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Acute Effects of Trichloroacetic acid on Serum Enzyme Levels and Erythrocyte Carbonic Anhydrase Activity in Rats

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Abstract: The aim of the present study was to investigate the effects of a sublethal dose of trichloroacetic acid (TCA) on serum enzyme levels and erythrocyte carbonic anhydrase (CA) activity in rats under laboratory conditions. Ten Sprague-Dawley albino rats were divided in 2 groups of 5 rats. A 200 mg/kg dose of TCA was administered intraperitoneally to the 5 rats in the treatment group. An equal amount of saline solution was injected into the control group.

Serum enzyme levels and erythrocyte CA activity of the rats were measured 1, 3, and 6 h after treatment. According to the results, while the TCA sublethal dose treatment caused significant increases in lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) after 1 and 3 h, aspartate aminotransferase (AST) significantly increased after 1, 3, and 6 h. No significant differences in the levels of alanine aminotransferase (ALT) and erythrocyte CA were observed in comparison to the controls. It was concluded that TCA may cause toxicity in rats.

Key Words: Trichloroacetic acid, Serum enzymes, Erythrocyte Carbonic anhydrase, Rats

Trikloroasetik asitin Akut Uygulamasının Sıçanların Serum Enzim Düzeyleri ve Eritrosit Karbonik Anhidraz Aktivitesi Üzerine Etkileri

Özet: Trikloroasetik asit (TCA) subletal dozda laboratuar şartlarında sıçanların serum enzim düzeyleri ve eritrosit karbonik anhidraz (CA) aktivitesi üzerine etkileri araştırıldı. 10 adet Sprague-Dawley albino iki gruba ayrıldı. Her grup için beş adet sıçan kullanıldı. Muamele grubuna 200 mg/ kg doz TCA periton altına (intraperitoneal) verildi. Kontrol grubuna ise aynı hacimde TCA'nın çözüldüğü % 0,9 luk fizyolojik su verildi.

Serum enzim düzeyleri ve eritrosit CA aktivitesi muameleden sonra 1, 3 ve 6 cı saatlerde belirlendi. Sonuçlara göre; TCA'nın akut subletal doz uygulaması laktat dehidrogenaz (LDH), kreatine posfokinaz (CPK) birinci ve üçüncü saat periyotlarında önemli düzeyde artışına neden olurken, aspartat aminotransferaz (AST) birinci, üçüncü ve altıcı saatlerde arttı. Alanin aminotransferaz (ALT) enzim düzeyi ile CA aktivitesi hiçbir periyotta kontrol grubuna göre önemli artış gözlenmedi. TCA sub letal dozda bile akut olarak sıçanlar üzerinde toksik etkisi olabileceği sonucuna varıldı.

Anahtar Sözcükler: Trikloroasetik asit, Serum enzimleri, Eritrosit karbonik anhidraz, Sıçan

Introduction

Environmental pollution caused by pesticide residues is a major concern due to their extensive use in agriculture and in public health programs (1). The environmental impact of pesticide use is related to several fundamental properties essential to their effectiveness as pesticides. Firstly, pesticides are toxicants, capable of affecting all taxonomic groups of biota, including non-target organisms, to varying degrees depending on physiological and ecological factors. Secondly, many pesticides need to be resistant to environmental degradation so that they persist in treated areas, thus enhancing their effectiveness. This property also results in long-term effects in the natural ecosystem (2). Since pesticides are recommended for plant protection, there has been an improvement in the control of pest populations and the spread of infection-born disease vectors. Public health programs in many developing countries, including Turkey, also utilize pesticides to control disease-transmitting organisms (3). There is

abundant evidence that many pesticides produce their acute toxic action by activating or inhibiting enzymes. In addition, chemicals, via the food chain, have harmed physiological mechanisms in humans. Additionally, many chemicals, even at relatively low dosages, disturb the metabolism of biota by altering normal enzyme activity (3-6). A considerable literature exists describing the effects of pesticides on populations and communities of organisms under field conditions. Major effects of pesticides on animal and insect populations result primarily in significant changes in species abundance and associated shifts in dynamics; thus they have resulted in an imbalance in the natural system. (7).

The effects of TCA in various vertebrates have already been investigated in several studies (8-12), but results concerning vertebrates are both very limited and conflict with each other. In the literature, it is reported that TCA causes changes in biochemical parameters of male rats following 7 days of drinking water exposure (8). Poon et al. (9) performed gross and microscopic examinations, serum chemistry, hematology, biochemical analysis, neurogenic amine analysis and serum TCA analysis at the end of the treatment period. Mather et al. (10) reported that TCA and DCA (dichloroacetic acid) produced substantial systemic organ toxicity to the liver and kidney during a 90-day sub-chronic exposure. Acharya et al. (11) showed that the interaction of TBA (tertiary butyl alcohol) and TCA did bring about an alteration in the biochemical parameters, which may play a pivotal role in toxic responses to long-term exposure. Bryant et al. (12) observed that monochloroacetic acid (MCAA), which is a derivative of TCA, caused an increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Despite the above reports, there is still great contradiction between results. Therefore, the purpose of this study was to assess the predictive toxic affects of TCA on warm-blooded animals. In order to evaluate the interactive toxicity of TCA, young male Sprague-Dawley albino rats were exposed to a dose injected intraperitoneally and then monitored for a period of 6 h. For this aim, TCA was injected intraperitoneally into rats because of the effect of chemicals represents a well characterized in vivo toxicity model system.

Material and Methods

Materials

The chemicals in the present study were obtained from the indicated sources: sodium carbonate, sodium bicarbonate, acetone, p-nitro phenyl acetate, sodium citrate dehydrate, NaCl, citric acid, and TCA were obtained from E. Merck.

Animals: Ten male rats (Sprague-Dawley albino), 8 weeks old and weighing 150-200 g were provided by the Medical School of Yüzüncü Yıl University, and were separated into 2 groups of 5 rats. All animals were group-fed a wheat-soybean-meal-based diet and water ad libitum in stainless cages, and received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institutes of Health (13). The animals were housed at 20 ± 2 °C and under a daily light/dark cycle.

Methods

Treatment of Rats: A 200-mg/kg dose of TCA was injected intraperitoneally into the 5 rats in the control group. The control rats were given only physiological saline.

Blood samples were obtained 1, 3, and 6 h after the treatment. The blood samples were obtained from tail arterial blood-vessel using a syringe. Blood samples were immediately put into disposable silicone coated glass tubes. The serum samples were obtained by centrifuging the blood samples at 3000 rpm for 15 min at 4 ºC, and enzyme levels were measured in these serum samples.

Preparation of hemolysates containing erythrocyte CA activity rat red blood cells: Rat blood samples were obtained using bottles with anticoagulator (ACD: acid citrate-dextrose). Blood samples were centrifuged at 1500 rpm for 20 min and the plasma was removed. After the packed red cells were washed 3 times with NaCl (0.9%), the erythrocytes were hemolysated with cold water. The cell membranes were removed by centrifugation at 14000 rpm (20000 xg) at

4 ºC for 30 min (14). The hemolysate was used for the determination of the activity of erythrocyte CA using the hydratase method (15) and hemoglobin (Hgb) was measured with a hematological assay apparatus (Coulter MAXM).

Measurement of Enzyme Levels: Serum enzyme levels were measured with an autoanalyzer (BM/HITACHI-911), using the enzyme kits.

Analysis of Data: All data were expressed as mean ± standard deviation (SD). For statistical analysis, the SPSS/PC+ package (SPSS/PC+, Chicago, IL, USA) was used. For all parameters, mean and SD were calculated according to the standard methods. Mann-Whitney U-test for differences between means of the treatment and the control rats was employed. The significance level was accepted as P = 0.05 for all tests.

Results

The treatment of rats with TCA produced change in the levels of some serum enzymes and erythrocyte CA (Table and Figures 1, 2, and 3). To determine the significance of increase or decrease in serum enzymes and erythrocyte CA following exposure to a 200-mg/kg dose of TCA, the data obtained were subjected to the Mann-Whitney-U test. While lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) both increased 1 and 3 h after TCA treatment, AST increased 1, 3, and 6 h after the treatment. As for the level of ALT and erythrocyte CA, no significant differences existed between the treatment and controls groups at 1, 3, or 6 h post-treatment.

Discussion

Pesticides are commonly used agricultural chemicals. The effects of pollutants on nature became a subject of interest for scientists beginning in the second half of the 20^{th} century, and, subsequently, investigations of the effects of these pollutants on human beings, plants, and animals were initiated. In our study, TCA was preferred because the information on its toxicological and biological effects on higher animals in vivo is very limited, and TCA and its metabolites also are found in wide variety of biologically active compounds.

The Table shows the changes in the level of AST, ALT, LDH, and CPK in serum, and the activity of erythrocyte CA. We observed that TCA caused significant increases in LDH and CPK 1 and 3 h after treatment, and AST increased 1, 3, and 6 h after treatment. No significant differences in the levels of ALT and erythrocyte CA were observed between the treatment and control groups. It was previously stated that TCA and its derivates have toxic effects on rats, altering some biochemical and physiochemical parameters (10-12).

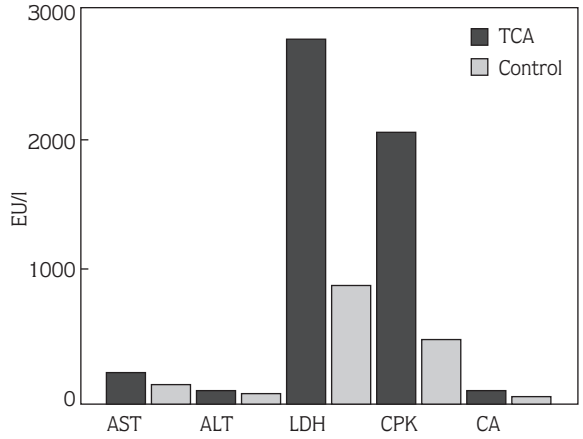
The present study revealed that TCA altered serum enzyme levels to a greater degree than erythrocyte CA activity. Thus, any external stressor, such as TCA, even at a sublethal dose can have a toxicological effect on the liver and other tissues. This is evidenced from our observation that, in vivo, a sublethal TCA dose increased the level of

Table. Effects of a sublethal dose of TCA on serum enzyme levels and carbonic anhydrase activity in rats.

ENZYMES	CONTROL (n = 5) X ± SD	TCA (n = 5) First hour X ± SD	TCA (n = 5) Third hour X ± SD	TCA (n = 5) Sixth hour X ± SD
AST (EU/l)	144.20 ± 18.73	224.80 ± 29.58*	232.80 ± 24.73*	272.40 ± 102.92*
ALT (EU/l)	64.20 ± 4.32	82.20 ± 35.14	71.60 ± 5.86	63.60 ± 0.55
LDH (EU/l)	879.40 ± 41.79	2766.80 ± 901.57*	4373.40 ± 220.46*	1260.60 ± 680.82
CPK (EU/l)	487.00 ± 214.30	2039.20 ± 266.21*	2954.40 ± 1151.23*	556.00 ± 109.22
CA (EU/mgHgb-1)	58.60 ± 29.64	79.80 ± 42.04	79.00 ± 44.16	63.20 ± 35.83

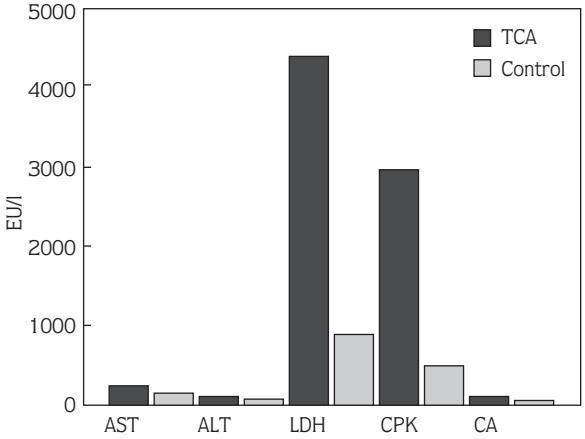
Each value represents the mean ± SD.

* Significantly different from control rats at P ≤ 0.05 (Mann-Whitney U-test).



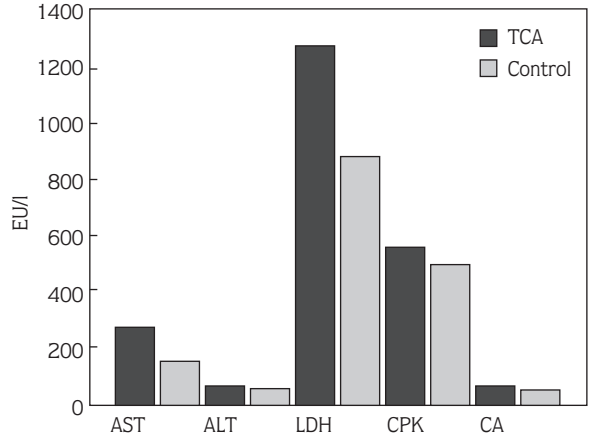
The levels of enzymes in rat serum and erythrocyte carbonic anhydrase 1 h post-treatment

Figure 1. Effects of a sublethal dose of TCA on serum enzyme levels and carbonic anhydrase activity in rats.



The levels of enzymes in rat serum and erythrocyte carbonic anhydrase 3 h post-treatment

Figure 2. Effects of a sublethal dose of TCA on serum enzyme levels and carbonic anhydrase activity in rats.



The levels of enzymes in rat serum and erythrocyte carbonic anhydrase 6 h post-treatment

Figure 3. Effects of a sublethal dose of TCA on serum enzyme levels and carbonic anhydrase activity in rats.

LDH, CPK, and AST, which all correlate to liver function and muscle lesions, including myocardial. As known, transaminases are intracellular enzymes, which exist in only small amounts in serum; therefore, damage to liver cells may result in leakage of the enzymes into plasma due to a large concentration gradient (16). The reasons for such an effect of TCA are not understood at the present, but it is conceivable that TCA, as a toxicological agent like other pesticides, might interact primarily with liver tissue cell membranes, resulting in structural damage and changes in metabolism of the constituents.

To date, no study examining the acute toxicity affect of TCA in vivo on rat serum enzymes has been performed, as in the present study. Therefore, we were unable to compare our findings. According to the present research, our findings are consistent with those of a previous (11) study that investigated the effect of orally consumed TCA on some serum enzymes. In addition, because of the high variability in analyzing enzyme-chemical interactions in vitro or in vivo, and inconsistent factors like treatment protocol, purity, and species tissue differences, it is difficult to compare data from different laboratories regarding the ranking of test chemicals for toxicological effect. Nonetheless, our data will provide a basis for further research, which would seek to understand the action of the in vivo damage to animal serum enzymes and erythrocyte CA. It is also postulated that liver and muscle damage indicator enzymes might be markers of choice for monitoring bio-toxicity of direct-acting compounds such as TCA.

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