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TRIAZOLE FUNGICIDE PROSARO EC 250 - INDUCED HEMATOLOGICALAND BIOCHEMICAL ALTERATIONS IN MALE RABBITS *(ORYCTOLAGUS CUNICULUS)*

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ABSTARCT

The study aimed to evaluate the toxic effects of fungicide Prosaro 250 EC on hematological profiles and renal functional-biochemical markers, as well as blood glucose level .Twenty-four male rabbits were equally and randomly divided into control untreated group (G1) (n =6) received distilled water and standard food, and three treated groups (n=6 each) received by oral gavage Prosaro 250 EC at three doses (mg/kg body weight (bw)), 0.093 (G2), 0.186 (G3) and 0.465 mg/kg bw (G4) for 21 days .Results revealed a significant decrease in the kidney weight, and a significant increase in the levels of serum urea, uric acid, creatinine, and glucose in Prosaro treated groups compared with the control group. Moreover, Prosaro-induced hematotoxicity was evidenced by a marked decline inred blood cell counts (RBC), hemoglobin (HGH) and hematocrit (HT) levels, along with a significant increase in white blood cell (WBC) and platelet count. Thus the sub-chronic toxicity of Prosaro 250 EC is proved to induce hematological and biochemical alterations in rabbits.

Keywords: Prosaro EC 250, rabbits, biochemical markers, hematological profiles, kidney.

INTRODUCTION

The rapid world population growth observed over the last few decades makes food production in high demands, and hence world nations would try to get over such a situation(Rehman et al., 2022).Accordingly, people are keen to enhance and/or to increase the agricultural production by using beneficial agricultural chemicals. Including pesticides which are world-widely used to preserve crops(Chen et al., 2018),and to sustain crops from hazardous living organisms and diseases(Nicolopoulou-Stamati et al., 2016). Additionally, pesticides can be categorized by chemical classes, functional groups, mode of action, and toxicity(Botitsi et al., 2017), as well as the pesticide target pests, classify pesticides into eight main groups, namely insecticides (kill insects), herbicides (killing herbs), fungicides (kill fungi), acaricides (kill mites), molluscicides (kill molluscs), bactericides (kill bacteria), avicides, and virucides(Azam et al., 2020; Ahmed et al., 2021). Unlike herbicides(the commonly used pesticides (47.5 %)), insecticides and fungicides are lesser used (29.5 and 17.5 %, respectively), and other pesticide classes like algicides and bactericides are rarely used (5.5 %) (Bojarski and Witeska, 2020). These chemicals act in disrupting the physiological functions of the target organisms by reducing their vitality(Jayaraj et al., 2016).

Humans might be exposed to pesticides from contaminated workplaces, and agricultural areas, and the consumption of contaminated water and

foodstuff(Anderson and Meade, 2014). Hence, pesticides can mainly enter the human body either orally via food ingestion(Damalas and Eleftherohorinos, 2011) , skin contact (Anderson and Meade, 2014), eye(Fareed et al., 2012), or via inhalation by respiratory tract(Gautam et al, 2022). Triazoles are commonly used fungicides in agriculture, due to their rapid diffusion through the soil, leading as a result to pollute the soil and groundwater. In addition, these chemicals have a variety of therapeutic and personal care items, such as shampoos, dermal creams, soaps, etc (Assress et al., 2020).

Recently, the agricultural triazole fungicides have sparked a growing number of concerns about their environmental safety and potential health dangers, and it has become one of the most important challenges in public and environmental health(Kukowski et al., 2017). Further, triazole fungicides were reported to have carcinogenic neurotoxic, organo-toxic, and genotoxic effects in animals(Knebel et al., 2018; Wu et al., 2021).According to the Environmental Protection Agency (EPA), Triazole fungicides are categorized as "human probable carcinogens" (Tan et al., 2018). In this context, this study aimed to evaluate the toxic effects of various doses of the fungicide Prosaro EC 250 on body weight, hematological profiles, and biochemical markers of kidney function- and blood glucose level in male rabbits *(Oryctolagus cuniculus).*

MATERIAL AND METHODS

Chemicals

A synthetic fungicide, Prosaro is composed of two active ingredients, namely prothioconazole (CAS 178928-70-6; 125 g/L) and tebuconazole (CAS 107534-96-3; 125 g/l), and inert ingredients as N, N-dimethyldecan amide (CAS 14433-76-2). Prosaro EC 250 was bought from SarlAgri-chem Company in Algeria.

Animals

Twenty-four male rabbits weighing (1,828± 0,143) kg were divided equally into four groups (six animals/group), and kept into. 76×54×47Cm^3metal cages in an animal house of our Institution maintained with standard temperature (22 ± 2 C), humidity (40%) and natural photoperiod cycle (12h dark/light cycle) with freely accessed to water and nutritionally balanced food containing all of the essential elements for the animals.

Experimental Design

The study experiments include a control untreated group (G1) receiving distilled water and standard regular food, and three treated groups. (G2, G3 and G4) receiving orally Prosaro at three doses (mg/kg body weight (bw)), 0.093, 0.186 and 0.465 mg/kg bw for 21 days. Animals were weighed after every three days of treatment and then were euthanized by decapitation at the end of the experiments. Blood samples were instantly collected into vacuum tubes containing EDTA for determining the hematological parameters(hemoglobin (Hb), hematocrit (HCT), red blood cells (RBCs), leukocytes (WBC),and platelets (PLT)) using BC-5380 Auto Hematology Analyzer by Mindray (UK) Ltd, and dried tubes which subsequently were centrifuged at 3000 rpm for 15 minutes at 4°C to get serum for the determination of biochemical kidney markers parameters (creatinine, uric acid and urea), and glucose level using Biochemistry Analyzer Cobas INTEGRA 400.

Statistical Analysis

The differences between the treated and control groups were tested by t-Student test using SPSS software (version 25). $P < 0.05$ was considered significant.

RESULTS

Prosaro Induced- Changes in Body Weight and Absolute Kidney Weight

Tables 1 and 2 indicate a significant increase in the body weight gains and a significant decrease in the absolute weight of the kidneys in Prosaro EC 250 treated groups compared with the control group.

Prosaro-induced Changes in Hematological profiles

As indicated in Table **3**, Prosaro treated resulted in a significant decrease in hemoglobin (HGB) and hematocrit (HCT) levels, and red blood cells (RBCs) count, along with a significant increase in white blood cells (WBCs) and platelet (PLT) counts as compared to these in the control group.

Prosaro-Induced Changes in Kidney Biochemical Markers

Data displayed in table **4** show a significant increase in serum levels of creatinine urea, and uric acid, as well as glucose levels in Prosaro treated rabbits compared to the controls group.

Table 1: Changes in body weight (BW) (g) in control and Prosaro EC 250 treated groups

.Parameters	Control (G1)	G2	G3	G4
Initial weight (g)	1762.33±104.007	1710±97 .297	1752±60.314	2009.33±60.833
Final weight (g)	2031.83±81.477	2074±114.990	2208 .83±65.147	2493.17±89 .399
Weight gain (g)	+269.50±43.592	+350.33±87.712	+460.67±71.536***	+458.17±84.440***

*Values are expressed means ± SD (N= Six rabbits for every group).*Significant (p<0, 05).*

Table 2: Changes in the absolute kidney weight control and Prosaro EC 250 treated groups.

Parameters	Control (G1)	G2	G3	G4
Left kidney (g)	6.596±0.346	5.995±0.191**	5.753±0.429**	5.718±0.411**

*Values are expressed means ± SD (N= Six rabbits for every group).*Significant (p<0, 05).*

Table 3: Changes in hematological profiles in control and Prosaro EC 250 treated groups.

Treatments	Control (G1)	G2	G3	G4
RBC (× 10^6/mm^3)	5.761±0.179	5.565±0.205	5.211±0.376**	4.801±0.267***
HGB (g/dl)	11.067±0.575	10.217±0.470*	9.267±0.516***	8.600±0.485***
HCT (%)	35.667±0.975	34.433±0.480*	32.617±1.435**	29.500±0.923***
WBC (× 10^6/mm^3)	8.733±0.979	9.733±0.621	10.317±0.534**	13.517±0.746***
PLT (× 10^3/mm^3)	420.33±27.384	433.50±53.309	464.50±30.989*	509.17±47.318**

*Values are expressed means ± SD (N= Six rabbits for every group).*Significant (p<0, 05).*

Table 4: Effect of Prosaro EC (250) on the serum blood level, and biochemical kidney markers.

Treatments	Control (G1)	G2	G3	G4
Glucose (g/l)	0.981±0.089	1.045±0.083	1.155±0.118*	1.301±0.065***
Creatinine (mg/L)	7.868±0.538	8.196±0.438	8.938±0.575**	9.816±0.620***
Urea (g/l)	0.388±0.030	0.388±0.042	0.448±0.044*	0.571±0.028***
Uricacid (mg/dL)	0.760±0.0981	0.925 ±0.081**	1.106±0.139***	1.290±0.165***

Values are expressed means ± SD (N= Six rabbits for every group).

DISCUSSION

In this study, our results show a significant increase in the body weight in animals exposed to Prosaro EC 250 at various doses compared to the control group was previously proved in some studies investigating the toxicity of fungicide maneb (MB)(Mallem et al., 2007), and 5 mg/kg bw Rivaneb® 80 in rabbits(Assia et al., 2020).

This finding is likely due to Dithiocarbamates' induced- reduction in thyroxin (T4) secretion in the thyroid gland, which somehow can result in changes in body weight gain following induction of thyroid hypoactivity associated with an increase in body fat (Assia et al., 2020). Opposingly, other studies(Ben Amara et al., 2015). Furthermore, a marked decrease in food consumption and body weight was observed in rats received rats 150 mg/kg bw of 2,4-dichlorophenoxyacetic acid (2,4-D) for four weeks as compared to the control group (Shafeeq and Mahboob, 2021). In addition, results showed a significant decrease in the kidney absolute weight in Prosaro 250 EC treated rabbits as compared with controls, and this has been previously reported in 0.01, 0.02 and 0.04 mg/kg bw brodifacoum treated rabbits for three weeks (Maamar et al., 2013). Conversely, asignificant increase in the left kidney weight was found in VT (Voliamtargo®) treated rabbits for three weeks compared to the control group (Bokreta et al., 2021).

In this study, the Prosaro EC 250 treatment led to a significant decrease in RBC count, hemoglobin (HGB), and hematocrit (HCT) levels compared to the control group. This result may refer to the onset of blood anemia. Additionally, the white blood cells (WBC) and platelets (PLT) count were significantly higher in Prosaro EC 250 treated animals compared to controls. Similarly, the increase in these parameters was previously found in fipronil (FIP) administrated orally to mice at 1/100, 1/50, and 1/30 of LD50 for 28 days(Abouelghar et al., 2020), and oral administration of mancozeb to rats at doses 500 and 1000 mg/kg bw for 8 weeks (Yahia et al., 2015), while PLT and WBC counts were decreased in fipronil treated rats for 4 weeks(Abouelghar et al., 2020).Pesticide-induced blood anemia can be associated with inhibition of hemoglobin synthesis and, thus reduction of the lifespan of circulating red cells. In this context, pesticides, including carbosulfan and diazinon treatments were found to alter the formation of red blood cells resulting in a change in hematological constituents (Zhang et al., 2019). Moreover, the increase in WBCs count indicates clearly the involvement of the white blood cells in immune system defense against tissue inflammations (Karimani et al., 2019).

On top of that, serum glucose level was significantly increased in groups of rabbits given different doses of Prosaro EC 250 when compared with control animals. This hyperglycemia has been previously reported in maneb exposed rabbits (Mohamed and Taha, 2018), and thiodicarbe treated rats (Ab. AL-Shinnawy, 2008).Moreover, results shown in Table 4 revealed a remarkable increase in serum levels of creatinine, urea and uric acid in Prosaro treated rabbits compared with controls. This result was similarly found in female rats orally administrated with 0.5, 25, and 125 mg/kg bw of atrazine for 28 days(Liu et al., 2014), rats exposed to systemic pesticides(El-Damaty et al., 2012), and mice and rats received metribuzin supplemented diet resulting in renal failure(Salem, 2011).Similar results were also previously reported rats received orally in 20 mg/kg bw fipronil (FIP) (Abdel-Daim and Abdeen, 2018)and 1 to 10 mg/l of FIP in their drinking water(Mossa et al., 2015), showing changes in serum renal biomarkers and kidney oxidative injury.

CONCLUSION

Prosaro 250 EC exposure caused marked alterations in kidney biochemical hematological profiles individuals should be very concerned about their health through consuming safe and healthy food. Therefore, severe rules ought to be put in place to avoid such careless application of pesticides in the agricultural sectors. In the future research plan, the study should be supported by the genotoxic investigation to provide more understanding of the mechanism underlying Prosaro toxicity.

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COMPETING INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Debabsa Rafika conducted the study as part of her thesis. Khaldi Fadila and Grara Nedjoud contributed in the study as supervisors, Gheid Abdelhakas laboratory director of *Laboratory of Sciences and Technology of Water and Environment, Mohamed Cherif Messadia University*, Guezgouz Noureddine for her contribution in bringing the chemical products and, Bouzahouane Hana with the cooperation of Debabsa Rafika in analyzing the results, all the authors contributed to produce and present this final manuscript**.**

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