© by PSP Volume 31– No. 08B/2022 pages 8962-8968 Fresenius Environmental Bulletin

TOXICITY OF TWO PESTICIDES(SEKATOR AND PROSARO EC 250) INDUCED ALTERATIONS IN LIVER HISTOPHYSIOLOGICAL AND BIOCHEMICAL MARKERS IN MALE RABBITS*(Oryctolagus Cuniculus)*

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ABSTRACT

Sekator and Prosaro EC 250 are common and widely used pesticides in agriculture in Algeria, but the health toxic effects limit their use. Therefore, the present study was undertaken to highlight the toxic effects of Sekator and Prosaro EC 250 on the liver biochemical markers, including serum total bilirubin, and enzymatic activity of transaminases (Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)), and liver histology in male rabbits *(Oryctolagus cuniculus).*A total of forty-two male rabbits were randomely allocated into seven groups of six animals each, including control group (G1), three groups treated orally with three doses of sekator (mg/kg body weight (bw)), namely G2 (0.213mg/kg bw), G3 (0.426mg/kg bw) and G4 (1.066 mg/kg bw), and three groups treated orally with three doses of prosaro EC 250: G5 (0.093mg/kg bw), G6 (0.186 mg/kg bw) and G7 (0.465 mg/kg bw) for three weeks (21 days).Results showed a significant ($P < 0.001$) increase in the liver absolute and relative weights in 1.066mg/kg bw sekator,and in 0.186 mg/kg and 0.465 mg/kg bw Prosaro treated animals compared with controls. Additionally, the enzymatic activity of transaminases (AST / ALT) and the level of serum total bilirubin were significantly increased ($P < 0.001$) in the high doses of both pesticides.The biochemical alterations of the liver induced by sekator and prosaro were supported by the histopathological observations showing venous dilation, inflammation, inflammatory infiltrates,congestion in the portal space, ballooned, vacuolated and necrotic hepatocytes, and sinusoidal dilatation.

KEYWORDS:
Sekator, Prosaro EC 250, Liver function, Biochemical markers, Liver Histopathology

INTRODUCTION

In the last few years, the increased global populationled accordingly to a slow increase in cultivated agriculture to reach only 12 percent. As a result, the continuous population growth estimated to be nearly 9.1 billion by 2050 would make the use of mechanized agro-technology, input-intensive, the soil nutrients supply as different ways to achieve environmental protection[1]. Moreover, the direct spray deposition of pesticides on farms or agricultural commodities is to kill pests (e.g, bugs and weeds) and disease vectors like mosquitos,to reduce or to eliminate production wastage, and to preserve high-quality products[2].World Health Organization (WHO) has reported that morethan 1000 of various pesticides used throughout the world are effective chemicals against pests. In fact, pesticides include insecticides, fungicides, herbicides, rodenticides, molluscicides, and others[3,4] and from which those used to inhibit the physiological activities of target organisms by causing malfunction and reduction of their vitality[5].Also, their resistance to heat, humidity, radiation, and biodegradation make them persistent chemicals in water and soil, harmful and toxicto humans and the environment[6].Further, herbicides and insecticides are the most commonly used pesticides, accounting, respectively 47.5% and 29.5% of the total used pesticide, while fungicides are the less used (17.5% of total pesticides)[7].Humans can be exposed to pesticides via inhalation (aerosol vapor), skin contact, or ingestion of contaminated water and foodstuffs (oral)[8].Interestingly, the exposuretime and exposure way, and the individuals' health physiology limit the severity effects of these chemicals on human and animal health[8].Sekator is a sulfonylurea, herbicide discovered in the 1970s and characterized by low toxicity to animals and low application levels. Sulfonylurea herbicides are strong inhibitors of acetolactate synthase (ALS), known as the first popular enzyme of branched-chain amino acids,

such as valine, leucine, and isoleucine biosynthesis[9], as well fungicide, protect plants, including trees and cereals in the field crops and the stored plant parts, such as seeds, vegetables, and fruits against fungal infections[10].Prosaro is a commercial fungicide containing two active ingredients tebuconazole and prothioconazole and is used on wheat and barley to control fusarium head blight, reduce mycotoxin levels in the grain, and protect against serious leaf diseases[11].Further, the carcinogenic, endocrine, reproductive, and cardiovascular effects of pesticides have been investigated[12]. It was reported that oral administration of tebuconazole induced developmental toxicity along with marked effects on developing nervous systems in rats, rabbits, and mice. In addition, tebuconazole was reported to induce no marked endocrine disruptor effects, but it is possiblya human carcinogen agent (group C), as proved by its ability to increase the incidence of hepatocellular adenomas, carcinomas, and combined adenomas/carcinomas in male and female mice[13]. In this regard, the levels of adrenaline, noradrenaline and cortisol, and the enzymatic activity of alanine (ALT) and aspartate transaminase (AST) (liver functional markers) were markedly increased in herbicide exposed fishes[7]. To our knowledge, we are the first to investigate the subchronic toxicity of a sulfonylurea herbicide(Sekator) and a triazole fungicide (Prosaro EC 250) on the major functional biochemical markers and the histology of the liver in male rabbits(*Oryctolagus cuniculus)*.

MATERIALS AND METHODS

Chemicals. The used chemicals in the present work were Sekator herbicide, including two active substances, namely amidosulfuron-sodium (CAS 596120-00-2 ; 100 g/L) and iodosulfuron-methyl-sodium (CAS144550-36-7 ; 25 g/l)) combined with a phytoprotectionmefenpyr diethyl (CAS135590-91-9 ;250 g/l), and fungicide Prosaro® 250composing of two active ingredients;prothioconazole (2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2- hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione; CAS 178928-70-6; 125 g/l)] and tebuconazole (α-[2-(4-chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H-1,2,4- triazole-1-ethanol; CAS 107534-96-3; 125 g/L)], and one inert ingredient named N, N-dimethyldecan amide (CAS 14433-76-2)(Bayer). Both chemicals (Sekator OD and Prosaro EC 250) were purchased from SarlAgrichem Company of Algeria.

Animals. This study was conducted ona total of forty-two domestic male rabbits (*Oryctolagus cuniculus*),obtained from a domestic animal breeding Centre in Constantine city (East Algeria), and weighing between 1600 and 2000 g. Animals were kept in $76\times54\times47cm^3$ metal cages in an animal house of our Institution under standard conditions of temperature, humidity and natural photoperiod cycle. Animals had free access to water and a healthy balanced diet composed of all the necessary nutrients for animal growth.

Experimental design. Forty-two male rabbits were divided into seven groups of six animals each, namely control untreated group (G1) received standard food and distilled water, three groups G2, G3 and G4 received, respectively three doses of sekator (mg/kg body weight (bw) 0.213, 0,426 and 1,066 mg/kg bw), and three other groups received prosaro at three doses(0.093 (G5), 0.186(G6) and 0.465 mg/kg bw (G7)). Treatments were given to animals by oral gavage for three weeks. After the treatment period, rabbits were euthanized by decapitation, and blood samples were collected in sterilized heparin tubes and centrifuged at 3000 rpm for 15 minutes at 4°C.The resulting serum samples were stored at -20^0C for further analysis. The liver of each animal was removed, weighted using a precision balance, and fixed in formalin 10% for the histopathological evaluation. The liver biochemicalmarkers (total bilirubin and transaminases (AST, ALT)) were determined using Biochemistry Analyzer Cobas INTEGRA 400.

Histology study. Histopathological examination of the liver was performed as previously described[14]. In brief, a liver fragment of each animal was dehydrated in gradient ethanol, hyalinized in xylene, embedded in paraffin wax, and cut into 5-mm thick paraffin slices. The liver tissues were then collected, stained with hematoxylin and eosin (H&E) on glass slides, and observed using a light microscope (Olympus CX23) supplied by an automated imaging system for image capture.

Statistical analysis. Data were presented as mean ± standard deviation (SD). The statistical significance of the differences between the treated and control groups was tested by t student test using SPSS software (vers.25).$P< 0.05$ was considered significant.

RESULTS

Organs Absolute and relative Weight: Sekator treatment caused a significant increase in the liver absolute and relative weights(Table 1) compared to the control group (G1), and this increase was remarkable, especially in the group exposed to the highest dose (G 4). Similarly, the absolute and relative weights of the liver were significantly increased in Prosaro EC 250 treated groups, in particular, those of G6 and G7 compared to control (Table 2).

Biochemical Studies. As indicated in Tables 3 and 4, the serum level of total bilirubin and the enzymatic activity of transaminases (AST, ALT) **w**as markedly increased in sekator and prosaro treated groups compared to control, and noteworthy, the activity of AST and ALT was highly significantly increased in the groups received the higher doses of both chemicals compared to control group.

Histopathology of liver: The liver histology from control rabbits revealed radially structured hepatic cords around the central vein. While, the liver tissues from treated animals with sekator at doses of 0.213,0.426 and 1.066 mg/kg bw showed marked histological alterations evidenced by vein dilatation, congestion, inflammation ,sinusoidal dilatation, inflammatory infiltrates, and congestion in the portal veins(FIGURE 1). Additionally, the liver histology of animals receiving the three doses of prosaro showed architectural disorganization of the hepatocytes associated with congestion and inflammation, sinusoidal dilatation,cellular vacuolation,ballooned and necrotic hepatocytes(FIGURE 2).

TABLE 1
Changes in the absolute and relative weights of the liver in sekator treated groups.

Parameters	Treatment groups			
	G1:Control	G2:Treated	G3:Treated	G4:Treated
Absolute liver weight (g)	48.351±5.672	55.348±3.267*	62.246±6.160**	69.761±8.995***
Relative liver weight (g/100g b.w.)	2.348 ±0.213	2.743 ± 0.096**	2.743 ± 0.329*	2.867±0.337**

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

TABLE 2
Changes in the absolute and relative weights of the liver in prosaro treated groups.

Parameters	Treatment groups			
	G1:Control	G5:Treated	G6:Treated	G7:Treated
Absolute liver weight (g)	48.351±5.672	59.081±7.080*	64.066±3.486***	73.253±6.824***
Relative liver weight (g/100g b.w.)	2.348 ± 0.213	2.844 ± 0.264**	2.903 ± 0.203***	2.933 ± 0.179***

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

TABLE 3
Total bilirubin level and the enzymatic activity ALT and AST in sekator treated groups.

Parameters	Experimental groups			
	G1:Control	G2:Treated	G3:Treated	G4:Treated
Bilirubin (mg/dl)	0.603±0.043	0.628±0.051	0.708±0.044**	0.776±0.054***
ALAT(U/l)	35.67±5.610	43.67±4.761*	47.33±6.713**	54.50±5.541***
ASAT(U/l)	33.50±6.473	40.50±3.391*	46.00±4.290**	52.83±4.021***

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

TABLE 4
Total bilirubin level and the enzymatic activity of ALT and AST in Prosaro treated groups.

Parameters	Experimental groups			
	G1:Control	G5:Treated	G6:Treated	G7:Treated
Bilirubin (mg/dl)	0.603±0.043	0.641±0.052	0.715±0.062**	0.943±0.091***
ALAT(U/l)	35.67±5.610	49.17±8.658**	56.33±9.585***	68.50±4.183***
ASAT(U/l)	33.50±6.473	40.50±4.764*	51.33±9.459**	70.67±11.96***

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

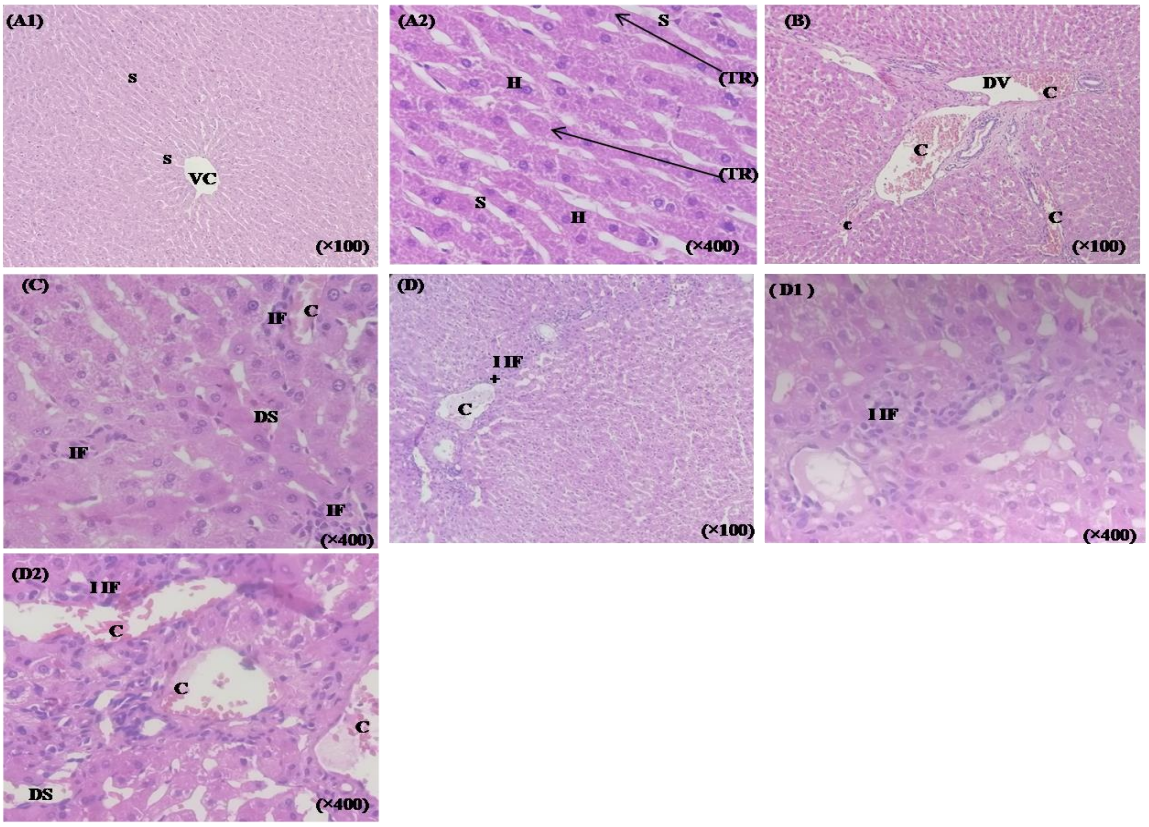

FIGURE 1

Cross-section of the liver from control and Sekator treated groups. Liver tissue of the control and treatment groups (G1-G4) are designated by (A1, A2), B, C and (D, D1, D2), respectively.

H: Hepatocyte,**VC**: Centrilobular vein, **S**: The sinusoidal capillary,**TR**: Spans of Remak,**DV**: Vein dilation, **DS**: sinusoidal dilatation, **C**: congestion, **IF**: Inflammatory, **IIF + C**: Inflammatory infiltrates +Congestion in the portal space.

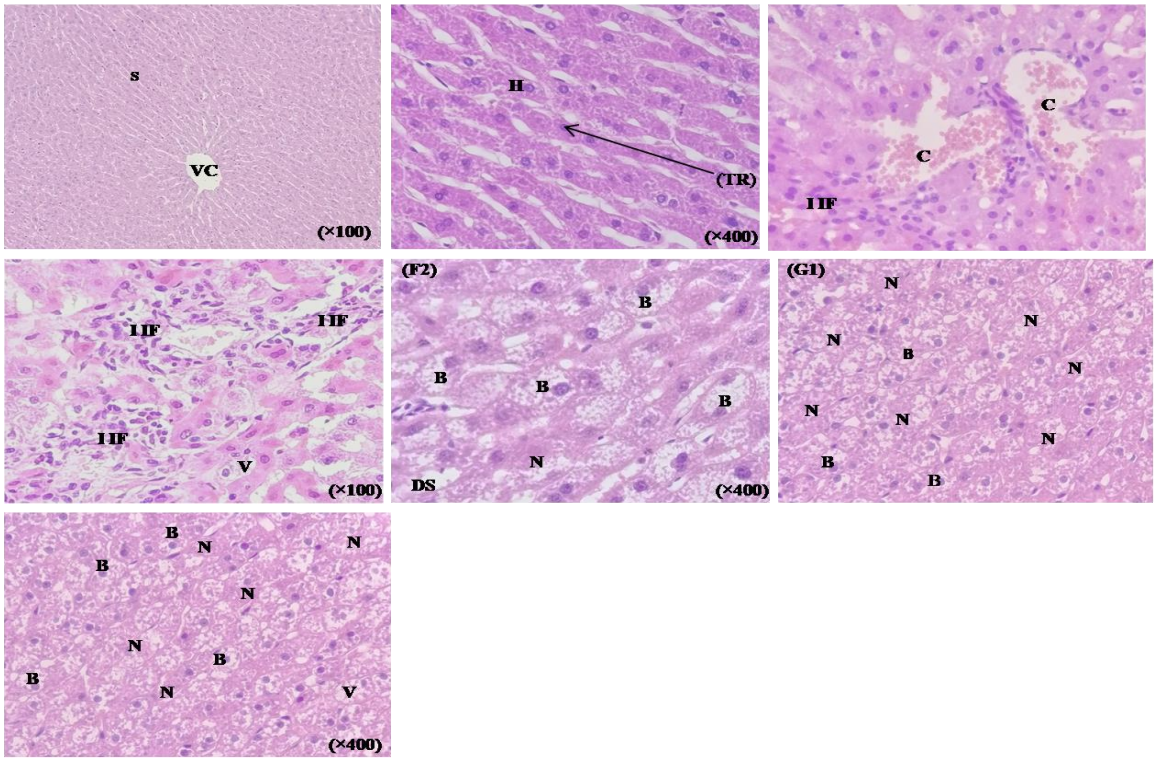

FIGURE 2

Cross- section of the liver from control and Prosaro treated groups. . Liver tissue of the control and the treatment groups (G1, G5, G6, G7) are designated by (A1, A2), E, (F1, F2) and (G1, G2) respectively.

IF: Inflammatory infiltrates, **C**: congestion,**DS**: sinusoidal dilatation, **B**:ballooned hepatocytes, **N**: necrosis.

DISCUSSION

In this study, a significant increase in absolute and relative weights of liver was noticed in rabbits treated with the Prosaro EC 250 compared to the control group, and this is in line with those previously reported in maneb treatment resulted in an increase in liver weight in rats received intraperitoneally of 30mg/kg bw[15] and in 1000 mg/kg/day mancozeb treated rats for 8 weeks [16]. Also, that propiconazole (100 mg/kg and 150 mg/kg), and tebuconazole (150 mg/kg) exposed female rats for 14 days were reported to induce a marked increase in the absolute liver weight[17]. Similarly, a significant rise in the liver weight was found in 5 mg/kg bw Rivanebe® treated *Cuniculus lepus*[18], and in 1.30 and 13 mg/kg bw metribuzin treated female rats for three months [19].This finding concords with another study showing that 90-day exposure to various doses of chlorpyrifos-ethyl,chlopyrifos-methyl (9.60 and 300 mg/kg) and methomyl (1.70 mg/kg) resulted in an increase in the liver relative weight[20].On the contrary,had a considerable drop in the absolute and relative liver weights related to severe hepatotoxic effect was found in experimental animals treated with high doses of MB(Maneb) [21].Further, our findings showed an increase in the hepatic mass in Sekator treated groups, and this concords with that previously reported [22].This hypertrophy is likely to the high accumulation of this chemical in the liver. In addition, 250ppm of metribuzin-treated male rats was found to increase the liver weight, along with a marked change in thyroid weight[22].As reported, the enzymatic activity of ALT and AST and serum bilirubin level are the most sensitive indicators for determining the severity of hepatic damage and toxicity caused by chemicals[23], as well as the transaminases are mainly found in the liver with extremely high concentrations (5,000 to 10,000 times the blood concentration)[24], and secondly found in other organs like, such as heart, kidney[19]. In this regard, our results showed a significant increase in the enzymatic activity of AST and ALT in rabbits treated with Sekator and Prosaro. This finding is in line with those previously reported, including the study investigating the short-term (15 days) toxicity of atrazine (300 ug/kg) in rats [25], and the toxicity of Rivaneb®80 at dose 5mg/kg bw administred orally to rabbits for 15 days [18]. Also, Fipronil(insecticide) treatment was reported to cause a significant elevation in the serum enzymatic activity of AST, ALT, and ALP in Wistar rats[26]. Similar results have been also found in Diazinon treated rats[27] and 20 mg/kg bw fipronil (FIP) treated rats for 15 days[28]. It was suggested in a study reporting an increase in transaminase activity in fungicide treated rats that serious liver injury can be evidently associated with toxic effects[29].Overall, the hepatotoxic effects may result in alterations of the plasma membrane permeability, which subsequently leads to the release of enzymes from the tissues into the blood stream[30]. On the other hand, the level of serum total bilirubin was significantly increased in Sekator and Prosaro EC 250 treated groups when compared with the control group. Similar results were found in rabbits receiving orally daily 5mg/kg of Rivaneb® 80 for 15 days[18], in dimethoate (DM) [O,O-dimethyl-S(N-methyl-carbomethyl) phosphorodithioate] treated rats[31], and in mancozeb administrated to rats orally for 8 weeks at 500 and 1000 mg/kg bw[16] and fipronil (COACH®) treated albino mice[32].The rise in bilirubin level might be due to increased bilirubin generation due to hemolysis or impaired liver absorption and/or conjugation[21]. Furthermore, pathological alterations such as liver necrosis may cause an increase in the permeability of the membrane of the hepatocyte, resulting in the release of bilirubin into the bloodstream[33]. In this study, the biochemical alterations caused by the two studied pesticides were supported by the histopathological observations showing marked histological damages as evidenced by vascular congestion, ballooned and necrotic hepatocytes, and inflammation. This result was similarly reported in some previous studies, including that investigating the hepatotoxicity in rabbits received 1/10 LD50 of imidacloprid for 37 days[34], and that investigating the liver histological changes in chlorpyrifos treated rats[35]. and on histological changes in the liver of rats treated with Fipronil[26].

CONCLUSION

The study proved that sekator and prosaro EC250 are potent hepatotoxic pesticide chemicals, especially at higher doses, able to induce liver dysfunction through alterations in liver biochemical markers. Hence, both pesticides induced an increase in liver mass, serum enzymatic activity of transaminases (ALT and AST) and bilirubin levels. Conclusively, the study suggests avoiding such casual application of herbicides and fungicides in the agricultural sector.

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Received: **25.04.2022**
Accepted: **27.06.2022**

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