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Protective effects of curcumin on liver damage in rats treated with chlorpyrifos during pregnancy

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Abstract

The purpose of this study is to examine the damage of chlorpyrifos, which is a type of pesticide commonly used in agriculture, will cause in the livers of rats during the gestation period and to examine to what extent and in which direction curcumin, an antioxidant, influences this damage. For this purpose, four groups of animals as the control, chlorpyrifos, curcumin, and chlorpyrifos+curcumin were used in the study. During their pregnancy, curcumin (100mg/kg) and chlorpyrifos (5 mg/kg) were given to rats (Wistar Albino) through lavage. After the birth, perfusion was performed to rats under deep anesthesia, and their livers were removed. After the livers were fixated with 10% buffered neutralized formalin, routine histological processes were performed All the tissues were embedded in paraffin and the tissue sections obtained were stained with hematoxylin-eosin, periodical acid-Shiff (PAS) and Masson 's trichrome staining. According to the results of the study, with the effect of chlorpyrifos, vesicular degeneration in the livers, enlargement, inflammation centers and necrotic cells in the sinusoids were observed. Also, especially the increase in the collagen fiber amount around the portal areas and the decrease in glycogen storages around the central veins were remarkable. In the groups, which were given only curcumin, no obvious changes were seen in the liver except for mild enlargements in the sinusoids. In groups, which were given chlorpyrifos and curcumin together, less histopathological changes were seen when compared with groups, which were given only chlorpyrifos. With these results obtained, it can be said that curcumin, which is a free radical scavenger decreases the oxidative damage of chlorpyrifos in the liver by inducing its free radical formation.

Keywords: Chlorpyrifos, curcumin, liver damage, pregnancy

Introduction

Pesticides are used widely in the world and our country to destroy pests in agriculture and to obtain high-quality products. While pesticides used in an agricultural fight can cause increases in the product by destroying the target organisms, they can also cause damages in living beings that are not targeted [1]. Organophosphate insecticides, which are widely used in the fight with a great number of pests, constitute a significant part of pesticides and today there are more than 200 organophosphate insecticides [2]. Pesticides and destruction products which stay in the soil without decaying for a long time and which enter the body through food chain cause negative changes in tissues and organs in time [3].

Chlorpyrifos-ethyl (CPF) [0,0'-diethyl 0-(3,5,6-trichloro2-pyridyl) phosphorothioate], is an organophosphate insecticide which is widely used against agriculture, forest and garden pests [4]. Since CPF is widely used in agricultural areas and homes, it is an important insecticide human and animals are frequently exposed to. CPF is transformed into chlorpyrifos-oxon, which is its metabolite, by cytochrome p-450 and CPF shows its toxic effect through chlorpyrifos-oxon. As in butyrylcholinesterase, this metabolite carboxyl esterase binds to acetylcholine esterase with a high affinity and shows a toxic effect by inhibiting acetylcholinesterase [5].

Pesticides are transferred to the human body through skin, respiration or digestion organ and metabolized in the liver with cytochrome P450 dependent monooxygenase system [6]. Pesticides have been shown to cause a decrease in cytochrome P450 and "hem" amount, a significant decrease in glucose 6-phosphatase and pirohospotase enzyme activities and stimulation of UDP-glucuronyl transferase enzyme by stimulating lipid peroxidation in hepatic microsomes [7].

One of the systems affected by pesticides is the antioxidant system, which is among the most important protective systems of the body [7]. Experimental studies conducted have shown that oxidative tissue damage plays a role in the pathogenesis of toxic effects that occur as a result of acute and chronic organophosphate applications (neurotoxicity, hepatotoxicity, immunotoxicity,

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embryotoxicity, genetic toxicity) [8, 4]. It has been reported that free radicals that occur as a result of oxidative stress, especially DNA, protein and cell phospholipids have an ability to react with a great number of organic and inorganic compounds, mainly multiple unsaturated fat acids [9, 10]. These free radicals cause the depletion of enzymatic and non-enzymatic antioxidant systems that protect the cell. It has been reported that changes occur in the lipid peroxidation, DNA damage and proteins of cells depending on the oxidative damage that occurs as a result of these effects. Antioxidants inhibit lipid peroxidation by preventing peroxidation chain reaction or collecting reactive oxygen types (ROT) [9]. The aforementioned pesticides and others increase DNA synthesis in the liver and thus cause increase in -OH-2-guanozin and lipid peroxidation and decrease in cellular antioxidants, which is an indicator of DNA damage [7].

Curcumin is the active agent of turmeric obtained from the roots of Curcuma longa, which is a plant of Zingiberaceae family found widely in India and China. Curcumin, which is used in the kitchen as a spice, in the cosmetic sector and as a drug in medicine, has effects in a wide spectrum as anti-inflammatory, antioxidant, anticancerogenic, antimutagenic, anticoagulant, antidiabetic, antibacterial, antiviral and nerve protective effects [11]. Curcumin eases the disposal of many reactive oxygen radicals, especially superoxide anions, nitrogen dioxide radicals and hydroxyl radicals [12].

Curcumin has been shown to decrease oxidative stress and tissue damages in damages that occur in kidney, heart, brain tissue and liver with its antioxidant characteristics [13]. Curcumin shows its antioxidant activity by preventing the transformation of xanthine dehydrogenase (XD) to xanthine oxidase (XO), preventing the formation of lipid peroxidation and collecting the superoxide radicals in the ischemic medium [14]. Curcumin increases the activity of catalase, superoxide dismutase (SOD) and glutathione peroxidase enzymes and decreases the peroxidation of lipids in the cell membrane [9].

Pregnancy is a very sensitive period in which significant physiological and metabolic changes occur in women. Changes in this period cause imbalance in the diet of the mother, deterioration in the sleep pattern, and psychological changes in the mother such as excitement, nervousness and fear of death especially in the first months and the last month of pregnancy. All these changes weaken the future mother's immune system and as a result of this the mother's sensitivity to the negative effects of chemical substances, drugs and other environmental pollutants increases [15]. Although there are many studies about the effects of being exposed to chlorpyrifos during pregnancy on the development of the fetus, there are no studies investigating what kind of histological changes occur in the livers of the pregnant.

The purpose of this study is to examine the damage that chlorpyrifos, which is a type of pesticide commonly used in agriculture, will cause in the livers of rats during the gestation period and to examine to what extent and in which direction curcumin, an antioxidant, influences this damage. We believe that the results of the study will show the utility of curcumin in the protection of environmental and human health against the harmful effects of pesticides and thus contribute to literature.

Material and Methods

Animals

This study was started with permission of Ondokuz Mayıs University Animal Local Ethical Committee of Experimental Animal Research. Wistar Albino rats used in the study were obtained from Ondokuz Mayıs University Experimental Animal Research (DEHAM). The rats were kept in plastic cages, at a room temperature of 18-22 oC and with 12 hours of light/dark and fed with pellet rat feed which included 20-22% raw protein, 4-5% raw fat, and 5-7 % raw cellulose produced by Samsun Feed Factory. Female rats with a weight of 250-300 gr from the same generation were used in the study.

Experimental Plan

The study consisted of 5 groups as control (C), chlorpyrifos (CPF), curcumin (CUR) and chlorpyrifos+curcumin (CPF+CUR) and corn oil (CO). Five female rats were used for each group in the study. Later, these animals were left to copulate under suitable conditions in the same cages with male rats for one day. The next day, vaginal smear samples were taken from the female rats and those which were found to have sperm were accepted to be on day 0 of their pregnancy and they were taken in separate cages according to groups. The rats in the control group did not undergo any interventions during their pregnancy. In the rats in CPF group, 5mg/kg chlorpyrifos was applied through gavage once every day from the first day of pregnancy to birth. In the rats in CUR group, 100 mg/kg curcumin was applied through gavage once every day from the first day of pregnancy to birth. In the rats in CPF+ CUR group, five hours after 100 mg/kg curcumin was applied through gavage, 5mg/kg chlorpyrifos was applied through gavage once every day from the first day of pregnancy to birth. Since curcumin and chlorpyrifos dissolved in corn oil, CO group was formed. Corn oil as much as calculated for per kg to be applied on the rats in these groups was applied through gavage. Cardiac perfusion was performed on rats which gave birth under anaesthesia applied with the injection of the mixture of Ketamine (50 mg/kg)/ Xylazine (10 mg/kg) prepared with a ratio of 5/1. Following this procedure, liver tissues were removed and taken in 10% buffered neutral formalin solution for light microscope examination. After routine histological follow ups were conducted on tissues, they were embedded in paraffin blocks. 5 μm thick sections were taken from the paraffin blocks obtained. Hematoxylin eosin, periodical acid-Shiff (PAS) and Masson's trichrome staining were performed for the light microscope examination of tissue samples [16].

Measuring the animals' weights

The female rats in all groups were weighed every day from day 0 of their pregnancy to the day they gave birth, and the total weight (gr) they gained during pregnancy was measured. The effect of chemicals applied in the experiment on the weights of rats during pregnancy was assessed with statistical analyses.

Histological Assessment

All livers were examined with Leica DM 1000 light microscope and photographed by using Leica DFC 290 digital camera and visualisation software. The following parameters were used in the assessments: H&E staining was performed for vesicular generation, sinusoidal enlargement, inflammation centres and necrotic cells; while Masson's trichrome staining was performed

for fibrosis and PAS staining was performed for cytoplasmic glycogen storages [16]. Semi quantitative analysis was conducted for histological assessment [17].

Statistical Analysis

All statistical analyses were conducted by using SPSS 21.0 program. Non-parametric tests one-way ANOVA and T-test were used for the comparison of groups. $p<0.05$ was considered as statistically significant

Results

As a result of the statistical analyses conducted, it was found that the weight increase in all groups given CPF was less when compared with all other groups and that this difference was statistically significant ($p<0.05$). In CPF+CUR groups, weight increase was higher when compared with the CPF group, and this difference was statistically significant ($p<0.05$). The weight increase difference in groups which were given only curcumin was not statistically significant when compared with the control group ($p>0.05$). Weight increase difference in CO group was not statistically significant when compared with the control group ($p>0.05$). No statistically significant difference was found between CUR and CO groups ($p>0.05$). Weight increase in CUR groups was statistically significant when compared with the CPF+CUR group.

The body weight increase in pregnant rats

Table 1. Average weight (gr) and SEM values of all pregnant rats in all groups during pregnancy

	Body weight increase (gr) ± SEM
C	49.00±1.00
CPF	30.20±0.66
CUR	49.40±0.81
CPF+CUR	42.80±1.15
CO	49.40±0.81

Histological Results

Table 2. Histopathological scores of liver tissues of all groups. In areas of central vein and around the portal area (+), in areas around and close to central vein and portal area (++), in areas of central vein and around the portal area and further (+++), whole liver (++++), no change (-)

	Control	CPF	CUR	CPF+CUR	CO
Hydropic degeneration	-	++++	+	+++	+
Sinusoidal enlargement	-	++++	+	+++	+
Inflammatory centre	-	++++	+	+++	+
Glycogen intensity in hepatocytes	++++	++	++++	+++	++++
Fibrosis	-	-	-	-	-

According to the results of the study, the liver had a normal histological appearance in C, CUR and CO groups (Figure 1A-C). No obvious histopathological changes were observed in these groups. In CPF groups and CPF+CUR groups, histopathological changes such as hydropic degeneration, inflammatory centers, sinusoidal enlargement, differences in glycogen intensity in hepatocytes were seen in the liver (Figure 1D-F).

In CPF groups, hydropic degeneration in hepatocytes in the whole liver, sinusoidal enlargement and a great number of inflammatory centers were seen, particularly around the central vein (Figure 1D-E). Also, when compared with the control groups, a decrease in glycogen intensity (Figure 2D-E) and fibrosis in portal areas was observed, especially around the central veins (Figure 3D-E).

When compared with the control group, hydropic degeneration in areas around central vein and portal area, inflammatory centers, and sinusoidal enlargement were seen in CPF+CUR group (Figure1F). Also, there was a decrease in glycogen intensity in the hepatocytes around central veins (Figure 2F) and fibrosis in portal areas (Figure 3F). However, when CPF+CUR group was compared with the CPF group, histopathological changes in the liver were less severe in CPF+CUR group (Table 1). It was found that curcumin reduced the histopathological changes caused by CPF.

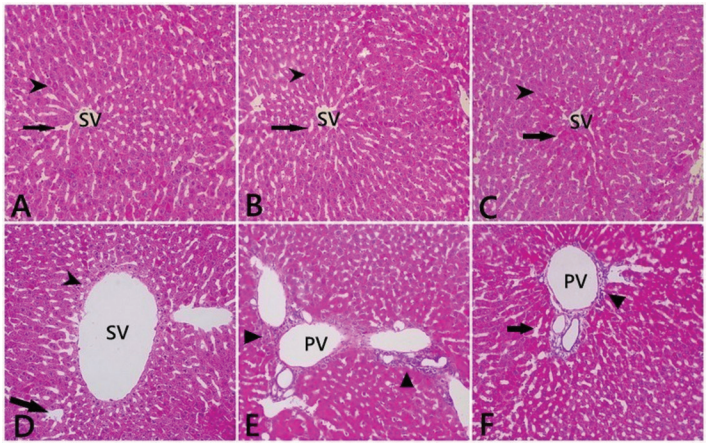


Figure 1. A. Histologic image of liver in C group. B. Histologic image of liver in CUR group. C. Histologic image of liver in corn oil group. D-E Histologic image of liver in CPF group. F. Histologic image of livers in CPF+CUR group. Hematoxylin&eosin x200 ➡ Sinusoid ► hepatocyte › Mononuclearcellular infiltration

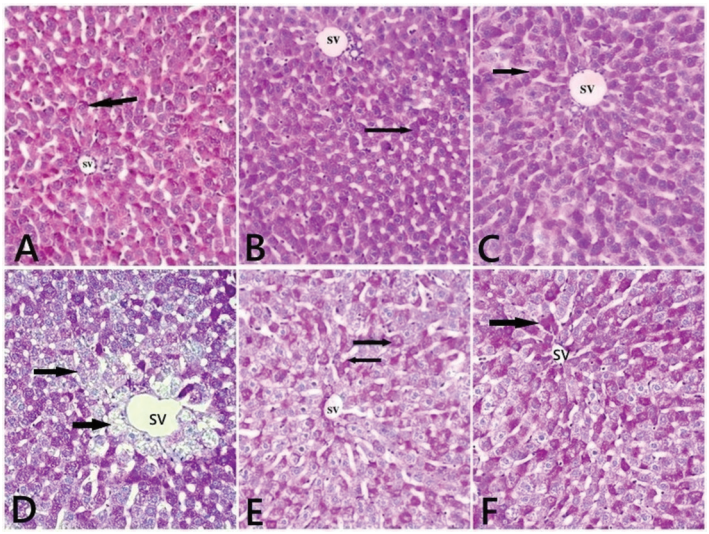


Figure 2. A. Histologic image of liver in C group. B. Histologic image of liver in CUR group. C. Histologic image of liver in corn oil group. D-E Histologic image of liver in CPF group. F. Histologic image of livers in CPF+CUR group. PASx200 ➡Glycogen particles in hepatoctyes

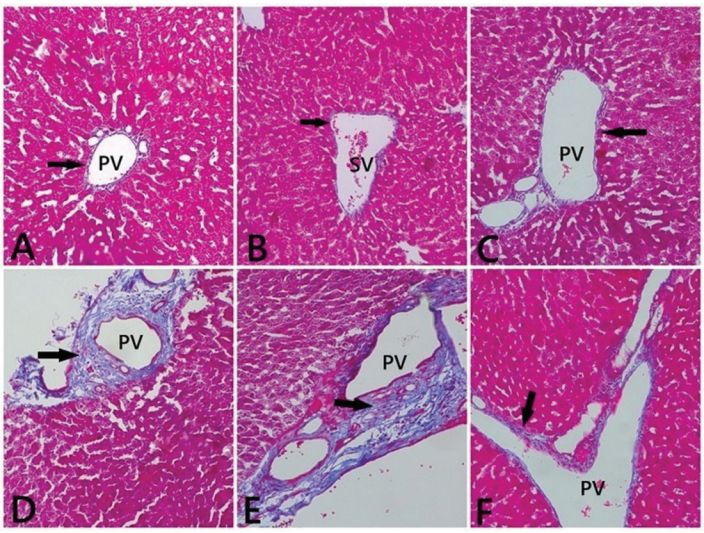


Figure 3. A. Histologic image of liver in C group. B. Histologic image of liver in CUR group. C. Histologic image of liver in corn oil group. D-E Histologic image of liver in CPF group. F. Histologic image of livers in CPF+CUR group Masson trichrome x 200 ➡ fibrosis around portal areas

Discussion

Pregnancy is a period in which significant physiological and metabolic changes occur in the future mother. These changes increase future mother's sensitivity against the negative effects of chemical substances, drugs and other environmental pollutants [15]. Although there are a great number of studies about the effects of exposure to pesticides on fetus development [8,35], there are no studies investigating what kind of histological changes exposure to pesticides causes in the livers of the pregnant during pregnancy period, which is a very sensitive and risky period for both the mother and the baby. In this study, we examined the effects of chlorpyrifos, a pesticide, and curcumin, an antioxidant, on the liver of pregnant women during pregnancy..

Organophosphate insecticides form a significant part of pesticides and today there are more than 200 organophosphate insecticides [2]. Organophosphate compounds have been reported to cause free radical production and thus cause the oxidative damage of important molecules in the metabolism, primarily membrane lipids [18]. Chlorpyrifos, which is an organophosphate insecticide, gets inside the cytoplasm from cells as a lipophilic molecule and harms cellular molecules inside the cell [19]. Oxidative damage first starts with the production of reactive oxygen types and causes damage in macromolecules such as lipid, DNA and protein [20]. It has been reported that free radicals that occur as a result of oxidative stress, especially DNA, protein and cell phospholipids have an ability to react with a great number of organic and inorganic compounds, mainly multiple unsaturated fat acids [9, 10]. These free radicals cause the depletion of enzymatic and non-enzymatic antioxidant systems that protect the cell. It has been reported that changes occur in the lipid peroxidation, DNA damage and proteins of cells depending on the oxidative damage that occurs as a result of these effects [9, 10].

Experimental studies conducted have shown that oxidative tissue damage plays a role in the pathogenesis of the toxic effects of chlorpyrifos [8, 4]. In their study. In their histopathological study, Tripathi and Srivastav [21] reported that depending on the application dose and period, chlorpyrifos caused hepatic vacuolisation, hepatocyte degeneration, nuclear degeneration, sinusoidal enlargement and hepatic necrosis in the liver. In their biochemical and histopathological study, Uzun and Kalender [22] reported that chlorpyrifos increased MDA, SOD and catalase activities in the liver. Mansour et al. [23] assessed the effects of chlorpyrifos in rat liver histopathologically and showed that chlorpyrifos increased lipid peroxidation; decreased plasma superoxide dismutase (SOD), glutathione-S-transferase (GST) and serum acetyl choline esterase (AChE) activities, and caused histopathological changes such as hepatocyte degeneration, focal inflammatory cell infiltrations and diffuse Kupffer cell proliferation.

Histological images obtained from our study showed sinusoidal enlargement, inflammatory cell infiltration and hydropic degeneration in the liver in CPF and CPF+CUR groups. We believe that these histopathological changes occurred as a result of chlorpyrifos causing oxidative damage in the liver.

One of the systems affected as a result of the oxidative stress caused by pesticides is the antioxidant system. Antioxidants are substances which protect cells against the adverse effects of xenobiotics, drugs, carcinogens and toxic radical reactions both directly and indirectly. Antioxidants inhibit lipid peroxidation by preventing peroxidation chain reaction or by collecting free radical species [9]. In our study, when compared with the CPF group, histopathological changes in the CPF+CUR groups were less severe. We believe that the reason for this is the fact that curcumin, which has been shown to have antioxidant characteristics with a great number of studies, reduces the oxidative damage created by chlorpyrifos.

In the histologic images we obtained in our study, sinusoidal enlargements, intensity of inflammatory centres and hydropic degenerations were more severe in the centrilobular area around the central vein in CPF and CPF+CUR group. We can say that the reason for this is that p450 enzyme which provides biotransformation of chlorpyrifos in the liver is more intense in the hepatocytes in centrilobular areas and creates an important source in the production of reactive oxygen types.

Another important finding of the study was the decrease in glycogen intensity in hepatocytes when CPF and CPF+CUR groups were compared with the control group. In their study, Goel et al. [24] reported that chlorpyrifos decreased the glycogen content in hepatocytes and increased the activity of glycogen phosphorylase enzyme. In another study conducted, it was shown that chlorpyrifos caused some histopathological changes in the liver and decrease in glycogen content [25]. By taking into consideration the results of previously conducted studies, the decrease in glycogen intensity in CPF and CPF+CUR groups in this study can be explained as chlorpyrifos application causing changes in the activities of carbohydrate metabolizing enzymes which have a vital significance in the regulation of energy metabolism as a response to increasing ATP requirement of the body under toxic conditions and in turn causing a decrease in glycogen content of hepatocytes.

On the other hand, when CPF+CUR group was compared with CPF group, the decrease in glycogen content of hepatocytes in CPF+CUR group was not as severe as CPF group. A great number

of studies conducted have shown that curcumin protects the blood sugar levels by regaining the changed activities of –phosphate dehydrogenase and glucose-6-phosphatase enzymes taking part in gluconeogenesis and glycogenolysis [26]. We believe that the curcumin used in this study can be responsible for there covery of the lost glycogen content in CPF groups back in the liver through the same mechanism.

Hepatic fibrosis is characterized with the extra production and accumulation of extracellular matrix (ECM) proteins by myofibroblast [27]. Although the mechanisms underlying the onset and advance of liver fibrosis are not fully understood, it is accepted that the activation and proliferation of hepatic stellate cells (HSC) is the factor which causes this process the most [28]. Following the liver damage, HSCs are activated and they proliferate by producing pro-inflammatory cytokines and chemokines, growth factors, pro-fibrogenic cytokines (including connective tissue growth factor) and metalloproteinase inhibitors and this in turn causes the formation of collagen-rich extracellular matrix in a path advancing to fibrosis.

A great number of experimental studies have reported both chlorpyrifos and other organophosphate insecticides to cause fibrosis in different tissues [3, 30]. It has been shown that oxidative stress can stimulate both in vitro and in vivo fibroblast and hepatic stellate cell proliferation and collagen synthesis [31].

HSC apoptosis induction is associated with the reversal of fibrosis and for this reason targeting the prevention of HSC activation and proliferation can help preventing or reversing fibrosis. Curcumin shows a great number of antioxidative, anti-inflammatory, antifibrogenic and antiproliferative effects in HSCs. Bruck et al. [32] showed that curcumin inhibits hepatic

Conclusion

The results obtained from the study show that chlorpyrifos, which is an insecticide widely used in agriculture and homes, causes damage by causing free radical formation in the liver during pregnancy and that curcumin, which has an antioxidant characteristic, has a protective effect on this oxidative damage.

We believe that our study will increase the awareness of people about pesticide use especially during pregnancy and contribute to the literature by showing the usability of curcumin during the gestational period.

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Ethical approval
Permission was obtained from the Local Ethics Committee of the Animal Experiments of Ondokuz Mayis University.

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