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Chemical Toxicity Assessment and Physiological Investigation in Rats Exposed to Pyrethroid Insecticides

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Abstract: Insecticides are chemicals that kill, restrict, or inhibit the reproduction and spread of creatures that compete for food, property, and human health. Rats were exposed to two insecticides, Pyrosol (P) (containing Pynamin forte 0.20% and Sumethrin 0.075%) and Kirox (K) (containing 0.25% ≈ 98% of the total contents of deltamethrin, and d-d- Trans cyphenothrine 0.12% ≈ 93% of the total contents) were investigated. After exposure to each pesticide (24 h), rats were weighed, blood samples were taken, and tissues were removed for analysis. The results indicated significant changes with an increase in liver and kidney functions, cardiac enzymes, acetylcholine esters in the brain, and thyroid hormones. There was also a significant increase in inflammatory markers (cytokines). On the contrary, a significant decrease in total antioxidants and body weight was observed in rats. Further, the histopathology study of different body organs was negatively affected by these pesticides. The results of the current study indicate that repeated, discontinuous inhalation of a commonly used insecticide containing Pynamin and deltamethrin can cause a variety of toxic effects to all body organs far from the lungs.

Keywords: insecticides; liver functions; kidney functions; cytokines; apoptosis; deltamethrin.

1. Introduction

Toxicity is becoming more of an issue daily, and heavy metals have acquired prominence due to their environmental risks [1–3]. To generate a clean environment, these metals must be removed from wastewater using sustainable novel approaches [4–6], followed by a more difficult step to stabilize and remediate the produced hazardous residues [7–9].

Humans began to manufacture and develop methods to control insect pests that compete for food in the last century on a large scale [10]. Where initially appeared a group of inorganic compounds such as arsenic compounds and compounds of plant origin such as (Rutonon - Nicotine - Parthrin), and in the early century used toxic gases such as hydrogen cyanide to smoke trees and at the same time emerged mineral oils tar and petroleum. After World War II, new industrial compounds such as compounds (organic chlorine or organic phosphorus) emerged, and those interested in pest control appeared to have achieved a tremendous victory and gave healing solutions to the eradication of pests and insects [10]. However, the repeated, excessive, and incorrect use of these pesticides revealed several problems that were not taken into account because the pesticide used at this stage was of a wide spectrum and very toxic to a large number of insect species, which led to the killing parasites and predators (vital enemies) and weakening their role in the process [11]. Pesticides no longer give the desired results, but

sometimes they also give the opposite result, especially when the characteristic of pesticide resistance appears in the behavior of the pest, such as the pesticide. It does not count the considerable damage pesticides cause to humans and the environment; these deteriorations are different in severity among living organisms, and talking about it requires all efforts to ban the use of well-known pesticides [12]. The insecticide particles enter the human body in the form of gases carried by air through breathing, and the effect of those harmful gases varies according to their chemical composition. The gases that dissolve in water also dissolve in the mucous fluid lining the upper part of the respiratory system, leading to acute infections [13]. Toxic pesticides penetrate the skin upon contact with it or enter the digestive system through contaminated vegetables and fruits that carry the remaining effects of these toxins and then reach the blood and to all parts of the body and settle in it and cause many serious diseases, including (liver diseases, kidney failure, and cancer) [14]. The results of scientific research also indicate that the residual effect of these pesticides leads to a weak sexual condition and ultimately causes infertility [15]. The current study aims to investigate the toxic effect of some insecticides, which harm humans and animals.

2. Materials and Methods

2.1. Pesticides.

Two commercial insecticides, namely Pyrosol (P) and Kirox (K), were used in the present study.

2.2. Animals.

Adult male albino rats weighing 170±10 g were obtained from the Animal Breeding House of the Nuclear Research Center, Cairo, Egypt, and maintained in clean plastic cages in the laboratory animal room (24±3 ^{o}C) on standard pellet diet and tap water ad-libitum. Rats were acclimatized for 1 week before the start of experiments. The experimental work on rats was performed with the approval of the Animal Care & Experimental Committee, Principles of laboratory animal care”” (NIH publication No. 85-23) [16] were followed, as well as specific national laws where applicable. All experiments were examined and approved by the appropriate ethics committee.

2.3. Study design.

Twenty-four rats were randomly divided into 2 main groups. Group 1: control (no exposure to pesticides) (n=8; 4 males, 4 females). Group II: It is divided into two subgroups (IIa and IIb), each consisting of 8 rats (4 males and 4 females). The rats in these subgroups were exposed to the aerosol preparation of the selected pesticides, namely, Pyrosol and Kirox. For exposure, the animal cage was sprayed (in all directions using a special attachment located on the ceiling in the center of the cage) with the selected insecticide for 30 seconds per minute for 15 seconds. Then, he kept the room closed for 15 minutes. After each spray separator, the rats were placed in the room for 30 minutes and transferred to a clean room. The exposure of the three groups was repeated three times. The interval between each cycle was 15 minutes of spray/15 minutes of the exposure stop for rats was 120 minutes. Twenty-four hours after the final exposure, the rats were weighed, and then blood samples and various body tissues were taken from the animals under light ether anesthesia; then, the serum was separated and used for

immediate determination of the biochemical parameters. The livers, kidneys, brains, spleens, testes, ovaries, and small and large intestines of all of the rats were removed for histopathological examination.

2.4. Collecting the blood samples.

The rats were sacrificed by cervical dislocation, the jugular vein was exposed and cut with a sterile scalpel blade, and the rats were bled into EDTA-coated specimen bottles. Plasma was obtained from a portion of the blood sample by centrifugation at 1500 xg for 5 min. All plasma samples were stored in the refrigerator at 4°C before analysis.

2.5. Measurements.

2.5.1. Measurement of biochemical parameters Liver and kidney functions.

Measurement of serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), bilirubin, Alkaline Phosphatase (ALP), albumin, and Gamma Glutamyl Trans-peptidase (GGT), blood samples were collected from the rats under anesthesia in clean, dry Wassermann tubes by puncture of the retro-orbital plexus using heparinized capillary tubes. The samples were allowed to stand for 15 min to clot at room temperature and then centrifuged (400 xg for 10 min). The serum was collected and used immediately for the determination of serum ALT, AST, ALP, and albumin using a Biomerieux kit (France) and total bilirubin using a Biocon kit (Biocon Diagnostik, Germany) and GGT (Fortress Diagnostics, Ltd., UK). Estimation of lipid peroxidation (TBARS) assay was carried out by following the method of [17]. Total protein concentration was determined by the method of [18] using Stanbio Laboratory kits. Acetylcholinesterase (AChE) activities were determined by [19] using Qumica Clinica Aplicada kits. Creatinine, BUN, and Kidney injury molecule-1 (KIM-1) concentrations were determined using Rat KIM-1 ELISA Kit (ab223858).

2.5.2. Detection of cytokines and total antioxidant capacity.

Serum cytokines levels such as interleukin-6 (IL-6), interleukin-12(IL-12), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (INF-γ) were determined by ELISA using commercially available kits (R&D systems, USA) according to the 'manufacturer's instructions. The cytokine concentrations were then calculated from the standard cytokine curve on the same sample plate. The total antioxidant capacity of serum was measured according to the method of Benzie and Strain [20]. Briefly, a working solution of FRAP (ferric reducing antioxidant power) was provided by mixing buffer acetate with TPTZ solution in HCl. After that, $FeCl_3$ was added and mixed. 8 µl of serum and 240 µl of the mentioned working solution were mixed and incubated for 10 min at room temperature. The optical density of samples was measured at 532 nm. Total antioxidant capacity was expressed as mmol/l.

2.5.3. Checks of programmed cell death.

The expression of caspase-3, Bax, and Bcl-2 was evaluated by staining with the ELISA kit according to manufacturer protocols.

2.5.4. Hematology parameters.

Hematological parameters (total white and red blood cells, hemoglobin concentration, platelet count, and PCV percentage) were determined using the Sysmex (Automated Hematology Analyzer Kx-2IN, Sysmex Corporation, Kobe-Japan). It employs a WBC detector block and a WBC/HGB lyse reagent to measure the WBC count and hemoglobin concentration, as described in Lopez-Rubio et al. [21]. Blood was collected into microfuge tubes, which contained EDTA; whole blood, 44.7 pl, for electronic measurements, was aspirated into a calibrated pipette and suspended in 10 ml Isotone I1 medium (Unopette micro collection system, Becton Dickinson, Rutherford, NJ). Another 5 pl of blood was diluted in 1 ml RBC suspension media for morphologic examinations, whereas the rest was aspirated into a thin, 12 cm long polyethylene capillary tube (code 19-0040-0 1, Pharmacia, Uppsala, Sweden). The capillary tube was sealed at one end by gently heating it over a flame. Each batch of tubes had been carefully calibrated by weighing the water contained by a certain length. The tubes with blood were centrifuged at 1500 x g for 15 min (4°C), supported by a rigid holder. The microhematocrit was recorded, and a length corresponding to 25.0 pl of packed RBC was cut from the mid-section of the RBC column and suspended in 50 ml of buffer to yield a hematocrit of 0.05%.

2.5.5. Detection of Rat Nuclear Factor-Kappa (NF-kB).

Plasma from all experimental animal groups was used to determine NF-kB activity using a commercially available nuclear extraction kit from (Wuhan Fine Biotech Co., Ltd. Catalogue No.: ER1186), sensitivity: 0.094 ng/ml.

2.5.6. Histopathological studies.

Liver and small intestine tissue samples were taken from the control and experimental groups, and the formalin fixation was performed 10% for approximately 24 hours. After several steps, according to the [22], paraffin sections were cut to 4 μm thick. These sections were then stained with hematoxylin-eosin dye and examined by light microscopy.

2.6. Statistical analysis.

The parameter values were all expressed as the mean ± SEM. Significant differences between the groups were determined by a Two-way analysis of variance (ANOVA) followed by 'Tukey's multiple comparison tests using the SPSS 23.0 software package program. The results were considered significant if $P < 0.05$.

3. Results and Discussion

Rats from the group exposed to insecticides, which were under study 24 hours after exposure, significantly decreased body weights in males and females. It was also found that the BW decreased in Pyrosol and Kirox (males: 22.40% and 25.30% respectively, $p < 0.05$, $n = 4$; females 21.02% and 24.63%, respectively, $p < 0.05$, $n = 4$) (Table 1).

The present study evaluated the toxicity and the effects of dietary exposure to the insecticides Pyrosol and Kirox. The animals ceased eating at higher concentrations of the insecticide. Still, enough was consumed to affect or kill the animals severely, confirming the findings that the chemically stable, lipid-soluble, organophosphorus insecticide is hazardous to

health [23]. Other studies have shown that chemicals accumulate rapidly in tissues, causing death and affecting growth in mallard ducklings [24]. The pattern of mortality in this study suggests that starvation was not the primary cause of death. Although the animals may have been killed by ingesting the active ingredients, the propellant chemical may have aggravated the toxic effects [25]. However, a summary of insecticides like Raid was presented, describing them as having acute dermal toxicity of 330 mg/kg bw/d for rats, which is considered extremely high [26].

Two-way ANOVA of cytokine concentrations of control and treatment groups, as shown in Table 2. A significant increase in pro-inflammatory cytokines was seen 24 hours after the experiment, which reflects the rapid severity of the inflammatory activity.

Regarding TNF-α, after the exposure to Pyrosol (P) and Kirox (K), the TNF-α concentration increased by 71.33% and 155.33% in males and 76.77% and 208.59% in females, respectively, when compared to the control. There was a significant increase in IFN-gamma after exposure to P and K. The highest values were observed in the case of K, either males or females; the level increased by 282.83% and 141.29%, respectively, compared to the control. Similarly, there was a significant increase in IL-12 in males and females after the exposure to P and K; the IL-12 concentration increased by 99.20% and 385.01% in males and 163.84% and 305.09% in females, respectively, when compared to the control.

Table 1. Effect of insecticides (P & K) on the body weight in the experimental rats.

		Body weight (gm)			
Groups	**Males**	**Females**	**F1 (Sex)**	**F2 (Treatments)**	**F3 (Sex* Treatment)**
GI GIIa	171.75± 2.67^{a} 133.31 ± 1.42^{b}	175.38± 5.43^{a} 138.51 ± 3.37^{b}	0.579 P= 0.452	44.39 P= 0.000	0.424 P= 0.73
GIIb	130.50 ± 2.12 ^{b}	134.70 ± 2.32^{b}			

Values are expressed as means ± SD. Different superscript letters in the row indicate significant differences at p< 0.05.

Table 2. Effect of pesticides on cytokines in the experimental rats.

Groups	**TNF- α (pg/mg)**		**INF-γ (pg/ ml)**		**IL-6 (pg/mg)**		**IL-12 (pg/ ml**	
	Males	Females	Males	Females	Males	Females	Males	Females
GI	37.50 ± 2.08^{d}	29.70 ± 1.23^{d}	209.69± 18.16^{d}	307.52 ± 21.07^{d}	30.66±2.10^{d}	39.50 ± 2.03^{d}	72.38± 4.05^{d}	83.50 ± 9.08^{d}
GIIa	64.25 ± 4.56^{c}	52.50 ± 3.01^{c}	446.30 ± 23.12^{c}	721.25 ± 14.50^{c}	225.95±13.27^{c}	326.30 ± 14.56^{c}	144.18± 8.04^{c}	220.31 ± 14.02^{c}
GIIb	95.75 ± 4.73^{a}	91.65 ± 5.79^{a}	802.75 ± 26.81^{a}	742.01 ±27.81^{a}	510.7±11.59^{a}	834.07± 24.13^{a}	351.05± 10.13^{a}	338.25± 16.24^{a}
F1 P-value	56.72 0.000		33.20 0.000		9.98 0.000		67.51 0.000	
F2 P-value	787.27 0.000		43.21 0.000		55.34 0.000		84.23 0.000	
F3 P-value	87.59 0.000		82.49 0.000		91.21 0.000		43.27 0.000	

Values are expressed as means ± SD. Different superscript letters in the row indicate significant differences at p< 0.05.

The main interest in our work was to evaluate pro-inflammatory cytokines (such as IL-1β, IL-12, IFN-γ, IL-6, and TNF-α), which enhance the proliferation of T- and B- lymphocytes, antibody synthesis, production of adhesion molecules, and synthesis of acute phase proteins.

Serum ALT, AST, γGT, and ALP activities, as well as total bilirubin concentration, was significantly increased (p<0.05) because of P and K administration. The total protein,

albumin, and globulin levels were increased in a dose-dependent manner (Table 3). Tissue insecticide concentration increased in lipid, muscle, brain, and liver. The insecticide accumulation in these organs may indicate that the active ingredients are also absorbed, resulting in decreased enzyme formation, which should accelerate immediate metabolic processes but instead remain complex in the tissue organs. Additionally, the retention time (within 10 min) may be increased because of its easy solubility and lower excretion rate, which leads to bioaccumulation [27]. Exposure to P and K resulted in hematological changes, such as decreased erythrocyte count in males and females. The liver synthesizes enzymes and structural proteins and detoxifies many internal and external products of the organism [28]. For these functions, hepatocytes use enzyme systems. The present study revealed increased liver enzymes, total bilirubin concentration, and plasma albumin and albumin/globulin ratio. Pesticides affect several human organs, resulting in various physiological changes. Compared with control participants, the pesticide sprayers showed significantly increased liver function marker enzymes, serum bilirubin, creatinine, and blood urea [29].

Table 3. Effect of pesticides on liver functions.

Groups	AST (U/l)		ALT (U/l)		GGT (U/l)		Bilirubin (U/ I)	
	Males	Females	Males	Females	Males	Females	Males	Females
GI	30.03 ± 2.46^{c}	26.01 ± 1.07^{c}	16.25± 1.63^{c}	20.75 ± 0.95^{c}	0.34±0.02 a	0.28 ± 0.01^{a}	0.32± 0.04^{d}	0.29 ± 0.01^{d}
GIIa	53.50 ± 3.11^{b}	42.25 ± 1.70^{b}	28.50 ± 1.91^{b}	38.50 ± 1.29^{b}	0.12±0.01 b	0.051 ± 0.007^{b}	1.34± 0.06^{c}	0.87 ± 0.03^{c}
GIIb	57.60 ± 3.41^{a}	70.20 ± 4.80^{a}	41.20 ± 2.52^{a}	49.30 ±0.95^{a}	0.02±0.00 4^{d}	0.018± 0.002^{d}	1.72± 0.03^{a}	1.58± 0.02^{a}
F1	14.82		199.19		108.86		40.496	
P-value	0.000		0.000		0.000		0.000	
F2	266.40		328.60		933.02		225.675	
P-value	0.000		0.000		0.000		0.000	
F3	32.904		12.43		12.45		6.293	
P-value	0.000		0.000		0.000		0.003	

Values are expressed as means ± SD. Different superscript letters in the same row indicate significant differences at p< 0.05.

Continuous Table 3. Effect of pesticides on liver functions.

Groups	ALP (U/l)		Total protein (g/dl)		Albumin (g/dl)		Globulin (g/	dl)
	Males	Females	Males	Females	Males	Females	Males	Females
GI	133.01 ± 8.93^{d}	127.00 ± 10.33^{d}	5.22± 0.37^{a}	5.26 ± 0.53^{a}	4.28± 0.28^{a}	4.68 ± 0.47^{a}	0.94± 0.09^{ab}	1.03 ± 0.21^{ab}
GIIa	325.75 ± 12.01^{c}	252.30 ± 26.60^{c}	4.13 ± 0.32^{b}	4.02 ± 0.30^{b}	3.27± 0.31^{b}	3.67 ± 0.55^{b}	0.85± 0.23^{a}	1.99± 0.67^{a}
GIIb	402.25 ± 17.81^{a}	366.41 ± 11.09^{a}	3.90 ± 0.09^{c}	3.88 ±0.40^{c}	3.03± 0.15^{b}	3.78± 0.30^{b}	0.85± 0.21^{c}	0.08± 0.03^{c}
F1	61.94			29.93		30.97	0.03	
P-value	0.000			0.000		0.000	0.86	
F2	453.18			46.51		23.46	10.63	
P-value	0.000			0.000		0.000	0.000	
F3	6.29			8.30		2.46	11.16	
P-value	0.003			0.001		0.087	0.000	

Values are expressed as means ± SD. Different superscript letters in the same row indicate significant differences at p< 0.05.

Table 4. Effect of pesticides on the LDH, CK-MB, LP, and TA.

Groups	LDH (U/l)		CK-MB(U/l)		LP (g/dl)		TA (%)	
	Males	Females	Males	Females	Males	Females	Males	Females
GI	225.50 ± 12.23^{c}	321.0 ± 9.81^{c}	201.86± 14.16^{c}	226.0±13.8 0 ^{c}	9.33±0.41 a	8.35 ± 0.66^{a}	1.41± 0.004^{d}	1.55 ± 0.02^{d}
GIIa	503.25 ± 12.31^{b}	784.5 ±20.10^{b}	408.50 ±20.10^{b}	613.25±17. 12 ^{b}	28.48±1.6 1^{b}	38.11 ± 1.33^{b}	0.35± 0.01^{c}	0.44 ± 0.01^{c}
GIIb	735.25± 11.21^{a}	789.0 ± 23.15^{a}	685.50 ±25.12 ^{a}	675.50 ±30.44^{a}	33.02±1.4 1^{d}	48.75± 1.94^{d}	0.13± 0.01^{a}	0.03± 0.003^{a}
F1	290.50		738.44		190.66		0.34	

Groups	LDH (U/l)		CK-MB(U/l)		LP (g/dl)		TA (%)	
	Males	Females	Males	Females	Males	Females	Males	Females
P-value	0.000		0.000		0.000		0.57	
F2	2033.46		553.61		1052.011		753.34	
P-value	0.000		0.000		0.000		0.000	
F3	115.86		283.46		42.80		10.01	
P-value	0.000		0.000		0.000		0.000	

Values are expressed as means ± SD. Different superscript letters in the same row indicate significant differences at $p< 0.05$

The increased liver enzymes because of the toxic sprays could be explained by inflammation and tissue damage induced by the previous toxic materials' effect on the liver hepatocytes.

The LDH, CK-MB, LP, and total antioxidants (TA) activities were increased in a dose-dependent manner in males and females (Table 4). While the low dose produced the most potent effect on AST activity, the high dose seemed to be the most effective on ALT, γGT, ALP, LDH activities, and total bilirubin concentration.

Serum creatinine, BUN, and KIM-1 levels were significantly increased because all testes of the previous toxic materials were administered to rats (Table 5). It was also found that the hematological counts decreased in males (RBC 31.22% and 43.19%); and in females (45.05% and 52.62%), $p < 0.05$, $n = 4$.

These results were consistent with the findings of [30] study on the toxicity and bioaccumulation of the insecticide P and K 'on rats, and their results suggested inhibition of some key metabolic enzymes resulting from the accumulation of the insecticide components in the tissues of rats were administered.

Rats from the group exposed to toxins, which were under study 24 hours after exposure, significantly decreased the RBC, PCV, MCH, and Hb and WBC counts in males and females (Table 6). PCV 60.36% and 61.91%; as well as, in females 64.69% and 74.46% $p < 0.05$, $n = 4$). MCH and Hb concentrations significantly decreased; the highest values were observed in the case of K in males and females (48.25% and 44.27%, respectively) compared to the controls. The white blood cells (WBC) or leucocyte count was significantly decreased due to the previous toxic materials; the percentage decreases were 26.804 and 40.689 %, respectively.

Table 5. Effect of pesticides on kidney functions.

Groups	BUN (U/l)		Creatinine (g/dl)		KIM-1 (pg/ml)	
	Males	Females	Males	Females	Males	Females
GI	27.19 ± 1.40^{d}	31.25 ± 1.26^{d}	0.34 ± 0.04^{d}	0.41 ± 0.06^{d}	322.50 ± 15.71^{e}	345.92 ± 20.55^{e}
GIIa	76.75 ± 2.28^{c}	55.52 ± 1.30^{c}	1.04 ± 0.03^{c}	1.28 ± 0.03^{c}	835.68 ± 17.11^{c}	954.78 ± 18.40^{c}
GIIb	114.26 ± 7.70^{a}	95.23 ± 4.12^{a}	1.75 ± 0.05^{a}	1.71 ± 0.05^{a}	1210.41 ± 30.00^{a}	1306.40 ± 28.20^{a}
F1	247.61		37.38		55.87	
P-value	0.000		0.000		0.000	
F2	319.95		586.29		67.12	
P-value	0.000		0.000		0.000	
F3	45.47		10.99		22.34	
P-value	0.000		0.000		0.000	

Values are expressed as means ± SD. Different superscript letters in the same row indicate significant differences at $p< 0.05$

The present study shows that the administration of P and K to albino rats significantly decreased red blood corpuscle count, white blood cells (WBC), hematocrit (PCV), and hemoglobin content at medium and high doses in a dose-dependent manner. However, the mean cell volume (MCV) of red blood corpuscles, as well as mean cell hemoglobin (MCH),

significantly increased as a result of the administration of the toxic materials used in a dose-dependent manner.

Table 6. Effect of pesticides on the hematological constituents.

Groups	RBCs (g/dl)		PCV (%)		MCH (%)		Hb		WBCs (x 10^6/mm^3)	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
GI	11.53 ± 0.75^a	12.01 ±74^a	44.47± 4.60^a	41.15 ± 2.14^a	29.43±1.68^d	25.18 ± 1.35^d	12.00 ± 1.05^a	13.10 ± 0.61^a	8.05± 0.36^a	7.32 ± 0.50^a
GIIa	5.37 ± 0.35^b	6.25 ± 0.81^b	17.63± 1.27^b	14.53 ± 1.28^b	41.01±1.82^c	45.30 ± 3.32^c	7.03 ± 0.43^b	10.00 ± 0.74^b	4.43± 0.29^b	5.35 ± 0.44^b
GIIb	6.55 ± 0.42^c	5.69 ± 0.33^c	16.94 ± 1.56^b	10.51 ±1.29^b	48.60±2.91^a	46.50± 3.43^a	6.21± 0.31^c	7.30± 0.62^c	4.20± 0.54^{bc}	4.25± 0.53^{bc}
F1 P-value	0.007 0.12		7.64 0.01		60.50 0.000		60.31 0.000		67.51 0.000	
F2 P-value	143.34 0.000		123.58 0.000		922.97	0.000	62.23 0.000		84.23 0.000	
F3 P-value	9.95 0.000		0.684 0.57		135.91 0.000		5.76 0.004		43.27 0.000	

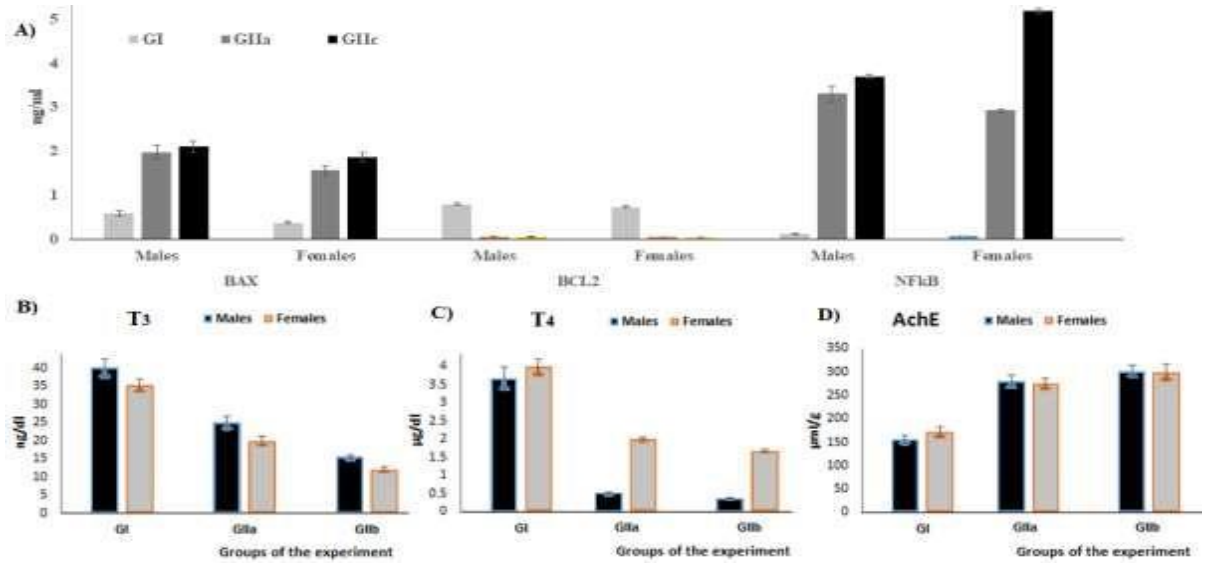

Figure 1. A) Effect of P and K on Bax, Bcl2, caspase-3, and NF-kB, B) T3, C) T4, and D) AChE in rats.

The extent of the negative effect of P & K administration on BAX, BCL2, and NF-KB was investigated in this study. These pesticides caused a substantial rise ($p < 0.05$) in both. BAX and NF-KB in males and females, whereas BCL2 increased by more than 95% in males and females (Fig. 1 A). In addition, the effects of P& K on T3, T4, and AChE (Figs. 1 B-D), respectively. T4 and AChE showed a dramatic increase ($p<0.05$) in males and females up to 60%, although T3 showed a significant decrease ($p<0.05$) when compared to the control.

The role of Bax and Bcl-2 in the process of apoptosis has been widely investigated in numerous in vitro. It has been widely investigated in numerous in vitro and in vivo systems. Previously, Zoja et al. [31] found that the up-regulation of caspase-3 at activity, protein, and mRNA levels positively correlated with apoptosis, inflammation, proliferation, and subsequent glomerulosclerosis, tubular atrophy, and interstitial fibrosis. In our current study, we have observed an increase in caspase-3 and Bax but a decrease in Bcl-2. Results in the present study also indicated a highly significant decrease in AChER among the exposed group compared to the control group. According to Sahota et al. [32], the pyrethroid deltamethrin caused a prolonged decrease in AChE activity in rats after single-dose administration [32]. These agents

increase Na^+ influx into synaptic terminals and create a hypopolarized and hyperirritable synaptic membrane, increasing the release of the neurotransmitter acetylcholine [32].

3.1. Histopathological findings.

Rats which administrated insecticide (K) revealed congestion in the central and portal veins of liver associated with oedema and inflammatory cells infiltration in the portal area (Fig. 2A).

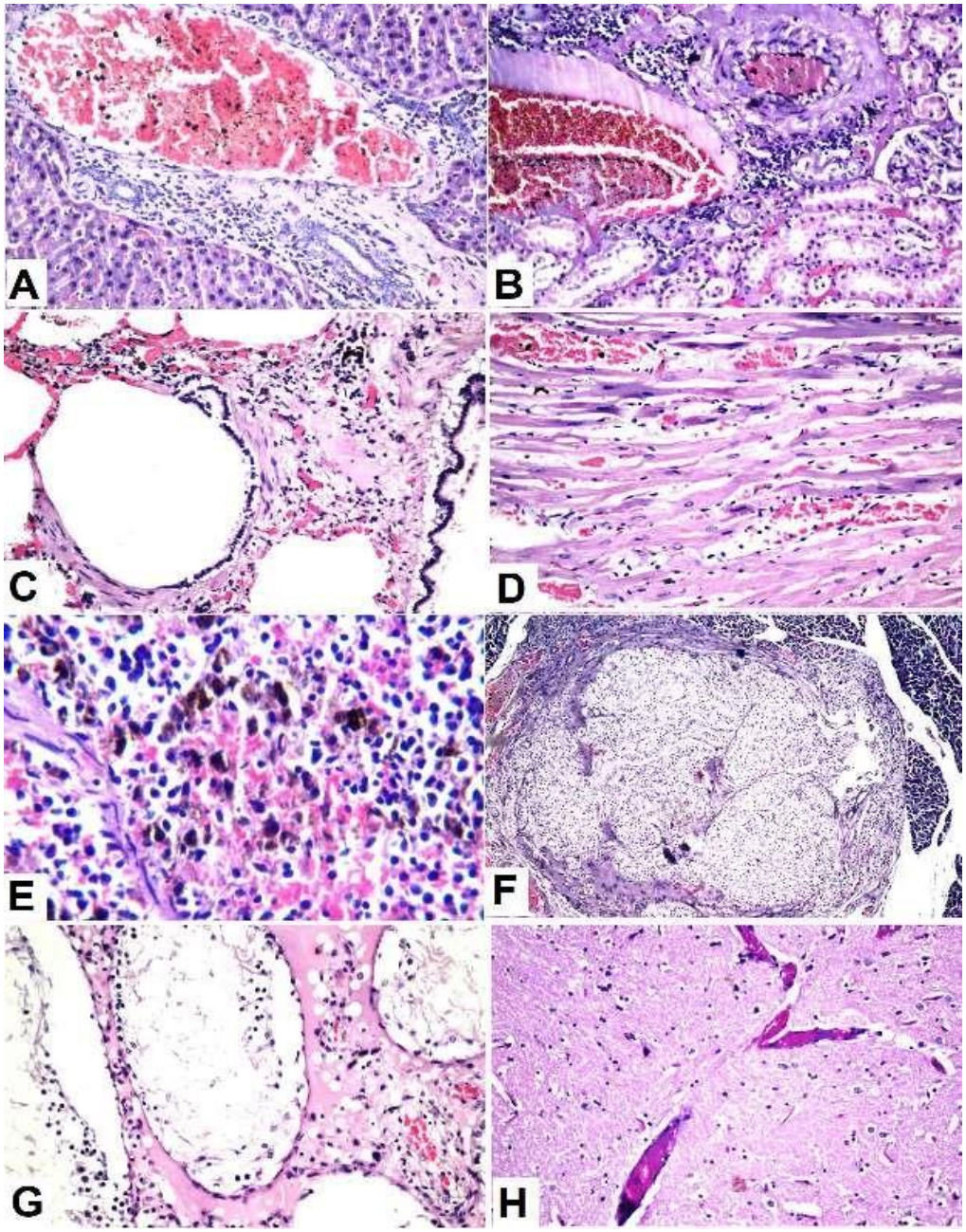

Figure 2. Effect of Kirox on the tissues of rats.
A) Liver: Congestion was detected in the central and portal veins with fibrosis in the portal area. B) Perivascular and perglomerular inflammatory cell infiltration in kidney. C) There was bronchiolar epithelial hyperplasia with peribronchiolar fibrosis in the lung. D) Sever congestion was observed in the blood vessels and capillaries in the myocardium. E) There was ischemia with severe hemosiderosis in the red. F) Circumscribed round anaplastic cells proliferation in the islet of Langerhans cells with atrophy in the surrounding acini (Adenoma). G) There was degeneration with loss of spermatogenic series in the lumen of the seminiferous tubules. H) There was diffuse gliosis with congestion of the blood vessels in the striatum.

There was perivascular inflammatory cells aggregation surrounding the congested cortical blood vessels in association with degeneration in the tubular lining epithelium of kidneys (Fig. 2B). The interstitial stromal tissue of lungs showed oedema, inflammatory cells infiltration and focal pigmentation (Fig. 2C). As well as, inflammatory cells infiltration with congestion in the blood vessels were detected in between the myocardial bundles (Fig. 2D). Additionally, there were ischemia with sever hemosiderosis in the red pulps of spleen (Fig. 2E). Circumscribed round anaplastic cells proliferation in the islet of Langerhans cells with atrophy in the surrounding acini (Adenoma) (Fig. 2F).

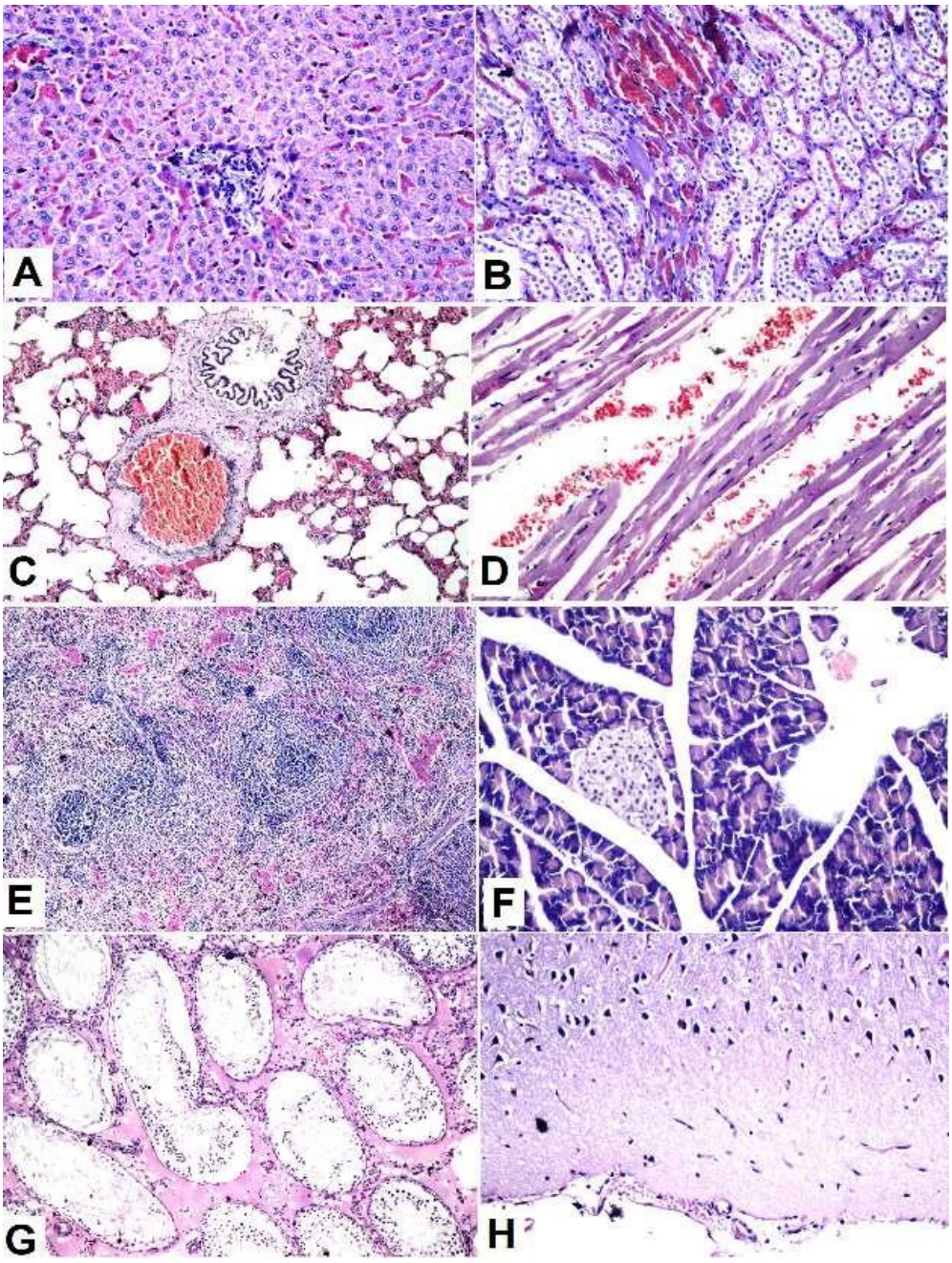

Figure 3. Effect of Pyrosol on rats.

A) The portal area showed edema, inflammatory cell infiltration with congestion in the portal vein. B) Focal hemorrhages with tubular degeneration were detected in the cortex of the kidney. C) Congestion was observed in the blood vessels associated with perivascular and peribronchiolar edema, as well as emphysema in the air alveoli. D) There were focal hemorrhages in between the myocardial bundles. E) [no histopathological alteration in the pancreas. G) There was degeneration with loss of spermatogenic series in the lumen of the seminiferous tubules associated with disintegration and edema in the interstitial Leydig cells in between. H) Nuclear pyknosis and degeneration were detected in the neurons of the cerebral cortex.

There was degeneration with lose of spermatogenic series in the lumen of the seminiferous tubules associated with disintegration and oedema in the interstitial Leydig cells in between (Fig. 2G). Nuclear pyknosis and degeneration were detected in the neurons of the fascia dentate in the hippocampus (Fig. 2H). There was diffuse gliosis with congestion of the blood vessels in the striatum. Similarly, a group of rats' administrated insecticide (P) revealed congestion in the central vein and sinusoids (Fig. 3A) associated with dark pigmented material between the hepatocytes all over the parenchyma. The portal area showed edema inflammatory cell infiltration with congestion in the portal vein. Focal necrosis was detected in the parenchyma. Focal hemorrhages with tubular degeneration were detected in the cortex of kidney, as well as in the corticomedullary junction (Fig. 3B). Congestion was observed in the blood vessels associated with perivascular and peribronchiolar edema as well as emphysema in the air alveoli (Fig. 3C). There were focal hemorrhages in between the myocardial bundles (Fig. 3D). Lymphoid depletion was detected in the white pulps. At the same time, the red one showed hemosiderosis of the spleen (Fig. 3E). There was no histopathological alteration in Pancreas (Fig. 3F). There was no histopathological alteration in the testes (Fig. 3G). Nuclear pyknosis and degeneration were detected in the neurons of the cerebral cortex (Fig. 3H).

Cell toxicity was increased in a dose-dependent manner after 24-hour exposure, indicating that P and K can activate cell death in rats. Apoptosis is programmed cell death that serves as a mechanism to maintain homeostasis in normal cells, and initiation of apoptosis may be a principal pathological event that causes cell alteration and diseases [33].

4. Conclusions

The administration of Pyrosol and Kirox to albino rats produced macrocytic anemia, leucopenia, elevated liver enzymes, and bilirubin, indicating hepatic damage. These changes are mainly dose-related, but there is no evidence of carcinogenic effect by tumor markers.

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Conflicts of Interest

The authors declare no conflict of interest.

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