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FUNCTIONAL AND HISTOLOGICAL CHANGES OF THE PANCREAS AND LIVER IN THE RATS AFTER 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 extensively used worldwide in agriculture and forestry, and their application represents a major health problem for humans and animals. The aim of this study is to investigate the possibility of adaptation of organism to prolonged administration of low dose of diazinon. **Methods.** The study was conducted on a total of 60 male Wistar rats. The first 30 rats was 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, activity of α-amylase and relative activity of LDH1-LDH5 isoenzymes in the blood were measured 24 hours after application. The remaining 30 rats was divided into two equal diazinon groups (n=10) and the control one (corn oil). The first group treated during 7 days, and second during 14 days with 55 mg/kg of diazinon (1/10 of previously determined LD_{50} value). The histopathology of the pancreas and liver and relative activities of LDH isoenzymes in the blood were determined after the completion of both time period. **Results.** Single administration of increasing doses of diazinon, results in a significant increase of concentrations of glucose, activity of α-amylase and LDH isoenzymes. Subacute application of low diazinon dose induces 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 subacute diazinon administration. **Conclusion.** Subacute administration of low dose of diazinon leads to the different degree adaptation of the organs and organ systems to toxic effects caused by this organophosphate.

Keywords:

Diazinon, Rats, Pancreas, Liver, Lactate dehydrogenase

Abstrakt

Uvod/Cilj. Organofosfatni pesticidi (OP) se intenzivno koriste širom sveta u poljoprivredi i šumarstvu, a njihova primena predstavlja značajan zdravstveni problem ljudi i životinja. Cilj ove studije je da se ispita mogućnost adaptacije organizma na prolongiranu primenu niskih doza diazinona. **Metode.** Studija je sprovedena na ukupno 60 muških pacova Vistar

soja. Prvih 30 pacova je podeljeno u četiri jednake diazinon grupe (n=6) i kontrolnu (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 (kukuruzno ulje). Prva grupa je tretirana 7 dana, a druga 14 dana sa 55 mg/kg diazinona (1/10 predhodno 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 rezultira statistički značajnim povećanjem koncentracije glukoze, aktivnosti α-amilaze i LDH izoenzima. Subakutna primena niske doze diazinona indukuje histopatološke promene u pankreasu manifestovane acinarnom nekrozom, a u jetri u vidu portalnog hepatitisa i multifokalne nekroze. Kumulativne doze rezultirale su statistički značajno nižom aktivnošću LDH izoenzima u poređenju sa jednokratnom primenom tih doza, što ukazuje 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 ovim organofosfatom.

Ključne reči:
Diazinon, Pacovi, Pankreas, Jetra, Laktat dehidrogenaza

Introduction

In the order to enhance food production and because of their broad-spectrum insecticidal activity organophosphate pesticides (OPs) are worldwide extensively used 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 activity leads to the accumulation of acetylcholine at nerve endings, resulting in overstimulation of the nicotinic and muscarinic receptors. Other mechanisms by which diazinon induced toxic effects in the organism are the oxidative stress and inflammation, leading to a histopathological lesions in the liver, pancreas, kidney and brain

[2, 3, 4, 5]. There are studies that suggest a correlation between oxidative stress and the anticholinesterase mechanism of action of OPs. Ranjbar *et al*. [6] proved that in organophosphorus-pesticide manufacturing workers, between inhibition of AChE in erythrocytes and increased concentration of thiobarbituric acid-reactive substances (TBARS), as an indicator of lipid peroxidation, there is a strong correlation. 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, 10, 11].

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

Methods

In this study was used diazinon (Makhteshim Chemical Works Ltd., Israel) minimum purity 95%, and corn oil (Uvita, Serbia) as a diazinon solvent. All animal procedures 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 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 all substances administered per oral to the rats did not exceed 0.1 ml/100 g of rat bw.

The first 30 rats was 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 application of diazinon, the concentration of glucose, activity of α-amylase and relative

activity of LDH1-LDH5 isoenzymes in the blood of rats were measured, in relation to a series of increasing doses of diazinon.

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

Activity of α-amylase in the blood 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 is 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 were analyzed using TotalLab TL 120 and the activity of each isoenzymes was expressed as band intensity.

The remaining 30 rats was divided into two equal diazinon groups, containing 10 animals each and the control one (corn oil). The group I was orally treated (by gastric sonde) during 7 days, and 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, animals anesthetized by diethyl ether and sacrificed immediately. The pancreas and liver removed and fixed by immersion in 10% neutral buffered formaldehyde (NBF) for histopathology. After fixation, samples of the pancreas and 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 incidence of histopathological lesions in the pancreas and liver of rats was performed in accordance with Ramos *et al.* [14] and Gülçubuk *et al.* [15], respectively.

In addition, in the rats of this group, the relative activity of LDH1-LDH5 isoenzymes in the blood plasma was determined on the day 7^{th} and 14^{th} day.

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

Results

The influence of diazinon on parameters of the pancreatic function

The results show 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 control (***ap<0.001), and the highest tested dose (800 mg/kg) leads to a significant increase compared to the previous doses (200, 400, 600 mg/kg) (***b,c,dp<0.001). The effects of diazinon on the concentration of glucose and activity of α-amylase between doses of 400 and 600 mg/kg did not reach statistical significance.

Table 1.

The influence of diazinon on the activity LDH1-LDH5 isoenzymes

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

Figure 1.

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

Figure 2.

In the group of rats that was treated with diazinon in a dose of 55 mg/kg for 7 and 14 days, the activity of isoenzymes LDH1-LDH5 was significantly higher than the control on the day 7th (***p<0.001) and 14th (***p<0.001, **p<0.01) (Figure 3). However, activity of isoenzymes LDH4/5 and LDH3 (indicates damage to the lungs) on the day 14th was statistically significant lower than their activity on the day 7th (###p<0.001). Activity of isoenzyme LDH2 on day 14th of the treatment was not statistically significantly different from activity on the day 7th. Only the LDH1 isoenzyme activity on the day 14^{th} was statistically significantly higher than the activity on day 7^{th} of treatment (***p<0.001) (Figure 3; Figure 4).

Figure 3.

Figure 4.

The activities of LDH1-LDH5 isoenzymes after a cumulative diazinon dose of approximately 800 mg/kg (within 14 days) was statistically significantly lower than the activities after 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) was statistically significantly lower than the activities after single dose of 400 mg/kg (++$p<0.01$) (Figure 5).

Figure 5.

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

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 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 portal spaces on day 14th. Normal histological architecture and presence of a small number of lymphocytes in the portal

spaces were features of liver in 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). 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 day 14th day, while in the control group was not prominent. Fibrosis was not noted in both experimental and control groups. The results of semiquantitative scoring of histopathological changes in the liver are presented in the Table 3.

Table 3.

Figure 6.

Discussion

The pancreas and 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, beside to the OPs dose level, also depends crucially on the exposition period. Therefore, we examined whether adaptation to toxic effects occurs during subacute diazinon poisoning. In these context, we compared the activities of LDH1-LDH5 isoenzymes after 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 days of treatment. Also, we compared our histopathological findings of the pancreas and liver (7 and 14 days) with the histopathological findings of these organs after 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 occurs. In the histopathological finding for a 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, after 7 days received a cumulative dose of approximately 400 mg/kg, and after 14 days treatment approximately 800 mg/kg. Histopathological findings

showed that subacute administration of diazinon (55 mg/kg) caused damage in both exocrine and endocrine pancreas in rats. We noted necrosis of acinar cells both 7th and 14th days of treatment (Table 2; Figure 6a, 6b), but the total histopathological changes were more pronounced on the day 14th (total score 3, range 1-2) compared with day 7th (total score 2, range 0-1) (Table 2). The linking the results of previous and our study, it is notable the progressive character of pancreatic changes in a function of time. It suggested that pancreatic BChE 7 and AChE 8 are target enzymes for of 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. Administration of increasing doses of diazinon in our case also resulted in a dose-dependent manner increase in serum α-amylase activity (Table 1). This finding is consistent with the results Gokcimen et al. 7.

Other mechanism by which diazinon induces pancreatitis may be oxidative/nitrosative stress, resulting in destruction of Langerhans islets cells 18, 19, 20. In our study, discrete degenerative changes in Langerhans islets were observed on the 14th day of treatment. Chronic administration of diazinon (10 mg/kg) to the rats for 2 months causes significant increase in the levels of malondialdehyde (MDA), activity of myeloperoxidase (MPO), as an indicator of inflammation and 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 dose-dependent manner increase in serum glucose level, with statistically significant differences between doses (Table 1). Our highest tested dose of the diazinon (800 mg/kg) increases the serum glucose level over 2 times compared to the control value. For the purpose of comparison, in rats receiving a cumulative dose of the diazinon of 980 mg/kg within 14 days, glucose levels were increased by about 2-fold compared with control rats 18. During four weeks of application, diazinon (70 mg/kg) in

rats induced instability in glucose homeostasis and diabetes 22. The limitation of our study is that plasma levels of α-amylase and glucose were not determined after 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 the control.

Histopathological findings of the liver in our study indicate portal hepatitis both 7th and 14th days (Figure 6d, Figure 6e). However, it was observed that the intensity of inflammation and necrotic changes in the liver was more pronounced on day 7th (median of degree 2) compared to day 14th (median of degree 1) (Table 3). This is supported by the finding that on the day 14th, the activity of LDH4/5 isoenzymes was significantly lower ($p<0.001$) compared to day 7th (Figure 3), implying a lower degree of hepatocyte damage on the day 14th. The same decrease in activity on day 14th compared to the day 7th of treatment ($p<0.001$) was observed at 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 at days 7th and 14th (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 damage to the heart, erythrocytes and brain. Comparing the activities of LDH1-LDH5 isoenzymes, a statistically significant difference is observed between the single and cumulative dose of 400 mg/kg (LDH1, LDH4/5) and between single and cumulative dose of 800 mg/kg (all five isoenzymes) (Figure 5). The findings that LDH isoenzymes activity was statistically significant lower at a cumulative dose of 400 mg/kg ($p<0.01$), and at a cumulative dose of 800 mg/kg, especially for LDH3 and LDH4/5 ($p<0.001$), indicates an adaptation of the organism to prolonged administration of low doses of diazinon.

We did not perform examination of pathological changes in the liver after single doses of 400 and 800 mg/kg, in order to compare them with changes after 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, 26, 27 and after subchronical 2 exposure of rats to the diazinon. The protocol and methodology that are the most similar to our experimental conditions was 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 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 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 an adaptation of the organism to the subacute administration of diazinon. Ivanovic et al. 28. have proved that during subchronic administration of diazinon in rats there is a downregulation of nicotinic and muscarinic receptor functions, indicating an adaptation of the peripheral cholinergic system.

In summary, a single administration of increasing doses of diazinon in rats, results in a significant increase of 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 statistically significantly lower on the day 14th compared to the day 7th. Also, decrease in activity on day 14th compared to the day 7th of treatment was observed at activity of the LDH3 isoenzyme, indicating 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 subacute diazinon administration.

Conclusion

Subacute administration of low dose of diazinon leads to the different degree adaptation of the 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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Figure titles

Fig. 1. Distribution of LDH1-LDH5 isoenzymes in rats treated with increased single doses of diazinon (200, 400, 600, 800 mg/kg)
(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 statistical significance between different doses)

Fig. 2. Representative PAGE of isoenzymes LDH1-LDH5 in relation to a series of increasing doses of diazinon
(column 1 – control rats; column 2 – 200 mg/kg, column 3, 4 – 400 mg/kg, column 5, 6 – 600 mg/kg, column 7, 8, 9 – 800 mg/kg of diazinon)

Fig. 3. Distribution of LDH1-LDH5 isoenzymes in rats treated with 55 mg/kg of diazinon during 7 and 14 days
(Mean ± SEM, **p<0.01; ***p<0.001 vs. control; ###p<0.001 statistical significance between 7th and 14th day)

Fig. 4. Representative PAGE of isoenzymes LDH1-LDH5 in relation to a 7th and 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)

Fig. 5. Comparison of distributions of the LDH1-LDH5 isoenzymes in rats treated with single doses of diazinon (400, 800 mg/kg) (left) and 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)
(Mean ± SEM, #p<0.05, ##p<0.01, ###p<0.001 statistical significance between single and cumulative dose of ≈800 mg/kg; ++p<0.01 statistical significance between single and cumulative dose of ≈400 mg/kg

Fig. 6. Histopathological changes of the pancreas and liver in rats treated with diazinon (HE, 400x)

a. Pancreas on the 7th day of treatment: necrosis of acinar cells (loss of nuclei) (arrows); b. Pancreas on the 14th day of 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 treatment: necrosis of hepatocytes (arrows) and regenerative changes represented with binucleated hepatocytes (arrowhead); e. Liver on the 14th day of treatment: hepatocytes show prominent microvesicularfatt 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

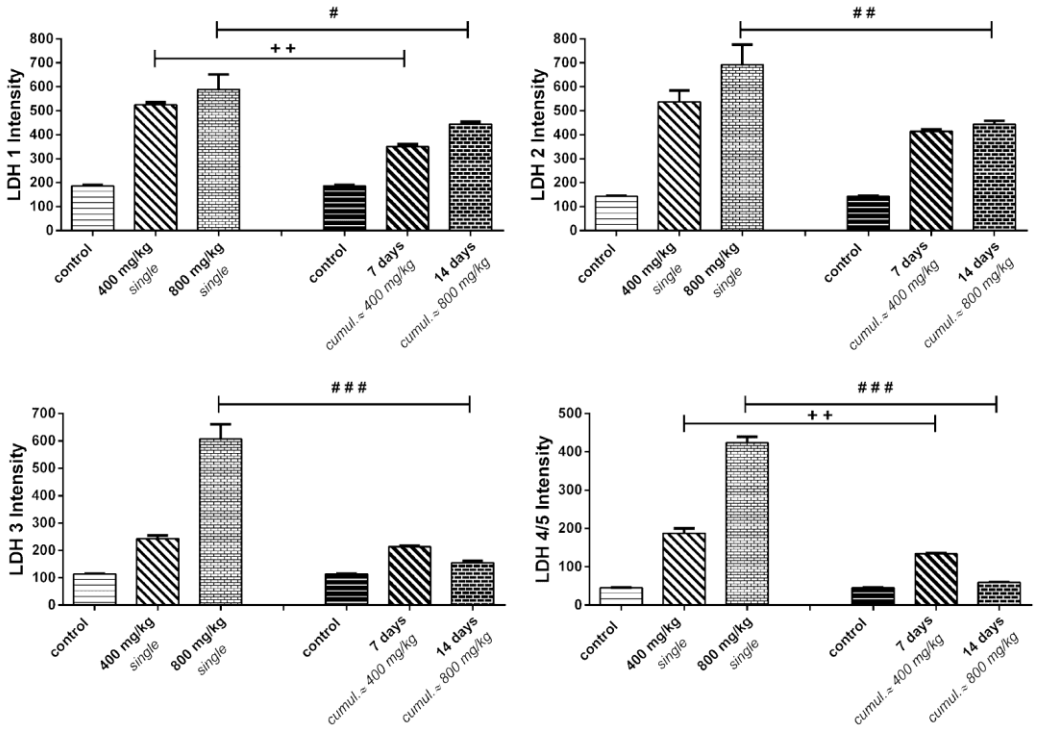

Table 1.

The concentrations of glucose and activity of α-amylase in the blood plasma of rats treated one-time p.o. with increasing doses of diazinon

		Glucose (mmol/L)	α-amylase (U/L)
Control group	corn oil	3.86±0.16	534±124
Doses of diazinon (mg/kg)	200	5.14±0.28a	1354±125a
	400	6.85±0.14a,b	1711±153a,b
	600	6.93±0.25a,b	1728±109a,b
	800	8.79±0.32a,b,c,d	1835±120a,b,c,d

Data expressed as mean ± SEM (Two-way ANOVA/Tukey test).

***ap < 0.001 compared with the control group; ***bp < 0.001 compared with the 200 mg/kg;

***cp < 0.001 compared with the 400 mg/kg; ***dp < 0.001 compared with the 600 mg/kg;

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 7^{th} day of diazinon treatment	1 (0-1)	1 (0-1)	2 (0-1)
Group II 14^{th} day of diazinon treatment	2 (1-2)	1 (1-2)	3 (1-2)
Control group (corn oil)	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 7^{th} day of diazinon treatment	2 (1-2)	2 (1-2)	4 (1-2)
Group II 14^{th} day of diazinon treatment	1 (0-2)	1 (0-1)	2 (0-2)
Control group (corn oil)	0 (0-1)	0	0 (0-1)

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