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Effects of Chlorsulfuron and Feed Containing Ascorbic Acid on Some Serum Parameters in Albino Rabbits

[1]Ahmet Uzatici, [2]Baver Coşkun, [2]Kemal Çelik and [3]Can Duman
[1]Department of Biga Vocational High School,
[2]Department of Animal Science,
[3]Department of Biochemistry, Faculty of Medicine Biochemistry, University of Çanakkale OnSekiz Mart, Çanakkale, Turkey

Corresponding Author: Ahmet Uzatici, Department of Biga Vocational High School,University of Çanakkale OnSekiz Mart, Çanakkale, Turkey

ABSTRACT

As all around the world, pesticides are used in our country to control the harmful organisms. As is known, however, use of pesticides takes a toll on humans, animals and the environment health-wise and insensible and intensive use causes residues in food, soil, water and air. Even though the use of pesticides creates an increase in quantity product-wise, they are potential toxic materials for humans and animals. In this study, New Zealand albino rabbits (75 days old, 2 kg average live weight) were given chlorsulfuron and vitamin C through feed for 45 days, their liver enzyme activities were monitored and it was checked whether or not the liver was damaged. In the research, levels of GGT and ALK.P in the serum samples received from trial group animals were observed to be sensible in comparison to the control group ($p<0.05$).

Key words: Herbicide, ascorbic acid, liver enzyme

INTRODUCTION

Pesticides are the most commonly used chemicals among the different agricultural combat methods against diseases and weeds. Generally speaking, in cases where pesticides are not used in the agricultural combat, losses due to weeds are estimated to be around 30-40% and quality issues arise. Therefore, the use of these chemicals to control harmful organisms is inevitable in our country just like it is all around the world. As is known, however, use of pesticides takes a toll on humans, animals and the environment health-wise and insensible and intensive use causes residues in food, soil, water and air. Insensibly used herbicides pose a significant health risk for living beings. Even though the use of pesticides creates an increase in quantity product-wise, they are potential toxic materials for humans and animals. Now-a-days residue issues might arise in almost every kind of agricultural product and residue level depends largely on the time of application. However, there are many factors that determine the amount of residue. In this regard, the highest chemical residue levels (MRL) in agricultural products are tested. MRL defines the officially accepted and allowed maximum concentration of a pesticide in foods, processed products or animal feeds. MRL is the definition of residue amount by milligram that is in 1 kg of food, processed product or animal feed. This is defined as ppm (part in million). In addition to MRL's individual determination by countries, it is also internationally put forth by the European Union (EU), World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO). In toxicity studies about these products, studies are carried out basing on the

assumption that humans are ten times more sensitive than the experimental animals involved in the toxicity tests. In order to make this possible and to determine the maximum exposure level allowed for humans, a 100 time more safety factor is applied to the lowest NOAEL (the safest maximum dosage level that does not cause a negative effect on living beings) value determined by trials. This determined value is defined as the dose that can be taken daily throughout life. A pesticide can be a biological agent like a chemical substance, a virus and a bacterium. Since most chemical pesticides cannot selectively affect the target organism, they cause various diseases in organisms other than the target one and they can even be deadly. Pesticides have been reported to cause intoxications and deaths in many countries. It is reported that every year 3.000.000 severe intoxication and 220.000 death cases are observed in the world due to pesticides (WHO, 1997). Today insecticides which are organic phosphate-involving pesticides that cause significant environmental pollution, are unfortunately not used sensibly enough in Anatolia. Pesticides penetrate human food chain as a result of plants' taking in the pesticide left in the soil directly or indirectly and these plants' being used as human food or animal feed. Therefore, they cause various problems. Residues of organic phosphate-involving pesticides have been detected in soil, water creatures, vegetables, seeds and various food products (John *et al*., 2001). Statistics show that world's pesticide production is 3 million tons and the annual turnover ranges between 25-30 billion dollars. An annual 1% tonnage-wise increase in world pesticide market is expected (Tiryaki *et al*., 2010). Annual pesticide consumption in Turkey is approximately 33.000 ton. This amount consists of 47% insecticides, 24% herbicides, 16% fungicides and 13% other groups. The approximate annual turnover of these pesticides is 230-250 million dollars (Tiryaki *et al*., 2010). On the other hand, there are 4100 authorised plant protection products in our country as from the end of year 2008. The number of authorised effective substances in our country is 418. However, pesticide consumption in Turkey is low in comparison to that in EU countries. Holland and Greece are the most; Belgium and Finland are the least pesticide-consuming countries in the EU (Durmusoglu *et al*., 2008). Turkey's consumption per hectare ranges between 400-700 grams depending on the year. While the effective substance amount per hectare was 400-500 grams in the 1990s (Tiryaki *et al*., 2010), it reached up to 705 grams in 2006 (Durmusoglu *et al*., 2008). These numbers show that Turkey consumes less pesticide when compared to EU countries. As it is known, however, there is a very heterogenic pesticide consumption in Turkey. In 1998, country's consumption was as follows: 42.02% in the Mediterranean and Aegean regions and 4.8% in Eastern Anatolia region. Furthermore, even though less pesticide is consumed in Turkey per hectare, most commonly consumed pesticides are the ones that pose a significant risk for the environment and health (Durmusoglu *et al*., 2008). Judging by this fact, to reduce the adverse effects of the pesticides is crucial. It is rather difficult to completely eliminate pesticide-related problems but in minimizing the harms of these toxic chemicals some vitamin supplements, one of the most substantial being vitamin C, are proven to be important. This vitamin is necessary for making collagen and this ligament protein is responsible for attaching cells and tissues. Thus, vein membranes become stronger, the absorption of iron in small intestine is regulated and what is more; vitamin C is necessary for the transformation of pholasin into folinic acid and the synthesis of some hormones (thyroxin, adrenalin and steroid). Vitamin C which has a part in amino acid metabolism, is also necessary for the healing of damaged tissues. Humans and some living beings (e.g., guinea pigs) are significantly damaged by the lack of this vitamin. Generally in our regions

where pesticides are relatively intensely and insensibly used, organic phosphorous and carbamate-involving ones cause intoxications. These chemicals cause acetylcholine accumulation by preventing cholinesterase enzyme in the body. In accidental intoxications, diseases may occur due to residues of pesticides in leaves and soil or their contact with toxic transformation products. It is rare of organic chlorinated pesticides to acutely intoxicate people unless they are taken in large doses. These compounds rather cause chronic intoxications, affect nervous system and damage the liver (Wefers and Sies, 1988). Researches have shown that agricultural labourers, who are exposed to the chronic effect of pesticides, have liver, kidney and muscle deformities along with many genetic damages. When looked at the serum FSH, LH and testosterone indications on labourers exposed to methamidophos, it was observed that LH levels significantly increased and in parallel with increased pesticide effect FSH levels increased and testosterone levels decreased (Padungtod *et al*., 1998).

In this study, rabbits' liver enzymes were monitored with the presence of chlorsulfuron and vitamin C, some biochemical parameters of liver function were determined and it was researched whether or not there was damage organ-wise.

MATERIALS AND METHODS

The University of Çanakkale OnSekiz Mart , Ethics committee decision No: 2011/08-04.

The research is carried out by the Division of Poultry Researches of the Department of Zootechnics of the Faculty of Agriculture of Çanakkale Onsekiz Mart University. In the study, 33 New Zealand albino rabbits supplied from the Centre of Experimental Animals of Uludag University were used. Animals were 2.0 kg and 75 days old on average and they were kept in individual cages. Groups were randomly formed as Control (C), Trial 1 (T1) and Trial 2 (T2). During the research, rabbit feed properties of which are shown at chart 1 was used and 50 g of clover per animal were given daily as supplement. When preparing feeds that contain pesticide, powdered/granule mixed feed was prepared on a scale of 1/500 g. Later on, the feeds were prepared through daily mixer aimed at 500/5000 g. While the animals in T1 group were given feeds containing 1.5 g 10% chlorsulfuron (750 mg kg^{-1} live weight), animals in T2 group were given 1.5 g 10% chlorsulfuron (750 mg kg^{-1} live weight) and 1.5 g ascorbic acid was also given to them through drinking water as supplement. Individual blood samples were taken from every group and then these samples were quickly put into an ice box and taken to University hospital's central laboratory in order to be separated into serums. The blood was centrifuged at 2500 rpm for 10 min and turned into serum. In the serum, Aspartate Aminotransferase (AST), Alanine Aminotransaminase (ALT) and Alkaline Phosphatase (ALK.P) levels of liver functions were completed through Roche Cobas 501 automatic analyser system and AST, ALT, ALK.P and Gamma Glutamyl Transferase (GGT) enzyme levels were analysed via enzymatic measurement method.

RESULTS

The rabbits used in the study are 75 days old on average, their live weight is 2 kg and pesticide application period is 45 days. Results obtained from trial animals and control group are presented in Table 1-4. Statistics obtained from T1 group animals show a significant increase in serum GGT and ALK.P levels in comparison to control group ($p<0.05$).

Table 1: Serum alanine aminotransferase (ALT) observations means (X) its Standard Errors (SE) as per groups and p values

	Control (C)		T1		T2		
Observations	Means X	SE	Means (X)	SE	Means (X)	SE	p
Trial start	60.1	8.05	-	-	-	-	-
1	115.2	19.93	211.7	68.38	117.0	21.54	0.2089
2	165.2	18.07	136.8	13.52	149.8	16.32	0.4631
3	138.0	11.20	113.9	10.42	132.2	12.99	0.3029
4	138.2	22.31	154.0	25.88	136.3	18.04	0.8423

Table 2: Serum aspartate aminotransferase (AST) observations, means (X) its Standard Errors (SE) as per groups and p values

	Control (C)		T1		T2		
Observations	Means X	SE	Means (X)	SE	Means (X)	SE	p
Trial start	64.1	6.62	-	-	-	-	-
1	106.1	22.81	78.8	16.68	60.8	12.6	0.2132
2	134.4	24.20	103.7	21.46	93.4	10.63	0.3324
3	116.2	21.20	88.0	15.52	66.6	7.30	0.1582
4	115.1	38.06	127.2	22.22	110.4	16.40	0.9210

Table 3: Serum alkaline phosphatise (ALK.P) observations, means (X) its Standard Errors (SE) as per groups and p values

	Control (C)		T1		T2		
Observations	Means X	SE	Means (X)	SE	Means (X)	SE	p
Trial start	-	-	-	-	-	-	-
1	43.8[a]	15.56	109.4[b]	6.02	113.2[b]	8.95	<0.0001
2	111.4[a]	11.62	82.2[b]	9.35	88.3[b]	4.03	0.0747
3	93.0[a]	11.58	59.6[b]	7.24	63.4[b]	4.88	0.0268
4	48.2	6.71	54.2	7.76	45.8	4.80	0.6608

Table 4: Serum gamma glutamyl transpeptidase (GGT) observations, means (X) its Standard Errors (SE) as per groups and p values

	Control (C)		T1		T2		
Observations	Means X	SE	Means (X)	SE	Means (X)	SE	p
Trial start	40.2	5.77	-	-	-	-	-
1	78.0[a]	17.74	20.4[b]	6.70	33.4[b]	10.81	0.0078
2	10.5	1.02	15.0	3.06	9.8	0.86	0.1251
3	9.1	0.88	12.2	2.59	7.8	0.76	0.2015
4	8.1	1.24	10.1	1.71	7.04	1.55	0.4581

DISCUSSION

The biggest criterion for evaluating liver activities is liver enzymes. It can be found out whether or not the liver functions normally by looking at liver enzymes. The serum is commonly used especially clinically in order to test organ functions in mammals by monitoring enzyme activities and to detect functional damages if there are any. The mentioned tests today are often used by environment biology operatives especially in order to find out the effects of environmental pollutants on living beings. In general, rabbits are used as test animals in determining the toxicity of a pesticide. When it is mentioned about a pesticide's acute toxicity, the following are stated: The amount of pesticide during the first 24 h of its intake into human body, temperature, contact time

with the pesticide, the amount of other chemical substances that the pesticide contains and the intoxications that occur as per animal kind. In the light of this information, we can say that chemical and non-chemical substances cause the emission of cellular enzymes and the increase in serum enzyme intensities by causing degeneration in tissues (Mongi *et al*., 2011). In this study, it was examined to what extent the livers of rabbits which were experimentally exposed to the chronic effect of pesticides (chlorsulfuron), were affected from pesticides in the presence of vitamin C. Particularly, there are numerous studies on the acute effects of many pesticides; however, information about these chemicals' chronic effects is yet not enough (Morowati, 1997; Bhatnagar *et al*., 2011). In mammals, liver is one of the vital organs in which many substances necessary for the organism are synthesised and metabolised and the morphologic changes in this organ also affect the metabolic instances in the organism (Sulak *et al*., 2005; Mongi *et al*., 2011; Wefers and Sies, 1988; Flanagan *et al*., 2007). For instance, the activities of cellular enzymes such as transaminases, alkaline phosphatase and lactate dehydrogenise in the serum increase due to some liver enzymes' penetration into blood as a result of the change in cell membrane permeability or the cell's rupture (Sulak *et al*., 2005; Padungtod *et al*., 1998). The results of this research showed that there was an increase in the enzyme activities of serum ALK, P and GGT as a result of giving the rabbits pesticides for 45 days. Similar results were also reported from another research (Yousef *et al*., 2003) and in that research cypermethrin, an insecticide from pyrethroides group, was orally applied to male rabbits in the dose of 1/100 LD_{50} for 12 weeks and free radicals in plasma, liver, brain and testicle were detected to increase as a result of the application. Decrease in Glutathione S Transferase (GST), AST, ALT and ALP activities, increase in plasma GST, AST, ALT and ALP activities and also a decrease in plasma total protein and albumin density were detected and it was observed that globulin density and albumin/globulin ratio were not affected ($p<0.05$). The increase in GGT and ALK.P enzymes obtained from the group members that were given pesticide is in compliance with this research's findings and indicates that the liver is exposed to the toxic effect of the pesticide. In a similar research, an organophosphate insecticide was used and changes in liver biochemistry and histopathology in albino mice on which application was performed were investigated. Findings have indicated that sub chronic hepatotoxirity occurred as from the fourth week. It was observed among the findings that a significant increase occurred in the AST, ALT and ALP values and that organophosphate and carbamate insecticides threatened living beings by showing their effects directly on peripheral and central nervous system (Morowati, 1997). Similar findings were observed in this research as well; serum ALK.P and GGT averages of trial group animals were detected to be significantly higher than that of control group ($p<0.05$). Researchers explained the findings as such: The livers of agriculture labourers who were exposed to pesticides' chronic effect showed some light deformities and as a result, the damaged liver cells released enzymes into blood and this caused the increase in serum activity. One of the important toxic materials for the liver is pesticides. In mammals, relatively toxic chemicals are significantly detoxicated through liver (Bhatnagar *et al*., 2011). Density and constant intake of toxic material hamper the liver's ability to detoxicate and prevent protein synthesis; thus, an increase is observed in activities of enzymes that are used to indicate the degeneration in liver cells (Mongi *et al*., 2011). It is thought that the increase in protein quantities might result from these substances' inhibition of DNA and RNA syntheses (Padungtod *et al*., 1998).

On the other hand, various antioxidant materials are applied today in order to reduce or completely eliminate the oxidative stress caused by pesticides. Vitamin C and E are among the most important of these materials. These vitamins are antioxidants that are not enzymatic. Vitamin C is a strong reductive agent in organism. It is also a strong antioxidant thanks to its strong reductive

activity (Lunec and Blake, 1990; Jialal and Fuller, 1993). Vitamin C has a hydrophilic quality and it cleans the free radicals in extra cellular liquid and the radicals that are in liquid phase and it takes action in order to protect bio membranes from peroxidative damage (Yavuz *et al*., 2004; Sulak *et al*., 2005). It cleans super oxide and hydroxyl radicals easily by reacting with them. In addition, vitamin C enables the tocopheroxyl radical in the membrane to be reduced to tocopherole (Stoyanovsky *et al*., 1995; Lunec and Blake, 1990; Jialal and Fuller, 1993). On the other hand, vitamin E and C are antioxidants that show synergic effect in organism (Bendich *et al*., 1986; Wefers and Sies, 1988; Chatterjee and Anuradaha, 1991; Prakasam *et al*., 2001). This is an important fact for the protection of functional coherence of cells that are under pesticide threat. Researches are about the fact that applications of C and E combination are protective against oxidative damage caused by various oxidants (Biri *et al*., 1998; Campisi *et al*., 1999). Gokalp *et al*. (2003) detected in a similar research that 25% or more of an organophosphate insecticide's LD_{50} dose might cause acute pancreas damage and that the insecticide did this damage probably with oxidative stress mechanism. Researchers indicated that vitamin E and C could prevent pancreas damage with their antioxidant effect.

Likewise, in a similar research (Ming *et al*., 2006) found out that vitamin E and C have protective effect against histopathological liver damage caused by thioacetamide (TAA), another pesticide. They observed inflammation and necrosis in the liver as a result of TAA application and they reported that there was an increase in AST and ALT levels of liver enzymes in the serum (Ming *et al*., 2006). Similar findings were also observed in this research. Study findings support each other. Uzunhisarcikli *et al*. (2007) reported that they investigated the protective role of vitamin C and E and the hepatotoxic effect of methyl parathion, an organophosphate insecticide, in one of their researches conducted on rats.

On the other hand, according to what Mohssen (2001), reported thimetin, an organophosphate insecticide, was given to albino mice and biochemical and histopathological changes that occurred in the liver were investigated. Findings have shown that a significant increase occurred in AST, ALT and ALK.P values due to sub chronic hepatotoxirity as from the fourth week and that organophosphate and carbamate insecticides threatened the animal's life by showing their effects directly on peripheral and central nervous system. These findings support the findings obtained from our research. Similar results were also reported from another research (Yousef *et al*., 2003) and in that research cypermethrin, an insecticide from pyrethroides group, was orally applied to male rabbits in the dose of 1/100 LD_{50} for 12 weeks and free radicals in plasma, liver, brain and testicle were detected to increase as a result of the application. Decrease in Glutathione S Transferase (GST), AST, ALT and ALP activities in liver, increase in plasma GST, AST, ALT and ALP activities and also a decrease in plasma total protein and albumin density were detected and it was observed that globulin concentration, albumin and globulin ratio were not affected. Similar findings were detected in both researches.

In another research, protective effects of vitamin C against haematological and biochemical toxicity were investigated on male rats which were given deltamethrin. Deltamethrin was given to the rats for 4 weeks and significant increases in ALT, AST, ALK.P, Lactate Dehydrogenise (LDH) and γ-glutamyl transpeptidase (γ-GT) levels were observed; and it was also observed that the levels of urea, creatine, serum cholesterol and lipid significantly increased. It was observed that all the parameters of the rats which were in the group that was given vitamin C appeared to be at levels close to normal. Thus, it has been reported that vitamin C gives hope in the protection against toxicity originating from deltamethrin (Mongi *et al*., 2011). Some findings obtained from this study are in compliance with the findings obtained from our study.

Following the studies carried out in our country, many chemical products, called POP (Permanent Organic Pollutants), commonly used in agriculture have been banned production-wise. In the scope of this study, aldrin, endrin, toxaphene, chlordane, dieldrin, DDT and industrial chemicals hexachlorobenzene and PCBs have been banned and their stocks have been monitored. In our country, where production of agriculture products is common, it is thought that companies that especially provide raw materials for feed sector will audit these materials more effectively in the future; because only 2-10% of the pesticides taken into animal body through feed are disposed of, the rest accumulates in organism. On the other hand, pesticide residues can pose a greater danger by becoming denser in the process of milk's turning into concentrated products such as cream, cheese and butter. An organophosphate insecticide chlorpyrifos was used for 10 weeks in a study called "Sub chronic chlorpyrifos intoxication in rats, haematological and serum biochemical changes and protective effect of vitamin C. In the group that was given vitamin C (100 mg kg^{-1}), results of urea, creatine (ALT), alanine aminotransferase, aspartate aminotransferase (AST) and alkaline phosphatase (ALK.P) were observed to be low. The study showed the protective effect of vitamin C against some organ damages in rats that were given chlorpyrifos and vitamin C and some important haematological and serum biochemical parameters were observed to have changed (Ambali *et al.*, 2007).

Findings obtained from this research show that pesticides containing chlorsulfuron change GGT and ALK.P, liver enzymes, activities significantly. In spite of the findings that show use of vitamin C partially reduces these effects, it is crucial to do other similar researches about this subject in our country, where agricultural combat pesticides are commonly used, for the sake of human and animal health.

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