Sub-Chronic Potential Effects of Opera Fungicide on Hematological and Liver Functions Parameters of Female Albino Rats

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ABSTRACT

Opera is a fungicide formulation contains a mixture of two-compounds, epoxiconazole and pyraclostrobin at the ratio of 50 and 133 g/L, respectively. This fungicide is widely applied in grain protection. However, details of Opera effects on liver biomarkers enzymes, hepatotoxicity, immunotoxicity and hematotoxicity have not been fully investigated. In this study, female albino rats were administered 287 mg/L Opera concentration in the water along 90 days (group I). The second group (group II) was administered 287 mg/L Opera for 90 days in drinking water, followed by untreated water for extra 30 days as a post exposure period. Our experiments revealed that this fungicide was able to induce some liver biomarker enzymes by elevated levels of alkaline phosphatase (ALP) and total antioxidants capacity (TAC) in plasma. While treatment decreased significantly the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and did not change catalase (CAT) during exposure period (90 days).The effect of Opera on hematological constituents; white blood cells (WBC), [total count and differential; lymphocytes (Lymph %), monocytes (Mon %) and granulocytes (Gran %)], red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), platelets (PLT) and plateletcrit (PCT). Erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cells distribution width (RDW), platelet distribution width (PDW), and mean platelets volume (MPV) of rats were also investigated under laboratory conditions. Results showed that Opera-treated group had a significantly higher in WBC count, Lymph%, Mon%, PLT, MCV, PCT and RDW. While, significantly lower in Gran% RBC, MPV, HCT% and PDW%. Our data showed that Opera at 287mg/L could suppress immune system in rat. This study may be helpful to determine the future risks of diseases in people who have been working with this fungicide.

Keywords: Opera, epoxiconazole, pyraclostrobin, biomarkers, Rats

INTRODUCTION

Opera is formulated as a suspo-emulsion containing two active ingredients; pyraclostrobin at 133 g/L and epoxiconazole at 50 g/L. Pyraclostrobin, methyl [2-[[[1 (4-chlorophenyl)-1Hpyrazol-3-yl] oxy] methyl] phenyl] methoxycarbamate has been widely used. Pyraclostrobin is one of the world's leading strobilurin fungicides. Epoxiconazole is also widely systemic fungicide (2RS, 3SR)-1-[3-(2-chlorophenyl)-2, 3-epoxy-2-(4-fluorophenyl) propyl]-1H-1, 2, 4-triazole) used to control a wide range of fungal diseases. Pyraclostrobin is an ideal partner to mix with epoxiconazole for use as a fungicide. The combination of two active ingredients in Opera fungicide has a high

efficiency (Nufarm, 2010). Epoxiconazole is a member of the triazole group of fungicides (Sunderland *et al*., 2014) and acts by inhibiting component of fungal membrane, biosynthesis of ergosterol, at the sterol. This fungicide has been on the market for almost 20 years and it has been used to protect cereals and oleaginous plants from diseases caused by Ascomycetes, Basidiomycetes and Deuteromycetes, which mainly affect plants. While the second active ingredient is Pyraclostrobin, a member of the strobilurin group of fungicides and acts effect on ATP production (Karadimos *et al*. 2005), by binding to cytochrome b, thus blocking electron transfer in the respiration pathway (Kraiczy *et al.*, 1996).

Regardless their effectiveness agricultural applications, Fungicides in general (and epoxiconazole in particular) can be extremely toxic to humans and wild life and have high stability in the environment (Gianfreda and Rao 2004; Veiga *et al*. 2006). In fact, epoxiconazole was prevented by the European Union in 1997 on account of its low degradability, high toxicity to aquatic organisms and human body, inclusive the development of hormone-associated cancers (European Commission 1997; Cocco 2002).

Inappropriate usage of pesticides by farmer leads to environmental contamination (Barcelo *et al.* 1994; Lartiges and Garrigues 1995; Vink and Vanderzee 1996). In addition, development of biomarkers to investigate the exposure level in occupationally exposed groups and in samples from the general population is warranted. Until more is known about the potential risk of these compounds, efforts should be taken to minimize the exposure of especially women in the childbearing age group as well as children to these compounds.

The toxicological and clinical pathology fields are of fundamental importance because many countries in the world have different regulatory authorities, whose duty is to evaluate toxicological data from pre-clinical toxicity and safety studies for any pesticides. Abnormal treatment related values could represent changes pertaining to pharmacological and/or toxicological effects. These changes could be regarding tissue morphology, detected by histopathological evaluation, and/or alterations in a series of *in vivo* analyzed parameters. Dose-related changes are also of crucial importance (Matsuzawa *et al*., 1995). Among these parameters, clinical chemistry and hematology data are of great importance for determining effects induced by treatment. Assessment of repeated dose toxicity and Specific target organ toxicity for the Opera formulation has not been tested (BASF, 2015). After repeated exposure of pyraclostrobin the prominent effect is local irritation and may cause damage to the olfactory epithelium after repeated inhalation. While, repeated exposure to large quantities of epoxiconazole may affect certain organs (BASF, 2015). Because pyraclostrobin fungicide inhibits cellular respiration in mitochondria by blocking electron transfer and reducing energy production, it may also impact non-target organisms (Bartlett *et al*., 2002). Toxicity of pyraclostrobin and its formulations have been reported in amphibians and aquatic invertebrates (Morrison *et al*.,

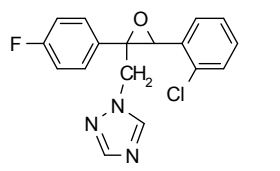
2013). Pyraclostrobin has high acute hazard index, aHI (Lozowicka, 2015).

Opera fungicide formulation has not yet been totally toxicologically investigated; only scarce information about the single active agents and/or their commercial formulations is available. Since there are no available data for epoxiconazole/pyraclostrobin effects on hematological parameter and hepatic enzyme levels in rat, we decided to assess the potential Hepatotoxicity and blood intoxication effect of Opera commercial fungicide formulation on rat**.**

MATERIAS AND METHODS

Testing fungicide

The selected fungicide was Opera ND 183 g/L, suspo-emulsion (SE). It is a product of BASF Company, Ludwigshafen, Germany. It consists of two active ingredients epoxiconazole 50 g/L (4.7% w/w) and pyraclostrobin 133g/L (12.5% w/w). LD_{50} rat (oral): 287 mg/kg (OECD Guideline 423, 2010). The chemical structure of pyraclostrobin and epoxiconazole were shown in Figure (1).



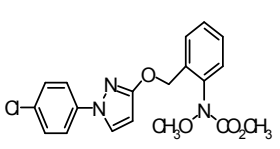


Figure (1): The chemical structure of pyraclostrobin and epoxiconazole

Animals

Six weeks old female albino rats were obtainedfrom faculty of veterinary medicine, Cairo University and acclimatized to the laboratory conditions for at least 2 weeks.Immediately after arrival, all animals were weighed, monitored fortheir health status and allocated in a randomized manner to thedifferent study groups. Rats were caged in groups of six per each cages and kept under standard laboratory conditions ata constant 12:12h light: dark cycle with controlled temperature (25 ?C±2 ?C) and humidity (50–60%). All animals had *ad libitum*access to tap water and diet. When treatment commenced, animals were atan age of 8 weeks and with an individual body weight rangeof 95–125g.

Experimental design

Twenty four female albino rats were involved in this study. Rats were randomly divided into four groups of six rats each. Two groups were used as a control. Another two groups, groups I was administrated with Opera (287 mg/L),which is equivalent to LD_{50} of rats, in drinking water for 90 days. Group II was administered with 287 mg/L Opera in drinking water for 90 days, followed by untreated water for extra 30 days as a post exposure period.The initial and final body weights were recorded every 10 days.

Twenty-four hours after completion of each period (90 or 120 days), the rats were anesthetized by diethyl ether. Blood was collected from all rats by cardiac puncture using a 30 gauge needle. Blood samples were divided into two aliquots in vacutainer blood collection tubes contained disodium salt of ethylene diamine tetra acetic acid (EDTA) as

anticoagulant. One aliquot was centrifuged immediately at 3000 rpm for 15 min at 4□C using cooling centrifuge (Model universal 30 RF, Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany) to obtain plasma. Plasma samples were stored at −20◦ C for further measurement of liver function. Second aliquot of blood samples were used immediately for determination hematological parameter. The relevant organs; liver, kidney and spleen were excised and cleaned in saline solution, dried on tissues paper and weighed at the end of each period.

Measurement of hepatic enzyme levels

Plasma enzyme activities: Aspartame aminotransferase (AST), Alanine aminotransferase (ALT), and Alkaline phosphatase (ALP) were measured using a biochemical analyzer SpinReact, Spain. The hepatic enzymes kits were obtained from Diasys Company, Germany. Catalase (CAT) and total antioxidant capacity (TAC) were measured using spectrophotometer (Shimadzu, Tokyo, Japan). The kits for CAT and TAC were obtained from Biodiagnostic Company, Egypt. All other chemicals were of reagent grades and were obtained from reputed companies. Hepatic enzyme levels, AST and ALT were determined according to the methods of Reitman and Frankel (1957), ALP according to Young *et al*. (1975), CAT was determined according to the method of Aebi (1980) and TAC was colorimetric determined according to Koracevic *et al*. (2001).

Determination of the hematological parameters

Blood samples were analyzed immediately for hematological parameter using an automatic hematological assay analyzer (Horiba medical, APX micros 60, Japan). Routine hematological parameters were assessed according to Dacie and Lewis (1986) as; white blood cells (WBC), [total count and differential; lymphocytes (Lymph %), monocytes (Mon %) and granulocytes (Gran %)], red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), platelets (PLT) and plateletcrit (PCT). Erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cells distribution width (RDW), platelet distribution width (PDW), and mean platelets volume (MPV).

Statistical analysis

The parameter values of control and treated animals were compared and presented as mean ± standard error (SE). Student's t-test was performed at $P<0.05$ and $P<0.01$ to determine the significant differences among the different treatments.

RESULTS AND DISCUSSION

Environmental toxicants, such as pesticides, may produce a variety of clinical manifestations in renal, hepatic and immune system. In daily diet, humans are continuously exposed to a variety of pesticide residues that might lead to cumulative toxicity and potentially affect human health (Hass *et al*., 2012; Rieke *et al*., 2014; Cedergreen, 2014; Kortenkamp, 2014; Schmidt *et al*., 2016). The fungicides have been applied worldwide to several cereals, as well as vegetables, fruits, and nuts.

Since triazole fungicides are designed to inhibit a fungal cytochrome-P-450 (CYP) enzyme, it is conceivable that an important part of their toxic effects in mammals is due to an unspecific

inhibition of mammalian CYP enzymes including some CYPs important for the biosynthesis of steroid hormones (Heise *et al*., 2015). On the other hand, strobilurins block the complex III at the mitochondrial electron transport chain (Bartlett *et al*., 2002; Reddy, 2013).

The hepatotoxic properties of many triazoles have been elucidated in a number of standard toxicity tests for their approval as active substances for pesticidal use summarized in Goetz and Dix 2009a, b; EFSA 2008a, b. Some triazoles like cyproconazole or epoxiconazole also cause hepatocellular tumours (EFSA 2008a, 2010; Hester et al., 2012). Even with the over use of Opera, few data have been obtained about its influence on the immune system. Moreover, sub chronic effect of Opera on the immune system has not been clearly defined. An extensive and systemic study on dose time relationship in different experimental animals appears to be essential to establish the definitive immunomodulatory role of Opera keeping this in view.

The effect of Opera on body and organs weights

No unscheduled deaths, no treatment-related clinical signs and no changes in behavior of tested animals were noted. A marked decrease in food consumption was initially observed during first 10 days of the treatment. When the Opera was administered (287 mg/L), body weight was reduced in the first 10 days of treatment in group I but was similar to the controls for the rest of the study. At termination, mean body weights in group I were still lower than in the controls but the difference was not statistically significant Figure (2). Similar result was observed also for group II during first 10 days of treatment but was typical to the control during the rest of exposure period (90 day). While during the post exposure period (30 day) the mean body weights in group II were higher than in the controls but the difference was not statistically significant (Figure 3).

Table (1) shows the body weight gain, liver, kidney and spleen weights of rats received 287mg/L Opera in drinking water. The body weight gain of both groups decreased slightly as compared with the control groups.

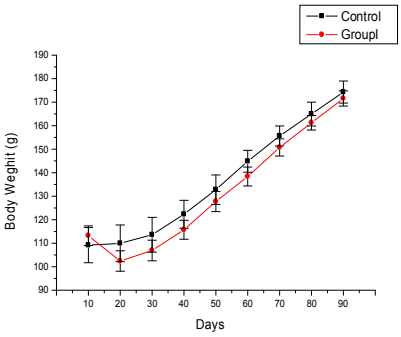


Figure (2): Body weight of rats treated with Opera for 90 days (group I).

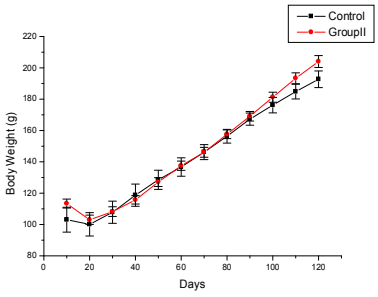


Figure (3): Body weight of rats treated with Opera for 90 days, followed by untreated water for extra 30 days (group II).

Opera concentration has no significantly effected on body weight gain. The treated rats, group I and group II showed a body weight gain of 50.57% and 75.71%, respectively, Table (1). Non-significantly changes were observed during 90 day of the treatment in absolute and relative weight of the organs, whereas significant decrease was observed in absolute and relative liver weight during post exposure period. In contrast the absolute and relative spleen weights were significantly increased, but absolute and relative kidney weights were not affected during post exposure period.

Table (1): The effect of Opera on the body and organs weights

Tested Groups	Parameter (mean±SE; n=6)						
	%Body weight gain	Liver weight		Kidney weight		Spleen Weight	
		Absolute(g)	Relative	Absolute(g)	Relative	Absolute(g)	Relative
Control	63.76±10.7	7.13±0.70	4.00±0.44	1.30±0.06	0.73±0.04	0.69±0.11	0.39±0.06
Group I	50.57±5.37	7.38±0.63	4.04±0.34	1.29±0.06	0.70±0.02	0.68±0.04	0.37±0.03
Control	84.98±4.93	7.67±0.22	4.04±0.20	1.33±0.07	0.73±0.01	0.65±0.04	0.34±0.01
Group II	75.71±1.84	6.27±0.37*	3.03±0.14*	1.38±0.06	0.66±0.02	0.86±0.05*	0.41±0.03*

Each value is a mean of 6 animals ± S.E.; significantly different from control: *$p < 0.05$, **$p < 0.01$. Body weight gain (%) = [(Final B. Wt. - Initial B. Wt.) / Initial B. Wt.]X100; relative weight = (Organ weight/ Body weight) X 100.

Some results in previously research are consistent with ours. Mellert *et al.* (1999a, 1999b) studied the effect of pyraclostrobin on organ and body weights after exposure for 90 and 120 days in a diet. They observed that pyraclostrobin caused a significant decreased in relative liver weight and body weight gain (8%) in male rats at 250 mg/kg bw/day. In the present study decreased organ weight by Opera exposure may be due to accumulation of its substitutes in the tissues. Liver receives up to 40% of dose (Mellert *et al*., 1999c). Liver concentration is influenced by humoral factor including adrenocorticotropic hormones. Pesticide interference with liver metabolism resulted in decreased liver weight. In contrast, the absolute and relative spleen weight were significantly increased. Spleen is the site of extramedullary erythropoiesis and removal of damaged blood cells, spleen is the major filter of blood borne antigens including toxicants bound with serum proteins. The spleen is an important part of the body's defense (immune) system. It contains special white blood cells that destroy bacteria and help the body fight infections. In this study correlation between increase of spleen weight and increase of WBC was observed (Mellert *et al*., 1999a, 1999b; Menges *et al*., 1999).

Liver function biomarkers

Few studies about Opera toxicity in animals, aquatic organisms and humans are available even with the wild use of Opera. Thus, we were unable to compare our results with previous ones. Our results were therefore compared to those studying toxic effect of epoxiconazole and pyraclostrobin as individual fungicides. Five serum hepatic

markers enzymes; AST, ALT, ALP, CAT and TAC were evaluated for hepatotoxicity of Opera on female rats.

The plasma biochemical parameters in this work revealed variations in Opera administrated groups compared to the untreated control groups. Results presented in Table (2) showed a significant decrease in the ALT and AST plasma activities due to the impact of Opera exposure. A significant increase in ALP and TAC was recorded in Opera treated group compared to untreated group. No significant change in catalase after 90 days exposure to Opera-in drinking water, when compared with the control group. In post exposure-group (group II), high significant changes in ALT plasma level was observed when compared with control. On the other hand, the AST, ALP, CAT and TAC did not significantly change in this group when compared with the control group, Table (2).Ours results corroborate with those found by Mellert *et al*. (1999a, 1999b).They indicated that the pyraclostrobin reduced AST and ALT in the 28^{th} day and long-term studies, 90 days in rats. Also, effect of Opera could take place via the absorption, distribution or elimination rates. In addition, induction and inhibition of the liver microsomal enzymes (cytochrom P450) could also play significant roles in pesticides toxicity (Dhasarathan et al., 2010). Whereas, Heise *et al*. (2015) noticed that no significant changes in any of the typical liver enzymes ALT and AST were observed after 28-days rat-feeding of epoxiconazole at high dose (900 ppm). Schmidt *et al*. (2016) found that epoxiconazole content was higher in liver tissues when administrated in combination with cyproconazole than after single substance administration.

Hematological parameters

Table (3) shows high significant increase ($p < 0.01$) in WBC, Lymph %, Mon%, PLT and MCV and significant increase ($p < 0.05$) was observed in PCT and RDW. On two hand, Gran% and PDW% were decrease highly significant ($p < 0.01$). Moreover, significant decrease ($p< 0.05$) was also recorded in RBC, HCT% and MPV. On the other hand, a non-significant decrease in treated rat HGB, MCHC and MCH levels was observed. After 30 day, post exposure period, group II revealed variations in Hematological results compared to control group. The PLT, Lymph % and Mon% were highly significant increase ($p<0.01$). Moreover, WBC and PCT showed significant increase ($p< 0.05$). In addition, RBC, HGB and all Erythrocyte indices were non-significant changed in comparison with control group. While, there was a high significantly decreased in Gran%.

Hematopoietic system is one of the most sensitive systems to assess the toxicity of environmental toxins and drugs in humans and animals (Yuan *et al*., 2014). The effects of Opera on rat hematology are not studied well (HRAU, 2016). However, some preliminary studies have indicated that occupational exposure to some pesticides can significantly affect the blood constituents (Khan *et al*., 2013) in human. Similarly, several studies have been conducted in animals which report that pesticides alter the hematology of animals (Khatun *et al*., 2014). The part of current study regarding the effects of Opera on hematology of rats showed an increase in the levels of WBC, lymph %, Mon%, PLT, MCV, PCT and

RDW, while the levels of Gran%, PDW%, RBC, HCT% and MPV were decreased (group I). However, the exposure to Opera did not dramatically alter the levels of HGB, MCHC and MCH. Interestingly, WBC and PLT were the most affected parameters (group I). White blood cell (WBC, leukocyte) count with White blood cell types (WBC differential) plays different role in protecting the body. The numbers of each one of these types of white blood cells give important information about the immune system. Too many or too few of the different types of white blood cells can help find an infection, an allergic or toxic reaction to chemicals. The increase of WBC values (Leukocytosis) could be normal reaction of white cells against any foreign substance.

Table (2): Clinical chemistry findings in rat's plasma exposed to Opera for 90 days and 30 days recovery period.

Enzymes	Experimental groups			
	Control	Group I	Control	Group II
ALT (U/L)	106.33 ± 23.88	39.20 ± 3.26**	84.67 ± 7.79	40.75 ± 6.26**
AST (U/L)	52.33 ± 1.85	19.64 ± 3.39**	26.2 ± 5.34	22.57 ±2.36
ALP (U/L)	262 ± 13.86	397.6 ± 40.41*	191.67 ± 12.17	217 ± 4.73
CAT (U/L)	555.94 ± 35.81	596.89 ± 40.89	514.42 ± 16.15	565.17 ± 21.70
TAC (mM/L)	0.73 ± 0.13	1.18 ± 0.06*	0.80 ± 0.18	0.74 ± 0.08

Each value is a mean of 6 animals ± S.E.; significantly different from control: *$p < 0.05$, **$p < 0.01$

Table (3): Hematology findings in rat's plasma exposed to Opera for 90 days and 30 days recoveryperiod.

Parameters	Units	Experimental groups			
		Control	Group I	Control	Group II
WBC	10^3 /mm^3	5.13±0.22	7.16±0.62**	6.17±0.6	7.48±0.33*
RBC	10^6 /mm^3	6.95±0.20	5.87±0.47*	6.63±0.22	6.38±0.36
HGB	g/dl	12.63±1.13	11.82±0.16	10.97±1.44	11.46±0.25
HCT	%	36.9±6.24	33.54±0.24*	35.46±1.76	34.34±0.86
PLT	10^3 /mm^3	186.00±23.28	283.40±27.37**	206.00±6.5	284.20±10.2**
PCT	%	0.13±0.01	0.23±0.02*	0.16±0.029	0.23±0.017*
Differential count					
Lymph	%	73.30±3.09	88.85±6.35**	73.48±1.39	85.68±1.59**
MON	%	11.27±0.09	15.38±1.02**	11.93±0.18	14.48±0.48**
Gran	%	13.10±0.23	9.36±0.38**	13.2±0.26	10.0±0.25**
Erythrocyte indices					
MCV	µm^3	53.00±0.58	57.16±0.31**	54.33±0.33	54.50±0.67
MCH	Pg	18.37±0.47	18.71±0.34	18.37±0.47	18.71±0.33
MCHC	g/dl	33.97±0.69	34.45±0.43	33.97±0.69	34.45±0.43
RDW	%	14.83±0.12	16.07±0.26*	14.83±0.12	15.23±0.46
MPV	µm^3	9.27±0.30	6.28±0.14*	8.27±0.98	8.28±0.39
PDW	%	2.77±0.13	1.55±0.13**	2.43±0.29	2.05±0.21

Each value is a mean of 6 animals ± S.E.; significantly different from control: *p < 0.05, **p < 0.01

White blood cell (WBC, leukocyte) count with White blood cell types (WBC differential) plays different role in protecting the body. The numbers of each one of these types of white blood cells give important information about the immune system. Too many or too few of the different types of white blood cells can help find an infection, an allergic or toxic reaction to chemicals. The increase of WBC values (Leukocytosis) could be normal reaction of white cells against any foreign substance. Leukocytosis is a usual body response to an underlying pathophysiological condition. The leukocytosis observed in this study probably points to proliferative response by the immune cells due to the presence of the fungicide residues in the blood stream of the Opera treated groups. Leukocyte increase demonstrated the guard of rat immune system after exposing to Opera. (HRAU, 2016) reported that Pyraclostrobin was increased white blood cell counts in oral toxicity studies at HED (human equivalent dose) doses > 100-200 fold higher than the short-term, subchronic and chronic RfDs (reference doses). The increase of WBC values seems to concern particularly neutrophils and could result from an inflammation caused by Opera general toxicity (Vial *et al*., 1996). Also, leukocytosis detected in rats exposed to Opera pesticide may be due to release of catecholamine as a stress hormone, which causes demargination of neutrophils, is often accompanied by lymphocytosis and sometimes by monocytosis and eosinophilia (Jain, 1993). The immune system is synonymous with circulating white blood cells, all of which derive from a single precursor, the pluripotential hemopoietic stem cells (Scott and Gordon, 1995).

Hematocrit (HCT) and packed cell volume (PCV): This test measures the amount of space (volume) red blood cells take up in the blood. The value is given as a percentage of red blood cells in a volume of blood. In this study, a hematocrit values was 33.54 means that 33.54 % of the blood's volume was made of red blood cells in group I. It was significant decreased than in control samples. Hematocrit and hemoglobin values were the two major tests that show if anemia is present.

Platelets (PLT) or thrombocytes are the smallest type of blood cell. They are important in blood clotting. If there are too many platelets, there is a chance of a blood clot forming in a blood vessel. Also, platelets may be involved in hardening of the arteries (atherosclerosis). Which is an important site for calcium storage. In the present study we demonstrated a highly significant increase in platelets. Which may be related to anemia. Mean platelet volume (MPV) measures the average amount (volume) of platelets, which used along with platelet count to diagnose some diseases.

After 90 days of treatment, a significant decline in erythrocyte counts count was observed. This disturbance of biochemical and physiological RBC function, low RBC (anemia), could be due to lipid peroxidation (Akhgari *et al*., 2003). In addition, oxidative damage in RBC may results from the presence of polyunsaturated fatty acid, heme iron and oxygen (Kale *et al*., 1999). Moreover, Muthuviveganandavel *et al.* (2008) found that red blood cells count significantly

decreased in rats treated with 5 and 25 mM Carbendazim (carbamate pesticide).

The hemolysis of Red blood cells have been reported in various physical and chemical stress (Safronov and Maiorava, 1978; Coakley *et al*., 1979). Under such condition the total red blood cell count is expected to show a decline in number. The observed decrease in the red blood cell count can be accounted for the possible mechanisms such as decrease production of renal erythropoietin which stimulates the bone marrow and spleen to release more erythrocytes (Mahananda and Mohanty, 2012). Moreover, severe oxidative stress can lead to hemolysis. In principle, the erythrocyte is very sensitive to peroxidative reactions. Under normal conditions, erythrocyte is very well protected against peroxidative reactions by the presence of catalase and glutathione. This balance, however, can be disturbed by different chemicals. Oxidative damage can result indirect injury to the cell membrane due to lipid peroxidation and bring about changes in membrane proteins and consequently, the permeability of such membranes of any erythrocyte (Bush, 1991; Kandil *et al*., 2006).

There are three red blood cell indices; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The MCV shows the size of the red blood cells. The MCH value is the amount of hemoglobin in an average red blood cell. The MCHC measures the concentration of hemoglobin in an average red blood cell. These numbers help in the diagnosis of different types of anemia. Red cell distribution width (RDW) can also be calculated, which shows if the cells are all the same or different sizes or shapes. Determination of erythrocytic indices, are helpful in classifying certain anemias (Pratt, 1985). From this study, the levels of MCV and RDW were significantly high in the group that was exposed to Opera treatment and the level of MPV and PDW were significant low in the same group. These findings may be due to the disruptive action of the Opera on the erythropoietic tissue or erythrocyte volume, alternatively, they can be an adaptive reaction of bone marrow to a reduced viability of circulating cells because of oxidative damage (Carmen *et al*., 2016). Earlier, humans exposed to 2, 4- D have suffered bone marrow depression and changes in erythrocyte volume and size (Brandt, 1971; Radinov *et al*., 1967).

The study revealed that toxicity of Opera significantly changes some of hematological parameters and liver enzymes levels in female rats. On the other hand, the recovery period lead to reverse some injury effect, which occurred during the period of treatment. Thus, exposure to pesticides may pose a serious threat for human health. The careful application of pesticides in agriculture and other practices may be highly recommended. However, the limited number of samples analyzed and the limited study area, the results may remain tentative. It may be desirable to carry out some further studies taking large study area, toxicokinetic interactions between the epoxiconazole and pyraclostrobin fungicides. And also to determine the Opera fungicide residues in the circulating blood of the

agriculture workers who have been exposed to this fungicide.

CONCLUSION

From the current study, it has been concluded that the exposure to Opera fungicide significantly affect liver biomarkers in rat and some of the hematological parameters. The alternations in the enzymes level and blood constituents might negatively impact human health, especially agriculture workers and may be alarming signals in these workers. This study may be helpful to determine the future risks of diseases in people who have been working with this fungicide.

Conflicts of interest

There are no conflicts of interest to declare.

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التأثير التحت مزمن المحتمل لمبيد اوبرا الفطرى على قياسات الدم ووظائف الكبد على إناث الفئران البيضاء

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اجريت هذه الدراسه بهدف دراسه التأثير التحت مزمن المحتمل لمبيد اوبرا الفطرى على قياسات الدم ووظائف الكبد على إناث الفئران البيضاء, حيث تم معامله مجموعه من الفئران بتركيز 287 مللجم/كلجم عن طريق الفم من خلال ماء الشرب لمده 90 يوم بالأضافه لمعامله مجموعه اخرى من الفئران بنفس التركيز ولمده 90 يوم تتبعها 30 يوم فترة شفاء تناولت الفئران خلالها ماء غير معامل وتم تقييم التأثير السام للمبيد للمجموعتين على وظائف الكبد من خلال تقدير الانزيمات فى البلازما مثل alkaline phosphatase (ALP) total antioxidants capacity (TAC) alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and did not change catalase (CAT).كذلك تم دراسه التأثير السام على قياسات الدم التاليه: white blood cells (WBC), lymphocytes (Lymph %), monocytes (Mon %) and granulocytes (Gran %)], red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), platelets (PLT) and plateletcrit (PCT) mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cells distribution width (RDW), platelet distribution width (PDW), and mean platelets volume (MPV)
وقد اظهرت النتائج زياده معدلات النشاط الانزيمى لكل من (ALP) و (TAC) وانخفاض مستوى كل من (ALT) و (AST) معنويا مقارنه بالفئران الغير معامله بينما لم يتأثر مستوى انزيم الكتالاز فى البلازما . وقد اظهرت النتائج ان فتره الشفاء (30 يوم-المجموعة الثانية) أعادت النشاط الانزيمى الى المستوى الطبيعى حيث لم تظهر اختلاف معنوى فى معدلات نشاطها فيما عدا نشاط انزيم (ALT) الذى استمر فى الانخفاض معنويا.كما اوضحت قياسات الدم ان معامله مبيد الأوبرا لمده 90 يوم قد اثرت بالزياده المعنويه على كل من WBC, Lymph%, Mon%, PLT, MCV, PCT and RDW بينما انخفض معنويا كل من Gran% RBC, MPV, HCT% and PDW% ولم تتأثر قياسات كل من HGB, MCHC and MCH. وقد اوضحت النتائج ان فتره الشفاء قد ساعدت فى تحسين بعض القياسات فى الدم مثل قياسات مؤشرات كرات الدم الحمراء. بينما ظلت قياسات كل من WBC and PCT PLT, Lymph % Mon% مرتفعه معنويا واستمر الانخفاض المعنوى لنسبه Gran% فى الدم. وتظهر هذه الدراسه ان المبيد الفطرى اوبرا يؤثر على نظام المناعه فى جسم الفئران وان فتره الشفاء لم تكن كافيه للحد من تأثير المبيد على بعض قياسات الدم ووظائف الكبد مما يترتب عليه ان تكون نتائج هذه الدراسه مفيده فى تحديد المخاطر المتوقعه من استخدام هذا المبيد وخاصه على عمال المزارع ومطبقى المبيد وكذلك السيدات والاطفال.
توصى الدراسه الحاليه باتباع التوصيات وإتخاذ الاحتياطات اللازمه عند تطبيق مبيد الاوبرا وعلى الرغم من ان عدد العينات خلال هذة الدراسه محدود فالمزيد من الدراسات مطلوب اجرائها