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RESEARCH ARTICLE

The Investigation of Genotoxicity and Oxidative Stress Potential of Luna Experience SC 400 Fungucide in Rat Liver and Blood Tissues

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Abstract

Fungicide is a general term given to all chemicals used to take under control the fungus that infect on plants. Luna Experience SC-400 is a new generation fungicide that combinated with Fluopyram and Tebuconazole. In this study, micronucleus assay and single cell gel electrophoresis (comet assay) were usedin rat liver and blood tissues. To determine the oxidative stress potential of the Luna Experience SC 400, catalase level (CAT) which is one of the antioxidant enzymes were measured. To determine the lipid peroxidation, malondialdehyde (MDA) levels were measured. Doses of Luna Experience SC 400 given to rats were determined as 5 mg/kg, 10 mg/kg and 20 mg/kg. MMC was used for positive control. The results of this study show that Luna Experience SC 400 fungicide has genotoxic effect and on CAT-dependent oxidative stress potential, but doesn't have MDA-dependent oxidative stress potential in rat liver and blood tissues.

Keywords: Single Gel Cell Electrophoresis; Fungucite (Luna Experience-EC 400); Micronucleus Assay; liver; Blood; Genotoxicity; Cytotoxicity

Introduction

Pesticides have been used by humans since 2000 BC to protect their crops. Increasing environmental pollution due to the use of pesticides and the widespread use of industrial products has caused pollution of food, water and soil by the residues of these chemicals and their metabolites [1]. In the 19th century, rotenone obtained from the roots of Pyrethrum and tropical vegetables obtained from Chrysanthemum was used as two other naturally occurring pesticides. Pesticides, which are economically beneficial for food preservation, cause pollution of water, soil and air due to their ability to withstand natural degradation and disrupt the ecological system [2].

When toxicological studies of pesticides revealed harmful effects at different levels, these levels of exposure were classified according to the amount received by the World Health Organization (WHO) [3]. Pesticides are used in agriculture in range 75% and 80%. But they are also used as wood preservatives, in dyes, in fabrics other from agriculture [4-6,7]. Fungicides are biocidal chemical compounds or biological organisms used to kill fungi or fungal spores. Luna Experience-SC 400 is a new generation fungicide with Fluopyram and Tebuconazole Combination. Luna Experience-SC 400 Luna Experience-SC 400 is a new broad spectrum, protective and therapeutic fungicide standard that combines different mechanisms with a mixture of tebuconazole, which has been proven by fluopyramine, one of the newest technological products produced by Bayer Crop Science. Luna Experience-SC 400 contains 200g/L tebuconazole and 200g/L fluoropyram as active substance [8].

Fluopyram is a pyridinyl-ethyl-benzamide fungicide developed by Bayer Crop Science in 2010. Has a structure that inhibits fluopyramine, succinate dehydrogenase enzyme, and fungal respiratory chain complex, which are effective in controlling the pathogens of more than 70 products. The kinetic behavior and metabolism of fluoropyram was investigated in rats. In subchronic studies, weight loss and nutritional problems were recorded. It has been determined that the liver is the target organ. Hepatotoxicity was evident due to dose increase. Another target organ has been found to have blood cells. Changes in the parameters of red blood cells and an increase in the platelet count were observed in studies performed [9]. In a chronic study in mice, 4 groups (0, 20, 60 and

180 ppm) were treated with 94.7% pure tebuconazole for 21 months and the results were reported. All doses have been reported to be the liver of the target organ due to fat accumulation in the liver [10].

In an *in vitro* study performed with tebuconazole in bovine lymphocyte cultures, cytotoxic and genotoxic effects have been studied and results have shown increased cytotoxicity and genotoxicity [11]. In a rat-based study in which 96.2% pure tebuconazole was used, it was stated that the liver is the target organ, the liver weight is increased and the serum enzyme levels are increased. Adenomas and carcinomas have been reported in the highest dose group (1500 ppm) [10]. As a result of study of Tebuconazole exposure, data obtained from *Cyprinus carpio* and *Rhamdia quelen* liver indicated that tebuconazole has been shown to be an effect of increasing oxidative damage potential [12,13]. The Comet assay, based on the principle of DNA (Deoxyribose Nucleic Acid) fractures at the gene level, has found widespread use in many DNA damage and repair determinations, biopsy studies and genetic toxicology [14,15]. Micronucleus (MN) is a small nucleus consisting of whole chromosomes that are not involved in the main nucleus, and which are visible in addition to the parent nucleus in the cytoplasm, due to fragments that have disappeared from the main chromosome or due to anaphase defects. Nowadays, as environmental pollution increases steadily due to rapid industrialization, living beings are exposed to more physical and chemical agents, so the need to detect and take measures to detect the adverse effects of strong toxic, mutagenic, carcinogenic and teratogenic factors is inevitable [16]. Free radicals occur as intermediatesin the reactions that occur in the normal metabolic pathways of cells.These free radicals lead to lipid peroxidation, oxidative damage in proteins, mutation in DNA and the activation in cell death receptors [17]. Lipid peroxidation causes significant changes in the function and structural organization of the cell membrane. In many studies, it is determined that there is relationship between lipid peroxidation and pesticide exposureand CAT is an antioxidant enzyme that catalyzes the decomposition of water and oxygen in H_2O_2 [12-18].

In this study, it was aimed to investigate the genotoxic effect and the potential of oxidative damage of Luna Experience SC 400 Fungicide in rat liver and blood. 1. Comet analysis and 2. Micronucleus analysis were performed for genotoxicity analysis. 3. CAT and MDA analysis were performed to determine the oxidative stress.

Material and Method

Chemicals

LUNA Experience SC-400 from produced by Bayer Crop Science (Tebuconazole CAS No.:107534-96-3, Fluopyram CAS No.: 658066-35-4, 619-797-7) was supplied as grade form and purchased from Sigma. NMA, LMA, PBS (Ca Mg free), Ethidium Bromide, NaOH and EDTA was supplied as grade form and purchased from Sigma. Acridine orange, MMC, DMSO, and RPMI 1640 purchased from Biochrom and was supplied as grade form and NaCl purchased from Merck Millipore.

Dose selection

In this study, doses were selected according to LD_{50} dose of Luna Experience SC 400 for rats. LD_{50} dose of Luna Experience SC 400 is 2000 mg/kg for rats (SAFETY DATA SHEET according to Regulation (EC) No. 1907/2006, Revision Date: 15.08.2017 102000032925 Print Date: 16.08.2017). Therefore, in this study, the highest dose was set at 20 mg/kg body wt, i.e. 1% of the LD_{50} dose. The lowest dose was determined via pre-study as 5 mg/kg body wt because this dose did not affect the animals neurologically and behaviorally. Five groups were used in the study. For this purpose, dose groups prepared by means of gavage were applied to female rats weighing 400 g and 12 months old at Mersin University Experimental Animal Lab at intervals of 48 hours for 30 days. In this study, mitomycin C (MMC) (2mg/kg), a single i.p. dose was used as a positive control.

It is acceptable that a positive control is administered by a route different fromor the same as the test substance and that is only a single time [19]. The positive control and untreated control rats were treated identically with equal volumes of Normal saline.

Animals and diet programme

In this study, healthy adult female Swiss albino rats (Wistar rat) [6–8 weeks of age and average body weight (body wt) of 180– 200g] were used. Rats were obtained from the Experimental Animal Center, University of Mersin, Turkey. The study was approved by the research and ethical committee at theUniversity of Mersin. The rats were randomly selected andhoused in polycarbonate boxes (seven rats per box) with steelwire tops and rice husk bedding. They were maintained with 12 h dark/light cycle in a controlled atmosphere of 22± 2 °C temperatures and 50–70% humidity with free access to pelleted feed and fresh tap water. The animals were allowed to acclimate for 14 d before treatment. In this study, rats were divided to five groups including seven rats (n=35). Therefore, this study was performed by five different diet programs one dose per 48 h for 4 weeks (≈30 d).

The rats were treated by gavage with
(i) 5 mg/kg body wt Luna experience SC400 dissolved in saline.
(ii) 10 mg/kg body wt Luna experience SC400dissolved in saline.
(iii) 20 mg/kg body wt Luna experience SC400dissolved in saline.
(iv) The untreated control rats were treated identically withequal volumes of normal saline only via gavage throughout the study
(v) Since positive controls may be administered by adifferent route and treatment schedule than the testagent [19]. In this study, a single doseof MMC (2 mg/kg, i.p.) was administered at the 10th week.

Experimental design

Comet assay in peripheral blood

Blood samples were obtained from rat heart by venipunctureat the end of the 4th week. Isolated blood cell suspension was washed two times with RPMI supplemented with 10% FBS. A part of the isolated cells was used directly for the comet assay. Comet assay was performed under alkaline conditions according to the method of Singh, *et al.* with slight modifications [20]. Completely frosted microscopic slides werecovered by a thin layer of 0.5% normal melting agarose (NMA) dissolved in Ca^2 and Mg^2 free phosphate buffer saline (PBS) at about 50 ºC. Eppendorf tubes were placed in water bath at 40 ºC. One hundred microliters of blood suspension were diluted with 1ml of PBS in eppendorf tube. Then 30 µl mixtures were mixed with 250 µl of LMA (0.5%). One hundred microliters of this mixture was spread on the NMA-coated slides using micropipette and immediately was covered with coverslips. Slides were preserved in refrigerators at +4 ºC for 15 min. The coverslips were slowly removed from top of slides. Then slides were placed in chalets including lysis solution and preserved for 2 h inrefrigerator in dark. Slides were washed with chilled distilled water and placed on a horizontal gel electrophoresis unitfilled with fresh electrophoretic buffer (0.3M NaOH+1mMEDTA) to allow DNA unwinding before electrophoresis for 20 min. Electrophoresis was conducted at 20 ºC using 25V and 300mA for 20 min. The above steps should be carried out in dark to avoid DNA damage. After electrophoresis, slideswere washed with chilled distilled water and placed in neutralizing buffer (for 5 min). Then again slides were washed with chilled distilled water and placed in chilled ethanol for 10 min. DNA was stained with ethidium bromide (0.1 mg/ml, 1:4) and the slides were examined with a fluorescent microscope (BX51, Olympus, Tokyo, Japan). Each treatment is having seven replicates.

Two different parameters were evaluated for genetic damage in comet assay:

- Genetic damage index was calculated as AU values.

The "Arbitrary units (AU)" were used to express the extent of DNA damage and calculated following formula:

$$AU = \sum_{i=0}^{4} i \times N_i$$

N_i is the number of scored cells in i level, i is the level of DNA damage (0, 1, 2, 3, and 4).
Genetic damage index: [(0xType 0)+ (1xType 1)+(2xType 2)+(3xType 3)+(4xType 4)]
AU values indicating the comet assay scores show levels of UD (undamaged, 0), Type 1 (low damaged, 1), Type 2 (moderate damaged, 2), Type 3 (high damaged, 3), and Type 4 (ultra high damaged, 4).

- Damaged cell percent (DCP): Type 2+Type 3+Type 4

MN assay in peripheral blood

Whole blood smears were collected on the day following the Luna Experience SC 400 administration, or 1 d after MMC treatment. Whole blood smears were prepared on cleanmicroscope slides, air dried, fixed in methanol, and stained with acridine orange (125 mg/ml in pH 6.8 phosphate buffer) for 1 min just before the evaluation with a fluorescence microscope using a 40xobjective [21]. The number of MNPCEs was determined using 2000 PCE per animal. Briefly, immature erythrocytes, i.e. PCEs were identified by their orange-red color, mature erythrocytes were identified by their green color, and micronuclei were identified by their yellowish color.

Comet assay in liver tissue

The experiment was terminated at the end of 30 days after completion of the diet program. Rats were anesthetized with Ketalar (Ketamine-HCl, Pfizer, Istanbul), sacrificed and their liver tissues were removed. For comet assay and micronucleus analysis, liver tissues were placed into falcon tubes. Liver samples (1 g) were transferred into dish and cut with scissors on the ice and wereminced, suspended at 1 ml/g chilled homogenizing buffer (0.075 MNaCl and 0.024 M EDTA, 10% DMSO) and gently homogenized onice for MN and comet assay analysis.

MN assay in Liver tissue

Liver homogenate was kept at +4 ºC for 24 h. Homogenate wasdiluted with Phosphate buffer saline (1:10) (PBS). 100 µl of diluted homogenate was dropped on to microscopy slides, air dried, fixedin methanol and stained with acridine orange (125 mg/ml in pH6.8 phosphate buffer) for 1 min just before the evaluation with afluorescence microscope using a 40× objective [22]. The number ofhepatocytes with MN was determined using 1000 hepatocyte peranimal. The method of the observation was barred in a blind wayduring which the observer had no knowledge of the identity of the slide.

Measurement of Catalase Enzyme Level in Blood and Liver Tissues

The catalase enzyme level was measured according to the method of Aebi, taking into account the reduction of hydrogen peroxide at 240 nm [23-24]. Samples were prepared in spectrophotometer cuvettes and examined at 240 nm and H_2O_2 was added last to the spectrophotometer bath and the bath was vortexed.

Measurement of Malondialdehyde Level in Blood and Liver Tissues

According to Ohkawa's method, the amount of malondialdehyde present in the medium was measured by reading on a

spectrophotometer at 532 nm [24-25]. Before attaching the TBA, the tubes were incubated for 15 minutes in a water bath at 95 °C. After the TBA was added, the tubes were incubated in a water bath at 95 °C for 60 minutes.

Statistical Analysis

Shapiro-Wilk, Kolmogorov-Smirnov, ANOVA, Kruskal Wallis, Pearson Correlation and Spearman Correlation methods were used in statistical analysis of the data obtained as a result of the test protocols. The doses applied to investigate whether there was a statistically significant difference between the results were compared between both the positive control values and the negative control values. When all analyzes were performed, the average of the data obtained from 35 individuals was used. When evaluations are made, *p value* (confidence interval) is taken as 0.05 and 0.001.

Results

In this study, the oxidative DNA damage data obtained from studies on blood and liver tissues from rats exposed to the specified dose groups were determined by the comet test method. For each group, the results of the comet analysis are shown in Table 1.

Groups	Rats	Type 0 Blood/liver	Type 1 Blood/liver	Type 2 Blood/liver	Type 3 Blood/liver	Type 4 Blood/liver
Negative Control	1	80/65	18 /28	1/6	1/1	0/0
	2	79/66	20/26	1/6	0/2	0/0
	3	77/67	22/26	1/6	0/1	0/0
	4	66/68	34/23	0/8	0/1	0/0
	5	69/67	30/24	1/8	0/1	0/0
	6	54/69	44/25	2/5	0/1	0/0
	7	62/65	37/25	1 /8	0/2	0/0
5mg/kg LunaExperince SC 400	1	42/56	52 /22	6 /12	0 /5	0/5
	2	33/57	60 /18	5 /14	2 /7	0/4
	3	43/60	47 /17	9 /12	1/6	0/5
	4	60/63	36 /16	1/9	3/8	0/4
	5	26/53	63 /23	11 /15	0/5	0/4
	6	48/56	40 /21	11 /13	1/7	0/3
	7	41/59	53 /24	6 /8	0 /6	0/3
10mg/kg LunaExperience SC 400	1	12/16	64 /38	20 /15	4/22	0/9
	2	28 /20	69 /45	18 /16	2/11	0/8
	3	26/22	60 /43	13 /15	1/12	0/8
	4	30/21	45 /47	23 /17	2/11	0/4
	5	3 /17	57 /49	31 /18	9 /8	0/8
	6	20/19	67 /43	9 /17	4/14	0/7
	7	7/23	83 /41	9 /16	1/12	0/8
20mg/kg LunaExperience SC 400	1	18/0	63 /9	21 /12	4 /26	4/53
	2	22/0	60 /8	13 /11	1/25	4/56
	3	5/1	66 /9	19 /14	8 /26	2/50
	4	19/0	53 /10	20 /13	6/24	2/53
	5	24/2	63 /11	8 /14	3/27	2/46
	6	29/0	55 /8	13 /16	1 /23	2/53
	7	17 /1	67 /9	13 /15	1/24	2/51
Positive Control MMC (2mg/kg)	1	0/0	2 /2	12 /6	28/28	58/64
	2	0/0	3 /3	8 /5	22/27	61/65
	3	0/0	2 /1	8 /6	28/27	62/66
	4	0/0	3 /2	12 /7	25/25	60/66
	5	0/0	1 /1	10 /8	24/24	65/67
	6	0/0	2 /1	12 /5	23/25	63/69
	7	0/0	2 /2	12/8	23/25	63/65

Table 1: The results of the comet test values (type 0-Type 4) obtained from damaged blood and livercells of rats exposed to Luna experience SC 400 by gavage for 4 week at 48 h intervals

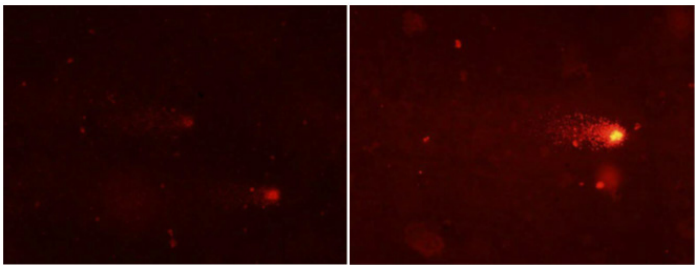
Results of Damaged Cell Percentage and Genetic damage Index in Blood and liver Cells

DCP and GDI values obtained from the data given in Table 1. are given in Table 2. Figure 1a-b represents Cometviews in liver and blood tissues of the animals under diet regimens, respectively. In general, DCP and GDI increased with dose increase of Luna Experience SC 400. There is a statistically significant difference between the groups in terms of values.

Groups	DCP Mean±SD		GDI Mean±SD	
	Blood	Liver	Blood	liver
Negative Control	01.14 ± 0.69	08.00±1.41	31.71±9.60	42.57±2.76
5 mg/kg	08.00 ± 3.00[b]	22.14±2.61[ab]	67.14±11.71[ab]	78.71±5.02[ab]
10 mg/kg	20.86±10.17[a]	36.57±4.54[ab]	108.57±18.86[a]	144.57±12.31[ab]
20 mg/kg	21.29 ± 7.06[a]	90.28±1.70[a]	112.14±17.49[a]	318.14±7.88[a]
Positive Control (MMC-2mg/kg)	97.00 ± 2.71[a]	98.28±0.75[a]	344.29±7.83[a]	356.14±3.13[a]

[a]*comparedto Negative Control (p <0.001),* [b]*comparedwith 20 mg / kg (p <0.001)*

Table 2: DCPand GDI values inblood and liver cells of rats exposed to Luna experience SC 400 by gavage for 4 week at 48 h intervals



Liver cells **(a)** Blood cells **(b)**

Figure 1: Comet views (Type IV) in ethidium bromide-stained liver hepatocytes **(a)** and blood cells **(b)** of rats treatedby gavage with Luna Experience SC 400

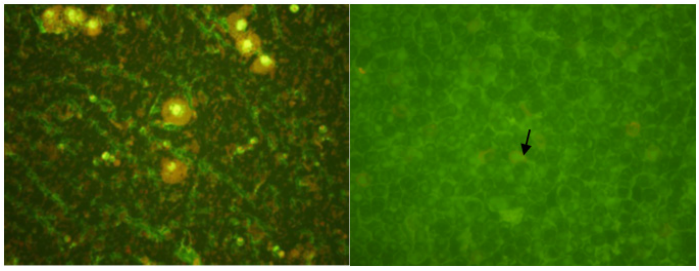
Results of MN analysis in blood and liver cells

There was an increase in MN values and a decrease in PCE values due to the dose increase of Luna Experience SC400 fungicide in blood cells (Table 3). In liver cells, there was an increase in MN values (Table 3). All doses varied according to negative control. Significant differences were also observed between the lowest dose group (5 mg / kg) and the largest dose group (20 mg / kg) in both blood and liver tissues. Figure 2c-d represents micronucleus image in liver and blood tissues of the animals under diet regimens.

Groups	Blood		Liver
	MN Mean±SD	PCE/total erythrocyte (‰) Mean±SD	MN (‰) Mean±SD
Negative Control	0.49±0.10	43.57±3.36	0.10±0.16
5 mg/kg	0.79±0.05[ab]	25.43±3.60[ab]	0.42±0.12[ab]
10 mg/kg	0.96±0.05[a]	18.00±2.38[a]	0.77±0.11[ab]
20 mg/kg	0.94±0.02[a]	15.29±1.50[a]	1.15±0.11[a]
Positive Control (MMC-2mg/kg)	1.29±0.06[a]	15.29±2.56[a]	1.38±0.03[a]

[a]compared with negative control (p <0.001), [b]compared with 20 mg/kg (p <0.001)

Table 3: Micronucleusfrequency in blood, liver cells and polychromatic erytrocyte frequency in blood cells of rats exposed to Luna experience SC 400 by gavage for 4 week at 48 h intervals



Liver MN **(c)** Blood MN **(d)**

Figure 2: Arrows indicates the MN in peripheral blood (c) and liver hepatocyte (d) of rats treated by gavage with Luna Expperience SC 400 *in vivo*

Results of Catalase (CAT) and Malondialdehyde (MDA)Measurement Assay Analysis in Liver and blood Cells

The data obtained from results of the MDA and CAT measurement test in blood and liver cells are shown in Table 4. There was no statistically significant difference between doses for MDA measurement in blood and liver cells. There was a significant difference between the highest dose group (20 mg / kg) and negative control ($p < 0.05$) for CAT enzyme measurement.

Groups	Blood Mean±SD		Liver Mean±SD	
	CAT	MDA	CAT	MDA
Negative Control	0.077±0.000	1.37±0.14	0.077±0.000	0.33±0.06
5 mg/kg	0.076±0.001	1.58±0.10	0.076±0.000	0.26±0.03
10 mg/kg	0.076±0.000	1.00±0.22	0.076±0.001	0.32±0.05
20 mg/kg	0.074±0.004*	0.91±0.23	0.071±0.008**	0.19±0.04

*$p<0.05$,**$p<0.001$ *compared with Negative control*

Table 4: Catalase and Malondialdehyde values in blood and liver cells of rats exposed to Luna experience SC 400 by gavage for 4 week at 48 h intervals

In this study, it was found that there is a very high correlation between the results of the comet and MN tests performed on the liver, and a moderate correlation between CAT and MDA results in both liver and blood cells. Correlation coefficient is 0.99 between GHI and DCP for liver and blood cells. In the blood cells, it is found that correlation coefficient between MN-GHI values is 0.87 and MN-DCP is 0.84. In liver cells, it is found that correlation coefficient between MN-GHI values and MN-DCP values is 0.95.

Discussion

In this study, Luna Experience SC 400, a commercial fungicide, was tested to determine the genotoxic damage and oxidative damage potential in liver and blood tissuesof rats. Genetic damage in the cells was determined using the Comet and MN methods. The CAT enzyme level was measured and lipid peroxidation was determined by measuring the oxidative damage potential and MDA level, respectively.

Luna Experience SC 400 fungicide showed a statistically significant genotoxic effect in rat liver and blood tissues due to the increase in the dose determined. The GDI and DCP values obtained as a result of the comet test increased with dose increase. When the administered doses are compared with the control group; P <0.001 for 5 mg / kg, $p < 0.001$ for 10 mg / kg and $p < 0.001$ for 20 mg / kg.

According to the results obtained from the MN test results, Luna Experience SC 400 showed statistically significant toxic effect on rat liver and blood tissues due to increase of doses of fungicide.

In our study, when comet and enzyme analyzes performed on liver and blood tissues were evaluated in general, a significant difference between measured CAT level and negative control indicated that genotoxic damage in DNA may be due to oxidative stress. No significance of the difference between the 5 mg / kg and 10 mg / kg dose groups and negative control suggests that the oxidative damage may be due to the dose increase.

An overall increase in MDA levels in the 5 mg / kg dose group was observed when the blood MDA measurements were generally evaluated, and the absence of this increase in other dose groups resulted in the rats being tolerant to lipid peroxidation.

There are similar *in vivo* and *in vitro* studies performed in different organism types, indicating the genotoxic effects of fungicides.

Sivikova, *et al*. have conducted an *in vitro* study to investigate whether a tebuconazole-based fungicide has an effect on bovine lymphocytes [11]. In the study, bovine lymphocytes were exposed to fungicide at doses of 3, 6, 15, 30 and 60 µL / mL for 24 and 48 hours. The study in the MN test was performed, an increase was noted due to the dose increase in MN frequency (p <0.05). The results of the study have shown that tebuconazole-based fungicide causes genotoxic and cytotoxic damage in bovine lymphocytes [11]. Although the organism used and the tests used in our study are different from this study, when the effect is evaluated in terms of genotoxicity; the two studies are parallel to each other. In our study, the decrease in blood PCE value due to the dose increase can be evaluated as a toxicity indicator. Bowen, *et al*. carried out a study including comet and MN tests [26]. In order to investigate the genotoxic effect of certain chemicals in rats. Doses of 1000 mg / kg, 1500 mg / kg and 2000 mg / kg were used in the study where benzimidazole group was used as a fungicide Carbendazim. As a result of comet test performed on the liver, there was a statistically significant increase due to dose increase ($p < 0,01$). There was no statistically significant increase in blood test results. There was a statistically significant increase ($p < 0.001$) due to the dose increase in the liver and blood MN test results. In this study, the experimental setup, the organism studied, the tests performed and the results of our study are similar. It has been shown that rats are susceptible to genotoxic damage, which is caused by fungicides.

Marques, *et al*. performed a study using *Anguilla anguilla* to determine whether the erythrocyte nuclear decay and comet analysis are complementary to each other in order to investigate the effect of Mancozan fungicide [27]. In this study, manzoceb, a fungicide as active substance, was applied to eel fish for 3 days at doses of 0.29 and 2.9 µg. As a result of this study with Comet test, statistically

significant increase in GDI values was observed due to dose increase ($p < 0.001$). Marques, *et al*. reported that Mancozan fungicide could cause genetic damage both at the DNA level and at the chromosomal level [27]. They stated that Comet and MN test systems would complement each other.In our study, two studies were parallel to each other, as we used the Comet test at the DNA level and the MN test at the chromosomal level to detect genetic damage. In our study as we used the comet test at the DNA level and the MN test at the chromosomal level to detect genetic damage, two studies are parallel to each other.

Sutiakova, *et al*. performed a study in sheep-derived lymphocyte cells in order to test the genotoxic effects of Euparin Multi fungicide.In the study, lymphocytes were exposed to fungus at a dose of 93 mg / kg for 28 days [28]. At the end of 28 days lymphocytes MN test was applied. It was found that the MN numbers of the experimental group were statistically significantly higher than the control group ($p < 0.05$) in the result of 1000 binuclear cells test. This study shows that although the organisms used in our study are different, the increase in MN frequency indicates that the two studies are supportive of each other.

Early cell death was reported in 80% of cells in the highest dose group. In our study, the Comet and MN test data that we have done to measure genotoxic effects support each other.In a study conducted by Schwarzbacherova, *et al* [29]. İt is reported that Tango Super fungicide caused early cell death in bovine lymphocytes owerlapped indicating the apoptosis of Type 4 data from the Comet test in Colo 320 cells in study performed by Hao, *et al.* [30]. In our also study, increase in the frequency of type 4 data of the Comet results in the liver support each other with the results have put forward by Hao, *et al.* [30]. The fact that Schwarzbacherova, *et al*. showed that the increase in genotoxic damage and mitochondrial superoxide formation, which Tango Super fungus has brought to the table, is a consequence of oxidative stress, also coincides with the significant increases in liver catalase assays in our study [29].

Ku-Centurión, *et al.* conducted a study to investigate the genotoxic effect of monocene 250 SC fungicide [31]. Funguicide-exposed zebrafish (*Danio rerio*) was used at doses of 250 µg / ml, 350 µg / ml, 625 µg / ml, 850 µg / ml and 1250 µg / ml for 2.5 hours. According to the results obtained from the comet test, the fungicide showed statistically significant increase in genotoxicity due to the dose increase. The data we obtained from this study and from our study show that fungicides can be occur similar effects in different organisms.

Jafari, *et al*. reported that Wistar rats were sensitive to pesticide exposure and that catalase levels decreased in this study when they investigated the oxidative damage potential of paraoxon pesticides [32]. Catalase is present in all major body organs, but is particularly concentrated in the liver. A decrease in liver catalase enzyme activity indicates oxidative damage to the liver.

In study conducted by Troudi, *et al*., rats exposed to 2,4 D showed a significant increase in malondialdehyde level and a decrease in non-enzymatic and enzymatic antioxidant system [33]. and also our study, the decrease in catalase enzyme activity in the enzymatic antioxidant system in funguicide-exposed rats results in the induction of oxidative stress by fungicide. To our knowledge, this study is the first report to investigate the genotoxic effect of Luna experience SC 400 fungicide and its relation to oxidative damage.

In conclusion, the present data showed that exposing adult rats to Luna Experience SC 400 funguicide led to increase in MN frequency and damage levels in Comet assay in both liver and blood tissues and decrease in catalase activity. This could be explained by oxidative stress induced by Luna Experience SC 400 in both blood and liver.The examination of the more detailed effects of fungicide tested in this study is important for the detection of harmful effects. For example, the investigation of the relationship with gene activity would provide clarity on the molecular basis.

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References

1. Ahmed FE (2001) Analyses of pesticides and their metabolites in food and drinks. Trends Anal Chem 20: 649-61.

2. Miller GT (2001) Living in the Environment.12.

3. Damalas CA, Eleftherohorinos IG (2011) Pesticide exposure, safety issues, and risk assessment indicators. Int J Environ Res Public Health 8: 1402-19.

4. Ridding The World of POPS: (2005) A Guide to The Stockholm Convention on Persistent Organic Pollutants. United Nations Environment Programme.

5. Soares WL, Porto MF (2012) Pesticide use and economic impacts on health. Rev Saude Publica 46: 209-17.

6. Benedetti D, Nunes E, Sarmento M, Porto C, Ioshims dos Santos, et al. (2013) Genetic damage in soybean workers exposed to pesticides: evaluation with the comet and buccal micronucleus cytome assays. Mutat Res 752: 28-33.

7. Cook SM, Khan ZR, Pickett JA (2007) The use of push-pull strategies in integrated pest management. Annu Rev Entomol 52: 375-400.

8. Haverkate F, Tempel A, Den Held AJ (1969) Interaction of 2,4,5-trichlorophenylsulphonylmethyl thiocyanate with fungal spores. Netherlands J Plant Pathol 75: 308-15.

9. Norwegian Scientific Committee for Food Safety, 19.02.2014. Secondary "Risk assessment of the fungicide Luna Privilege with the active substance fluopyram".

10. Shah PV, Yoshida M, Tasheva M (2010) Tebuconazole, WHO/JMPR, 503-64.

11. Sivikova K, Dianovsky J, Holeckova, B, Galdikova M, Kolesarova V (2013) Assessment of cytogenetic damage in bovine peripheral lymphocytes exposed to in vitro tebuconazole-based fungicide. Chemosphere 92: 555-62.

12. Ferreira D, da MottaAC, Kreutz LC, Toni C, Loro VL, et al. (2010) Assessment of oxidative stress in Rhamdia quelen exposed to agrichemicals. Chemosphere 79: 914-21.

13. Toni C, LoroVL, Santi A, de Menezes CC, Cattaneo R, et al. (2011) Exposure to tebuconazol in rice field and laboratory conditions induces oxidative stress in carp (Cyprinus carpio). Comp Biochem Physiol C Toxicol Pharmacol 153: 128-32.

14. Çavaş T, Könen S (2007) Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (Carassius auratus) exposed to a glyphosate formulation using the micronucleus test and the comet assay. Mutagenesis 22: 263-68.

15. Eke D, Çelik A, Yılmaz BM, Aras N, Kocatürk Sel S, et al. (2017) Apoptotic gene expression profiles and DNA damage levels in rat livertreated with perfluorooctane sulfonate and protective role ofcurcumin. Int J Biol Macromol 104: 515-20.

16. Eke D, Çelik A (2016) Curcumin prevents perfluorooctane sulfonate induced genotoxicity and oxidative DNA damage in rat peripheral blood. Drug Chem Tox 39: 97-103.

17. Buonocore G, Perrone S, Tataranno ML (2010) Oxygen toxicity: chemistry and biology of reactive oxygen species. Semin Fetal Neonatal Med 15: 186-90.

18. Alpertunga B, Catalgol B, Catalgol S (2009) Effects of benzimidazole fungicides, benomyl and carbendazim, on lipid peroxidation and antioxidant system in rat. Toxicol Lett 189: 212.

19. Hayashi M, Tice RR, Macgregor JT, Anderson D, Blakey DH, et al. (1994) In vivo rodent erythrocyte micronucleus assay. Mutat Res 312: 293-304.

20. Singh NP, McCoy MT, Tice RR, Schneider ELA (1988) Simple technique for quantitation of low levels of DNA damage in individuals cells. Experimental Cell Res 175: 184-91.

21. Holden HE, Majeska JB, Studwell D (1997) A direct comparison of mouse and rat bone marrow and blood as target tissues in the micronucleus assay. Mutat Res 39: 87-9.

22. Weber K, Goerke H (2003) Persistent organic pollutants (POPs) in antarctic fish: levels, patterns, changes. Chemosphere 53: 667-67.

23. Aebi HE, Bergmeyer J, Grabl M (1983) "Catalase. In: Methods of enzymatic analysis". Eds. Verlag Chemie, Weinheim 3: 273-86.

24. Güler G, Çelik A (2016) 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Genotoxicity and Oxidative Damage Potential in Human Peripheral Blood Lymphocytes. J Carcinogene Mutagene 7: 1-6.

25. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351-8.

26. Bowen DE, Whitwell JH, Lillford L, Henderson D, Kidd D, et al. (2011) Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the Comet assay and the flow-cytometric peripheral blood micronucleus test. Mutat Res 722: 7-19.

27. Marques A, Rego A, Guilherme S, Gaivão I, Santos MA, et al. (2016) Evidences of DNA and chromosomal damage induced by the mancozeb-based fungicide Mancozan(R) in fish (Anguilla anguilla L.). Pestic Biochem Physiol 133: 52-58.

28. Sutiakova I, Kovalkovicova N, Pisti J, Novotny J, Legath J (2006) Chromosomal aberrations and frequency of micronuclei in sheep subchronically exposed to the fungicide Euparen Multi (tolylfluanid). Ecotoxicol Environ Saf 64: 312-20.

29. Schwarzbacherová V, Wnuk M, Lewinska A, Potocki L, Zebrowski J, et al. (2017) Evaluation of cytotoxic and genotoxic activity of fungicide formulation Tango(R) Super in bovine lymphocytes. Environ Pollut 220: 255-63.

30. Hao H, Lei F, Nancai Y, Wen S, Xiaojuan Q (2009) The single-cell gel electrophoresis assay to determine apoptosis induced by siRNA in Colo 320 cells. African J of Biotech 8: 3731-3.

31. Ku-Centurión M, Calderón-Segura ME, Maldonado E (2016) Detection of DNA damage in zebrafish (Danio rerio) embryos exposed to Monceren® 250 SC fungicide through alkaline comet assay. Toxicol Lett 259: S183.

32. Jafari M, Salehi M, Asgari A, Ahmadi S, Abbasnezhad M, et al. (2012) Effects of paraoxon on serum biochemical parameters and oxidative stress induction in various tisuues of Wistar and Norway rats.Environ Toxicol Pharmacol 34: 876-87.

33. Troudi A, Sefi M, Amara BI, Soudani N, Hakim A, et al. (2012) Oxidative damage in bone and erythrocytes of suckling rats exposed to 2,4-dichlorophenoxyacetic acid. Pestic Biochem Physiol 104: 19-27.