

Study Of Changes In Blood Parameters And Calculation Of PCT, MPV And DPW For The Platelets Of Laboratory Females And Males Of Albino Mice During Exposure To Doses Of PyrethriodPesticide (Alphacypermethrin)

Saphia .Ali Aitte* And MysoonHussain.Zain **

*Community Health Department, Health and Medical Technical College, Southern Technical university, Basra –Iraq. **,Biology Department College of Education for pure of sciences - University of Basra –Iraq .
Corresponding Author: Saphia .Ali Aitte

Abstract:The aim of this work to investigated the effect of Pyrethriod pesticide alpha cypermethrin insecticide on the hematological parameters as the branch of physiological organs, were female and meal exposure for albino mice exposed to sub –lethal doses(2.5mg/kg bw and 4.75mg/kg bw) for a period 5 weeks to measure blood parameters. The decrease in R.B.C count from (8.57,to5.91($10^6/mm^3$), Hb from(11.7 to8.0%) and HCT, from (47.85to 29.91%)observed could be described shrinkage ,distraction and hemolysis of RBC.,MCV,MCH and MCHC then showed significant decrease ($p \leq 0.05$) in the males more than in the females compared with control group. And the significant increase was found ($p < 0.05$) in the value of Platelets count in all dose of both sexes with increase in males was higher in females compared to control . We found platelet count, PCT and the ratios of MPV count, MPV/PCT, PDW/Platelet count, were significantly different for male and female of mice compared with females in table 3 and control .W.B.C count was increased significantly in experiment period , and a decrease percentage of neutrophils ,basophils , eosinophil's and monocyte cells , while increased in the lymphocyte cells , these values vary by doses and by sex .

Key words: Albino mice, Hematological, pesticide, sub lethal effects , Differential erythrocytes

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I. Introduction

Pesticides are generally used extensively in the control of agricultural pests and increase in agricultural yield as they are considered as chemical fertilizers due to population increase. [1] These chemicals are widely used in industry, agriculture, home and gardens for many different purposes, including the protection of seed grain during storage and germination (2). Many pesticides can be grouped into chemical families. ([3]). Pesticide Profiles: toxicity, environmental impact, and fate. Insecticide families include organochlorines, organophosphates, and carbamates. Organ chlorine hydrocarbons (e.g., DDT) could be separated into dichlorodiphenylethanecyclodiene compounds, Pesticides may cause acute and delayed health effects in people who are exposed[4,5] Pesticide exposure can cause a variety of adverse health effects, ranging from simple irritation of the skin and eyes to more severe effects such as affecting the nervous system, mimicking hormones causing reproductive problems, and also causing cancer(6). Toxicity can be defined as the relative ability of a substance to cause adverse effects in living organisms. This "relative ability is dependent upon several conditions. As Paracelsus suggests, the quantity or the dose of the substance determines whether the effects of the chemical are toxic, nontoxic or beneficial. In addition to dose, other factors may also influence the toxicity of the compound such as the route of entry, duration and frequency of exposure, variations between different species and variations among members of the same species .The dose rate refer to the quantity of pesticide to which an animal is subjected orally, dermally or through inhalation . A small dose of a more toxic chemical may be more damaging than a large dose of a less toxic chemicals .Dosages can be measured as the weight of toxicant per unit kilogram of body weight .Pesticides may cause acute and delayed health [7]. Pesticides are beneficial chemicals .they are used to help reduce malnutrition and starvation of humans and animals pesticides . Blood as the fluid that circulates through the heart, arteries, capillaries, and veins and is the chief means of transport within the body. It transports oxygen from the lungs to the body tissues, and carbon dioxide from the tissues to the lungs. It transports nutritive substances and metabolites to the tissues and removes waste products to the kidneys and other organs of excretion. It has an essential role in the maintenance of fluid balance[8]. Blood is a body fluid circulate in the principal vascular system of human beings and other vertebrates in humans consisting of plasma in which the red blood cells, white blood cells, and platelets are suspended. It was necessary for the delivery of oxygen to the tissues. The major function of red blood cells, also known as

erythrocytes, is to transport hemoglobin, which in turn carries oxygen from the lungs to the tissues. The red blood cells have other functions besides transport of hemoglobin. Blood is composed of two parts: the fluid portion is called plasma, and the solid portion or formed elements suspended in the fluid) consists of the blood cells (erythrocytes) and the platelets. Plasma accounts for about 55 per cent of the volume and the formed elements account for about 45 per cent. cells.[9] Due to this cumulative extension as negative reflection on the blood contents and other physiological things, the effect of which effects that human who feeds on agricultural crops and albino mice as a vital indicator to explain these effects on public health [10]. Depending on the physiological composition of blood and formation in the body, many studies have dealt with the effect of pesticide on the blood cells during the exposure of albino mice to the organo phosphorous pesticides, which caused different effects [11] found a significant decrease in the RBC count (5.2 ± 0.24), hemoglobin content (11.5 ± 2.3) and the number of blood platelets (543 ± 15.3). In contrast, metalaxyl treatment induced a significant increase in the WBC count (5.8 ± 0.4), while [12], showed the results of the hematological parameters dichlorovos treated rats found a significant decrease ($p < 0.05$) in the mean value of red blood cells, hemoglobin and packed cell volume as a significant increase ($p < 0.05$) in the WBC count and platelets which was dose dependent, and many alteration found by ([13]) the stress on hematological profile, decreases in TEC, DLC, TTC, ESR and Hb concentration, but increases in TLC and lymphocytes. Therefore, studies on the effect of pesticide on the blood parameters of albino mice varied, depending on the dose and the duration of administration, in addition to the age and sex of the animal, but in general, leading to decreases and increase in the measurements of blood [14,15]. A lot of studies examined the effect of these pesticides and their toxicity on the biochemical parameters [16, 17] and physiological aspects histological studies [8,19] and blood chemistry [20]. The aim of the current study the effect of alpha-cypermethrin on blood profile of albino mice within laboratory study and determined the toxicity of this pesticide according to its effect on the blood parameters [21].

II. Materials and Methods

2.1 Test animals and chemical treatment

Animals: Male and female of Albino mice obtained from the animal house of University of Basra Biology Department College of and then housed in plastic cages (40x30x10)cm in the laboratory at constant temperature (28 ± 2) C°. They were allowed to acclimatize for two weeks before the experiment. They were kept maintained under standard conditions with 12hday /light cycle and fed with standard bush and water was available libitum, during the period of work from December 2017 to May 2018.[22] Chemical treatment :- Albino mice were exposed to alpha cypermethrin C22H19Cl2NO3 pesticide containing active martial 50%, animals were treated with pesticide solution cardio intraperitoneally injection (2-3) times each week with (2.5mg/kg and 4.75mg/kg) of tested solution for 5 weeks period of exposure. Albino mice were divided (5) replicates each one contain (5) animal (n=25), at the same time control were not treated was given 2.5mL, distal water

2.2. Collection of blood samples :- The albino mice anesthetized with acetone within 2hr of anesthetic chemical, the blood samples were collected by cardiac puncture then put the blood into appropriate specimen of anticlotting tubes, hematological parameters was carried out as explained in studies[23]

2.3. Statistical analyses Analysis were done by SPSS software. The results of hematological profile were presented as the mean \pm S.D. Comparing between un treatment control and treatment groups using One-way analysis of Anova with values $P \leq 0.05$ were regarded as statistically significant

III. Results

The results of this study, on the effect of pesticide of cardio dosing on hematological profile in the albino mice laboratory is presented in tables(1, 2), were showed the differences in R.B.C count, Hb, HTC and Platelets, values between female and male of mice after exposed to sub lethal dose of pesticide (for 5 weeks) periods. Data indicates there is graduated decrease in R.B.C count, Hb, HTC values compared with control group, depending on the dose for pesticide. Found that there was a varied in blood parameters between male and female of albino mice, where the mean was decrease in male is higher than in females compared to the control. The value of erythrocyte in females was ($6.37 \text{ R.B.C} \cdot 10^6/\text{mm}$) while in males $5.19 \text{ R.B.C}(10^6/\text{mm})$ for 2.5mg/kg dose of pesticide compared to control ($8.57 \text{ R.B.C} \cdot 10^6/\text{mm}$), and during measurement of Hb, HTC values, there were also decrease in the values of Hb in male 8.0 mg/L and females was 9.55 gm/L as well as the values of HTC in males was (29.91 where as in females (33.2 mg/L) blood value, in addition there was a significant increase in Platelets was high in both sexes (Table 1,2) In the case of blood corpuscular, there were clear decreases in the values of MCV. MCN.MCHC for the control values where recorded decrease (of 9.75%) at dose 4.75 mg/kg bw during the exposure of male albino mice, while the MCHC recorded of (2.5mg/kg bw) at 033.75%) compared with the male record at the same dose where it was (30.55) as compared to the control as shown in (Figure - 1-). Table 1 and 2 indicated a significant increase in blood platelets values in

both doses and both sexes of laboratory mice compared to controls . The increase in males was also higher than in females .However, the study showed that there are values associated with the platelets count , table 3and 4 with doses females and males where the count different compared to control , where the highest PCT % count high was found in males 1.698 and lost count in the p- lcr measurement , females were 16.87 during MPV count , it was high in males and PDW count was low compared with control .The RDW-CV platelet large cell ratio count was 27.6 in males table 4 and 24 in females , We found platelet count, PCT and the ratios of MPV count, MPV/PCT, PDW/Platelet count, were significantly different for male and female of mice compared with females in table 3 and control

Tabl-1- Effect of AlphaCypermethrin) pesticides on hematological parameters in male of albino mice

Parameter	Control	2.5mg/kg bw	4.75mg/kg bw	F VAUES
R.B.C($10^6/mm^3$)	8.57±0.752	7.15±0.12	5.91±0.298	10.38
HB (gm. %)	11.712±0.932	10.3±0.50	8.0±0.93	5.36
HTC (%)	47.074±2.273	41.45±2.128	29.91±3.39	12.71
PLT (mm^3)	636±33.3	735.2±42.2	983.5±55.7	19.77

(P<0.05)

Tabl-2- Effect of AlphaCypermethrin) pesticides on hematological parameters in female of albino mice

Parameter	Control	2.5mg/kg bw	4.75mg/kg bw	F VAUES
R.B.C($10^6/mm^3$)	8.77±0.41	8.33±0.48	6.37±0.48	8.25
HB (gm %)	12.35±1.52	11.56±1.36	9.55±0.70	3.70
HTC (%)	44.85±3.18	43.4±2.45	33.22±2.40	9.96
PLT(mm^3)	898±63.32	1267 ±81.15	1441±94.69	14.13

(P<0.05)

Table - 3- Effect of Alpha Cypermethrin) pesticides on platelets counts in female of albino mice

Parameter	Control	2.5mg/kg	4.75mg/kg	F value
PCT(%)	0.281±0.32	0.341±0.18	0.918±0.23	0.465
P-LCR (%)	38.8±3.87	22.73±9.18	16.87±5.34	1.809
MPV (fl)	12.72±0.12	8.88±1.26	6.9±1.79	0.500
PDW(fl)	17.08±2.64	15.44±2.43	9.7]±1.3	1.44
RDW-CV(%)	11.0±1.25	20.84±1.40	24±1.54	1.353
RDW-SD(fl)	35.7±3.30	32.96±2.61	26.63±4.94	6.83

(P<0.05)

Table - 4- Effect of Alpha Cypermethrin) pesticides on platelets counts in males of albino mice

Parameter	Control	2.5mg/kg	4.75mg/kg	F value
PCT %	0.10±0.02	0.83±0.12	1.57±0.32	1.698
P-LCR %	36.45±5.11	28.02±4.98	20.5±217	0.1212
MPV(fl)	9.12±3.97	11.36±2.04	14.0±3.40	3.835
PDW(fl)	13.72±4.16	10.06±0.53	7.56±1.07	1.062
RDW-CV%	12.89±3.22	24.09±2.86	27.6±1.56	10.26
RDW-SD(fl)	41.55±4.07	36.38±6.39	31.3±3.45	6.84

(P<0.05)

Abbreviations : PCT: Plateletcrit, MPV: Mean Platelet Volume, PDW: Platelet distribution Width, Red cell dis distribution, P-LCR platelet large cell ratio,

During measurement of white blood cells, a significant increase was observed in the calculation of W.B.C. , which was dependent on the effective dose for both sexes .Table 5,6 indicated to a difference in these values between females and males treated with the alpha -cypermethrin pesticide , where the values of W.B.C. ($10.9710^3/mm^3$) at the higher dose and in female ,($8.5910^3/mm^3$) .And reduce in the values of differential Leucocyte count Basophil and Eosinophil compared to the control, on other hand found an decrease in the values of Neutrophil cells where the most numbers among the other granules cells of W.B.C. and these variables significant .As for the monocyte and lymphocyte cells values , the current study showed significant decrease in the monocyte cells of the albino mice recorded at a mean value of (0.57%) compared with control (2.48%), also observed significant increase in lymphocyte values as found in (Table 5.6) .In addition there is a clear differences between males and females animals. The results show that there are he values of the

components of the lymphatic system
Tabl-6- Effect of Alpha Cypermethrin) pesticides on Differential Leucocyte count in female of albino mice

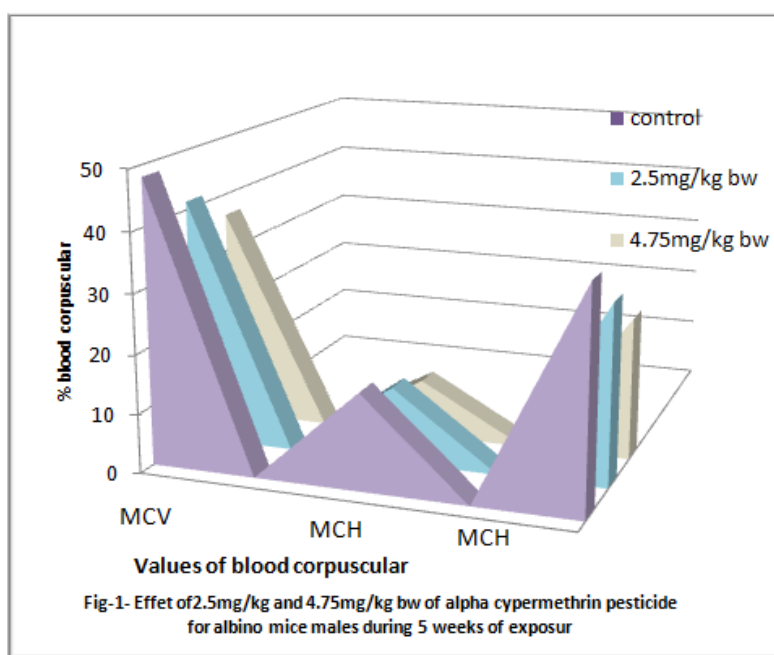
Differential Leucocyte count	Control	2.5mg/kg bw	4.75mg/kg bw	F VAUES
W.B.C. (10 ³ /mm ³)	5.78±0.38	6.77±0.97	8.59±0.98	5.7
Basophil%	1.43±0.63	0.92±0.06	0.46±0.04	12.6
Eosinophil%	1.98±0.50	1.68±0.54	0.62±0.03	5.81
Neutrophil %	21.43±0.65	15.88±2.38	12.64±2.15	3.42
Lymphocyte (%)	37.22±3.21	77.36±5.02	80.5±7.64	13.24
Monocyte (%)	2.75±0.45	0.7±0.25	0.57±0.34	3.37

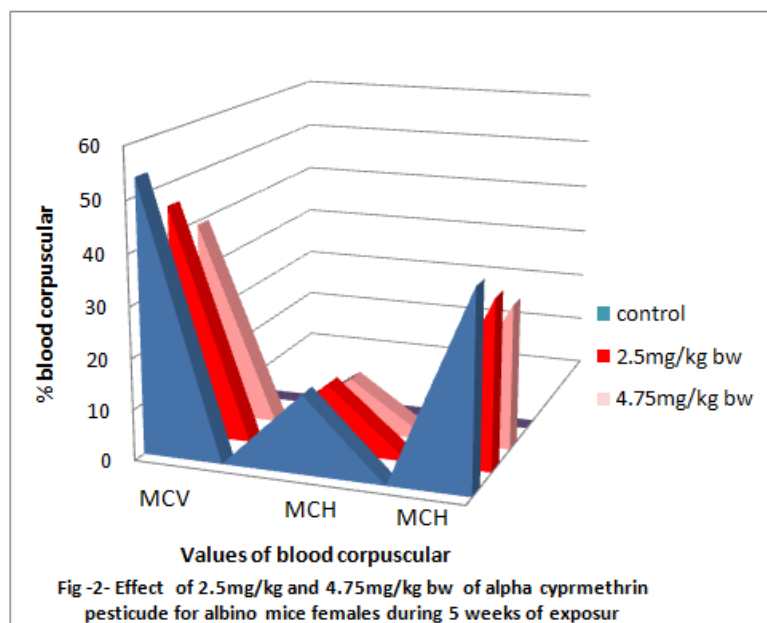
Tabl-5- Effect of pesticides Alpha Cypermethrin) on Differential Leucocyte count in male of albino mice

Differential Leucocyte count	control	2.5mg/kg bw	4.75mg/kg bw	F VAUES
W.B.C. (10 ³ /mm ³)	6.53±0.231	7.79±	10.97±0.12	9.58
Basophil%	2.24±1.06	1.06±0.08	0.94±0.15	8.9
Eosinophil%	1.09±0.067	0.857±0.063	0.15±0.024	7.67
Neutrophil %X	23.37±035	19.56±0.253	15.61±0.33	8.50
Lymphocyte (%)	38.3±2.65	79.2±4.211	82.12±6.25	34.84
Monocyte (%)	2.48±0.61	0.63±0.44	0.35±0.23	7.36

(P<0.05)

And this confirms that .The toxicity of the pesticide has an active role in different values about their natural values . The values of the factor (F) analysis of variance indicated that there were significant differences under level (P<0.05) in all blood variables ,whether R.B.C or W.B.C, as shown in tables (1,2,3,4 .5and 6)





IV. Discussion

The damage caused by pesticides and other chemicals substances during their presences n the environmental for long periods of months and years ,(24)). The (tables 1-4)show the data of the current study where the alpha cypermethrin pesticide was used to explain its toxicity to physiological processes of the mammals by studying the blood profile for albino mice . In our results the decrease in the RBC, Hb and PCV's was found in the sample treated to with control. Previous studies [25,26] they found that the direct effect of pesticides is a significant reduction in the total number of RBC, Hb content and HCT ,reduction in TEC, HB, HCT , lead to breaking the erythrocyte cells causes changes in the size and shapes and occurrence of the processes of hemolysis [27] .These alternation occur shrinkage and break of erythrocytes count causes hypoxia ,thus RBC and HB are very important in transporting respiratory gasses and the process of hemolysis are also reflected in the lack of efficiency of the transfer of oxygen thus (28) observed also suggests that there is change in oxygen – carrying capacity of the blood and the amount of oxygen delivered to the tissues , lead to respiratory disorder [29] . Present study found significant decrease $p \leq 0.05$ in the level of the Hb content, and PCV .All of this decreases in the value of red blood cells is the result of its inability to carry oxygen, so the case of suffocation , which in turn affects the process of activating the process of the composition of red blood cell during which the condition of inhibition in the inability of the work of the bone marrow to release red blood cells and to confirm that the flair of these organs physiological functions are a clear result of the effectiveness of the toxic substance , in which blood is considers the vital artery all of organs of the body . The recent study describe the effect in mice male was higher than that of females and the dose 4.72mg/kg was more effect than (2.5mg/kg) compared with control ,thus decrease in Hb values is due to an increase in the rate which Hb destroyed status agreement with study by (30) they examined the blood variables in the rats during treated with pesticide .The decrease in the number of red blood cells disrupts the physiological system of the animal . This change is followed by a decrease in the hemoglobin content. In this way , hypothermia is reduced by the decline in the imports of oxygenated substance throughout the body . Therefor the black of oxygen or hemoglobin is not less than the effect of red blood cells breakdown but it is actually physiological linked .Iron is obtained from stored ferritin and a dietary source which is essential for synthesis Hb . Intoxicated mice reduce the food intake capacity and there is no other source of iron take, might be the reason for iron deficiency .The toxicity by pesticide restudies leads to the development of a anima due to interfere of Hb biosynthesis and shortening of the life spine circulation erythrocyte. (31). while reduced number of Hb and HCT may also be a consequence of severe hemorrhage which results in dilution of blood ,and. hemoglobin , hematocrit values are directly correlated with RBC count, due to the synergistic link among these blood parameters in all vertebrates. [32]These declines in the values of erythrocytes cells is the result of inhibition in the bone marrow and its inability to put red blood cells and to confirm that the failure of these organs of physiological functions is a clear result on the effectiveness of the toxic substance and its impact on blood profile .Hematocrit is attributed to the destruction in size this also observed by[33]. Impact the pesticide in Hb content and HCT could be due to the impaired biosynthesis of the harem in bone marrow, The RBC count and Hb content could also be due to disruptive action of the pesticides on the erythropoietin tissue as a result of which the viability of the cells might be affected. and [34]. also reported a decrease in some hematological

parameters (RBC, Hb, Ht. As a result of these changes are anemia followed by reduction in iron and folic acid [35]. Anemia, defined clinically as a decrease in hematocrit or Hb concentration, may be caused by blood loss, excessive hemolysis, or deficient [36,37]. Studied the decline in RBC count, hemoglobin concentration and may be PCV presumably reflects erythrocyte hemolysis and due to increase in the rate at which hemoglobin concentration may be destroyed or a decrease in the hemoglobin synthesis. Results from this study imply that alpha cypermethrin do alter neither RBC size nor relative Hb content. [38] reported that high dose of Cypermethrin significantly lowered RBC count, the PCV, the MCHC and prospectively. Alpha Cypermethrin treated obviously became progressively anemic in the rabbits [39]. The experiment was evidence by significant decrease in RBC count, Hb and PCV levels in comparison to control mice, significant decrease in all doses in female and male for albino mice. In addition, the reductions by sex are shown in fig 1 and 2 in the most of the males blood parameters showed more reduction than females. Red blood Cells (RBC), Hemoglobin (Hb), Hematocrit (HCT) in the blood of mammals are reported to vary with sex. [40, 41], Decrease in MCV, MCH and MCHC was observed in the current study this means lower than normal value when red blood cells are too small or destruction, This condition is called microcytic anemia may be caused by iron deficiency, iron is important for the production of Hb. Then struggle to provide enough oxygen to all its tissues. As results, these tissues are deprived of oxygen and unable to get rid of carbon dioxide. [42,43]. Hematological changes in rats study by [44] was explained the decline in MCV, MCH and MCHC in mice treated with lambda cyhalothrin. Decrease in MCV, MCH and MCHC shows distraction of RBC and reduction in hemoglobin synthesis and content [45]. Evaluation of hematological parameters for laboratory, estimation of Platelets count used in the assessment of hematoma's. Platelets are produced in the bone marrow by megakaryocytes, which are large precursor cells. The platelets themselves, which are released into the bloodstream from the bone marrow, are actually pieces of the megakaryocytes. Increase in platelet count is called thrombocytosis is a condition in which there is an excessive number of platelets in the blood. Platelets are blood cells in plasma that stop bleeding by sticking together to form a clot. And involved in coagulation which is requires that the platelets should be in sufficient size, number and formation vary according to the health status of age and sex. Too many platelets can lead to certain conditions, including stroke, heart attack, or a clot in the blood vessels. There are two types of thrombocytosis: primary and secondary. Primary thrombocytosis, also known as essential thrombocythemia, is a disease in which abnormal cells in the bone marrow cause an increase in platelets. In the recent findings the significant increase ($p < 0.05$) in the value of Platelets count in all dose of both sexes and the increase in males was higher in females compared to control as in the table number as indicated by many studies [46, 47], where they confirmed in his study on the effect of pesticide on the blood measurement of mice pointed to an increase in the number of Platelets due to the fracture of red blood and the decrease in the value of Hb, so this increase is to compensate for physiological imbalance that occur in as a result of these changes and to prevent of clotting that affects the blood vessels. Usually, younger platelets are larger in size than older platelets, and MPV is often considered a reflection of the average age of your platelets. Where the tables recorded values to calculate platelet variables, it was found that RDW has values that confirm the iron deficiency or microcytic anemia present while there is increasing evidence that platelet indices, such as mean platelet volume (MPV), platelet distribution width (PDW), and platelet large cell ratio (P-LCR), have a significant role in the discrimination between hyper destructive thrombocytopenia and hypo productive thrombocytopenia, confirmed in his study on the effect of pesticide on the blood measurement of mice pointed to an increase in the number of Platelets due to the fracture of red blood and the decrease in the value of Hb, so his increase is to compensate for physiological imbalance that occur in as a result of these changes and to prevent of clotting that affects the blood vessels, and the differences between females and males as found in the calculation of values shown in table (3, 4) due to causes of toxicity substance injected into the bodies of laboratory mice. The increase in WBC's might be due to activation of animal's defense mechanism and immune system (Table 2). Elevated WBC count has been suggested to be due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymph myeloid tissue. Such a response might arise due to presence of toxic substances and associated with pollutant induced tissue damage and severe disturbance of the non-specific immune system leading to increased production of leucocytes. Several authors [48, 49] have noted such an increase in WBC of animals repeatedly treated with sub lethal doses of insecticides. Thus, these immune cells. where are part of the immune system. Have not been able to perform their functions as a defensive cells. This increase was found to be ineffective by substituting the numerical plaques of the W.B.C to counteract the high toxicity of the alpha-cypermethrin insecticide. Increase in leukocytes was also reported by [50, 51], they demonstrated similar situation in albino rats exposed to cypermethrin. In this study male and female mice poisoned with alpha-cypermethrin, mobilization of leucocyte system took place, manifested by a statistically significant increase in the number of leucocytes. Increase was particularly evident in case of lymphocytes as reported in our study. Depending upon the function, lymphocytes are divided into two types: T lymphocytes: Cells concerned with cellular immunity. B lymphocytes: Cells concerned with humeral immunity, thus lymphocyte response might be due to the presences of toxic substances may be associated with pollutants induced tissues damage of

immune system[52,53] . Several authors have noticed an increase in lymphocyte cells in animals repeatedly treated with Lambda cyhalothrin and alpha-cypermethrin by [54,55] noted that a blood analysis of rabbits treated with lambda-cyhalothrin revealed a significant decrease in red blood cell, hemoglobin concentration and monocyte. The differential count regarding neutrophils showed a significant decrease in all doses. Neutrophils are known to be sensitive to stress hormones and various stress agents .A decreased percentage of neutrophils in peripheral blood observed in animals poisoned with alpha cypermethrin may suggest, eutrophils involves in phagocytosis during xenobiotic intoxication, during which some of the neutrophils might have ruptured. Therefore the neutrophils count consistently reduction during different doses of alpha cypermethrin intoxication in mice in the present investigation. Based on the studies on humans (males and females) participating in the production of liquid pesticides, a significant decrease was noted in the number of neutrophils from (23 to 15 %).Thus in this work show the decline in the eosinophil's and basophils cells at type of white blood cells , and a low number of eosinophil's and basophils in the blood called esinopenia and basopenia ,Where the values in the eosinophil's cells of the female mice were (from 2.98to 0.62 %) and basophils values of the males of mice were(from 2.24 to 0.94 %) ,it can be caused by infections, sever toxic can occur blood stream infection, as well as decline in the number of monocyte in the blood called monocytopenia can be caused by anything that decrease , were the values from (2.75 to 0.57%) for the mice female ,and increase lymphocyte found from (37.22 to 80.5 %) for the mice female. Thus ,it is clear from the effect of alpha cypermethrin pesticide has a significant changes on the physiological aspects due to the effect on the function of immune system and blood circulatory and changes in the normal values of the blood .Changes in blood values, which are a complete blood profile in animal such as a laboratory albino mice, are considered to be a vital indicator of human in case of exposure to toxins at work or at home. **Acknowledgement**

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