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THE EFFECTS OF PENTACHLORONITROBENZENE AND PENTACHLORONITROBENZENE +VITAMIN E ON SERUM TOTAL PROTEIN LEVEL AND TRANSAMINASE ACTIVITIES IN RATS

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S U M M A R Y

Pentachloronitrobenzene (PCNB) is a fungicide used for seed and soil treatment in agriculture. In this study, PCNB's effect on serum alanine (ALT) and aspartate (AST) transaminases activities and serum total protein levels of female rats were examined biochemically. Besides, whether vitamin E, a strong antioxidant, might have a protective effect or not on probable toxicity of PCNB in rats was also investigated. Based on these biochemical observations, it was concluded that vitamin E has a protective effect against PCNB toxicity.

Ö Z E T

Pentaklornitrobenzen (PCNB) ziraatte, toprak ve tohum uygulamalarında kullanılan bir fungusittir. Çalışmada, dişi sıçanların serum alanin (ALT) ve aspartat (AST) transaminaz aktiviteleri ve total protein miktarları üzerine PCNB'nin etkileri biokimyasal olarak incelendi. Ayrıca, kuvvetli bir antioksidan olan vitamin E'nin PCNB olası toksisitesine karşı koruyucu bir etkisinin olup olmadığı da araştırıldı. Elde edilen biokimyasal sonuçlara dayanılarak, PCNB toksisitesine karşı vitamin E'nin koruyucu etkisinin olduğu sonucuna varıldı.

Key words: Serum, pentachloronitrobenzene, total protein, serum transaminase

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INTRODUCTION

Pentachloronitrobenzene (PCNB) is a fungicide commonly used for root rotting and damping-off disease on a wide variety of crops including turf, bulbs, cauliflower, mushrooms and seedling (1,2). It is marketed under a variety of names such as PCNB, Botrilex®, Folosan®, Terraclor®, Brassico®, Tritisan®, Tilicarex® and Quintozene® (1). PCNB can be produced by chlorination of nitrobenzene or by nitration of pentachlorobenzene (3). PCNB and its technical impurities have been reported to be persistent in soil (4). Beck and Hansen have estimated that residues may be detected up to 3 years following treatment (5). PCNB is metabolized to pentachloroaniline (PCA) and pentachlorothianisole (PCTA) in the rat, dog and cow (6). Grain is a major component of various animal feeds. Some of the grains used in animals feeds may have residues of PCNB due to unintentional contamination (4). The residuses of this drug are contaminated to humans by plants through animal food chain (7,8). The humans are subjected to this pollution by food substances and in during seed and soil treatments (8). This kind of environmental pollution lead to free radical production which induce damages in the cell, when metabolized in the liver (9).

Antioxidants such as vitamin E, selenium, glutathione are used to prevent the formation of free radicals or to quench their cell damaging effects (10-12). Free radicals causing lipid peroxidation in cell membranes are hold by the antioxidant effect of vit.E. This property of vit. E, results in the protection of living organisms against disorders resulting from oxidant, as well as liver injury caused by carcinogenes (13, 14).

In this study, PCNB and PCNB+vitamin E effects on serum alanine and aspartate transaminases activities and serum total protein levels of female rats were examined biochemically.

RESULTS AND DISCUSSION

Table 1 presents the results for serum ALT, AST activities and total protein levels in the control and experimental groups.

According to Table 1, a significant difference in the ALT activities of 4 groups were observed (P_{ANOVA}=0.0001). A significant increase of serum ALT activities in the group administered PCNB was determined in comparison with the control group ($P_{t\text{-}test}$=0.0001), and when ALT activities of the group administered PCNB+vitamin E were compared with the group only PCNB, a statistically significant decrease were determined ($P_{t\text{-}test}$= 0.002). There was a significant increase in the serum ALT activities of control+vitamin E group as compared to control group ($P_{t\text{-}test}$ = 0.0001).

Table 1: Serum alanine (ALT) and aspartate (AST) activities and total protein levels for all groups*.

Groups	n	ALT (U/L)	$P_{t\text{-test}}$	AST (U/L)	$P_{t\text{-test}}$	Total Protein (g %)	$P_{t\text{-test}}$
Control	7	24.80 ± 0.57	0.0001	87.26 ± 1.45	0.0001	8.17 ± 0.68	0.009
Control + Vit.E	7	59.29 ± 1.10		96.45 ± 1.16		9.43 ±0.47	
PCNB	11	30.82 ± 0.87	0.002	101.47 ± 1.88	0.0001	7.62 ± 0.61	0.238
PCNB + Vit. E	10	28.72 ± 0.53		70.18 ± 1.87		7.93 ± 0.40	
PCNB	11	30.82 ± 0.87	0.0001	101.47 ± 1.88	0.0001	7.62 ± 0.61	0.151
Control	7	24.80 ± 0.57		87.26 ± 1.45		8.17 ± 0.68	
P_{ANOVA}		0.0001		0.0001		0.0001	

n= number of animals
* Mean ± SD

When AST values of rats administered PCNB were compared with the values of the control group, a statistically significant increase was determined ($P_{t\text{-test}}$=0.0001). After the administration of PCNB +vitamin E a decrease in AST values in comparison to the group that was administered only PCNB was observed, a statistically significant decrease was determined ($P_{t\text{-test}}$ =0.001). There was a significant increase in the serum AST level of control +vitamin E group as compared to control groups ($P_{t\text{-test}}$ =0.0001). A significant difference in the AST activities of 4 groups were observed (P_{ANOVA}=0.0001).

In this study, it was observed that an insignificant decrease in the serum total protein levels of the group administered PCNB, in comparison with the control group ($P_{t\text{-test}}$= 0.151) and an insignificant increase in the group administered PCNB+vitamin E in comparison with PCNB administered group occured ($P_{t\text{-test}}$ = 0.238). It was observed that a significant increase in the serum total protein levels of the group administered vitamin E in comparison with control groups ($P_{t\text{-test}}$= 0.009). There was a significant difference in serum total protein levels of 4 groups (P_{ANOVA} = 0.0001).

The liver is the most important organ in the metabolism of drugs and other toxic substances (15). Morphological and biochemical alterations that occur in the liver affect many metabolic process in the metabolism. Acute and chronic administration of toxic substances to rats and other animals could lead to a variety of pathological changes and

ultrastructural abnormalities in liver (16). Liver cell destruction shows its effects by an impairment in liver cell membrane permeability and a result release of some enzymes into blood plasma and increase in their activities (17). The increase in ALT and AST activities in serum is an indicator of liver destruction and is directly proportional to the degree of cellular damage. Vitamin E, which is a strong antioxidant is known to hold free radicals causing lipid peroxidation and to be effective against various toxic materials including CCl_4 hepatotoxicity (18). However, this preventive effect is probably dependent upon the dosage of the toxic material, the amount and the duration of administration of vitamin E. In our study, the administration of vit. E, although increasing serum transaminase activities in controls, significantly decreased serum ALT and AST in rats given PCNB (Table 1). This finding is in accordance with our previous studies, in which it was observed that vitamin E had a protective effect against coumarin, propanil and pentachloronitrobenzene hepatotoxicity (7,9,19).

Several researches point out that race, age, environment, physiological condition and encountering any kind of antigen, influence the level of total protein in serum (20). There must be a statistically significant change in the amounts of total protein in serum in cases of cellular damage. After injection of pentachloronitrobenzene, the average serum total protein of rat in the drug treatment group decreased insignificantly, 6.73 % compared to the control group. Total protein levels are likely to be decreased if there is inhibition of protein synthesis or if degradation of protein is promoted (21). In our study, total protein levels increased by administration of vitamin E. Determination of decrease in the amount of serum ALT and AST and increase in the amount of serum total protein by the treatment of vitamin E, shows reduction in liver damage.

As a result of all these biochemical findings, it is concluded that vitamin E has a protective effect against PCNB toxicity.

EXPERIMENTAL

Animal groups

35 female 4.5-5 months old Wistar albino rats [Istanbul University Centre for Experimental Medical Research and Application (DETAM)] were used in this study. Their diet consisted of standart animal pellet food and tap water *ad libitum*. The animals were separated into four groups, as follows. The first group (7 rats) to which 2 mL corn oil was injected subcutaneously, as a single dose was defined as the control group. 7 rats were injected 300 mg vitamin E, dissolved in 2 mL corn oil (control +vitamin E group). 11 rats were injected 400 mg /kg PCNB, dissolved in 2 mL corn oil (PCNB group). 10 rats were injected 300 mg of vitamin E dissolved in 1 mL of corn oil, one hour after the

injection of 400 mg/kg PCNB dissolved in 1 mL corn oil (PCNB+vitamin E group). The animals were starved overnight, were dissected under ether anesthesia 24 hours after the injection and blood samples were taken for biochemical investigation.

Biochemical assays

Serum ALT and AST activities were measured according to Reitman and Frankel (22). Serum total protein levels were determined by the Lowry method (23).

Statistical analysis

The results were evaluated using an unpaired T-test and ANOVA variance analysis using the NCSS statistical computer package (24).

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