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EFFECTS OF ORGANOPHOSPHATE AND PYRETHROID INSECTICIDES MIXTURE ON THE HAEMATO-BIOCHEMICAL AND HISTOLOGICAL FUNCTION OF MALE ALBINO RATS

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ABSTRACT

Pesticides have been used to treat agricultural lands since ancient times. The effects of an organophosphate (OP) and pyrethroid combination on the haemato-biochemical and histological functions of male albino rats were investigated. Male albino rats (total 18 rats, 12 are treated and 6 are control) were dermally treated with two organophosphate and pyrethroid combinations for 8 weeks (chlorpyrifos, chlorpyrifos-methyl, and lambda-cyhalothrin). We also looked at organ weights, haematological and histological changes, and clinical symptoms. Except for RBCs, total protein, and haemoglobin, all haematological measures rose in treated rats after 8 weeks. After 4 weeks, Runsave and Lambaphos treatments had RBC counts of 5.02 and 5.15 10⁶/mm³, respectively, compared to 6.1 10⁶/mm³ in the control group. Haemoglobin values for Runsave, Lambaphos, chlorpyrifos, chlorpyrifos-methyl, and lambda-cyhalothrin were 13.53, 14.27, 14.60, 15.03, and 15.27 g/100 ml after toxicant administration, compared to 16.53 in the control. Haematological parameters change correlated positively with exposure time. A histological examination on liver and kidney found that Runsave-treated rats had an uneven structure, severely congested blood arteries, necrotic areas, fatty liver, pyknotic nuclei, and cytoplasmic vacuolation in hepatic cells. Lambaphos-treated liver shows haemolysis, cytoplasmic vacuolation, pyknotic nuclei, and partial structural disruption. Bowman's capsule, glomeruli, and proximal tubule are normal in the control kidney. Runsave had abnormal renal anatomy. Lambaphos kidneys are asymmetrical. This study demonstrated that albino rats subjected to an insecticide mixture of organophosphate and pyrethroid had significant histological and haemato-biochemical changes compared to the control group.

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INTRODUCTION

Organophosphate (OP) compounds are most commonly used insecticides which non-competitively inhibit the enzymatic activity of acetylcholine esterase which metabolizes acetylcholine. The enhanced cholinergic activity of muscarinic and nicotinic acetylcholine receptors causes the acute symptoms of OP poisoning (Zhang *et al.*, 2021; Yongcai *et al.*, 2020 and Minton and Murray, 1988). Pyrethroids are ion channel poisons that prolong neuronal excitement without being cytotoxic. Two basic poisoning syndromes might be seen. Reflex hyperexcitability and fine tremor are caused by type I pyrethroids, while salivation, hyperexcitability, choreoathetosis, and seizures are caused by type II pyrethroids (Ray *et al.*, 2000, Hossain *et al.*, 2017, Hossain *et al.*, 2021). Paresthesia and respiratory irritation are common side effects of pyrethroid exposure, which are likely caused by the recurrent firing of sensory nerve endings (Rahman *et al.*, 2016, Mahbub *et al.*, 2018, Karim *et al.*, 2022).

The mixtures of insecticide are generally applied in the field so that they can enhance the spectrum of the control when multiple pests are attacking simultaneously. Since the mid-1980s, organophosphorus (OP) and pyrethroid (PYR) insecticides have been frequently used to manage pest complexes of cotton and other crops in Egypt. Pesticide companies sell these mixtures as premixes, or farmers can mix them in their tanks. In theory, insecticides with diverse modes of action should be used together to complement each other's actions in killing the target pest (Abd El-Mageed & Shalaby, 2011; Ahmad, 2004). Different chemicals and co-exposure to a mixture of two insecticides can result in various physiological, behavioural and haematological changes in living cells (Karim *et al.*, 2022; Hasan *et al.*, 2022; Rahman *et al.*, 2016). The majority of prior studies found that (OP/PYR) combinations were more hazardous to insects and mammals than their individual parts (Ahmad *et al.*; 2007; Latuszynska *et al.*, 2003; Latuszynska, 1999; Latuszynska, 2001).

Inhalation or dermal exposures are the most common methods of pesticide exposure in the workplace (Sullivan *et al.*, 1992). However, Dermal exposure was larger than inhalation, accounting for around 73 percent of total exposure. The majority of occupational exposures are caused through absorption through exposed skin, such as the face, hands, forearms, neck, and chest (Fenske, 1990). Human skin exposure to pesticide mixes occurs during activities such as preparing working solutions, spraying, and cleaning equipment. As a result, occupational poisoning is caused by improper handlings, such as spraying without a protective mask and gear, spraying with a higher concentration or for a longer period of time, and cleaning sprayer obstructions with the mouth (Luty, 1998).

The literature lacked sufficient data on the harmful effects of pesticide mixtures (OP/PYR) administered topically on mammals. The current study compared the toxicological properties of two commercial preparations of OP/PYR mixtures (namely Runsave and Lambaphos) to adult male rats treated dermally with sublethal doses to their individual insecticides for 8 weeks because the majority of spray workers and pest control exterminators in Egypt are men and occupational poisoning is primarily caused by dermal exposure (Al-Ansary *et al.*, 2016). Hazard assessments could be performed employing hematological and histological examinations, as well as weighing the relative weights of various critical organs in comparison to a control group. Scientists have been more concerned in recent years that human-made toxins might impair physiological and haemato-biochemical functions in both wildlife and humans. It has been seen that long-term exposure to OP/PYP mixture can result in cancer, neurological disorders, reproductive and developmental damage, and respiratory issues (Bassil, 2007; Zaganas, 2013; Parrón *et al.*, 2011; Hanke & Jurewicz, 2004; and Mahananda & Mohanty, 2012). In general, this mixture of compounds binds to the amino acid serine, rendering it incapable of engaging in a catalytic activity inside the enzyme, as well as the further blockage of the active site by the organophosphate residue, results in high toxicity (Atamanalp and Yanik, 2003). As a result, the current research comprises examining the histological and haemato-biochemical effects of the two OP/PYR mixes on male rats.

MATERIALS AND METHODS

All the experiments of the present study were conducted at the Laboratories of Plant Protection Department, Faculty of Agriculture, Al-Azhar University, Cairo. These experiments were focused on investigating the adverse effects of two commercial preparations of OP/PYR mixtures (Runsave and Lambaphos) if spray workers were exposed to them at different times. Therefore, the experiments were carried out on adult male albino rats after the Ethics Committee of the Faculty of Agriculture, Al-Azhar University, Egypt, approved the protocol of experiments and procedures used in the study.

The experiments were divided into two parts:

Part I: To study the toxicological properties of Runsave (30% chlorpyrifos + 3% lambda-cyhalothrin) and Lambaphos (50% chlorpyrifos-methyl + 0.5% lambda-cyhalothrin) and their individual insecticides to rats treated dermally with sublethal doses of them for 8 weeks. Clinical manifestations were recorded. At the termination of experiments numbers of rats were sacrificed for haematological and histopathological studies as well as measuring the relative weights of many internal organs, all concerning those of individual insecticides and corresponding controls. The number of rats was sacrificed after 4 weeks from their exposure to these mixtures in order to study the effect of exposure time on the aforementioned measurements. Thus, the side-by-side comparison would be accurate since it had occurred under identical test conditions.

Part II: "Applied part". To study the effects of candidate mixtures on the haemato-biochemical and histological properties of male albino rats which were exposed dermally to sublethal doses of these mixtures for 8 weeks.

Test Animals

Thirty-six adult male Sprague-Dawley rats *Rattus norvegicus albinus*, their weights ranged from (190-210 g), were purchased from the Biological Products and Vaccines Holding Company, Helwan farm, Cairo, Egypt. Rats were maintained under the laboratory conditions of $25\pm 5\text{ }^{\circ}\text{C}$ and $65\pm 5\%$ R.H for two weeks before starting the experiments for acclimatization. They were housed in metal cages (65 x 25 x 20 cm) with a 12:12 hr - light/dark cycle and maintained on *ad Libitum* diet and water.

Experimental design

Table 1. Experimental design and number of rats used

For chlorpyrifos, chlorpyrifos-methyl, and lambda-cyhalothrin the selected sublethal doses were 1/30 of their dermal LD_{50} values as reported in the Pesticides Manual (Tomlin, 1997). Thus, the estimated sublethal doses used as formulated products for them were 139, 246.6, and 442 mg/kg, respectively. For OP/PYR mixtures (Runsave and Lambaphos), the chosen sublethal doses of their formulated products were 121.2 and 146.5 mg/kg, respectively. The selection of these sublethal doses was based on both preliminary experiments (which didn't cause any rat mortality after 8 weeks) and in the neighbourhood of those reported previously with OP/PYR mixtures (Latuszynska, 1999; Noaishi *et al.*, 2013). The experiments were divided into two parts:

Haematological study

This study was undertaken of male rats to demonstrate the effects of the candidate toxicants on their blood pictures. Thus, at each time interval blood samples were collected from each sacrificed rat and divided into two parts. The first part was used for counting the red blood corpuscles (RBCs), white blood cells (WBCs) (Dacie and Lewis, 1984), and the determination of haemoglobin content (Van Kampen & Zijlstra, 1961). The second part was allowed to stand at room temperature for 10 mins, and then centrifuged at 3000 r.p.m for 15 mins. The supernate serum was removed and kept under freezing conditions until used for the determination of plasma aminotransferases activities (AST and ALT), total protein, creatinine, and urea levels activity in plasma.

Runsave and Lambaphos were obtained from Chema Industries, Cairo. Six Commercial kits used for determination of AST, ALT, creatinine, urea, and total protein concentration were purchased from Diagnostic Company, while those used for determination of haemoglobin was purchased from Diamond Diagnostic Company, and other chemicals (NaCl, MgCl_2 , Na_2HPO_4 , and HCl) were purchased from El-Nasr Pharmaceutical Chemicals Company. Acetylthiocholine iodide and 5, 5-Dithiobis-2-nitrobenzoic acid was purchased from Pasteur Laboratory Company, Egypt.

Histopathological study

The present work was planned to investigate the influence of candidate toxicants on the histology of many organs such as the liver (the essential organ for drug metabolism), and kidney (the essential organ for drug excretion). These organs were fixed by immersion in 10% formalin for two days, washed in tap water, then all organs dehydrated in ascending grad of ethyl alcohol and finally cleared with xylene and embedded in melted paraffin wax. The paraffin block was six-micron cut and stained by haematoxylin and eosin to show the abnormalities in structure between treated and control sections (Everson, 1968). These sections' outcomes were photographed under the microscope.

RESULTS

Haematological study

The effect of candidate toxicants on many haematological parameters (i.e., counts of R. B. Cs and W. B. Cs, the levels of haemoglobin (Hb), AST, ALT, creatinine, urea, and total protein) were listed in Tables (2, 3) and Figures (1, 2). These results showed that all the parameters examined increased in treated rats except R. B. Cs counts, Hb, and total protein contents which decreased, all about those of the corresponding control.

Results listed in Tables (2, 3) and Figures (1, 2) indicated that there was a positive correlation between the magnitude of haematological parameters change (increase or decrease) and the length of exposure time for OP/PYR mixtures. For example, in rats treated with Lambaphos mixture for 4 and 8 weeks, the RBCs counts were 5.15 and $4.89 \times 10^6/\text{mm}^3$, respectively, compared to about $6.1 \times 10^6/\text{mm}^3$ in control. On contrary, WBCs counts were 5.93 and $6.9 \times 10^3/\text{mm}^3$, respectively, compared to about $5.13 \times 10^3/\text{mm}^3$ in control. For R. B. Cs counts, results of Tables (2, 3) and Figures (1, 2) indicated that after 4 weeks from Runsave and Lambaphos treatments, the R. B. Cs counts were 5.02 and $5.15 \times 10^6/\text{mm}^3$ while become 4.70 and $4.89 \times 10^6/\text{mm}^3$ after 8 weeks, respectively, comparing to about $6.1 \times 10^6/\text{mm}^3$ in control.

For haemoglobin content results listed in Table (3) and Figure (2) showed that after 8 weeks from toxicant administration the haemoglobin contents were 13.53 , 14.27 , 14.60 , 15.03 , 15.27 g/100 ml for Runsave, Lambaphos, chlorpyrifos, chlorpyrifos-methyl, and lambda-cyhalothrin, respectively, comparing to 16.53 g/100 ml in control. For W. B. Cs count, data of Tables (2, 3) & Figures (1, 2) showed that, after 4 weeks from Runsave and Lambaphos administration, the W. B. Cs counts were 6.27 and $5.93 \times 10^3/\text{mm}^3$, while they were 7.27 and $6.90 \times 10^3/\text{mm}^3$ after 8 weeks, respectively, compared to about $5.13 \times 10^3/\text{mm}^3$ in control.

Results listed in Tables (2, 3) and Figures (1, 2) showed that after 4 and 8 weeks from Runsave administration, the levels of creatinine were 2.02 , 4.59 mg/dl, respectively compared to about 1.39 mg/dl in control. The same trend was observed with urea levels which were 58.7 , and 80.92 mg/dl, respectively compared to about 50.65 mg/dl in control.

Histological study

After 4 and 8 weeks from dermal exposure to sublethal doses of OP/PYR mixtures (Runsave and Lambaphos), sections of vital organs (livers and kidneys) isolated from control and treated rats were stained with eosin and haematoxylin to demonstrate the abnormalities in each organ structure. Sections of these organs were obtained also from rats treated with individual insecticide after 8 weeks. Thus, the side-by-side comparison would be accurate since it had occurred under identical test conditions. Despite the great number of specimens collected from each organ intoxicated with each toxicant, only one photomicrograph was selected to represent changes that occurred in that organ concerning its corresponding control organ after a definite exposure time.

Table 1. Experimental design and number of rats used

Total rats	Number sacrificed after		Treatments
	8 weeks	4 weeks	
8	4	4	Control
8	4	4	Runsave(mixture)
8	4	4	Lambaphos(mixture)
4	4	-	Chlorpyrifos
4	4	-	Chlorpyrifos-methyl
4	4	-	Lambda-cyhalothrin
36	Total		

Table 2. Haematological parameters of male rats treated dermally with sublethal doses of OP/PYR mixtures for 4 weeks

Treatments	Counts		Concentration					
	R. B. Cs 10 ⁶ /mm ³ ↓	W. B. Cs 10 ³ /mm ³ ↑	AST U/ml ↑	ALT U/ml ↑	Creatinine mg/dl ↑	Urea mg/dl ↑	Total protein g/100ml ↓	Hb g/100ml ↓
Control	6.45	5.00	115.67	59.00	1.49	51.64	11.58	16.53
Runsava Mixture	4.70 (27%)	7.27 (45%)	165.33 (42%)	86.30 (46%)	4.59 (208%)	80.92 (56%)	7.54 (34%)	13.53 (18%)
Lambaphos Mixture	4.80 (25%)	6.90 (38%)	153.67 (32%)	80.33 (36%)	4.17 (179%)	79.69 (54%)	8.33 (28%)	14.27 (13%)
Chlorpyrifos	4.89 (24%)	6.57 (31%)	149.50 (29%)	75.32 (27%)	3.81 (155%)	71.86 (39%)	9.04 (21%)	14.60 (11%)
Chlorpyrifos Methyl	5.01 (22%)	6.43 (28%)	140.67 (21%)	72.67 (23%)	3.16 (112%)	68.53 (32%)	10.18 (12%)	15.03 (9%)
Lambda- cyhalothrin	5.15 (20%)	6.33 (26%)	131.00 (13%)	68.67 (16%)	2.03 (36%)	56.39 (8%)	10.32 (10%)	15.27 (7%)
L. S. D at 5%	0.64	0.55	4.16	3.96	0.12	1.38	0.45	1.22

Table 3. Haematological parameters of male rats treated dermally with sublethal doses of OP/PYR mixtures and their individual insecticides for 8 weeks

Parameters	Concentration of:					Counts of:		
	Hb g/100ml ↓	Total protein g/100ml ↓	Urea mg/dl ↑	Creatinine mg/dl ↑	ALT U/ml ↑	AST U/ml ↑	W. B. Cs 10 ³ /mm ³ ↑	R. B. Cs 10 ⁶ /mm ³ ↓
Control	16.53	11.58	51.64	1.49	59.00	115.67	5.00	6.45
Runsava Mixture	13.53 (18%)	7.54 (34%)	80.92 (56%)	4.59 (208%)	86.30 (46%)	165.33 (42%)	7.27 (45%)	4.70 (27%)
Lambaphos Mixture	14.27 (13%)	8.33 (28%)	79.69 (54%)	4.17 (179%)	80.33 (36%)	153.67 (32%)	6.90 (38%)	4.80 (25%)
Chlorpyrifos	14.60 (11%)	9.04 (21%)	71.86 (39%)	3.81 (155%)	75.32 (27%)	149.5 (29%)	6.57 (31%)	4.89 (24%)
Chlorpyrifos Methyl	15.03 (9%)	10.18 (12%)	68.53 (32%)	3.16 (112%)	72.67 (23%)	140.67 (21%)	6.43 (28%)	5.01 (22%)
Lambda- cyhalothrin	15.27 (7%)	10.32 (10%)	56.39 (8%)	2.03 (36%)	68.67 (16%)	131.00 (13%)	6.33 (26%)	5.15 (20%)
L. S. D at 5%	1.22	0.45	1.38	0.12	3.96	4.16	0.55	0.64

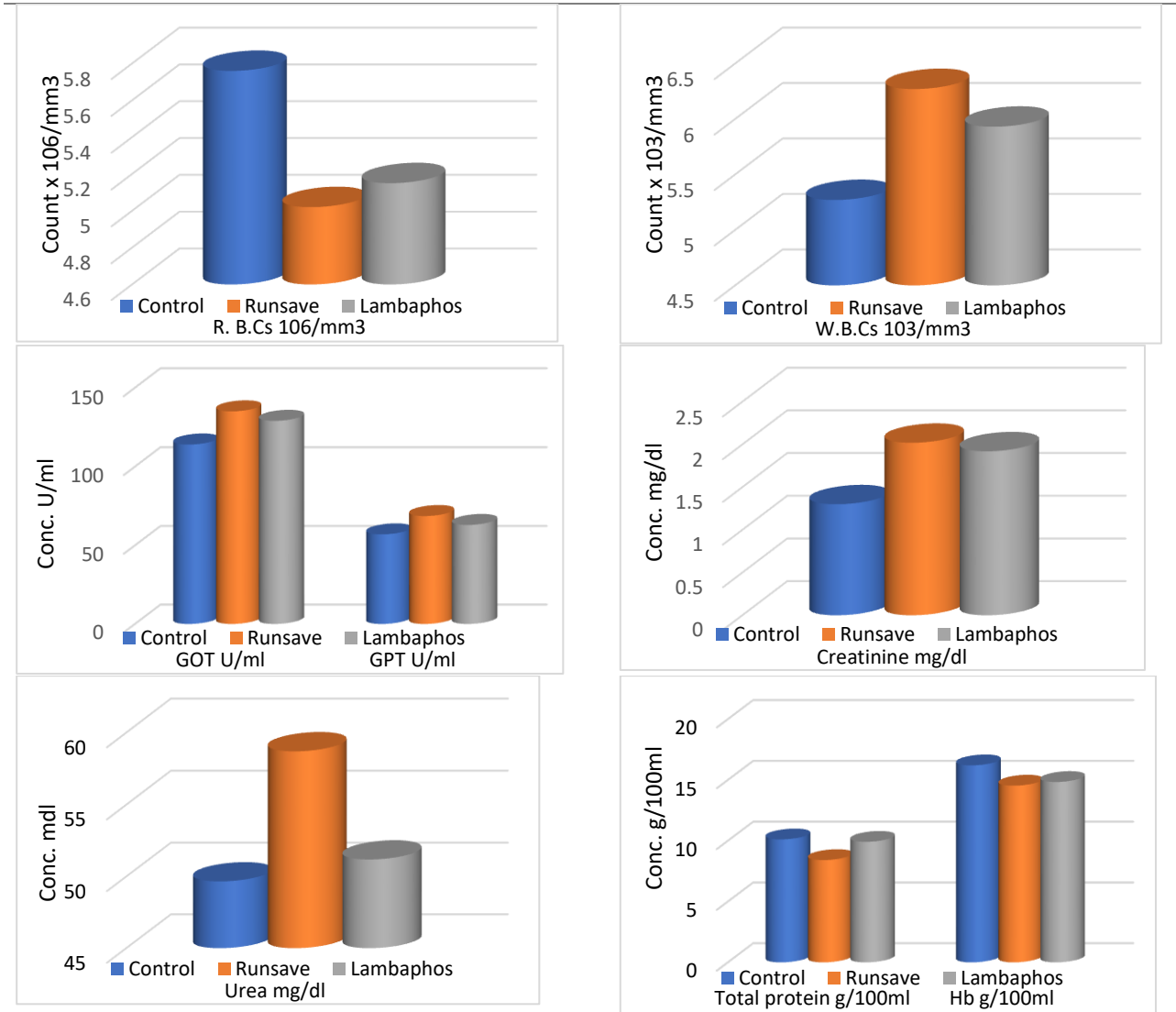


Figure 1. Haematological parameters of male rats treated dermally with sublethal doses of OP/PYR mixtures for 4 weeks

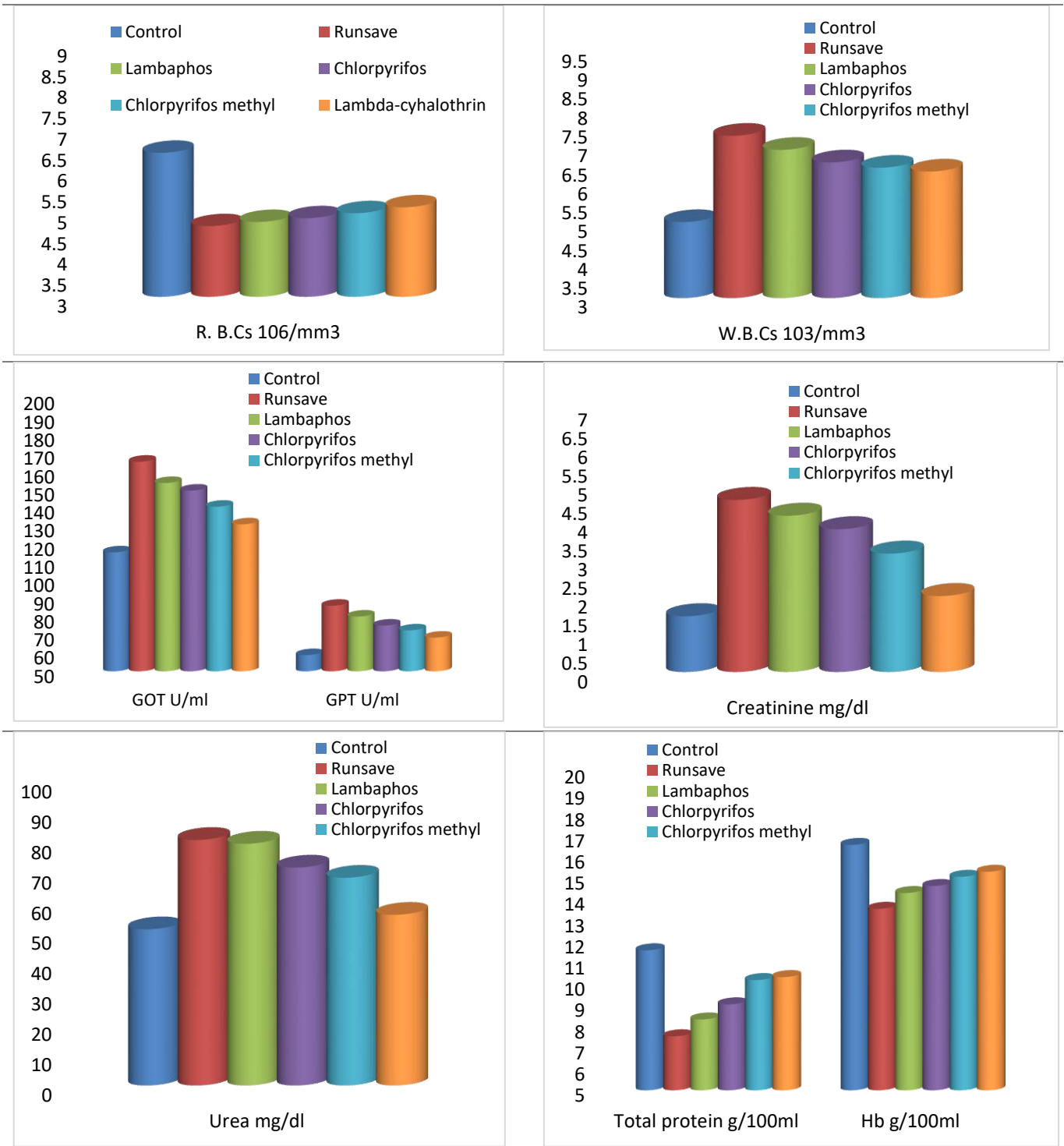


Figure 2. Haematological parameters of male rats treated dermally with sublethal doses of OP/PYR mixtures and their individual insecticides for 8 weeks

Liver histology

The specimens collected from control rats either after 4 or 8 weeks showed the classic hepatic lobules in which the hepatocytes were arranged in cords radiating from the central veins to the periphery of the lobules. Specimens collected from rats treated with OP/PYR mixtures after 4 weeks is presented in Figure 3, while their effects with their individual insecticides after 8 weeks are presented in Figure 4. The hepatocytes were polyhedral in shape with eosinophilic cytoplasm and central large vesicular nuclei. The cell cords are separated by blood sinusoids (Figure 3 (A)).

Figure 3 (A) depicts the typical architectural organization of hepatic tissue, hepatic polygonal cells (HC), normal central vein (CV), and blood sinusoids in the control liver (BS). Figure 3 (B) shows that rats treated with Runsave (121.2 mg/kg) have an irregular structure, severely congested blood vessels (CV), necrotic regions (N), fatty change liver (FL), pyknotic nuclei (P), and cytoplasmic vacuolation in many hepatic cells. While Figure 3 (C) demonstrates that rats treated with Lambaphos (146.5 mg/kg) have a partial disruption of normal structure, haemolysis (H), cytoplasmic vacuolation, and pyknotic nuclei (P).

In these preparations, the hepatocytes have exhibited some degree of cytomegaly being somewhat hypertrophied and contained a homogeneously eosinophilic cytoplasm without any definite bounding membranes. Furthermore, many of these cells have designated a rather diffused and structures less appearance manifesting a certain stage of cloudy swelling eventually leading to cellular necrosis. Dilation and congestion of liver blood vessels (central vein, blood sinusoids, and portal tracts) were a common phenomenon in all specimens. Most of the hepatic cell nuclei became relatively small, less vesicular, and deeply stained (pyknotic), while the nuclei of other hepatic cells were completely absent and fewer ones had fragmented nuclei. Cellular infiltration was a common phenomenon in degenerated specimens. Such cellular infiltration was most probably lymphocyte in nature.

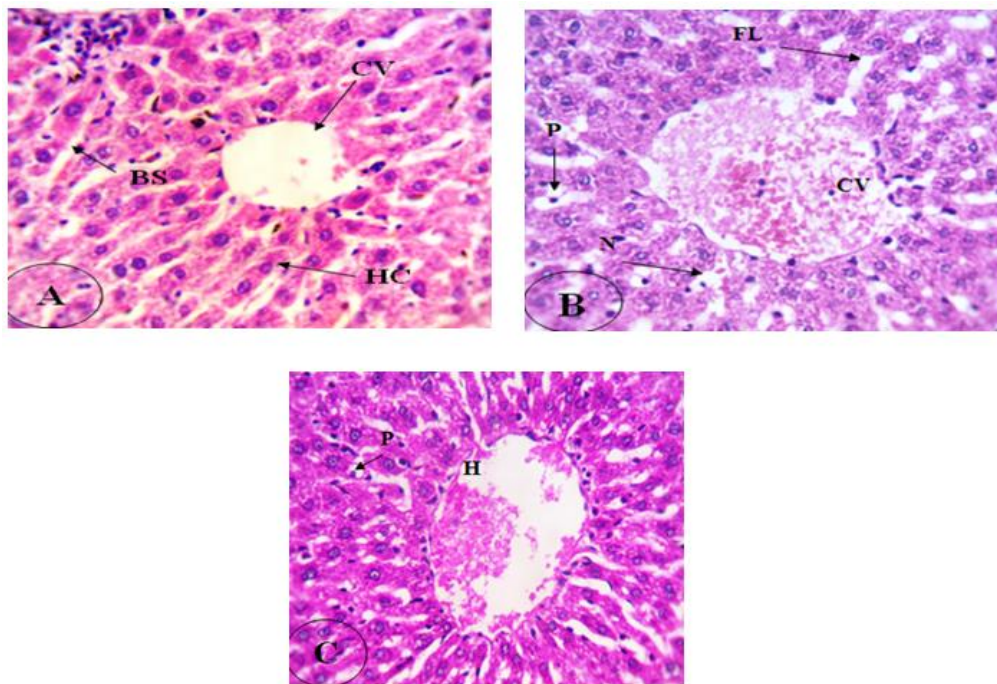


Figure 3 (A, B, C). Photomicrographs of liver of control and treated rats with OP/PYR mixtures after 4 weeks (E/H x400)

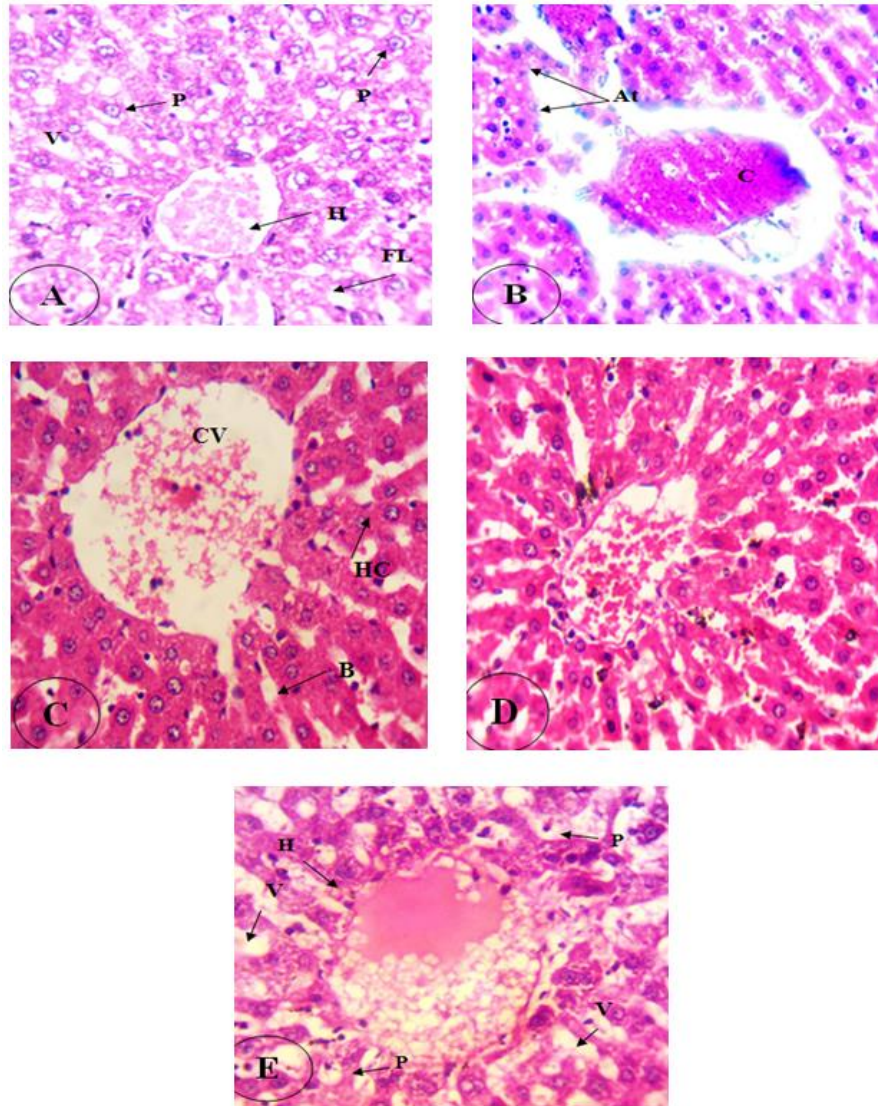


Figure 4 (A, B, C, D, E). Photomicrographs of rats' livers intoxicated with OP/PYR mixtures and their individual insecticides after 8 weeks (E/ H X 400)

Figure 4 (A) demonstrates that rats treated with Runsave (121.2 mg/kg) had hemolysis (H), cytoplasmic vacuolation (V), fatty altered liver (FL), pyknotic nuclei (P), and an increase in inflammatory cells. Figure 4 (B) depicts a section of the liver from rats treated with Lambaphos (146.5 mg/kg) that demonstrates congestion in the central vein (C), as well as hepatocyte shrinkage (At). When the liver sections of rats treated with Lambda-cyhalothrin (442 mg/kg) exhibit a moderate improvement in structure, hepatic polygonal cells (HC) with round nuclei, normal central vein (CV), and blood sinusoids (B) are present Figure 4 (C). Figure 4 (D), on the other hand, demonstrates that the liver section of rats treated with Chlorpyrifos-methyl (246.6 mg/kg) has a moderately aberrant shape. Finally, Figure 4 (E) indicates that the liver section of rats treated with Chlorpyrifos (139 mg/kg) has anomalous structural hemolysis (H), cytoplasmic vacuolation (V), and many pyknotic nuclei (P).

Accurate microscopic examination of the intoxicated liver specimens indicated that, in case of OP/PYR mixtures, the severity of hepatic changes increased with increasing the exposure time Figures 3 and 4, and Runsave exhibited more pronounced impairments than Lambaphos. Liver sections of Figure 4, indicates that liver changes associated with OP/PYR mixtures were more apparent than those of their individual compounds. Kidney histology

The present study includes the study of kidney alterations as it is the essential organ of drug excretion. The specimens collected from the control rat showed the normal structure of renal tissue (Figure 5 A). The section of the control rat kidney in Figure 5 (A) shows a normal Bowman's capsule (BC), normal glomeruli (G), and proximal tubules (PT). Figure 5 (B) shows that rats given Runsave (121.2 mg/kg) had abnormal kidney structure, with significant necrosis in the renal tubules (N), severe atrophy in the glomeruli leading to decreasing glomeruli (SG), pyknotic glomeruli (PG), and an increase in Bowman's gap (BS). Figure 5 (C) shows that rats treated with Lambaphos (146.5 mg/kg) have irregular kidney structure, severe atrophy in the renal tubules, an increase in Bowman's space (BS), and shrinkage with the fragmentation of glomeruli (FG), as well as necrotic regions (N).

The study showed extensive collapsed glomeruli, congested glomerular capillaries, thickened Bowman's capsule, invasions of endothelial cells, and inflammatory cell infiltration within the glomeruli have prevailed. Degeneration of renal epithelium and presence of amyloid substance within the renal tubular epithelium Figure 6 (C). Degeneration in the cortical compartment as in cortical glomeruli and some convoluted tubules. Demonstrated glomeruli with segmental necrosis, fibrinoid changes, and acute interstitial nephritis. In addition, lobulated glomeruli, thick Bowman's capsule, complete disintegrated renal tubules, necrotic areas, and haemorrhage were also perceptible as in Figure 6 (D).

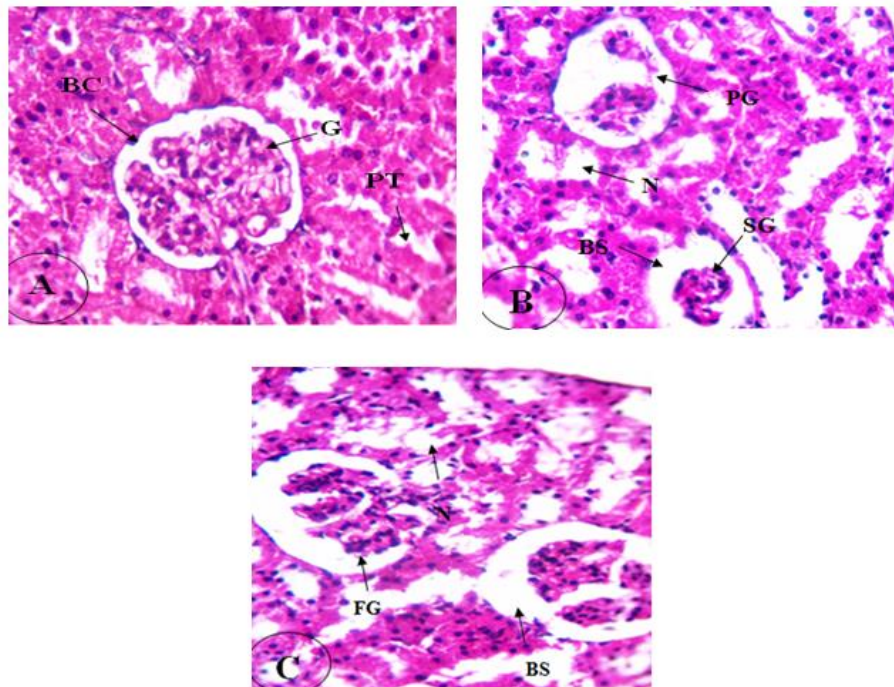


Figure 5 (A, B, C). Photomicrographs of kidney of control and treated rats with OP/PYR mixtures after 4 weeks (E/ H X 400)

Figure 6 (A) shows that rats treated with Runsave (121.2 mg/kg) had substantial damage in their renal tubules, with tubular atrophy (AT) resulting in pyknotic glomeruli (PG), decreasing glomerular (SG), necrotic renal cells (N), and an increase in Bowman's space (BS). Figure 6 (B) revealed that the kidney sections of rats treated with Lambaphos (146.5 mg/kg) showed minor damage, shrinkage with lobulation, or fragmentation of glomeruli, as seen in (A) (Fr). Figure 6 (C) demonstrates that the kidney structure of rats treated with Lambda-cyhalothrin (442 mg/kg) improved when compared to the control. A section of the kidney from rats treated with Chlorpyrifos-methyl (246.6 mg/kg) revealed moderate damage in the renal tubules, an increase in Bowman's space (BS), shrinking of the glomeruli (SG), Myelomatosis (Me), or neoplastic overgrowth of plasma cell series cells, and acute tubular necrosis (AC) (Figure 6 (D)). Finally, Figure 6 (E) demonstrated that the kidney sections of rats treated with Chlorpyrifos (139 mg/kg) showed significant damage, atrophy in the renal tubules (At), and shrinking of the glomeruli (SG), and necrotic renal cells (N). Accurate microscopic examination of intoxicated kidney specimens indicated that the severity of changes in kidneys of rats exposed to OP/PYR mixtures increased with increasing the exposure time Figure 6 (A, B), and Runsave produced more severe changes than Lambaphos. Also, these changes were more apparent with the two mixtures than with their individual compounds.

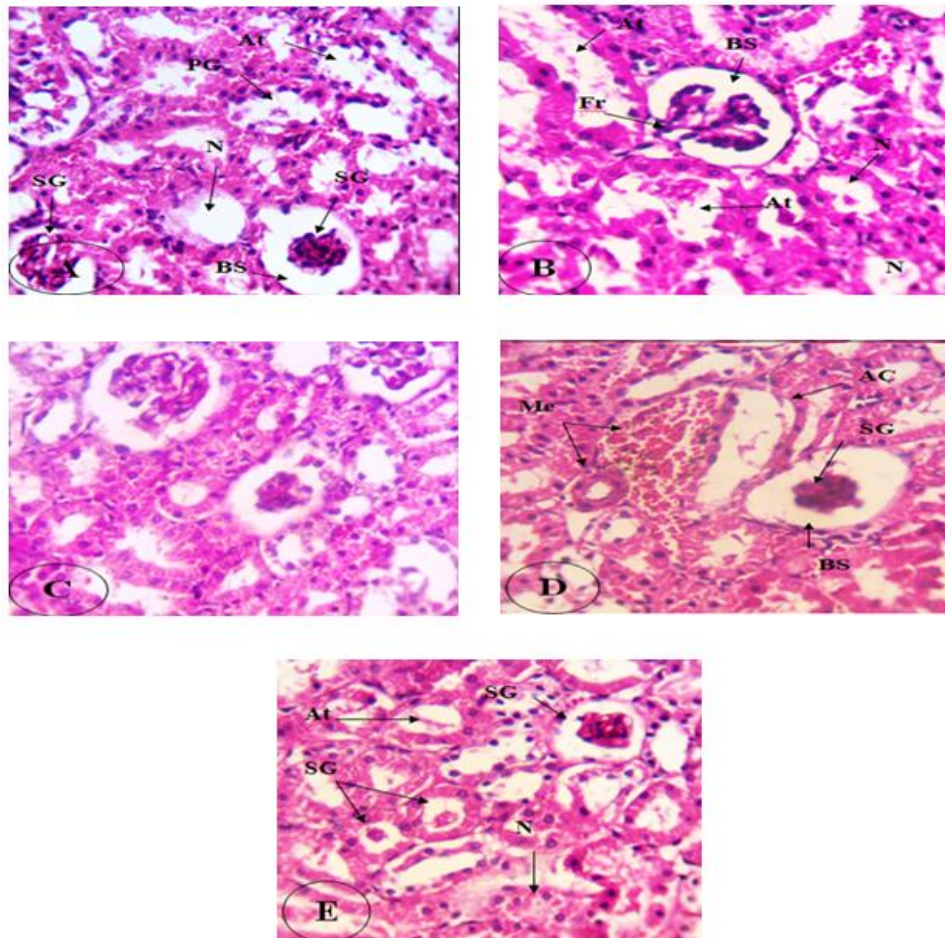


Figure 6 (A, B, C, D, E). Photomicrographs of rat's kidneys intoxicated with OP/PYR mixtures and their individual insecticides after 8 weeks (E/ H X 400)

DISCUSSION

The OP/PYR mixtures are widely used as pesticides and they are harmful to humans not just at high concentrations, causing acute poisonings, but also at low doses. As a result, the current study will monitor changes in the haematology and histology of the liver and kidney in male albino rats to better understand the problems in humans exposed to this pesticide mixture.

Blood findings are significant for examining numerous systemic functions and animal health under a variety of environmental situations, as well as for diagnosing medication or chemical-induced hemolysis (Tomlin, 1997). To estimate the haematological changes, the haematological parameters (i.e., counts of R. B. Cs and W. B. Cs, the levels of haemoglobin (Hb), AST, ALT, creatinine, urea, and total protein) were measured. Except for R. B. Cs counts, Hb, and total protein content, which diminished in contrast to the control, all of the measurements analysed elevated in treated rats. The results of these haematological parameters are congruent with those previously described findings (Shalaby, 2010; Yousef et al., 1999; Jacobsen, 2004; El-Tawil, 2014; Soliman *et al.*, 2007; Ambali *et al.*, 2011; El-Magad, 2011; and Sangha, 2011). The study shows that there was a positive correlation between the magnitude of changes in haematological parameters (increase or reduction) and the length of the period of exposure for OP/PYR mixes. Also, Runsave had a greater effect than Lambaphos in general, and the two combinations had a greater influence on the blood picture of treated rats than their separate components. This set of findings corresponds to those previously reported (Noaishi *et al.*, 2013; El-Tawil, 2014; Chauhan *et al.*, 2005).

The decrease in R. B. Cs numbers could be due to a variety of factors. First, the failure to replenish the circulation with cells from hematopoietic organs that may have been damaged by the toxicant (Goel et al., 2006). The second factor is the potential for toxicants to harm red blood cell membranes. Linman claims that increased erythrocyte destruction causes haemoglobin catabolism (Linman, 1975). RBCs count is directly related to haemoglobin concentration and haematocrit values, as per the findings. This is due to the synergistic link among these blood parameters in all vertebrates including man (Harris, 1972). The reduction in haemoglobin content may be due to either an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis or may be due to hydration, anaemia, and transfusion of fluids (Reddy & Bashamohidden, 1989; Barna-Lloyd et al., 1990; Barna-Lloyd et al., 1991). In a similar vein, Goel et al. revealed a fall in serum iron concentration, which leads to decreased haemoglobin synthesis (Goel et al., 2006). Anaemia observed in treated rats was also symptomatic of hepatic failure. The previous study describes that the significant decline in RBCs and Hb could be attributable to erythropoiesis and haemo synthesis suppression, as well as an increase in the rate of erythrocyte destruction in hemopoietic organs.

The current investigation found that after administering Runsave and Lambaphos, the amount of W. B. Cs in male albino rats elevated as a result of the inflammatory response generated as a defence mechanism that indicated detrimental effects on the body. W. B. Cs are the first line of defence against infectious agents, tissue injury, parasites, and inflammatory or foreign materials and exert their activity by eliminating foreign materials by phagocytosis (Udall, 1981). Increased WBC count may also be found in leukaemia, a disease in which uncontrolled abnormal proliferation of hemopoietic cells leads to progressive infiltration of the bone marrow, where a large number of immature forms are produced, and these immature forms eventually escape into the peripheral blood, resulting in a very high leukocyte count (Saxena, 2003).

The current study's serum transaminases (AST & ALT) data are consistent with those obtained by Shalaby and Abd EL-Mageed, who discovered that rats treated with an OP/PYR mixture known as "Chlorosan" had significantly higher serum AST and ALT activities than rats treated with either insecticide alone. Similar results were obtained by Ambali et al. and Fetoui et al., when rats were treated with chlorpyrifos and lambda-cyhalothrin, respectively (Ambali *et al.*, 2011; Fetoui *et al.*, 2009). Aspartate transaminase (AST) increases in plasma are strongly associated with myocardial infarction, whereas Alanine transaminase (ALT) increases in plasma are strongly associated with liver cirrhosis. A significant increase in the activities of these enzymes has been proposed as a diagnostic value in determining liver dysfunction (Cornelius, 1980). Transaminase disruption from normal values indicates biochemical impairment and lesions of tissues and cellular function because they are involved in the detoxification process, metabolism, and biosynthesis of energetic macromolecules from various essential functions (Tordoir, 1980). This experiment also observed an increase in creatinine and urea levels after administration of the Runsave or Lambaphos mixture, and Guyton stated that an increase in serum creatinine and urea is an indication of glomerular failure (kidney damage) (Khonsary, 2017). Study suggested that the reduction in serum total protein concentration was due to the elevation of protease activity (Savithri & Seethamma, 2014). According to Abdel-Tawab and Abbassy, the decrease in protein content could be due to an imbalance between the rate of protein synthesis and the rate of protein degradation in the liver (Mossa and Abbassy, 2012).

In the present study, rats treated with OP/PYP mixtures were stained with eosin and haematoxylin to show the abnormalities in each organ structure. So, it focused on liver modifications because it is the primary site of xenobiotic metabolism and kidney alterations. After all, it is the primary organ of drug excretion. Hepatocytes in the current study display some degree of abnormality when treated with OP/PYP mixes, such as hypertrophy, congested blood vessel, and fatty change liver. The most obvious indicator of tissue damage was cytoplasmic vacuolization, which was found in the great majority of the constituent hepatocytes. The hepatic alterations observed in the present work were also noticed previously with varying degrees, with OP/PYR mixtures (Noaishi *et al.*, 2013; Latuszynska, 1999), chlorpyrifos (Savithri *et al.*, 2010; Akhtar *et al.*, 2009), and pyrethroids (Luty, 1998; Fetoui *et al.*, 2009; Yavaşoğlu *et al.*, 2006).

Martin and his associates suggested that the higher values of transaminases (AST & ALT) enzymes were the indication of various degenerative events in the liver (Harper *et al.*, 1985). Again, the haematological data obtained from the present investigation support this speculation in which the blood pictures of treated rats showed an elevation of these transaminases (AST & ALT). A study proposed that these changes were caused by cypermethrin's inhibitory effect on the activity of total adenosine triphosphates in rat liver, which disrupted active transport of Na⁺, K⁺, and Mg⁺ ions and resulted in pathological abnormalities in liver cells (El-Toukhy, 1993). Fetoui and his team explained liver injury in lambda-cyhalothrin-treated rats as a series of events: decreased antioxidant defense mechanisms (by decreasing antioxidant enzyme activities), increased lipid peroxidation, initiated free radicals that damage the hepatocellular membrane, and thus led to hepatic damage (Fetoui *et al.*, 2009).

The histological examination of the intoxicated liver showed that many hepatocytes exhibited cytoplasmic vacuolization. Previous researchers proposed a variety of causes for vacuolar genesis. For example, Pilat regarded this feature to represent the initial stages of cell degeneration (Pilat, 1935). Grasso et al., reported that such cytoplasmic vacuolization might be a consequence of the destruction of lysosomal particles which occurred under the effect of the rodenticide (Grasso et al., 1974). Thus, the swelling and vacuolation of the hepatic cells, noticed in the present study, are most probably due to the retention of fluid inside the hepatocytes resulting in what is known as cloudy swelling or hydropic degeneration. Such degeneration might proceed to necrosis due to partial or complete loss of cell boundaries, huge vacuolation, and fragmentation of the nuclei. Patches of lymphocyte aggregation identified along some portal tracts and infiltrating adjacent hepatic lobules. Study found that lymphocytic aggregation in portal sections of rat liver intoxicated with a mixture of chlorpyrifos and cypermethrin (Latuszynska, 1999). Such cellular infiltration was suggested by Savithri et al., to be due to the presence of necrotic cells which acted as an irritant substance attracting the inflammatory cells (Savithri and Seethamma, 2014). The abundance of lymphocytes and lymphocytic infiltration was a prominent response of body tissues facing any injurious impacts. Accurate microscopic examination of the intoxicated liver specimens indicated that, in case of OP/PYR mixtures, the severity of hepatic changes increased with increasing the exposure time and Runsave exhibited more pronounced impairments than Lambaphos. Liver sections of Fig. (4), indicates that liver changes associated with OP/PYR mixtures were more apparent than those of their individual compounds.

In the current study, sections of kidneys from treated rats showed glomerular abnormalities such as atrophied, shrinking and thickening of glomerular capsules. The histological changes in kidneys observed in this work agree with those obtained previously, with varying degrees (Luty, 1998; Akhtar *et al.*, 2009 and Luty *et al.*, 2000). Study found that rats exposed dermally to α -cypermethrin caused widening of endoplasmic reticulum and Golgi apparatus in the epithelial cells of the proximal tubule, in other words, caused parenchymatous degeneration in single cells in the proximal tubule. They also noticed a considerable number of auto phagocytic vacuole. The necrotic changes and cellular infiltration observed in intoxicated kidneys in the present work are in accordance with Walter and Israel who stated that the necrotic cells led to the release of different chemotactic factors which attracted different inflammatory cells (Walter, 1974). Research stated that the interstitial lymphocytic infiltration was an indication of the inflammatory process, while the swelling of the epithelial cells of proximal convoluted tubules could be an indication of regenerative changes in these tubules (Abdel-Dayew, 1990). So, the severity of kidney alterations in rats exposed to OP/PYR combinations increased with exposure duration, with Runsave producing more severe changes than Lambaphos. Furthermore, the alterations were more obvious in the two mixtures than in the individual components.

CONCLUSION

In comparison to the control group, albino rats subjected to an insecticide mixture of organophosphate and pyrethroid showed significant changes in their histological structure and haemato-biochemical markers. Due to the effects of the organophosphate and pyrethroid mixture, three crucial haemato-biochemical markers—RBCs, total protein contents, and hemoglobin level—have decreased in the case group compared to the control group. When organophosphate and pyrethroid mixes were used, accurate microscopic analysis of the intoxicated liver specimens showed that Runsave showed more pronounced impairments than Lambaphos and that the severity of hepatic alterations increased with exposure time.

CONFLICT OF INTEREST

The authors confirm that conducting this study is free of any conflicts of interest.

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