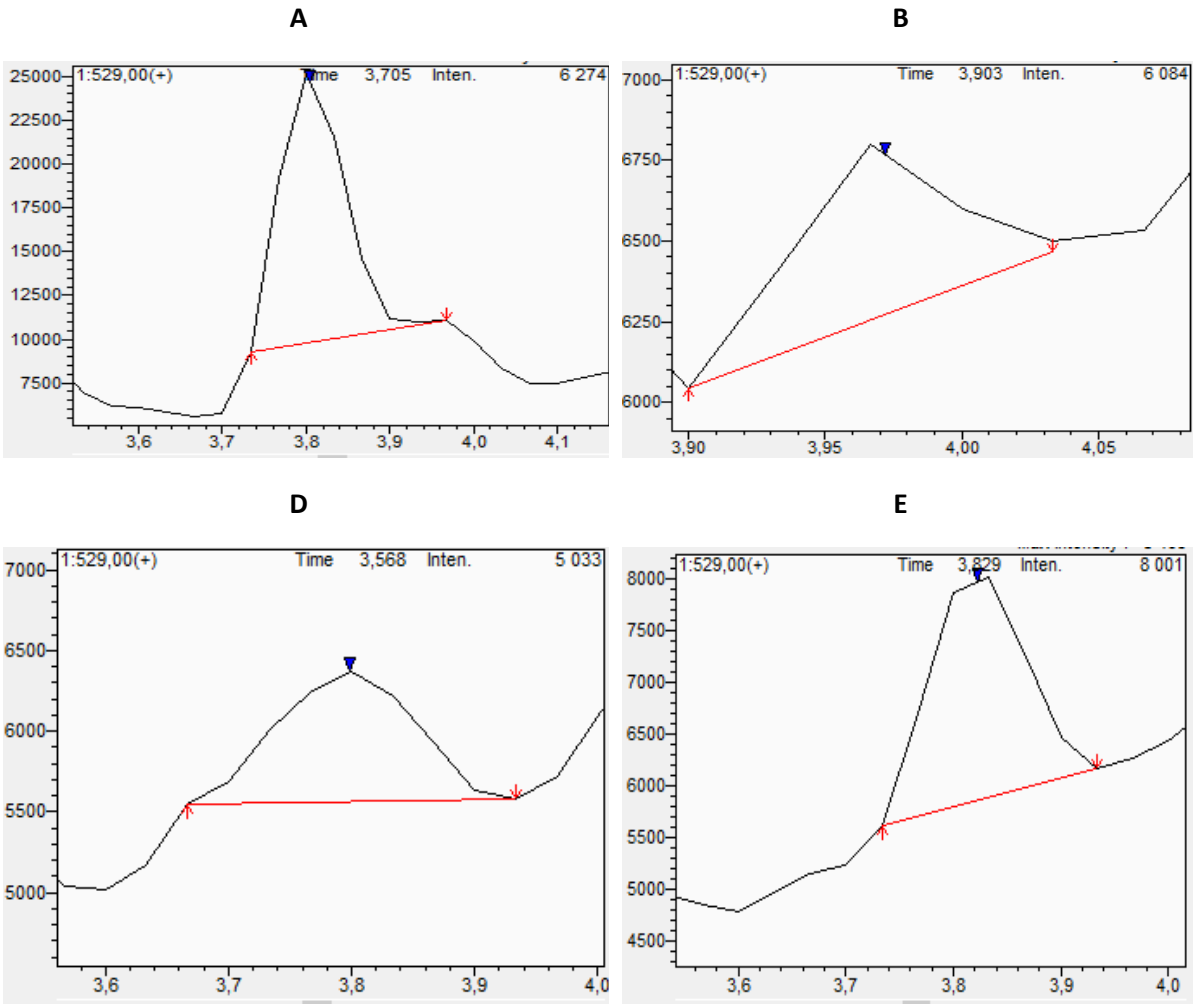


Fig. 2. Chromatograms of the amount of residual pesticides in the organs (liver, lungs, spleen, brain) of rats poisoned with indoxacarb on the 3rd day of pregnancy. Liver (A), lungs (B), spleen (D), brain (E).

In the organs of pregnant rats poisoned with indoxacarb on the 17th day of pregnancy: in the liver - 66.54446 ± 4.4 mg kg^{-1} (Fig. 4A), in the lungs - 13.5132 ± 1.2 mg kg^{-1} (Fig. 4B), in the spleen - 21.25212 ± 1.8 mg kg^{-1} (Fig. 4D), in the brain - 30.51392 ± 2.1 mg kg^{-1} (Fig. 4E; Table 1). In the literature, it has been established that the effect of pesticides at concentrations above the maximum permissible limits also affects the course of pregnancy (Ferguson *et al.* 2019). At the same time, cypermetrin and its metabolites are present in the amniotic fluid of the fetus and can affect the fetus and its development (Bossi *et al.* 2013). Slow removal of residual pesticides from the cells and tissues of living organisms (Juliana *et al.* 2012; Cao *et al.* 2024) on the 3rd and 13th days, the residual pesticide content in the organs of poisoned animals was found to be lower than in the organs of poisoned animals on the 17th day. This can cause a number of biochemical changes in the pregnant organism and its embryo (Parpieva *et al.* 2023).

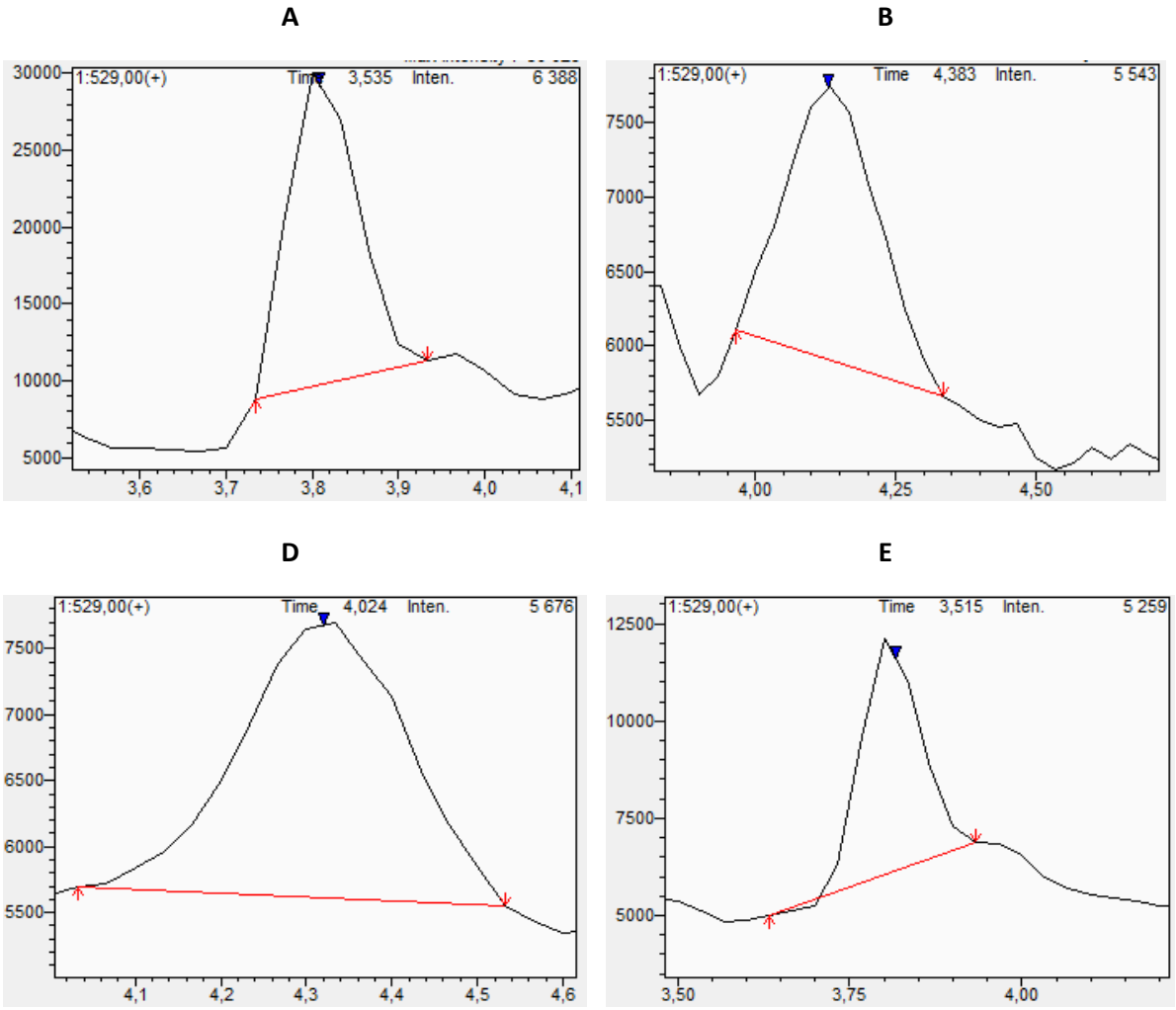


Fig. 3. Chromatograms of the amount of residual pesticides in the organs (liver, lungs, spleen, brain) of rats poisoned with indoxacarb on the 13th day of pregnancy. Liver (A), lungs (B), spleen (D), brain (E).

To confirm the development of intoxication in rats under the influence of pesticides, it is necessary to determine the changes in enzymes and some types of proteins in their blood. Enzymes ALT and AST, released into the blood as a result of damage to liver and heart tissues, are actively involved in metabolism. Changes in the co' indicators of this enzyme under the influence of pesticides signal the beginning of necrosis in cells, damage to hepatocytes of the liver. Therefore, determining the content of aminotransferases in blood serum is an important diagnostic test for a number of diseases, including viral hepatitis, cirrhosis of the liver, and damage caused by other xenobiotics. Therefore, initially, in our experiments, the influence of flavonoid compounds on changes in the content of ALT, AST and bilirubin in blood plasma on the 20th day of pregnancy in rats intoxicated with indoxacarb on the 3rd day of pregnancy was determined. According to the obtained results, the level of ALT in the blood of healthy rats of group I, taken as a control, was 54.5 E L^{-1}, the level of AST - 38.4 E L^{-1}, and the level of total bilirubin - 3.0 mg dL^{-1} (Table 2). In pregnant rats of group II, in whom indoxacarb was administered, the level of ALT in blood plasma was 187.3 E L^{-1}, AST 70.5 E L^{-1}, and total bilirubin 11.7 mg dL^{-1}, which is 3.4 times higher in ALT, 1.8 times higher in AST, and 3.9 times higher in total bilirubin, respectively, compared to the control co'. An increase in ALT and AST levels under the influence of indoxacarb determines the degree of liver cell damage. The increase in AST levels is slightly lower than ALT, which is explained by their relatively low content in the liver. Bilirubin is a pigment formed during the breakdown of hemoglobin, myoglobin, and cytochromes in the reticuloendothelial system of the liver and spleen. The causes of elevated total bilirubin levels are increased red blood cell breakdown (hemolysis) under the influence of indoxocarb, toxic liver damage. In our subsequent experiments, rats that received the

pesticide indoxacarb on the 3rd day of pregnancy were administered the flavonoid SFL to group III and the flavonoid narcissin to group IV for 7 days from that day. Thus, on the 20th day of pregnancy, the levels of ALT, AST, and total bilirubin in the blood plasma of experimental rats were determined. According to the results obtained, the level of ALT in the blood plasma of pregnant rats poisoned with indoxacarb, in whom the flavonoid SFL was administered, was 104.1 E L^{-1}, that of AST was 53.5 E L^{-1}, and that of total bilirubin was 6.8 mg dL^{-1}, which is 1.8 times lower in ALT, 1.3 times lower in AST, and 1.7 times lower in total bilirubin, respectively, compared to the indicators of group II poisoned pregnant females. The level of ALT, AST and total bilirubin in the blood plasma of pregnant rats poisoned with the flavonoid narcissin were 95.6 E L^{-1}, 48.7 E L^{-1}, and 4.2 mg dL^{-1}, respectively which is 1.9 times lower than the co' indicators of group II poisoned pregnant rats, 1.4 times lower than the level of ALT, 1.4 times lower than the level of AST, and 2.8 times lower than the level of total bilirubin (Table 2). Thus, flavonoid compounds reduced the levels of ALT, AST, and total bilirubin in pregnant rats poisoned with indoxacarb.

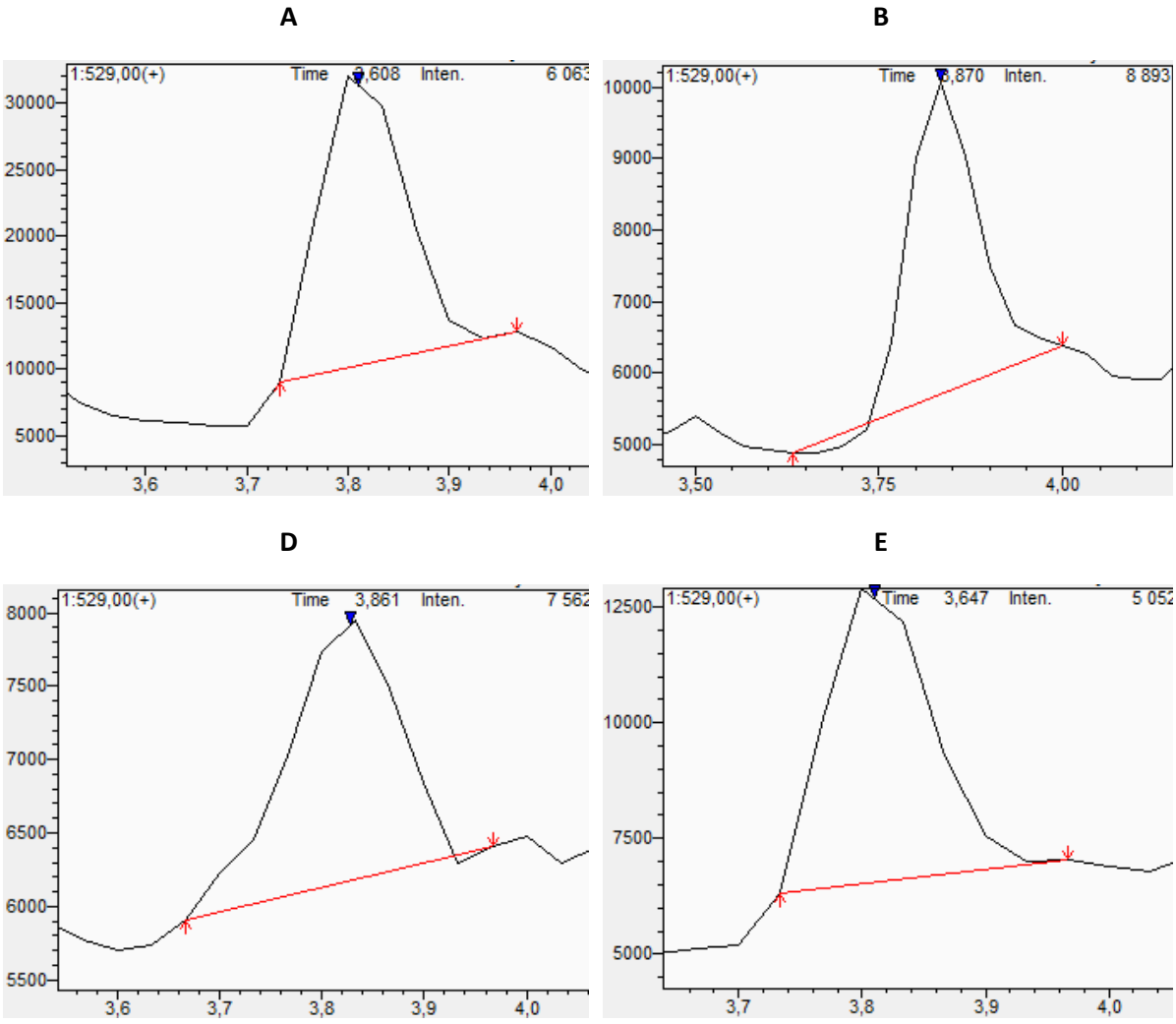


Fig. 4. Chromatograms of the amount of residual pesticides in the organs (liver-A, lungs-B, spleen-D, brain-E) of rats poisoned with indoxacarb on the 17th day of pregnancy.

In our subsequent experiment, the influence of flavonoid compounds on changes in the content of ALT, AST and bilirubin in blood plasma on the 20th day of pregnancy in pregnant rats intoxicated with indoxacarb on the 13th day of pregnancy was determined. According to the obtained results, the level of ALT, AST and total bilirubin in the blood of healthy rats of group I, taken as a control, were 57.4 E L^{-1}, 43.1 E L^{-1}, and 3.3 mg dL^{-1} (Table 3). The level of ALT, AST and total bilirubin in the blood plasma of pregnant rats of group II, in whom indoxacarb was administered, were 195.6 E L^{-1}, 77.8 E L^{-1}, and 13.5 mg dL^{-1} respectively, exhibiting 3.4 times higher in ALT, 1.8 times higher in AST, and 4.1 times higher in total bilirubin compared to the control co' of group I,

(Table 3). Pesticide intoxication in experimental animals led to a sharp increase in the levels of ALT, AST, and bilirubin in their blood plasma as a result of damage to the liver tissue.

Table 2. Changes in the level of ALT, AST enzymes and bilirubin in blood plasma as a result of the administration of SFL and narcissin from 3 days to 7 days in pregnant rats intoxicated with indoxacarb.

Experimental groups	ALT (E L^{-1})	AST (E L^{-1})	Total bilirubin (mg dL^{-1})
Control group I (healthy)	54.5 ± 3.5	38.4 ± 3.1	3.0 ± 0.28
Group II indoxacarb	187.3 ± 10.6**	70.5 ± 5.3**	11.7 ± 1.5**
Group III indoxacarb + SFL	104.1 ± 8.4*	53.5 ± 3.3*	6.8 ± 0.54*
Group IV indoxacarb+narcissin	95.6 ± 7.5**	48.7 ± 3.1*	4.2 ± 0.37**

Note: $^{*}p < 0.05$; $^{**}p < 0.01$; n = 5.

Table 3. Changes in the level of ALT, AST enzymes and bilirubin in blood plasma as a result of the administration of SFL and narcissin from 13 days to 7 days in pregnant rats intoxicated with indoxacarb.

Experimental groups	ALT (E L^{-1})	AST (E L^{-1})	Total bilirubin (mg dL^{-1})
Control group I (healthy)	57.4 ± 4.1	43.1 ± 3.5	3.3 ± 0.31
Group II indoxacarb	195.6 ± 11.8**	77.8 ± 4.7*	13.5 ± 1.4**
Group III indoxacarb + SFL	97.5 ± 7.3**	57.6 ± 3.6*	7.5 ± 0.48*
Group IV indoxacarb+narcissin	84.8 ± 6.7**	50.1 ± 4.0*	6.1 ± 0.53**

Note: ($^{*}p < 0.05$; $^{**}p < 0.01$; n = 5).

As a result of administering the flavonoid SFL at a dose of 10 mg kg^{-1} for 7 days from the same day to rats of group III, intoxicated with indoxacarb on the 13th day of pregnancy, the level of ALT, AST and total bilirubin in their blood plasma were 97.5 E L^{-1}, 57.6 E L^{-1}, and 7.5 mg dL^{-1} respectively. This indicates a decrease in the levels of ALT, AST, and total bilirubin by 2.0, 1.3, and 1.8 times (Table 3). The levels of ALT (84.8 E L^{-1}), AST (50.1 E L^{-1}) and bilirubin (6.1 mg dL^{-1}) in blood plasma of rats of group IV, intoxicated with indoxacarb on the 13th day of pregnancy, were 2.0, 1.5, and 2.2 times (Table 3). Thus, the natural compounds SFL and narcissin flavonoids, isolated from plants, showed pharmacological corrective properties by reducing the content of ALT, AST and total bilirubin in the blood of intoxicated pregnant rats. It has been proven that the action of the flavonoid narcissin on the content of ALT, AST and bilirubin in the blood of pregnant rats under conditions of pesticide intoxication is more active than SFL. Aminotransferases catalyze intermolecular reactions in the transport of amino groups from amino acids to keto acids. Determining the activity of ALT and AST enzymes under the influence of pesticides is important, since these enzymes differ in their activity in different organs and tissues. Thus, for example, the activity of ALT in the liver is somewhat higher than in cardiac tissues, while AST, on the contrary, has higher activity in the heart muscle. The activity of aminotransferases in blood plasma is very low, and their amount increases significantly in various pathological conditions when liver cells or cardiomyocyte membranes are damaged and their integrity is compromised. The increase in the pesticide effect of ALT and AST in the conducted experiment also confirms the above ideas.

CONCLUSION

SFL and narcissin flavonoids reduced the levels of ALT and AST enzymes in blood plasma and liver tissue of pregnant rats poisoned with the pesticide indoxacarb. SFL and narcissin effectively suppressed the increase in total bilirubin levels under the influence of pesticides. It was established that the effect of narcissin on the levels of ALT, AST and total bilirubin is more effective than that of SFL.

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