Acta Veterinaria (Beograd), Vol. 57, No. 5-6, 497-507, 2007.

DOI: 10.2298/AVB0706497D UDK 619:616.37-008.6

HEPATOTOXICITY OF DINITRO-O-CRESOL IN RATS (*RATTUS NORVEGICUS*)

DERE E, OZDIKICIOGLU FERDA and TOSUNOGLU H

Uludag University, Faculty of Science and Art, Department of Biology, Bursa

(Received 14. January 2007)

In this study we investigated the hepatotoxic effect of DNOC in rats, using biochemical serum parameters as indicators of liver disease. Experimental groups were injected intraperitoneally with 2.8 mg/kg DNOC.

While an increase was determined in LDH activities for all periods, an increase was determined for urea values only up to 16 hours. In general, increased AST and ALP activities were recorded up to the 8th hour. Thereof, at later periods inhibition was observed. Inhibition was observed in female rats, while ALT activation was observed in male rats. Amylase activity inhibition was observed in both groups. Increased values were observed for cholesterol and HDL at the initial hours. No significant changes were observed in triglyceride levels.

As a result, it has been observed that DNOC caused changes in serum enzyme activities and in biochemical parameters in female and male rats.

Key words: DNOC, rat, LDH, AST, ALT, ALP

INTRODUCTION

One of the pesticides frequently used in agriculture is 4,6 Dinitro-o-cresol, (DNOC) which is used as a herbicide, insecticide, acaricide, larvicide, ovicide, fungicide and in plastic industry as an inhibitor of the polymerization of styrene and vinyl aromatic compounds. As a consequence of using DNOC in the agricultural and chemical industry workers are affected directly by respiration and dermal absorption. Other populations are affected indirectly owing to the fact that DNOC mixes with soil, water, and air (WHO, 2000).

When someone is exposed to DNOC via digestion, respiration or skin, acute (short-term) toxicity symptoms such as, excessive perspiration, thirst, fatigue, insomnia, rapid breathing, nausea, headache, anorexia, and greenish yellow pigmentation appear. Damage to the liver, kidney, and nervous system has been reported in humans following acute exposure. Dermal contact may lead to local necrosis. Chronic (long-term) exposure results in the same toxicity symptoms in humans (US EPA, 2005). Bilateral cataracts and blindness have been observed in individuals chronically exposed to DNOC by ingestion (OEHHA, 2003). Effects to

the cardiovascular, gastrointestinal, and central nervous systems and changes in blood counts of chronically exposed workers have been reported. Decreased weight gain and food consumption have been observed in rats chronically exposed to DNOC by ingestion. Changes in the blood and urine, decreased liver enzyme activity, and changes in the absolute and relative organ weights were also reported in rats (US EPA, 2005).

DNOC may indicate a toxic effect by binding to cell macromolecules. Luk'ianchuk (1983) showed that DNOC causes toxicity by being bound to human serum albumin. Hrelia *et al*. (1994) recorded that DNOC increases structural chromosome damage in bone marrow cells of rats. Moreover, DNOC creates chain breakages in DNA, thus DNA damages (Grilli *et al*., 2003). Ken *et al*. (2003) stated that DNOC causes damage in germ cells of rats.

Serum enzymes including ALT, AST, ALP, GGT and LDH are mainly used in the evaluation of hepatic damage. Although these enzymes are not completely specific, an increase in the activities reflects active liver damage (Nemcsok *et al*., 1987; Asztalos *et al*., 1990).

Due to the fact that the toxic effects of DNOC had been seen and resulted in variations in energy metabolism, in this study, we aimed to describe the effect of DNOC on enzymes and biochemical parameters in the blood serum of rats in regard to sex differences.

MATERIAL AND METHODS

Wistar rats (*Rattus norvegicus*) weighing 200-250 g were used in this study. Animals were obtained from the laboratory at Uludag University. Eight rats for the control group and eight in the DNOC treated group for each trial period were used. Experiments were conducted on a total of 128 rats. Four male and female rats were used at all investigated periods.

Control groups were treated with saline while experimental groups were injected intraperitoneally with 2.8 mg/kg dose of DNOC (1/10 LD_{50}) using 1 mL sterile injectors. DNOC is 90% pure and purchased from Aldrich. The rats were left without food and water for 24 hours before injection. Therefore, equal starting points in the metabolic profiles of the animals in the control and experimental groups were ensured. Following DNOC injection, food and water were regularly given to the animals until the trial was completed. Animals were sacrificed by cervical dislocation 0, 2, 4, 8, 16, 32, 64 and 72 hours after DNOC injection. Blood was obtained from the heart, and centrifuged in a Nüve NT 201 centrifuge at 12000 rpm for 15 min. Samples were used to determine enzymes activities. The biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase, cholesterol, high density lipoprotein (HDL), triglycerides and urea were determined using a Hitachi 911 autoanalyser with the aid of Hitachi kits.

Statistical analysis

Data were analyzed using SPSS 11.0 for Windows. Independent t-test was applied between control and experimental groups. The significance was

calculated using one-way analysis of variance (ANOVA) and Student's *t*-test. A value of $P<0.05$ was taken as statistically significant. The results were calculated as mean with standard error (±SE) values. The results are given in and figures.

RESULTS

The obtained results of plasma enzyme activities are shown in Figures 1-5.

DNOC treated male rats had higher serum ALT activities throughout the trial (Figure 1). Plasma AST activity showed no consistent pattern of change. However for both sexes is evident that highest values in treated animals were reported for the period between 32 and 64 hours after DNOC treatment (Figure 2).

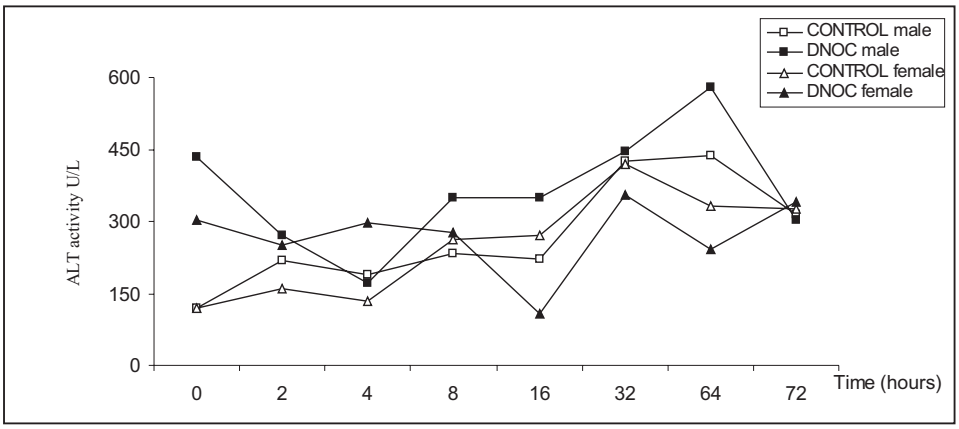

Figure 1. Changes in serum ALT activity in DNOC-treated animals with respect to time and sex

Changes in serum ALP activities were studied for the same time period as the changes of serum transaminases' (ALT and AST) activities, i.e. 72 hours. During this period is evident that DNOC treated male rats had higher serum ALP activities when compared with female treated rats. The highest values for both sexes were reported 32 hours after DNOC application (Figure 3).

Serum LDH activity increased in all treated groups (Figure 4). Highest serum LDH activity was obtained 72 hours after treatment, and was mainly (except at 32 hours) higher in males.

Studies of changes in serum amylase activity (Figure 5) have shown a clearly distinctive pattern when compared to changes in serum AST, ALT, ALP and LDH. The most outstanding difference is reflected in the lower amylase activity in DNOC treated rats when compared to the untreated animals. However, there were no differences in the enzyme activity between treated males and females.

Changes in the serum lipid profile (cholesterol, HDL and triglycerides) are described in Figures 6 to 8.

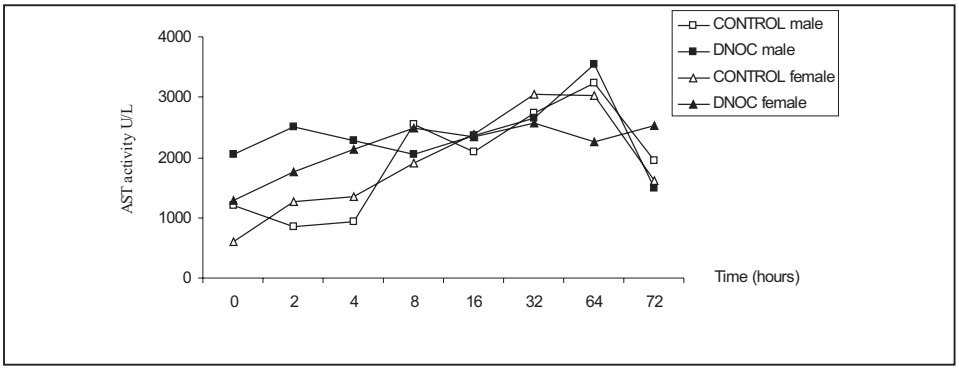

Figure 2. Changes in serum AST activity in DNOC-treated animals with respect to time and sex

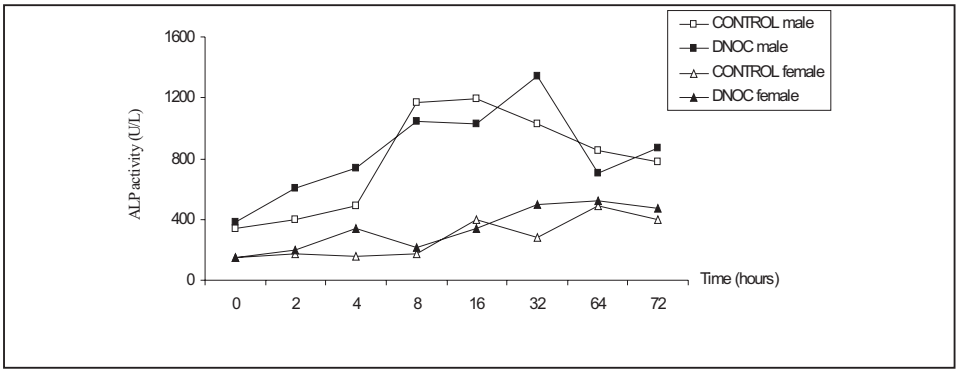

Figure 3. Changes in serum ALP activity in DNOC-treated animals with respect to time and sex

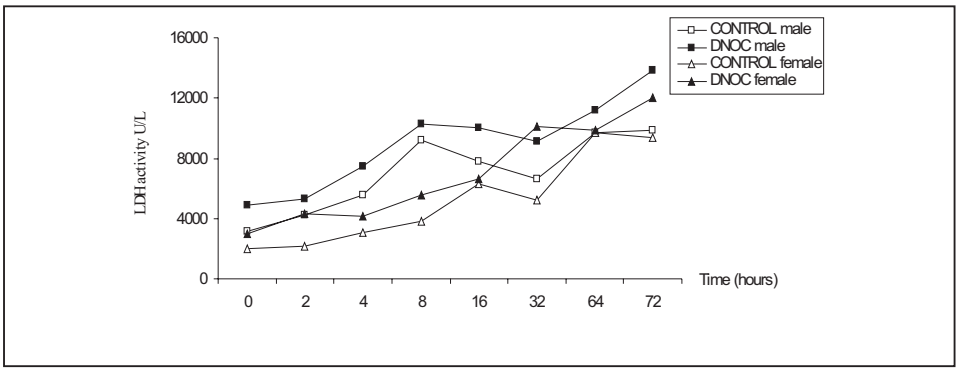

Figure 4. Changes in serum LDH activity in DNOC-treated animals with respect to time and sex

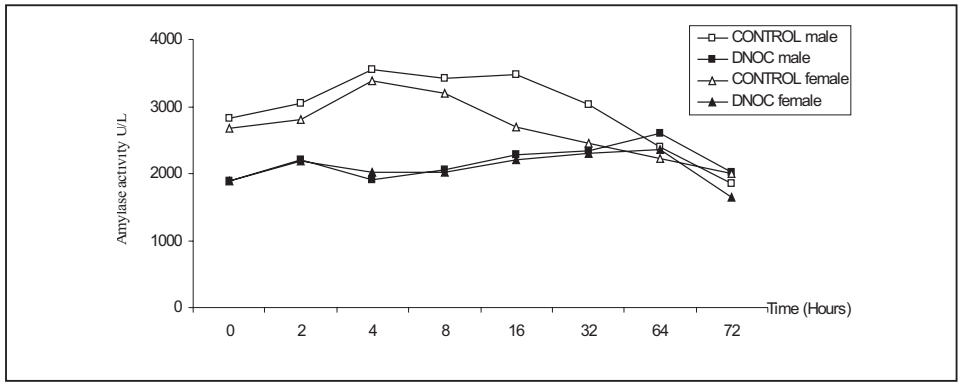

Figure 5. Changes in serum amylase activity in DNOC-treated animals with respect to time and sex

Higher cholesterol values (Figure 6) are reported only during the first hours after DNOC treatment. Thereof, these values plateau at a common value and there is no difference between treatments or sex. A very similar pattern is maintained for the changes in HDL levels (Figure 7). Serum triglycerides levels show an increase in treated rats in the first hours after DNOC application. At the end of the trial equal values were obtained for the treated and untreated male rats, while at this time the untreated females has slightly lower plasma triglyceride levels (Figure 8).

Serum urea levels (Figure 9) increased in both sexes during the first 10 hours after DNOC application. After this period the values maintained a steady level with decreasing differences between treated and untreated groups.

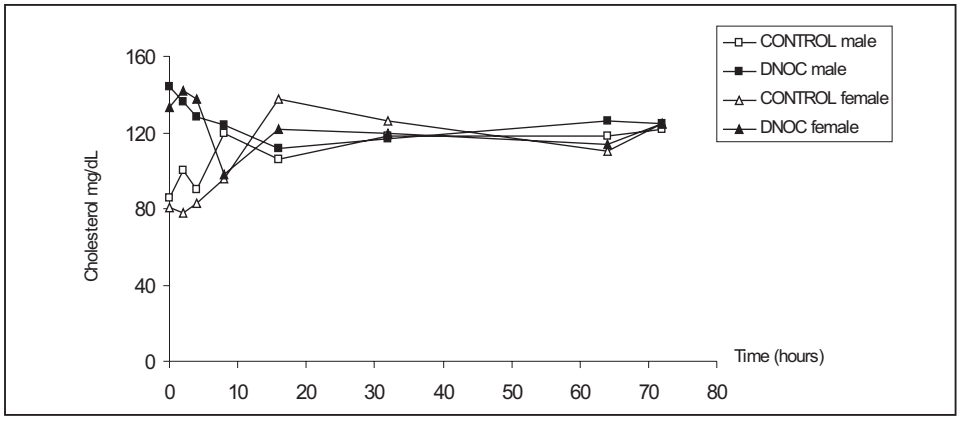

Figure 6. Changes in cholesterol levels in serum of DNOC-treated animals with respect to time and sex

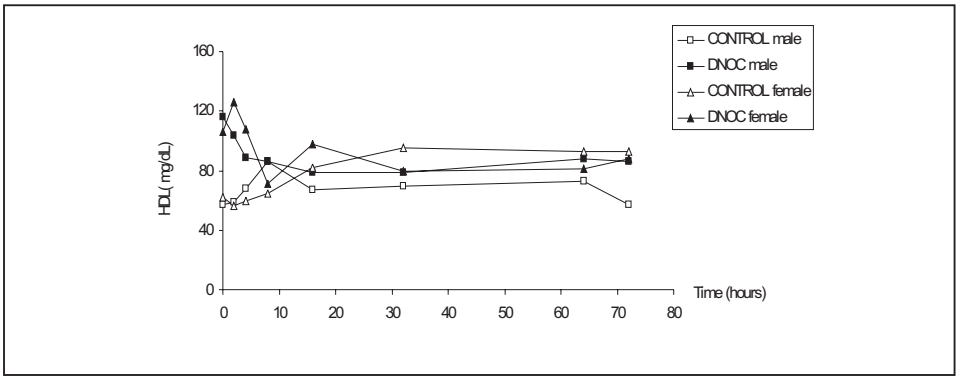

Figure 7. Changes in HDL levels in serum of DNOC-treated animals with respect to time and sex

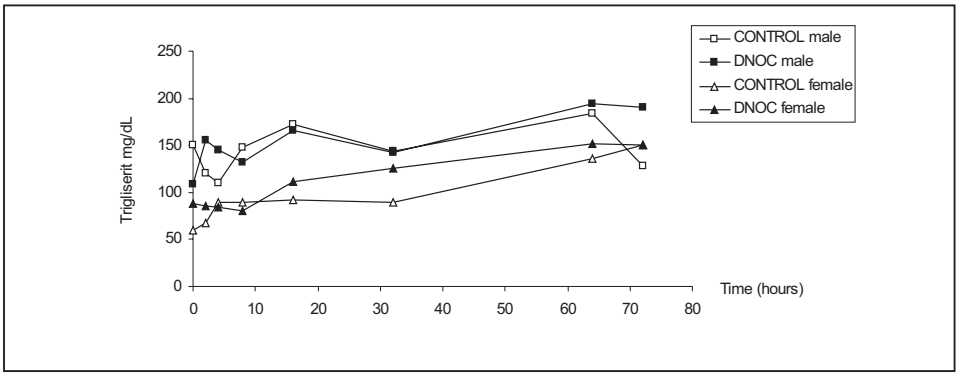

Figure 8. Changes in triglyceride levels in serum of DNOC-treated animals with respect to time and sex

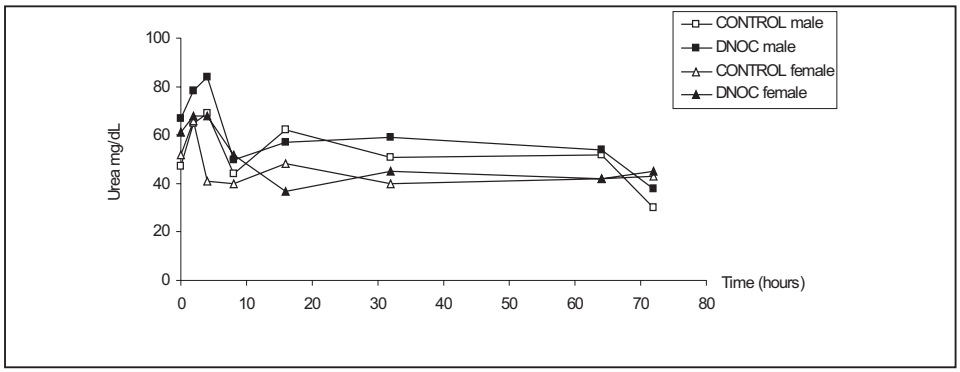

Figure 9. Change in Urea level in serum of DNOC-treated group animals with respect to different time and sex

DISCUSSION

Acute pesticide poisoning is one of the most common reasons of fatality. It is known that, nearly 3 million people seek medical assistance because of acute pesticide poisoning. Nearly 7% of these poisonings result with fatality. 95% of the pesticide poisonings are observed in developing countries. The causes of pesticide poisonings are mostly insecticides, herbicides, and rodenticides. The fatalities caused by pesticides are often due to suicidal intentions.

According to our studies the liver enzymes activities like ALT, AST, LDH and ALP increased in the observed sera. The one reason for these increases may be cellular tissue damage. According to WHO (2000) DNOC causes cellular damage in hepatocytes. When the cellular membrane is damaged, the content of the cell enters the blood circulation. After the irreversible ischemic damages, the main molecules that enter the blood are; aldolase, myokinase, CK, ALT, AST and LDH (Chapman *et al*., 1996). In another study, benzene (which has similar chemical properties to DNOC) given intraperitoneally caused a decrease in the activities of LDH, PK, ALT and AST in liver tissue (Dere *et al*., 2003). These decreases may occur as enzymes enter the serum after cell damage. In a study done with Glyphosate-Biocarb, a pesticide, an increase in the activities of these enzymes is reported (Benedetti *et al*., 2004). In another study, it is seen that paraquat used as a herbicide, causes increases and decreases of CK, ALT, AST, LDH and GGT activities in liver, kidney and lung of mice (Dere and Polat, 2001). These results support our study.

We saw that ALP activity generally increases in the treated group. The enzyme activity in males is higher than in the females. Rahman and Siddiqui (2003) reports are similar to our results on ALP activity (Rahman *et al*., 2003).

In our study, we observed that there are increases and decreases of enzymes activities in both male and female rats. Differences may occur by means of sex hormones being affected by DNOC. We observed that enzyme activities of female rats were lower than male rats. Gordon *et al*. (1996) showed that female rats were generally more sensitive than males to chlorpyrifos, which is a commonly used organophosphate pesticide. The differences in sensitivity to chlorpyrifos between male and female rats may be attributed to testicular function (Gordon *et al*., 1996). In another study, it was observed that serum and intratesticular levels of testosterone were significantly reduced by atrazine in male rats (Friedmann, 2006). In addition DNOC affects the germ cells of males and causes reproductive toxicity (Ken *et al*., 2003). Dere *et. al*. (2003) showed that benzene decreased esteradiol activity in females.

Enzymes activities results show that DNOC may cause acute pesticide poisoning and coronar instability. It is shown that DNOC inhibits the electron transfer by damaging mitochondrial oxidative phosphorylation, and as a result of this it moderates the synthesis of ATP by decreasing oxidative phosphorylation (Castýlho *et al*., 1997). The generated energy turns into heat due to high usage of oxygen in DNOC, which is toxic for cells (WHO, 2000). As a result of these effects, activities of enzymes like ALT, AST and LDH may increase.

The most important toxic effect of DNOC is on energy metabolism. This effect comes into action by separating oxidation from phosphorylation in the respiratory chain. When the phosphorylation of ADP is inhibited, electron transfer is affected as well. Energy can not be stored as ADP for a long time so it should be converted into ATP. When this conversion is inhibited by DNOC, ATP deficiency in crucial organs, such as the heart and muscle, may lead to paralysis, early *rigor mortis* and even death at high dosages (WHO, 2000). In a study done by Ozdikicioglu and Dere (2004) it was shown that as a toxic agent benzene affects carbohydrate metabolism, causing significant increases and decreases in glycogen content in liver and muscles. DNOC may also affect carbohydrate metabolism by alteratering enzymes activities, thus these effects of DNOC may cause cellular damage in the liver. Enzyme activity changes which shown in our study may be related with these damages.

In our study a significant inhibition of amylase activity of both males and females is observed. These results support in one respect that DNOC causes liver damage. Amylase activity decreases especially in liver diseases and infections (Chun and McGee, 2004). In another study, when DNOC 1/3 LD_{50} is given to guinea pigs intraperitoneally for 30 days, 6 times in a week, it is seen that there is an important increase in levels of amino sugars and sialic acid in liver and serum. As a result, it was suggested that the stability of the lisosomal membrane is damaged and glycolysis speeds up (WHO, 2000).

One of the reasons of these increases and decreases of the activities of enzymes may be the inhibition of protein synthesis by binding DNOC to DNA and RNA, or causing damage in these molecules indirectly. In a study performed by Hrelia *et al*. (1994) it was shown that DNOC, or its metabolites, cause chromosome damages and mutations (Hrelia *et al*., 1994). In addition to this it is suggested that DNOC inhibits mitosis, slows down and delays mitosis, and causes different cell damages (Parent-Massin and Thouvenot, 1993).

In our study, an increase of cholesterol and HDL at the start of the trial was observed in both sexes. The reason of these increases may be the deceleration of lipogenesis. Gasiewicz (1991) suggests that degradation of fatty acids and glycogenolysis was accelerated, but lipogenesis was inhibited by the effect of DNOC. This result supports our opinion.

Among the biochemical parameters we studied, there are increases and decreases, one of the reasons of these increases and decreases may be the effect of DNOC on organelles. Its effect on organelles may cause changes in the activities of enzymes and biochemical parameters, indirectly. In a study, it is suggested that DNOC damages the stability of the lysosome membrane (WHO, 2000). However, the hepatotoxic effects of diazinon, an insecticide, on biochemical indices in serum (ALP, ALT, AST, total cholesterol, triglyceride and VLDL) and liver ultrastructural changes were investigated by Kalender *et al*. (2005). It was explained that in their electron microscopic investigations, swelling of mitochondria and breaking up of the mitochondrial cristae of hepatocytes in diazinon-treated groups were observed. In another study, Vicente *et al*. (1998) showed that liver mitochondria of rats are much more sensitive than the

mitochondria of plants. All these studies show that DNOC affects enzyme activityes.

We described urea level increases until 16 hours and decreases after 32 hours in both males and females. Fujitani *et al.* (1993) showed that serum urea levels increased in rats treated with piperonyl butoxide (insecticide). In one respect, this study seems to support our findings. Gulec *et al*. (2006), shows that serum levels increase in plasma BUN, creatinine levels and urine total protein levels, leading to acute renal failure in rats treated with Cisplatin.

Our results suggest that DNOC causes cellular damage at vital organs such as liver, kidneys, and lungs, thus inducing changes in the physiological and metabolic activities of the individual. Cellular damages in vital organs cause enzymes activity and biochemical parameters in serum. All metabolisms and organisms may be affected by alteration in serum such as ALT, AST, LDH, ALP, amylase, cholesterol, HDL, triglyceride, urea.

ACKNOWLEDGEMENT

We are thankful to unit of Scientific Research Project Uluda| University (project no: 2003/65) for financial support and also to the staff of the Uluda| University, Faculty of Medicine, Department of Pharmacology and Sami AYDIN for their help and cooperation.

Adress for correspondence:
Egemen DERE,
Uludag University, Faculty of Science and Art,
Department of Biology, Bursa
Turkey
E mail: edere@uludag.edu.tr

REFERENCES

1. *Asztalos BJ, Nemcsok I, Benedeczky G R, Szabo A, Refaie OJ*, 1990, The effects of pesticides on some biochemical parameters of carp (*Cyprinus carpio* L.), *Arch Environ Contam Toxicol*, 19, 275-82.
2. *Benedetti AL, Vituri, CL, Trentin AG, Domingues MA, Alvarez-Silva M*, 2004, The effects of sub-chronic exposure of wistar rats to the herbicide Glyphosate-Biocarb, *Toxicol Lett,* 153, 227-32.
3. *Castýlho RF, Vicente JAF, Kowaltowski AJ, Vercesý AE,* 1997, 4,6 Dinitro-o-Cresol uncouples oxidative phosphorylation and induces membrane permeability transition in rat liver mitochondria, *Int J Biochem Cell Biol*, 29, 1005-11.
4. *Chapman JF, Christenson RH, Silverman LM*, 1996, Cardiac and muscle disease. In : Kaplan, L.A, Pesce A.J, eds. Clinical Biochemistry, 3rd ed, USA, 593-612.
5. *Chun AA, McGee SR,* 2004, Beside diagnosis of coronory artery disease a systemic review, *Am J Med*, 117, 334-43.
6. *Dere E, Giborova S, Aydýn H,* 2003, The effect of benzene on the activity of some enzymes and hormones in different tissues of rats, *Acta Vet (Belgrade)*, 53, 1, 87-101.
7. *Dere E, Polat F*, 2001, The effect of paraquat on the activity of some enzymes in different tissues of mice (*Mus musculus* – swiss albino), *Tr J Biol*, 25, 323-32.
8. *Friedmann SA*, 2002, Atrazine inhibition of testosterone production in rat males following peripubertal exposure, *Repr Toxicol*, 16, 275-9.
9. *Fujitani T, Tada Y, Yoneyama M,* 1993, Hepatotoxicity of piperonyl butoxide in male F 344 rats, *Toxicol*, 84, 171-83.

10. Gasiewicz TA, 1991, Nitro compounds and related phenolic pesticides, 3rd edition In: Hayes WJ Jr, & Laws ER Jr ed. Handbook of Pesticide Toxicology, San Diego, CA Academic Press, 3, 1191–269.
11. *Gordon CJ, Grantham TA, Yang Y,* 1996, Hypothermia and delayed fever in the male and female rat exposed to chlorpyrifos, *Toxicol*, 118, 149-58.
12. *Grilli S, Ancora G, Raný P, Valentý A, Mazzullo M, Colaccý A,* 1991, *In vývo* unwýndýng fluorometrýc assay as evýdence of the damage induced by fenarýmol and DNOC in rat-liver DNA, *J Toxýcol Envýron Health*, 34, 485-94.
13. *Gulec MM, Iraz HR, Yýlmaz H, Ozyurt I, Temel A,* 2006, The effects of ginkgo biloba extract on tissue adenosine deaminase, xanthine oxidase, myeloperoxidase, malondialdehyde, and nitric oxide in cisplatin-induced nephrotoxicity. *Toxicol Ind Health,* 22, 125-30.
14. *Hrelia P, Vigagni F, Maffei F, Morotti M, Colacci A, Perocco P et al,* 1994, Genetic safety evaluation of pesticides in different short-term tests, *Mut Res*/*Gen Toxicol*, 321, 219-28.
15. *Kalender S, Ogutcu A, Uzunhisarcikli M, Açýkgöz F, Durak D, Ulusoy Y et al,* 2005, Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes, *Toxicol*, 211, 197-206.
16. *Ken LT, Hiroaki A, Kunio K, Shoji T*, 2003, Effects of dinoseb, 4,6-dinitro-*o*-cresol, and 2,4-dinitrophenol on rat Sertoli-germ cell co-cultures*, Repr Toxicol*, 17, 247-52.
17. *Luk'ianchuk VD,* 1983, Molecular mechanisms of interaction between blood serum albumin and dinitroorthocresol, *Vopr Med Khim*, 29, 8-11.
18. *Nemcsok J, Asztalos B, Vig E, Orban L,* 1987, The effect of an organophosphorus pesticide on the enzymes of carp (*Cyprinus carpio* L.), *Acta Biol Hung*, 38-1, 77-85.
19. OEHHA, 2003, Office of Environmental Health Hazard Assesment. No significant risk levels (NSRLS) for the proposition 65 carsinogen benzene, California Environmental Protection Agency.
20. *Ozdikicioglu F, Dere E,* 2004, Effects of benzene on glycogen levels of liver and muscle týssues and on blood glucose of rats, *Acta Vet (Belgrade)*, 54, 379-94.
21. *Parent-Massin D, Thouvenot D,* 1993, In vitro study of pesticide hematotoxicity in human and rat progenitors, *J Pharm Toxicol Meth,* 30, 203-07.
22. *Rahman MF, Siddiqui MKJ*, 2003, Biochemical effects of vepacide (from *Azadirachta indica*) on Wistar rats during subchronic exposure, *Ecotoxicol Envir Saf*, 59, 332-9.
23. U.S. Environmental Protection Agency, 2005, Health and Environmental Effects Profile for 4,6 Dinitro-o-Cresol (DNOC).
24. *Vicente JAF, Santos MS, Vercesi AE, Madeira VMC,* 1998, Comparative effects of the herbicide dinitro-o-cresol on mitochondrial bioenergetics, *Pest Sci*, 54, 43-51.
25. WHO, 2000, Dinitro-*ortho*-Cresol Environmental Health Criteria , 220, World Health Organization, Geneva.

HEPATOTOKSIČNOST DINITRO-O-KREZOLA KOD PACOVA

DERE E, OZDIKICIOGLU FERDA i TOSUNOGLU H

SADRŽAJ

U ovoj studiji su ispitivani hepatoksični efekti dinitro-O-krezola (DNOC) kod pacova određivanjem vrednosti nekih biohemijskih parametara kao indikatora oštećenja jetre.

Tokom ogleda je registrovano kontinuirano povećanje aktivnosti LDH dok je koncentracija uree bila povećana samo do 16. sata nakon trovanja. Aktivnost AST I ALT je bila povećana sve do 8. sata nakon tretmana a kasnije su registrovane nepravilne oscilacije ovih parametara. Kod ženki pacova se aktivnost ALT smanjivala dok je kod mužjaka pacova ona rasla. Smanjenje aktivnosti serumske amilaze registovano je kod životinja oba pola. U početnim satima ogleda dolazilo je do povećanja koncentracije holesterola I HDL, a nije bilo promena u koncentraciji triglicerida.