

Biochemical, Hematological and Pathological Effects of Bispyribac Sodium in Female Albino Rats

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Abstract

Bispyribac-sodium (nominee (used extensively as a herbicide in rice fields in Egypt. We investigate the cytotoxic effect of bispyribac-sodium in albino rats that intubated orally with 0, 16.47, 32.94, 65.88 mg/kg of bispyribac-sodium (equivalent to 1/160 1/80 and 1/40 of the LD50 of bispyribac-sodium) for three months twice per week. It was found that bispyribac-herbicide caused non-significant decrease in the body weight gain ratio of albino rats. Moreover, bispyribac-sodium elevated significance levels of ALT, AST, urea, creatinine and LDH. In contrast, bispyribac herbicide decreased significant levels of glucose, cholesterol, total protein and albumin. Bispyribac-sodium showed histopathological changes in liver, lungs, kidney and spleen, but had no significant effect on RBCs count while showing significant increases in leukocyte count. On the conclusions, bispyribac sodium had a toxic effect on liver, lungs and kidney of female albino rats.

Keywords: Bispyribac sodium; Liver; Kidney function

Introduction

Pesticides considered as heterogeneous chemicals used for controlling of plant diseases (pests or weeds) to enhance the productivity of agriculture and yield [1]. As herbicides are some of the most used pesticides worldwide, sub-lethal concentration detection can act as sensitive early alarm tools to indicate their presence and can support for regulatory assessments and monitoring to be not harmed to aquatic life [2].

Herbicides constitute more than 60% of pesticides that used in agriculture. Most herbicides have low mammalian toxicity due to all herbicides that has hazardous effect on human or animals has been rejected [3]. Bispyribac-sodium (sodium 2, 6-bis [(4, 6-dimethoxy-2-pyrimidinyl) oxy] benzoate) belonged to a pyrimidinyl thiobenzoate herbicide [4], is a post emergent herbicide according to Health Canada Pest Management Regulatory Agency (2008) and used mainly to control weeds in rice cultivation (EPA, 2001) [5,6].

The oral LD50% of bispyribac-sodium in rats was measured to be 4111 mg/kg body weight in male, while 2635 mg/kg body weight in female. While its tissue distribution in male larger than female due to superiority in the excretion in female besides repeated administration not affect absorption nor metabolism and excretion [7]. Bispyribac mostly absorbed from gastrointestinal tract completely, had no accumulation effect and excreted mostly as the parent compound and its elimination in male more than female (Australian Pesticides & Veterinary Medicines Authority 2011) [8].

Technical bispyribac-sodium had low toxicity and increased liver enzyme activity and proliferation of the gall bladder and bile duct where the liver considered the main target organ based on the NOEL in rats (7.2 mg/kg bw/day) (Health Canada Pest Management Regulatory Agency, 2008) [5].

Hiroyuki reported that bispyribac-sodium at a dose of 20,000 ppm and/or 50,000 ppm in a four-week study in albino rats, decreased body weight and feed consumption, on necropsy dilatation of the cecum with the proliferation of bile duct and increase in the level of total cholesterol, GPT, GOT, and ALP activities [9]. In a sub-chronic study carried out by the California Environmental Protection Agency, (2001)

found that at dose level 1538 mg/kg/day for 13 weeks on female rats increased relative liver, kidney and spleen weight with microscopic inter and intralobular bile duct proliferation and lymphocytosis in urinary bladder and different body organs [10]. The aim of this study was to evaluate the biochemical parameter changes, histopathological changes and hematological parameters of commercial bispyribac-sodium toxicity in albino rats

Materials and Methods

Experimental animals and grouping

Female albino rats obtained from experimental unit, Faculty of pharmacy, Mansoura university; weighted from 95 to 115 GM. Animals were apparently healthy and housed in plastic cages contain wood shaving as a bedding material. Animals accommodated for 2 weeks before the experiment and maintained on a balanced ration also feed and water given ad libitum throughout the experiment. Rats divided into four groups each one contains eight rats weighted 115 ± 5 GM; intubated orally with 0, 16.47, 32.94, 65.88 mg/kg of bispyribac-sodium (equivalent to 1/160, 1/80 and 1/40 of the LD50 of bispyribac-sodium) for three months twice per week [7]. Animals weighted twice per week before dosing to maintain constant dose throughout the experimental period.

Body weight gain determination

the body weight was recorded throughout the experiment before each administration; the body weight gain % calculated according to the following formula [11]:

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Body weight gain%=(Final body weight-Initial body weight)/(Initial body weight) × 100

Biochemical analysis

Serum of all rats were analyzed for ALT, AST, LDH, total protein, total albumin, serum Glucose, urea according to Burtis at al., serum creatinine and cholesterol levels [12-19].

Hematology

Blood taken from the apex of the left ventricle of the heart with a syringe, then collected in gel tubes contain EDTA as an anticoagulant and the blood sample analysis was carried out by hematological analyzing. Where hemoglobin (HGB), red blood cell count (RBC), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) also white blood cell count (WBC), differential white blood cell count neutrophils, monocytes, lymphocytes, basophils and eosinophils were evaluated; The mean value and the standard deviation calculated for each parameter according to Agbasi et al. [20].

Histopathological studies

Specimens from liver, spleen, kidney and testes were fixed in formalin 10% and section of 5 μ thickness prepared from specimens and stained with hematoxylin and eosin (H&E) and examined under the microscope.

Ethical approval

The research and ethics committee of our faculty of veterinary medicine, Mansoura University, Egypt approved the research methodology including the use and method of handling the animals

Statistical analysis

Data statically analyzed by for variance by one-way ANOVA and least standard difference LSD that as described by Snedecor and Cochran through using computerized SPSS program version 13.0 [21].

Conflicts of Interest

The authors have no conflicts of interest

Results

Clinical signs and PM changes ascribed to bispyribac sodium administration

Rats showed dullness, decrease the motor activity with abdominal distention, decrease the feed consumption and the body weight gain with increasing of the dose and anogenital staining at all doses (1/160, 1/80 and 1/40 of the LD50) but signs more severe at higher doses. In addition to death of one rat after the second day of administration of dose 1/40 with signs of abdominal distention and difficult breathing in addition to distention in intestinal tract and lesions on liver and lungs

Body weight gain determination

The results showed that there was a drastic decrease in body weight gain % in groups 1/160, 1/80 and 1/40 of the LD50 (equivalent to 65.88, 32.94 and 16.47 mg/kg) respectively in respect to control value (Table 1).

Biochemical analysis

All doses of bispyribac sodium (1/160, 1/80 and 1/40 of the LD50)

showed a significant increase in ALT, urea, creatinine and LDH and both doses 1/80 and 1/40 of the LD50 showed significant increase in AST in respect to control level after treatment of bispyribac sodium orally for 3 months twice per week.

On the other hand, All doses of Bispyribac sodium (1/160,1/80 and 1/40 of the LD50) decreased significantly in glucose, protein, albumin and cholesterol level in respect to control value after administration of bispyribac sodium orally for 3 months twice per week (Table 2 and Figure 1).

Hematology

The results showed that there was no significance change in the total erythrocyte count, HGB, PCV, MCV, MCH and MCHC in respect to control values, but there was a significant increase in total and differential leukocytes count in group 1/40 of LD50 in comparison with the control group (Table 3).

Histopathological findings

Liver: The liver showed intralobular fibroblastic proliferation with congestion of the hepatic sinusoid and hyperplasia of bile ductules at dose level 1/40 of the LD50 (equivalent to 65.88 mg/kg) and lymphohistiocytic infiltrate in hepatic parenchyma with congestion of the portal blood vessels at dose level 1/80 and 1/160 of the LD50 (equivalent to 32.94 and 16.47 mg/kg), results illustrated in Figure 1a and 1b.

Lung: Lung showed round cell infiltration with thickening of interstitial tissue besides alveolar atelectasis and round cells consolidation in pulmonary alveoli at dose level 1/40 of the LD50 (equivalent to 65.88 mg/kg) and hyperplasia in peribronchial lymphoid tissue with congestion of peribronchial blood vessels at dose level 1/80 and 1/160 of the LD50 (equivalent to 32.94 and 16.47 mg/kg), results illustrated in Figure 1c and 1d.

Kidney: Kidney showed cloudy swelling of the renal tubular epithelium with lymphocytic infiltration at dose level 1/40 of the LD50 (equivalent to 65.88 mg/kg) and congestion of renal glomeruli with lymphocytic infiltration in interstitial tissue vessels at dose level 1/80 and 1/160 of the LD50 (equivalent to 32.94 and 16.47 mg/kg), results illustrated in Figure 1e and 1f.

Spleen: The spleen is showing marked lymphocytic depletion and necrosis of lymphocytes in white pulp with marked hemosiderosis of the red pulp at dose level 1/40 of the LD50 (equivalent to 65.88 mg/kg) and lymphoid depletion with mild lymphocytic necrosis in white pulp at dose level 1/80 and 1/160 of the LD50 (equivalent to 32.94 and 16.47 mg/kg), results illustrated in Figure 1g and 1h.

Discussion

Body weight gain % of bispyribac-sodium

There was a drastic decrease in body weight gain % in groups 1/160, 1/80 and 1/40 of the LD50 (equivalent to 16.47, 32.94 and 65.88 mg/

	Mean final body weight	Body weight gain%	Body weight gain%
Group 1	97.50 ± 1.33	188.63 ± 12.39	93.47
Group 2	114.45 ± 2.72	200.25 ± 8.94	74.97
Group 3	126.75 ± 1.29	200.75 ± 13.02	58.38
Group 4	138.50 ± 4.27	192.63 ± 8.73	39.08

Table 1: Showed mean initial and final weight and body weight gain % in rats exposed orally to bispyribac sodium.

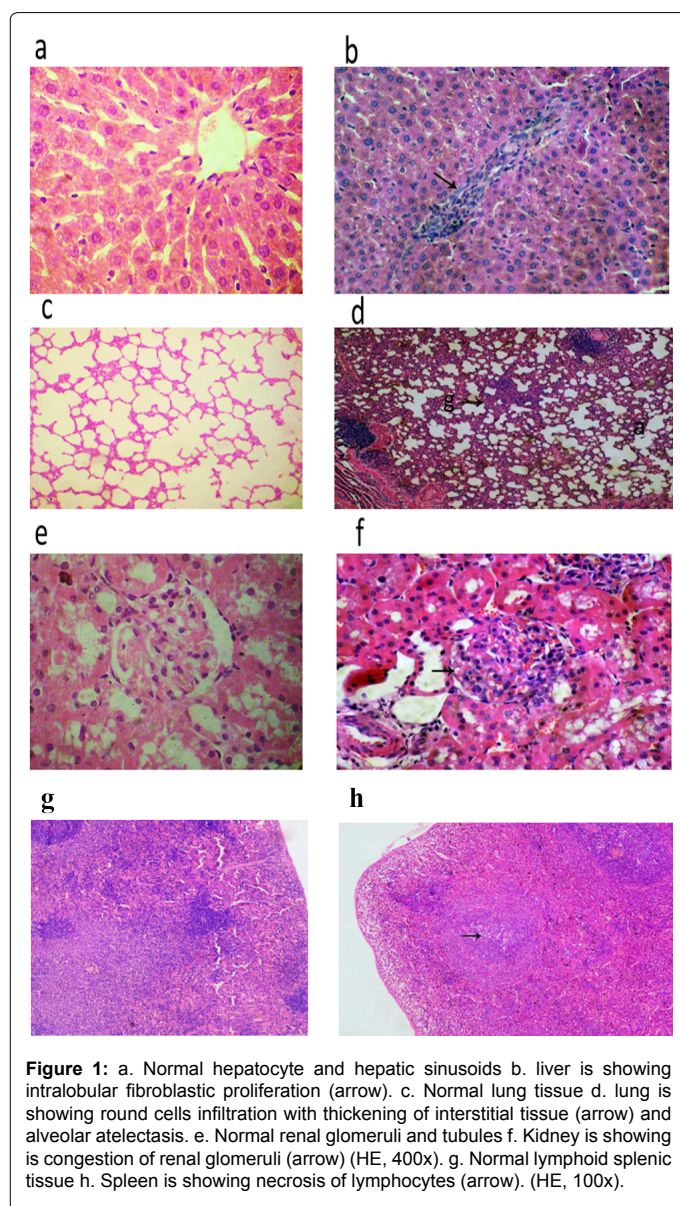
	ALT	AST	Glucose	Cholesterol	Albumin	Protein	Urea	Creatinine	LDH
G1	23.99 ± 0.52 ^c	36.68 ± 1.49 ^c	155.98 ± 5.72 ^a	92.05 ± 8.97 ^a	5.52 ± 0.12 ^a	8.36 ± 0.28 ^a	54.58 ± 1.15 ^d	0.42 ± 0.01 ^d	654.21 ± 63 ^d
G2	32.33 ± 2.98 ^b	44 ± 1.53 ^c	121.79 ± 7.06 ^b	75.89 ± 2.46 ^b	4.71 ± 0.16 ^b	7.07 ± 0.01 ^b	63.13 ± 2.19 ^c	1.11 ± 0.04 ^c	1344.90 ± 105.44 ^c
G3	36.05 ± 3.75 ^{ab}	72.5 ± 3.27 ^b	110.06 ± 2.97 ^{bc}	67.77 ± 1.88 ^{bc}	3.77 ± 0.15 ^c	6.54 ± 0.19 ^b	71.37 ± 1 ^b	1.39 ± 0.05 ^b	1925.03 ± 71.36 ^b
G4	42.34 ± 2.46 ^a	106.62 ± 4.12 ^a	106.36 ± 2.82 ^c	53.33 ± 3.34 ^c	2.94 ± 0.17 ^d	5.23 ± 0.17 ^c	78.78 ± 1.45 ^a	1.66 ± 0.05 ^a	2073.20 ± 104.30 ^a

a, b, c, d: Different letters are significantly different between groups at $P \leq 0.05$

Table 2: Showed the biochemical changes ascribed to administration of bispyribac sodium to female albino rats.

	RBCs	HGB	PCV	MCV	MCH	MCHC	Total WBCs	Deferential WBCs				
								LYMPHO	Neutrophil	MONO	ESINO	BASO
G1	8.03 ± 0.38	14.70 ± 0.42	45.35 ± 0.86	56.90 ± 3.12	18.50 ± 1.36	32.44 ± 1.08	7.07 ± 0.26 ^b	4.52 ± 0.10 ^c	1.32 ± 0.04 ^b	0.42 ± 0.16 ^b	0.05 ± 0.02	0
G2	8.26 ± 0.37	14.97 ± 0.64	46.32 ± 0.60	56.51 ± 3.28	18.30 ± 1.45	32.29 ± 1.06	6.87 ± 0.31 ^b	4.92 ± 0.21 ^{bc}	1.42 ± 0.04 ^{ab}	0.50 ± 0.07 ^b	0.02 ± 0.02	0
G3	8.00 ± 0.27	15.55 ± 0.40	46.45 ± 0.62	57.30 ± 2.46	19.47 ± 0.56	33.48 ± 0.87	7.45 ± 0.16 ^{ab}	5.05 ± 0.6 ^{ab}	1.55 ± 0.06 ^{ab}	0.80 ± 0.04 ^a	0.05 ± 0.02	0
G4	7.95 ± 0.29	15.80 ± 0.42	45.47 ± 0.31	57.40 ± 1.91	19.93 ± 0.75	34.74 ± 0.85	8.05 ± 0.18 ^a	5.52 ± 0.10 ^a	1.57 ± 0.13 ^a	0.85 ± 0.02 ^a	0.07 ± 0.02	0

Table 3: Showed the hematological parameters in rats exposed orally to bispyribac sodium twice per week for three months.



kg) respectively in respect to control value that attributed according to Glusczak et al. [22] proposed that the usage of energy to detoxify compounds and alteration in protein and carbohydrate metabolism, and so less energy from food was available for promotion of growth also decrease in food consumption and such results agree with Hiroyuki who reported that female rats at dose level 0, 100, 500, 20000 and 50000 ppm signs of decrease body weight and feed consumption and on necropsy, dilatation of cecum with histopathological bile duct proliferation has been observed also [23].

Biochemical changes

All doses of bispyribac sodium (1/160, 1/80 and 1/40 of the LD50) showed a significant increase in ALT value and both doses 1/80 and 1/40 of the LD50 showed significant increase in AST that considered according to Daabees et al. [24]. To be diagnostic to liver diseases and reflect the hepatotoxicity that in turn lead to liver enzymes liberation in the blood, and such results agree with Hiroyuki who reported that in four week study carried out in female rats administered orally bispyribac sodium at dose level 0, 100, 500, 20000 and 50000 ppm increase in the concentration of total cholesterol, GPT, GOT, and ALP activity of rats receiving 20,000 ppm and/or 50,000 ppm than control groups ; also such results confirmed by the histopathological change in the present study where Liver showed intralobular fibroblastic proliferation with congestion of the hepatic sinusoid and hyperplasia of bile ductules [9] (Table 2).

All doses of bispyribac sodium (1/160, 1/80 and 1/40 of the LD50) showed a significant increase in urea and creatinine that considered according to Liu et al. to be diagnostic to nephrotoxicity and defect in elimination through both which in turn lead to decrease in clearance of creatinine and elevation of creatinine and urea in the blood [25]; such results agree with Hiroyuki who founded that In a sub chronic study for 13 weeks rats dosed orally different doses of bispyribac sodium 0, 100, 1000, 10000 and 20000 ppm increase GOT, ALP, gamma-GTP activities [23], BUN content also agree with Hiroyuki who reported that in chronic study bispyribac sodium administered in female rats for 104 week at dose level 0, 20, 200, 5000 and 10000 ppm clinically triglyceride, glucose, and phospholipid decreased while creatinine, ALP, gamma-GTP, GOT, GPT, and total cholesterol were increased and such results confirmed with the histopathological change in the present study where kidney showed cloudy swelling of the renal tubular epithelium with lymphocytic infiltration (Table 2) [26].

All doses of Bispyribac sodium (1/160, 1/80 and 1/40 of the LD50) showed significant decrease in Glucose, protein, albumin and cholesterol level in respect to control value that attributed according to Zaahkook et al. [27] to liver disease and disturbance in metabolism of protein, carbohydrates and lipid also according to Gawarammana et al. acetoacetate synthase inhibited by bispyribac may inhibit branched chain amino acids bio synthesis as leucine in human that leads to metabolic disorders [28]; such results agree with Hiroyuki where In a sub chronic study for 13 weeks rats dosed orally different doses of bispyribac sodium 0, 100, 1000, 10000 and 20000 ppm albumin concentration, total protein and cholesterol concentration less than normal control (Table 2) [23].

All doses of Bispyribac sodium (1/160, 1/80 and 1/40 of the LD50) showed significant increase in LDH that attributed according to Tripathi and Shukla [29] to release of isozymes of LDH (five isoenzymes) from destroyed tissues of lungs, liver, muscles, reticuloendothelial system and heart and such results confirmed with the histopathological changes in the present study in lungs that showed that thickening of interstitial tissue besides alveolar atelectasis and round cells consolidation in pulmonary alveoli (Table 2).

Hematological finding for bispyribac sodium

There was no significance change in total erythrocytic count, HGB, PCV, MCV, MCH and MCHC in respect to control values but there was significant increase in total and differential leukocytic count in group 1/40 of LD50 in comparison with the control group where according to Witeska [30] White blood cell affected by a variety of environmental and physiological factors especially exposure to toxicant and such results agree with California Environmental Protection Agency, that at dose level 1538 mg/kg/day for 13 week on female rats causing increase in relative liver, kidney and spleen weight with microscopic inter and intralobular bile duct proliferation and lymphocytosis in urinary bladder and different body organs (Table 3) [10].

Histopathological findings ascribed to Bispyribac-sodium administration

Liver showed intralobular fibroblastic proliferation with congestion of the hepatic sinusoid and hyperplasia of bile ductules at dose level 1/40 of the LD50 (equivalent to 65.88 mg/kg) and lymphohistiocytic infiltrate in hepatic parenchyma with congestion of the portal blood vessels at dose level 1/80 and 1/160 of the LD50 (equivalent to 32.94 and 16.47 mg/kg) (Figure 1a and 1b) such results according to Sancho et al. attributed to that the liver perform various functions that associated with the metabolism of xenobiotics; and considered the main detoxifying organ and affected after stress that caused by toxicants [31].

Moreover, these results agree with Hiroyuki [9] who founded that rats dosed orally for 13 week different doses of bispyribac sodium 0, 100, 1000, 10000 and 20000 ppm where feed consumption decreased throughout the study, furthermore dilated lumen of the cecum observed at necropsy with proliferative bile ducts, slight liver fibrosis on the other hand lung showed round cells infiltration with thickening of interstitial tissue besides alveolar atelectasis and round cells consolidation in pulmonary alveoli at dose level 1/40 of LD50 (equivalent to 65.88 mg/kg) and hyperplasia in peribronchial lymphoid tissue with congestion of peribronchial blood vessels at dose level 1/80 and 1/160 of LD50 (equivalent to 32.94 and 16.47 mg/kg) (Figure 1c and 1d) that may attributed according to Lee et al. [32] to the adjuvant and surfactant effect on the same way as glyphosate surfactant that leads to Respiratory distress, pulmonary pneumonia, metabolic acidosis,

hyperkalemia and shock with poor outcome in a study carried out on a 57-year-old woman case admitted to the emergency after ingestion of surfactant of glyphosate and such results agree with Gawarammana et al. [28] who proposed that surfactant and solvents significantly contribute in poisoning mortality and severity in a case study human self poisoning with Bispyribac containing herbicide (Nominee® that contain surfactant poly ethylene glycol besides other solvents) also surfactant Tergitol® that used in formulation is a poly ethylene glycol considered toxic and irritant to GIT.

Also kidney showed cloudy swelling of the renal tubular epithelium with lymphocytic infiltration at dose level 1/40 of LD50 (equivalent to 65.88 mg/kg) and congestion of renal glomeruli with lymphocytic infiltration in interstitial tissue vessels at dose level 1/80 and 1/160 of LD50 (equivalent to 32.94 and 16.47 mg/kg) (Figure 1e and 1f) where Chaudhuri et al. [33] reported that kidney considered the main excretory organ and Because the divalent ions excretion considered as a major function of the renal tubules so heavy metals and pesticides affect these cells and hyaline droplets and degeneration in renal tubules indicate renal toxicity and such result agree with Jayasumana [34] who proposed that Bispyribac increase the risk of the chronic kidney disease CDKu that caused due to drinking polluted water with previous exposure to such herbicides seven time than normal in epidemiological study.

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