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Functional and histological changes of the pancreas and the liver in the rats after the acute and subacute administration of diazinon

Funkcionalne i histološke promene pankreasa i jetre kod pacova posle akutne i subakutne primene diazinona

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Abstract

Background/Aim. Organophosphate pesticides (OPs) are used extensively worldwide in agriculture and forestry, and their application represents a major health problem for humans and animals. The aim of this study was to investigate the possibility of the adaptation of an organism to the prolonged administration of a low dose of diazinon. **Methods.** The study was conducted on a total of 60 male Wistar rats. The first 30 rats were divided into four equal diazinon groups (n = 6) and the control one (corn oil). Diazinon was orally administered once at doses: 200, 400, 600, 800 mg/kg (one dose – one group). The concentration of glucose, the activity of α-amylase and the relative activity of LDH1-LDH5 isoenzymes in the blood were measured 24 hours after the application. The remaining 30 rats were divided into two equal diazinon groups (n = 10) and the control one (corn oil). The first group was treated during 7 days, and the second during 14 days with 55 mg/kg of diazinon (1/10 of previously determined LD_{50} value). The histopathology of the pancreas and the liver, as well as the relative activities of LDH isoenzymes in the blood, were determined after the completion of both time periods. **Results.** Single administration of increasing doses of diazinon resulted in a significant increase in the concentrations of glucose, activity of α-amylase and LDH isoenzymes. Subacute application of a low diazinon dose induced histopathological changes in the pancreas manifested by acinar cell necrosis, and in the liver in the form of portal hepatitis and multifocal necrosis. The cumulative doses resulted in statistically significantly lower activities of LDH isoenzymes compared with the single administration of these doses, indicating a lower degree of the cells damage after the subacute diazinon administration. **Conclusion.** Subacute administration of a low dose of diazinon leads to a different adaptation degree of organs and organ systems to toxic effects caused by this organophosphate.

Key words:
diazinon; histological techniques; liver; organophosphorus compounds; pancreas; rats; toxicity test, subacute.

Apstrakt

Uvod/Cilj. Organofosfatni pesticidi (OP) se intenzivno koriste širom sveta u poljoprivredi i šumarstvu, a njihova primena predstavlja značajan zdravstveni problem kod ljudi i životinja. Cilj ove studije bio je da se ispita mogućnost adaptacije organizma na prolongiranu primenu niskih doza diazinona. **Metode.** Studija je sprovedena na ukupno 60 pacova muškog pola Vistar soja. Prvih 30 pacova je podeljeno u četiri jednake grupe tretirane diazinonom (n = 6) i kontrolnu grupu (kukuruzno ulje). Diazinon je primenjivan jednokratno peroralno u dozama: 200, 400, 600, 800 mg/kg (jedna doza – jedna grupa). Koncentracija glukoze, aktivnost α-amilaze i relativna aktivnost LDH1-LDH5 izoenzima u krvi, određivani su 24 sata nakon aplikacije. Preostalih 30 pacova je podeljeno u dve jednake diazinon grupe (n = 10) i kontrolnu grupu (kukuruzno ulje). Prva grupa je tretirana 7 dana, a druga 14 dana sa 55 mg/kg diazinona (1/10 prethodno određene vrednosti LD_{50}). Histopatologija pankreasa i jetre i određivanje relativne aktivnosti LDH izoenzima u krvi urađeni su po završetku oba vremenska perioda. **Rezultati.** Jednokratna primena rastućih doza diazinona rezultirala je statistički značajnim povećanjem koncentracije glukoze, aktivnosti α-amilaze i LDH izoenzima. Subakutna primena niske doze diazinona indukovala je histopatološke promene u pankreasu manifestovane acinarnom nekrozom, a u jetri promene su se ispoljile u vidu portalnog hepatitisa i multifokalne nekroze. Kumulativne doze diazinona rezultirale su statistički značajno nižom aktivnošću LDH izoenzima u poređenju sa jednokratnom primenom tih doza, što ukazuje

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na niži stepen oštećenja ćelija posle subakutne primene diazinona. **Zaključak.** Subakutna primena niske doze diazinona dovodi do različitog stepena adaptacije organa i organskih sistema na toksične efekte izazvane tim organofosfatom.

Ključne reči:
diazinon; histološke tehnike; jetra; organofosforna jedinjenja; pankreas; pacovi; toksičnost, subakutna, testovi.

Introduction

In the order to enhance food production and because of their broad-spectrum insecticidal activity organophosphate pesticides (OPs) are used extensively worldwide in agriculture and forestry. However, only a very small amount of the applied pesticides reaches the target pests, and the rest spreads through water, soil, and food [1]. Therefore, their application represents a major environmental, as well as a health problem for humans and animals.

In humans and animals, diazinon is metabolized to the more toxic metabolite − diazoxone. Its anticholinesterase (AChE) activity leads to the accumulation of acetylcholine at nerve endings, resulting in overstimulation of the nicotinic and muscarinic receptors. Other mechanisms by which diazinon induces toxic effects in the organism are the oxidative stress and inflammation, leading to a histopathological lesions in the liver, pancreas, kidney and brain [2–5]. There are studies that suggest a correlation between oxidative stress and the AChE mechanism of action of OPs. Ranjbar et al. [6] proved that in OPs manufacturing workers, there is a strong correlation between inhibition of AChE in erythrocytes and increased concentration of thiobarbituric acid-reactive substances (TBARS), as an indicator of lipid peroxidation. In addition, intoxication with diazinon results in increased activity of total lactate dehydrogenase (LDH) [7, 8]. Increase in total LDH is rather nonspecific parameter, and because of these, we conducted measurement of its isoenzymes. LDH is an intracellular enzyme, biomarker of energy metabolism, which exists in the 5 isoforms, localized particularly in the heart, erythrocytes and brain (LDH-1), reticuloendothelial system (LDH-2), lungs (LDH-3), pancreas and kidneys (LDH-4), liver and striated muscle (LDH-5). When the cells damaged, there is a "leaking" of LDH from the cells to the bloodstream, where its elevated level is identified. Therefore, LDH isoenzymes are useful biomarkers because they serve as indicators of disturbances integrity of the cells in the different tissues and organs induced by pathological conditions [9–11].

The aim of this study was to investigate the possibility of the adaptation of an organism to the prolonged administration of a low dose of diazinon (1/10 of LD_{50} value).

Methods

In this study we used diazinon (Makhteshim Chemical Works Ltd., Israel) minimum purity of 95%, and corn oil (Uvita, Serbia) as a diazinon solvent. All animal procedures were conducted in accordance with the Directive 2010/63/EU on the protection of animals used for study and other scientific purposes and in accordance with the requirements of the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade.

The study was conducted on a total of 60 male Wistar rats, weighing 200 ± 20 g. Maximum volume of all substances administered perorally to the rats did not exceed 0.1 mL/100 g of rat bw.

The first 30 rats were divided into four equal diazinon groups, containing 6 animals each and the control one (corn oil). Diazinon was orally administered at increased single doses: 200, 400, 600 and 800 mg/kg (one dose – one diazinon group). Twenty-four hours after the application of diazinon, the concentration of glucose, the activity of α-amylase and the relative activity of LDH1-LDH5 isoenzymes in the blood of the rats were measured, in relation to a series of increasing doses of diazinon.

The glucose concentration in the plasma was determined using glucose assay kit (Linear Chemicals S.L., Spain), in the reaction of glucose oxidation by the glucose oxidase (GOD) and the concentration was expressed in mmol/L [12].

The activity of α-amylase in the plasma was assayed using α-amylase assay kit (Linear Chemicals S.L., Spain) with 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNP-G3) as a substrate. The enzyme activity was expressed in U/L.

Isoenzymes LDH1–LDH5 in the blood plasma were detected by PAGE technique using Tris–glycine buffer (25 mM Tris, 192 mM glycine pH 8.3) and sodium lactate as a substrate in the presence of nitroblue tetrazolium chloride 13. LDH1–LDH5 isoenzyme bands intensity was analyzed using TotalLab TL 120 and the activity of each isoenzyme was expressed as band intensity [13].

The remaining 30 rats were divided into two equal diazinon groups, containing 10 animals each and the control one (corn oil). The group I was orally treated (by gastric tube) during 7 days, and the group II during 14 days with 55 mg/kg of diazinon (1/10 of previously determined LD_{50} value). The control group was administered with corn oil with the same procedure. After the completion of treatments, the animals were anesthetized by diethyl ether and sacrificed immediately. The pancreas and the liver were removed and fixed by immersion in 10% neutral buffered formaldehyde (NBF) for histopathology. After fixation, the samples of the pancreas and the liver were dehydrated and embedded in paraffin. Paraffin tissue blocks were cut into 5 μm thick sections, routinely processed and stained with hematoxylin and eosin (HE). Histological preparations were examined using a microscope Olympus BX51 (Tokyo, Japan). Semiquantitative scoring of the severity and the incidence of histopathological lesions in the pancreas and the liver of the rats was performed in accordance with Ramos et al. [14] and Gülçubuk et al. [15], respectively.

In addition, in the rats from this part of the experiment, the relative activity of LDH1-LDH5 isoenzymes in the blood plasma was determined on the 7th and 14th day.

The statistical analysis was performed using a two-way (ANOVA) followed by Tukey's multiple comparisons test. Values $p < 0.05$ were considered significant. All experimental results are shown as the mean ± standard error of the mean (SEM).

Results

The influence of diazinon on the parameters of the pancreatic function

The results showed that the increase in glucose concentration and α-amylase activity are dose-dependent (Table 1). All four tested doses of diazinon resulted in a significant increase in both parameters relative to the control ($p < 0.001$), and the highest tested dose (800 mg/kg) led to a significant increase compared to the previous doses (200, 400, 600 mg/kg) ($p < 0.001$). The effects of diazinon on the concentration of glucose and the activity of α-amylase between doses of 400 and 600 mg/kg did not reach statistical significance.

The influence of diazinon on the activity of LDH1-LDH5 isoenzymes

Diazinon at doses of 400, 600 and 800 mg/kg significantly increased the relative activity of all five isoenzymes of LDH compared to the control ($p < 0.001$), except for the LDH3 isoenzyme, where the dose of 400 mg/kg achieved a lower statistical significance compared with the control ($p < 0.01$) (Figure 1). For the LDH4/5 isoenzymes, which indicate the damage of the pancreas and the liver, the effects of the doses of 400, 600 and 800 mg/kg were also statistically significantly higher than the dose of 200 mg/kg ($p < 0.001$).

At the dose of 800 mg/kg, the activity of LDH4/5 was significantly higher than the activity recorded with the doses of 400 mg/kg and 600 mg/kg ($p < 0.001$) (Figures 1 and 2).

In the group of rats that was treated with diazinon at the

Table 1

The concentrations of glucose and the activity of α-amylase in the blood plasma of the rats treated one time *per os* with increasing doses of diazinon

Treatment	Glucose (mmol/L)	α-amylase (U/L)
Corn oil (control group), 1 mL/kg	3.86 ± 0.16	534 ± 124
Diazinon (mg/kg)		
200	5.14 ± 0.28[a]	1,354 ± 125[a]
400	6.85 ± 0.14[a,b]	1,711 ± 153[a,b]
600	6.93 ± 0.25[a,b]	1,728 ± 109[a,b]
800	8.79 ± 0.32[a,b,c,d]	1,835 ± 120[a,b,c,d]

Data are expressed as mean ± standard error of the mean (SEM) (Two-way ANOVA/Tukey).
[a]$p < 0.001$ compared with the control group; [b]$p < 0.001$ compared with the 200 mg/kg; [c]$p < 0.001$ compared with the 400 mg/kg; [d]$p < 0.001$ compared with the 600 mg/kg.

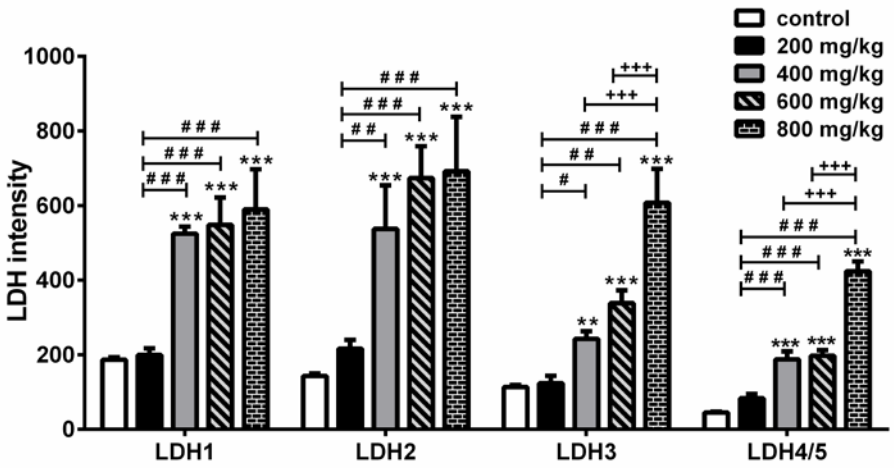

Fig. 1 – Distribution of LDH1-LDH5 isoenzymes in the rats treated with increased single doses of diazinon (200, 400, 600, 800 mg/kg).
Data are expressed as mean ± standard error of the mean (SEM).
****p < 0.01, *****p < 0.001 vs. control; #p < 0.05, ##p < 0.01, ###p < 0.001 and +p < 0.05, ++p < 0.01, +++p < 0.001 between different doses.**

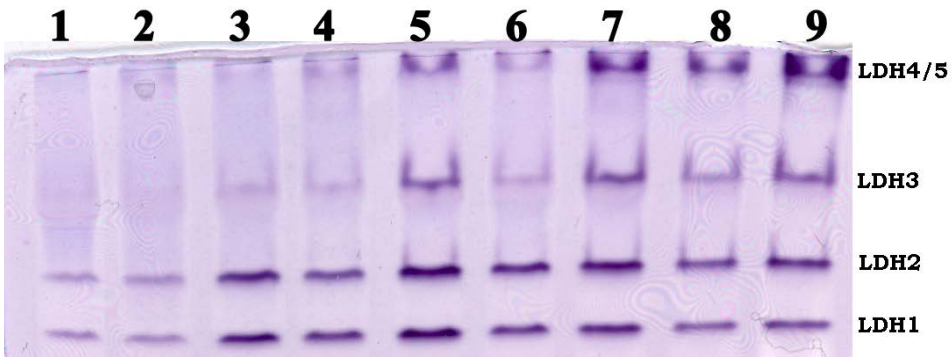

Fig. 2 – Representative polyacrylamide gel electrophoresis (PAGE) of isoenzymes LDH1-LDH5 in relation to a series of increasing doses of diazinon.
Column 1, control rats; Column 2, 200 mg/kg; Columns 3 and 4, 400 mg/kg; Columns 5 and 6, 600 mg/kg; Columns 7– 9, 800 mg/kg of diazinon.

dose of 55 mg/kg for 7 and 14 days, the activity of isoenzymes LDH1-LDH5 was significantly higher than that of the control group on the 7th ($p < 0.001$) and 14th day ($p < 0.001$, $p < 0.01$) (Figure 3). However, the activity of isoenzymes LDH4/5 and LDH3 (indicates damage to the lungs) on the 14th day was statistically significantly lower than their activity on the 7th day ($p < 0.001$). The activity of isoenzyme LDH2 on day 14 of the treatment was not statistically significantly different from the activity on the 7th day. Only the LDH1 isoenzyme activity on the 14th day was statistically significantly higher than the activity on the 7th day of the treatment ($p < 0.001$) (Figures 3 and 4).

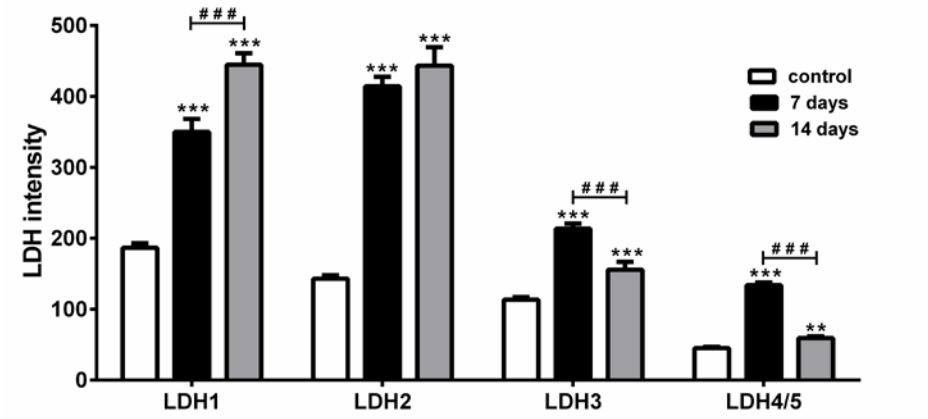

Fig. 3 – Distribution of LDH1-LDH5 isoenzymes in the rats treated with 55 mg/kg of diazinon during 7 and 14 days.
Data are expressed as mean ± standard error of the mean (SEM).
****p* < 0.01, *****p* < 0.001 vs. control; ###*p* < 0.001 between the 7th and the 14th day.**

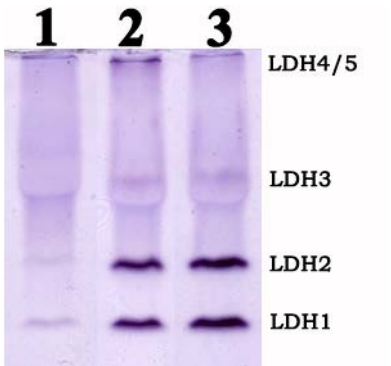

Fig. 4 – Representative polyacrylamide gel electrophoresis (PAGE) of isoenzymes LDH1-LDH5 in relation to the 7th and the 14th day of the treatment with 55 mg/kg of diazinon.
Column 1, control rats; Column 2, 7 days treatment; Column 3, 14 days treatment.

The activities of LDH1-LDH5 isoenzymes after a cumulative diazinon dose of approximately 800 mg/kg (within 14 days) were statistically significantly lower than the activities after the single dose of 800 mg/kg: LDH1 ($p < 0.05$), LDH2 ($p < 0.01$), LDH3 ($p < 0.001$) and LDH4/5 ($p < 0.001$) (Figure 5). In addition, the activities of LDH1 and LDH4/5 isoenzymes after a cumulative diazinon dose of approximately 400 mg/kg (within 7 days) were statistically significantly lower than the activities after the single dose of 400 mg/kg ($p < 0.01$) (Figure 5).

Histopathology of the pancreas and the liver

Histopathological findings of the pancreas revealed necrosis of acinar cells in both analyzed periods: on the 7th (Figure 6a) and 14th day (Figure 6b), but slightly more pronounced on the 14th day (total score 3, range 1–2), which is associated with larger number of macrophages (Table 2). Edema and hemorrhage were discrete in all experimental group samples, while fat necrosis and fibrosis were not noted at all. Also, some discrete degenerative changes in cells within Langerhans

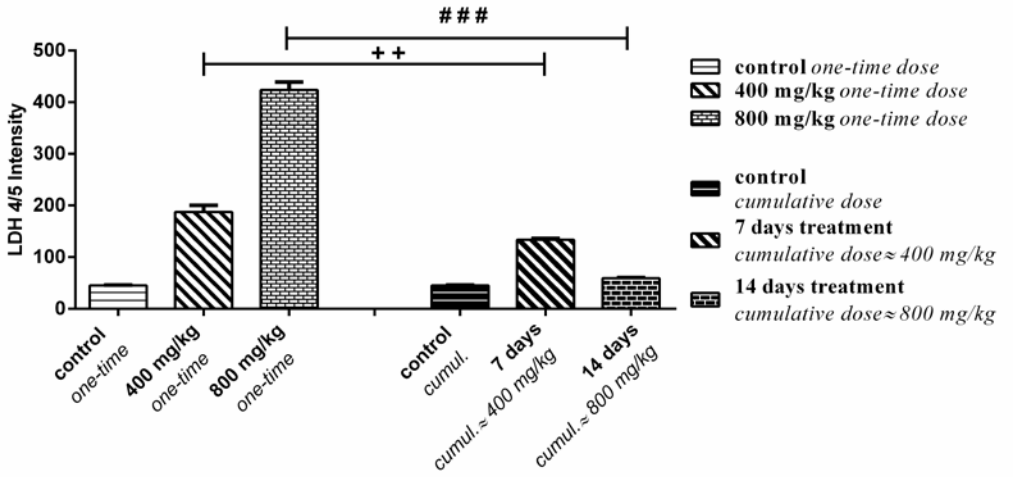

Fig. 5 – Comparison of the distribution of the LDH1-LDH5 isoenzymes in the rats treated with single doses of diazinon (400, 800 mg/kg) (left) and the rats treated with 55 mg/kg of diazinon during 7 days (cumulative dose of ≈ 400 mg/kg) and 14 days (cumulative dose of ≈ 800 mg/kg) (right). Data are expressed as mean ± standard error of the mean (SEM). ###$p < 0.001$ between single and cumulative dose of ≈ 800 mg/kg; ++$p < 0.01$ between single and cumulative dose of ≈ 400 mg/kg.

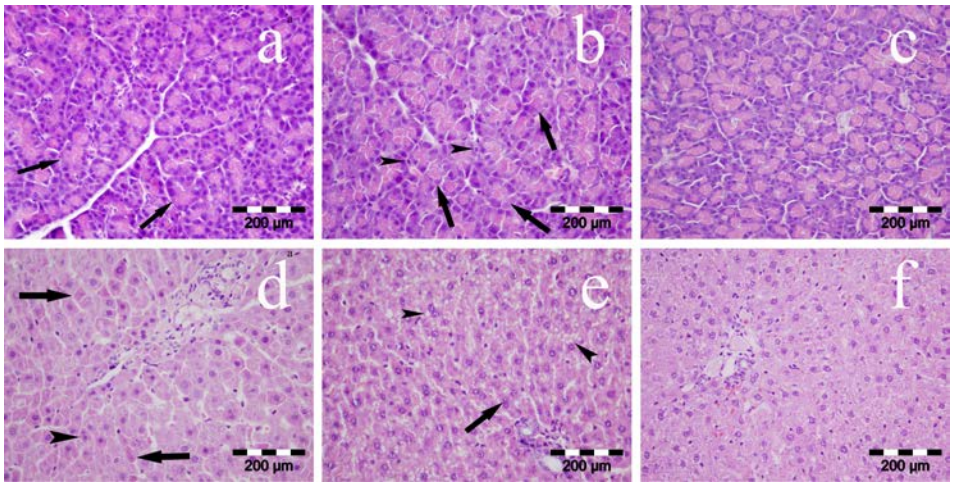

Fig. 6 – Histopathological changes of the pancreas and the liver in the rats treated with diazinon (hematoxilin-eosin staining, 400x). a) Pancreas on the 7th day of the treatment: necrosis of acinar cells (loss of nuclei) (arrows); b) Pancreas on the 14th day of the treatment: necrosis of acinar cells (arrowheads) and numerous macrophages (arrows); c) Pancreas in the control group (corn oil): normal histological pattern; d) Liver on the 7th day of the treatment: necrosis of hepatocytes (arrows) and regenerative changes represented with binucleated hepatocytes (arrowhead); e) Liver on the 14th day of the treatment: hepatocytes show prominent microvesicular fat change (arrowheads) and regenerative changes represented with binucleated hepatocytes (arrow); f) Liver in the control group (corn oil): normal histological architecture, presence of a small number of lymphocytes in the portal spaces.

islets were noted on the 14th day. All morphological features of the pancreas showed normal histological pattern in the control group treated with corn oil (Figure 6c). The results of semiquantitative scoring of histopathological changes in the pancreas are presented in the Table 2.

Liver histopathology showed moderate mononuclear cell infiltration of the portal spaces, both on the 7th (Figure 6d) and 14th day (Figure 6e), with the median of degree of portal inflammation 2 (range 1–2) and 1 (range 0–2), respectively (Table 3). A slightly higher proportion of macrophages were present within the portal spaces on day 14. Normal histological architecture and the presence of a small number of lymphocytes in the portal spaces were features of the liver in the control group with corn oil (Figure 6f). Hepatocytes damage revealed hydropic degeneration, multifocal necrosis and apoptosis on the 7th day (Figure 6d), while microvesicular steatosis and hydropic degeneration were more common histopathological findings on the 14th day (Figure 6e). The median of degree of necroinflammatory activity was 2 (range 1–2) on the 7th day and 1 (range 0–1) on the 14th day (Table 3). The regenerative response of the liver (binucleated hepatocytes) was more pronounced on the 14th day, while in the control group it was not prominent. Fibrosis was noted neither in the experimental groups nor in the control one. The results of semiquantitative scoring of histopathological changes in the liver are presented in Table 3.

Table 2

Degree of leukocyte infiltration and acinar necrosis of the pancreas

Groups	Degree of leukocyte infiltration, median (range)	Degree of acinar necrosis, median (range)	Total histopathological score, median (range)
Group I 7th day of diazinon treatment (55 mg/kg)	1 (0-1)	1 (0-1)	2 (0-1)
Group II 14th day of diazinon treatment (55 mg/kg)	2 (1-2)	1 (1-2)	3 (1-2)
Control group (corn oil, 1 mL/kg)	0	0	0

Table 3

Degree of portal inflammation and necroinflammatory activity of the liver

Groups	Degree of portal inflammation, median (range)	Degree of necroinflammatory activity, median (range)	Total histopathological score, median (range)
Group I 7th day of diazinon treatment (55 mg/kg)	2 (1-2)	2 (1-2)	4 (1-2)
Group II 14th day of diazinon treatment (55 mg/kg)	1 (0-2)	1 (0-1)	2 (0-2)
Control group (corn oil, 1 mL/kg)	0 (0-1)	0	0 (0-1)

Discussion

The pancreas and the liver are among the main targets of the toxic effects of OPs, which lead to their damage and dysfunction. The degree of damage to these organs, besides the OPs dose level, also depends crucially on the exposition period. Therefore, we examined whether the adaptation to toxic effects occurs during subacute diazinon poisoning. In this context, we compared the activities of LDH1-LDH5 isoenzymes after a single and cumulative administration of approximately the same dose, and compared the activities of LDH1-LDH5 isoenzymes for the cumulative doses on the 7th and 14th day of the treatment. Also, we compared our histopathological findings of the pancreas and the liver (7 and 14 days) with the histopathological findings of these organs after the acute diazinon administration, obtained by other authors in test protocols similar to ours.

Acute toxicity studies of diazinon in rats, after single oral administration of increasing doses (25, 50, 100, 200, 300 mg/kg) showed that histopathological changes were not recorded up to a dose of 200 mg/kg, when expressed pancreatitis occurred. In the histopathological finding for the dose of 200 mg/kg, fat necrosis, cellular and glandular degeneration, and congestion were observed [7]. In our study, the rats treated with diazinon at a dose of 55 mg/kg, received a cumulative dose of approximately 400 mg/kg after 7 days, and approximately 800 mg/kg after 14 days of the treatment. Histopathological findings showed that the subacute administration of diazinon (55 mg/kg) caused damage to both exocrine and endocrine pancreas in the rats. We noted necrosis of acinar cells both on the 7th and on the 14th day of the treatment (Table 2; Figures 6a and 6b), but the total histopathological changes were more pronounced on the 14th day (total score 3, range 1-2) compared with the 7th day (total score 2, range 0-1) (Table 2). Linking the results of the previous and our study, the progressive character of pancreatic changes is notable in the function of time. It suggested that pancreatic butyrylcholinesterase (BChE) 7 and AChE 8 are target enzymes for OPs toxicity. The inhibition of pancreatic cholinesterases causes cholinergic overstimulation, resulting in ductular hypertension. Pancreas is a very vulnerable gland and any increased internal pressure can consequently cause severe tissue damage, which leads to acute pancreatitis and dysfunction [16, 17].

When we applied a series of increasing doses of diazinon to a separate group of rats, as a result, we obtained a

significant increase in α-amylase activity with all doses compared to the control value (activity increased in the range from 2.5 to 3.5 times) (Table 1). Acute pancreatitis is diagnosed when the α-amylase is 3 or more times higher than physiological values [16]. The administration of increasing doses of diazinon in our case also resulted in a dose-dependent increase in the serum α-amylase activity (Table 1). This finding is consistent with the results of Gokcimen et al. [7].

Another mechanism by which diazinon induces pancreatitis may be oxidative/nitrosative stress, resulting in the destruction of Langerhans islets cells [18–20]. In our study, discrete degenerative changes in Langerhans islets were observed on the 14th day of the treatment. Chronic administration of diazinon (10 mg/kg) to the rats for 2 months causes a significant increase in the levels of malondialdehyde (MDA), the activity of myeloperoxidase (MPO), as an indicator of inflammation, and the serum glucose levels. In the same study, histopathological examination showed destruction in pancreatic tissues, and the β-cells were the most affected cells among the injured islets [21]. At increasing doses of diazinon, as well as in the case α-amylase, we recorded a dose-dependent manner increase in the serum glucose level, with statistically significant differences between the doses (Table 1). Our highest tested dose of diazinon (800 mg/kg) increases the serum glucose level over 2 times compared to the control value. For the purpose of comparison, in the rats receiving a cumulative dose of diazinon of 980 mg/kg within 14 days, the glucose levels were increased by about twofold compared with the control rats [18]. During the four weeks of application, diazinon (70 mg/kg) induced instability in glucose homeostasis and diabetes in rats [22]. The limitation of our study is that the plasma levels of α-amylase and glucose were not determined after the subacute administration of diazinon. However, based on the higher histopathological score on the 14th day compared to the 7th day, we assume that these values would still be statistically significantly higher than those in the control group.

Histopathological findings of the liver in our study indicate portal hepatitis both on the 7th and on the 14th day (Figures 6d and 6e). However, it was observed that the intensity of inflammation and necrotic changes in the liver were more pronounced on day 7 (median of degree 2) compared to day 14 (median of degree 1) (Table 3). This is supported by the finding that on the 14th day, the activity of LDH4/5 isoenzymes was significantly lower compared to day 7 (Figure 3), implying a lower degree of hepatocyte damage on the 14th day. The same significant decrease in the activity on day 14 compared to day 7 of the treatment was observed in the activity of LDH3 isoenzyme (Figure 3), indicating reduced lung damage even though the cumulative dose was doubled. The finding that the LDH2 isoenzyme activity (biomarker for reticuloendothelial system) was not statistically significantly different on days 7 and 14 (Figure 3) also indicates some form of adaptation of the organism to subacute diazinon poisoning. This result did not exist in the case of the LDH1 isoenzyme, which indicates the damage to the heart, erythrocytes and brain.

Comparing the activities of LDH1-LDH5 isoenzymes, a statistically significant difference is observed between a single and cumulative dose of 400 mg/kg (LDH1, LDH4/5) and between a single and cumulative dose of 800 mg/kg (all five isoenzymes) (Figure 5). The findings that LDH isoenzyme activity was statistically significantly lower at the cumulative dose of 400 mg/kg, and at the cumulative dose of 800 mg/kg, especially for LDH3 and LDH4/5, indicates the adaptation of the organism to the prolonged administration of low doses of diazinon.

We did not perform the examination of pathological changes in the liver after single doses of 400 and 800 mg/kg, in order to compare them with the changes after the subacute administration of diazinon (7 and 14 days). However, hepatotoxicity and pathohistological changes are described by different authors after the acute [23, 24], subacute [25–27] and subchronical [2] exposure of rats to diazinon. The protocol and methodology that are most similar to our experimental conditions were conducted by Beydilli et al. [23]. In that study, diazinon (in the corn oil) was administered orally to rats in a single dose of 335 mg/kg. For the assessment of histopathological changes in the liver, was used a range of 3 grades (1–3), based on the intensity and prevalence of lesions. The liver tissue was significantly damaged, and assessed with the maximal grade 3. The histopathological findings were dominated by: severe sinusoidal dilatations, moderate disrupt radial alignment of hepatocytes, severe vacuolization of hepatocyte cytoplasm and centrilobular necrosis. If we make an analogy with our results, we can say that the pathohistological changes of the liver after the cumulative dose ≈ 800 mg/kg (median of degree of portal inflammation 1, range 0–2 and necroinflammatory activity 1, range 0–1), have a lower intensity compared to the single dose of 335 mg/kg (grade 3, range 1–3). This finding also suggests the existence of the adaptation of the organism to the subacute administration of diazinon. Ivanović et al. [28] have proved that during subchronic administration of diazinon in rats there is a downregulation of nicotinic and muscarinic receptor functions, indicating the adaptation of the peripheral cholinergic system.

In summary, single administration of increasing doses of diazinon in rats results in a significant increase in the concentrations of glucose, activity of α-amylase and LDH1-LDH5 isoenzymes in the blood plasma. Subacute application of diazinon (7 and 14 days) at a low dose (55 mg/kg) induces histopathological changes in the pancreas manifested by acinar cell necrosis, and in the liver in the form of portal hepatitis and multifocal necrosis of the hepatocytes. Histopathological findings of the pancreas were more pronounced on the 14th day. Contrary to that, the histopathological changes in the liver were less pronounced, and the activity of the LDH4/5 isoenzymes was statistically significantly lower on day 14 compared to day 7. Also, a decrease in the activity on day 14 compared to day 7 of the treatment was observed in the activity of the LDH3 isoenzyme, indicating a reduced lung damage, even though the cumulative dose was doubled. Furthermore, the cumulative doses of ≈ 400 and ≈ 800 mg/kg resulted in lower activities of LDH1-LDH5 isoenzymes

compared with the single administration of these doses, indicating a lower degree of the cells damage after the subacute diazinon administration.

Conclusion

Subacute administration of a low dose of diazinon leads to a different adaptation degree of organs and organ systems to toxic effects caused by this organophosphate.

Conflict of interest

The authors declare no conflict of interest.

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REFERENCES

1. *Karami-Mohajeri S*, *Ahmadipour A*, *Rahimi HR*, *Abdollahi M*. Adverse effects of organophosphorus pesticides on the liver: a brief summary of four decades of research. Arh Hig Rada Toksikol 2017; 68(4): 261−75.
2. *Kalender S, Ogutcu A, Uzunhisarcikli M, Acikgoz F, Durak D, Ulusoy Y*, et al. Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes. Toxicology 2005; 211(3): 197−206.
3. *Gokalp O, Buyukvanli B, Cicek E, Ozer MK, Koyu A, Altuntas I,* et al. The effects of diazinon on pancreatic damage and ameliorating role of vitamin E and vitamin C. Pestic Biochem Physiol 2005; 81(2): 123−8.
4. *Yehia MA, El-Banna SG, Okab AB*. Diazinon toxicity affects histophysiological and biochemical parameters in rabbits. Exp Toxicol Pathol 2007; 59(3−4): 215−25.
5. *Shah MD, Iqbal M*. Diazinon-induced oxidative stress and renal dysfunction in rats. Food Chem Toxicol 2010; 48(12): 3345−53.
6. *Ranjbar A*, *Pasalar P*, *Abdollahi M*. Induction of oxidative stress and acetylcholinesterase inhibition in organophosphorous pesticide manufacturing workers. Hum Exp Toxicol 2002; 21(4): 179−82.
7. *Gokcimen A, Gulle K, Demirin H, Bayram D, Kocak A, Altuntas I*. Effects of diazinon at different doses on rat liver and pancreas tissues. Pestic Biochem Phys 2007; 87(2): 103−8.
8. *Costa MD*, *Gai BM*, *Acker CI*, *Souza AC*, *Brandão R*, *Nogueira CW*. Ebselen reduces hyperglycemia temporarily-induced by diazinon: a compound with insulin-mimetic properties. Chem Biol Interact 2012; 197(2−3): 80−6.
9. *McKenzie D, Henderson AR*. Electrophoresis of lactate dehydrogenase isoenzymes. Clin Chem 1983; 29(1): 189−95.
10. *Drent M, Cobben NA, Henderson RF, Wouters EF, van Dieijen-Visser M*. Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. Eur Respir J 1996; 9(8): 1736−42.
11. *Bisgaard HC*, *Thorgeirsson SS*. Evidence for a common cell of origin for primitive epithelial cells isolated from rat liver and pancreas. J Cell Physiol 1991; 147(2): 333−43.
12. *Trinder P*. Determination of glucose in blood using glucose oxidase with on alternative oxygen receptor. Ann Clin Biochem 1969; 6(1): 24−7.
13. *Yoshida M, Takakuwa Y*. Method for the simultaneous assay of initial velocities of lactate dehydrogenase isoenzymes following gel electrophoresis. J Biochem Biophys Methods 1997; 34(3): 167−75.
14. *Ramos CAF, Sá RCDS, Alves MF, Benedito RB, de Sousa DP, Diniz MFFM*, et al. Histopathological and biochemical assessment of d-limonene-induced liver injury in rats. Toxicol Rep 2015; 2: 482-88.
15. *Gülçubuk A, Sönmez K, Gürel A, Altunatmaz K, Gürler N, Aydın S*, et al. Pathologic alterations detected in acute pancreatitis induced by sodium taurocholate in rats and therapeutic effects of curcumin, ciprofloxacin and metronidazole combination. Pancreatology 2005; 5(4−5): 345−53.
16. *Sahin I, Onbasi K, Sahin H, Karakaya C, Ustun Y, Noyan T*. The prevalence of pancreatitis in organophosphate poisonings. Hum Exp Toxicol 2002; 21(4): 175−7.
17. *Harputluoğlu MM*, *Kantarceken B*, *Karincaoglu M*, *Aladag M*, *Yildiz R*, *Ates M*, et al. Acute pancreatitis: an obscure complication of organophosphate intoxication. Hum Exp Toxicol 2003; 22(6): 341−3.
18. *Khaksar MR*, *Rahimifard M*, *Baeeri M*, *Maqbool F*, *Navaei-Nigjeh M*, *Hassani S*, et al. Protective effects of cerium oxide and yttrium oxide nanoparticles on reduction of oxidative stress induced by sub-acute exposure to diazinon in the rat pancreas. J Trace Elem Med Biol 2017; 41: 79−90.
19. *Nurdiana S*, *Goh YM*, *Ahmad H*, *Dom SM*, *Syimal'ain Azmi N*, *Noor Mohamad Zin NS*, et al. Changes in pancreatic histology, insulin secretion and oxidative status in diabetic rats following treatment with Ficus deltoidea and vitexin. BMC Complement Altern Med 2017; 17(290): 1−17.
20. *Ghafour-Rashidi Z, Dermenaki-Farahani E, Aliahmadi A, Esmaily H, Mohammadirad A, Ostad SN*, et al. Protection by cAMP and cGMP phosphodiesterase inhibitors of diazinon-induced hyperglycemia and oxidative/nitrosative stress in rat Langerhans islets cells: Molecular evidence for involvement of non-cholinergic mechanisms. Pestic Biochem Physiol 2007; 87(3): 261−70.
21. *El-Medany A, El-Medany J*. Effect of chronic exposure to diazinon on glucose homeostasis and oxidative stress in pancreas of rats and the potential role of mesna in ameliorating this effect. J Pharma Care Health Sys 2015; 2(4): 71.
22. *Pakzad M*, *Fouladdel S*, *Nili-Ahmadabadi A*, *Pourkhalili N*, *Baeeri M*, *Azizi E*, et al. Sublethal exposures of diazinon alters glucose homostasis in Wistar rats: Biochemical and molecular evidences of oxidative stress in adipose tissues. Pestic Biochem Physiol 2013; 105(1): 57−61.
23. *Beydilli H*, *Yilmaz N*, *Cetin ES*, *Topal Y*, *Celik OI*, *Sahin C*, et al. Evaluation of the protective effect of silibinin against diazinon induced hepatotoxicity and free-radical damage in rat liver. Iran Red Crescent Med J 2015; 17(4): e25310.
24. *Hassani S, Maqbool F, Salek-Maghsoudi A, Rahmani S, Shadboorestan A, Nili-Ahmadabadi A,* et al. Alteration of hepatocellular antioxidant gene expression pattern and biomarkers of oxidative damage in diazinon-induced acute toxicity in Wistar rat: A time-course mechanistic study. Excli J 2018; 17: 57−71.
25. *Al-Attar AM*. Effect of grapeseed oil on diazinon-induced physiological and histopathological alterations in rats. Saudi J Biol Sci 2015; 22(3): 284−92.

26. *Lari P*, *Abnous K*, *Imenshahidi M*, *Rashedinia M*, *Razavi M*, *Hosseinzadeh H*. Evaluation of diazinon-induced hepatotoxicity and protective effects of crocin. Toxicol Ind Health 2015; 31(4): 367−76.

27. *Pourtaji A*, *Robati RY*, *Lari P*, *Hosseinzadeh H*, *Ramezani M*, *Abnous K*. Proteomics screening of adenosine triphosphate-interacting proteins in the liver of diazinon-treated rats. Hum Exp Toxicol 2016; 35(10): 1084−92.

28. *Ivanović SR, Dimitrijević B, Ćupić V, Jezdimirović M, Borozan S, Savić M*, et al. Downregulation of nicotinic and muscarinic receptor function in rats after subchronic exposure to diazinon. Toxicol Rep 2016; 3:523-30.

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