Effects of Aluminum Chloride on the some Blood Parameters and Histological Spleen in White Male Rats

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Abstract

Aluminum exists in numerous produced foods, medicines and likewise added to drinking water for refining purpose. Its existence has so heavily contaminated with the surroundings that exposed to, it is almost inescapable. This survey was aimed at evaluating the possible effects that Aluminum chloride could exposure have in the blood parameters and histopathology of spleen twenty four rats were used and divided into four groups; "first group was the control injected with normal saline, group II injected into subcutaneous with (240) ppm from Aluminum chloride ($AlCl_3$), group III injected with (320) ppm from ($AlCl_3$), group IV injected with (400) ppm from ($AlCl_3$) for 45 days. This study showed a significant decrease ($P<0.05$) in red blood cell count, hemoglobin concentration, packed cell volume, mean corpuscular volume and mean corpuscular hemoglobin when compared to control group, while there was a significant increase ($P<0.05$) in total leucocyte count (TLC) and Differential leucocytes count (DLC) especially in lymphocyte. The results showed a significant elevated ($P<0.05$) in ESR value. Changes increased with increase in concentration of Aluminum chloride injected. Observation of blood parameters allows the most rapid detection of changes in wistar rats after the exposure of poisonous($AlCl_3$)".

Keywords: Aluminum chloride, Blood parameters, histology, Rats.

الخلاصة

يوجد الالمنيوم في بعض الصناعات الغذائية والدوائية ويضاف الى مياه الشرب في اثناء عملية التنقية. الهدف من الدراسة الحالية هو تسليط الضوء لمعرفة تأثير التعرض المزمن لكلوريد الالمنيوم على معايير الدم الحيوية والتغيرات النسيجية للطحال.

استخدم اربعة وعشرون جرذاً ابيض وقسمت الى اربعة مجاميع المجموعة الاولى حقنت بالمحلول الملحي الفسيولوجي والمجموعة الثانية حقنت بـ (240) جزء بالمليون من كلوريد الالمنيوم ($AlCl_3$) والمجموعة الثالثة حقنت بـ (320) جزء بالمليون من كلوريد الالمنيوم ($AlCl_3$) والمجموعة الرابعة حقنت بـ (400) جزء بالمليوم من كلوريد الالمنيوم ($AlCl_3$) تحت الجلد ولمدة خمسة واربعون يوماً (45) اشارت الدراسة الى حدوث انخفاض معنوي ($P<0.05$) في العدد الكلي لكريات الدم الحمراء (RBC) ، تركيب الهيموكلوبين(Hb) ومعدل كريات الدم المضغوطة (PCV)، ومعدل حجم كريات الدم الحمراء (MCV) ومعدل هيموكلوبين الدم (MCH) عند مقارنتها مع مجموعة السيطرة.

كمل ولوحظ هناك زيادة معنوية ($P<0.05$) في العدد الكلي لكريات الدم البيضاء (TLC) والعدد التفريقي لكريات الدم البيضاء (DLC) خصوصاً في الخلايا اللمفاوية . كذلك لوحظ زيادة معنوية في معدل الترسيب لكريات الدم الحمراء (ESR) لمجاميع المعاملة مقارنة مع مجموعة السيطرة.

اما بالنسبة الى التغيرات النسيجية فقد لوحظ حدوث نزيف دموي وتحلل وزيادة في المادة البيضاء مقارنة مع المادة الغامقة نتيجة التعرض لكلوريد الالمنيوم وقد ازدادت التأثيرات مع زيادة التراكيز المستخدمة.

الكلمات المفتاحية: كلوريد الالمنيوم، معايير الدم، الانسجة، جرذان.

Introduction

Aluminum is known to cause toxic effects to variety of organ, including brain as well as bone, kidney, liver, spleen and circulatory system (blood) (Sarin *et al.*, 1997). It has been proposed as an environmental factor that may contribute to some neurodegenerative disease, and effects on several enzymes and biomolecules relevant to Al-Zhemer's disease (Ferreya-moyah & Barragan, 1994; Naya& Chatteryjee, 2001).

"Aluminum is a ubiquitous element found in every food product. The source of Aluminum cosmetics, pesticide, medicines and is also added to drinking water for purification purposes (Rudenko, 1999). The rapid development of industry and especially chemical industry has created a serious problem of water pollution. Human destructive influence on the aquatic environment is in the form of sublethal pollution, which results in a chronic stress condition that have negative effects on aquatic life (Mason, 1991). Hematological parameters are used as an index of rats health status in a number of laboratory animals to detect physiological changes following different stress conditions like exposure to pollutant, disease, metals, hypoxia, etc. Therefore, hematological techniques are the most common method to determine the sub lethal effects of pollutants (Larason *et al.,* 1985). Aluminum may enter natural waters via coal strip mining activities, water treatment facilities using Aluminum sulphate (Alum) as a coagulant for suspended solid particles, industrial wastes and acid rainfall. Thus, when Aluminum becomes available to organisms through acidification of surface water, it is toxic to fish and human (Driscoll *et al.,* 1980). Histopathological investigations have long been recognized to be reliable biomarkers of stress in fish (Vanderoost *et al.,* 2003). Besides, the dangerous involved from the presence of metal in the environment derive not only from their persistence and toxicity, but also from the remarkable degree of bioaccumulation they undergo through the tropic chain, thus becoming a serious danger to man (Bishop, 2000). Heavy metals are considered the most important form of pollution in aquatic environments because of their toxicity and accumulation by organisms such as fish (Emami, 2005).

The elemental Aluminum doesn't occur in its pure state, but always united with other factors such as chloride, hydroxide, silicate, sulphate and phosphate. The broad distribution of this element ensures the potential for causing human exposure and damage (Berthon, 1996; Zhang&Zhou, 2005)".

Epidemiological surveys have indicated a link between "Aluminum in drinking water and Al Zheimer's disease (AD) and a sort of human and animal studies have implicated learning and memory deficits after Aluminum exposure (Buraimoh *et al.,* 2011; Yoke, 2000). Aluminum chloride was implicated to have negative effects on behavioral end point of wistar rats (i.e alters behavior), have negative effects on anxiety-related behavior of rats as it increase the average of anxiety in Aluminum treated rats, have neurodegenerative effects on the histology of the cerebral cortex of adult Wister rats and also decreases in red blood cell count and hemoglobin concentration, also decrease the mean of number of sperm (Buraimoh *et al.,* 2011, Buraimoh *et al.,* 2012).

The cause of tissue injuries induced by several heavy metals is thought to result in the formation of lipid peroxidation in membrane (Ohtawa *et al*., 1983). This survey was aimed at evaluating the possible effects that Aluminum chloride could have on blood parameters and the histology of the spleen of adult Wister rats.

Materials and methods

The study was conducted at a central animal house of college of Science, Babylon university. Wistar albino Sprague Dawley rats (Rattus norvegicus var. albino) are reproduced continuously at a central animal house. All experiments were carried out at this center where animal reproduced, only male rats were used in experiments.

The rats were allocated into 4 cages applying a photoperiod 12 hr. of light at 22±1.5 ºC and moisture of laboratory was kept between 48% and 56% during the experiments, rats were fed with standard food. Weight of rats ranged between 200-230 gm. Each of experimental groups consist of 6 rats and total of rats 24 animals used in the experiment, including control group. Rats received aluminum chloride (240, 320 and 400) ppm and injected subcutaneous for 45 days, while control group received normal saline and injected subcutaneous for 45 days.

Hematological parameters

At the end of the experimental period, rats blood was collected in tubes have anticoagulant to study blood parameters, "total leucocytes count (TLC), differential leucocytes count (DLC), red blood cell count (RBCs), packed cell volume (PCV), hemoglobin concentration (Hb), R.B.C. indices (MCV) and (MCH) and erythrocytes sedimentation rate (ESR)" and study histopathology of spleen.

1. Total Erythrocytes count

We used the haemocytometer method and Hyme's fluid as a diluted fluid to count the total erythrocytes (Sood, 1989).

2. Hemoglobin estimation

We used the cyanomethegolobin to estimation the concentration of hemoglobin in blood sample and measured. The coloric density by using spectrophotometer in the wavelength 540 nm.

3. Packed cell volume measurement

In this method used the capillary tubes nonheparinized and micro centrifuge and hematocrit reader to measure the percentage (%) of packed cell volume (Powers,1989).

4. Red blood corpuscles indices

We calculated R.B.C indices by using the information obtained from PCV and the concentration of hemoglobin and total erythrocytes count by the equations:

a. Mean corpuscular hemoglobin (MCH)

It means the average weight of hemoglobin in red blood cell for studying a sample and measured by pg (Shirlyn;2004)

MCH(Pg)=hemoglobin in gm/dL/red cell count per liter ×(10)

b.Mean corpuscular volume (MCV)

It refers to volume of red blood cell femto liter (FL) (Shirlyn;2004)

MCV (FL)=packed cell volume (%)/red cell count per liter ×10

5.Erythrocyte sedimentation rate measurement

We used Westergreen's method to estimate erythrocyte sedimentation rate (Brawn., 1976).

6. Estimation of leucocytes count

a.Total leucocytes count

We used haemocytometer method and Turk solution to count total leucocytes number (Humanson., 1978).

b.Differential leucocytes

In this method we prepare a blood smear and stained with leishman stain and examined by oil emersion to count differential leucocytes granular and a granular (Bancroft&Steven;1982).

Preparation of histological sections

Histological section prepare from the organs taken from sarcifice animals to knew the changes of this tissue because of injected with aluminum chloride, in this preparation we used the method from (Bancroft&Steven;1982).

The organs fixed with Bouns solution, and washed more than one by ethyl alcohol 70%, and dehydration with serial steps by ethyl alcohol (70, 80, 90, 95 and 100%) for 2 hour to every concentration of them and clearing with xylene for 2 hour melting point (56-58) ºC, and then embedding with paraffin wax and then prepare serial section with rotary microtome, and mounting on the slide, when put it on the water bath (42-50) ºC for 2 min. and staining with hematoxylin and eosin and put a cover slide by Canada balsam and examined by microscope compound to recognize the histological changes(Humanson., 1978).

Statistical analysis

Statistical package for the social sciences (SPSS) were used for the analyses of data. Data of blood parameters were presented as means ±standard error of the mean. One w2ay ANOVA testing was applied to data if they were normally distributed and means were tested using least significant difference (LSD) test and significant differences are indicated as follows ($P<0.05$).

Results

This study showed that 45 days of injection $AlCl_3$ (240,320,400) ppm in male rats was not considerably lethal. There were changes in the hematological parameters showing a significant decrease ($P<0.05$) in total number of erythrocytes on the treated group (240,320,400) ppm as compared to control group table (1). So the results for packed cell volume (PCV) and hemoglobin concentration (Hb) showed a significant decrease ($P<0.05$) in treated groups as compared to control group table (1). While the values of erythrocyte sedimentation rate (ESR) showed a significant increase in treated groups as compared to control group table (2).

In addition, this study showed a significant decrease ($P<0.05$) in mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) in treated group (240,320,400) ppm as compared to control group table (2), (3) showed a significant increase in total leucocytes (TLC) in treated groups as compared to control group, this results' reflect on the results of DLC, differential leucocytes count exactly in lymphocytes, monocytes and granular cell.

The histopathological study in the spleen was showed an increase in white pulp and degeneration in treating group figure (2,3,4) as compared to control group.

Table (1): Effect of Aluminum chloride ($AlCl_3$) on the blood parameters.

Treatment	Total erytrhrocytes count cell/mm^3	Packed cell volume (PCV) %	Hemoglobin concentration g/100ml
Control	5.100±0.163	36±0.215	11.3±0.225
240ppm	5.116±0.163	21±0.215[ab]	8.617±0.225 [ab]
320ppm	4.5±0.163[a]	19±0.215 [ab]	8.00±0.225 [ab]
400ppm	3.787±0.163[ab]	18±0.215 [ab]	7.217±0.225 [ab]
LSD	**0.370**	**0.500**	**0.574**

LSD: Least Significant Difference (P<0.05)
N: 6 animals

Table (2): Effect of Aluminum chloride ($AlCl_3$) on the erythrocyte sedimentation rate and red blood cell indices.

Treatment	Mean corpuscular volume (MCV) FL	Mean corpuscular hemoglobin (MCH) pg	Erythrocyte sedimentation rate (ESR) mm/hour
Control	72.2±0.668	22.30±0.429	1.5±0.189
240ppm	65.158±0. 668[a]	19.667±0. 429 [a]	3.917±0. 189 [a]
320ppm	64.942±0. 668 [a]	18.892±0. 429[ab]	4.33±0. 189 [ab]
400ppm	64.217±0. 668 [ab]	16.792±0. 429 [ab]	4.90±0. 189 [ab]
LSD	**1.550**	**0.88**	**0.430**

LSD: Least Significant Difference (P<0.05)
N: 6 animals

Table (3): Effect of Aluminum chloride ($AlCl_3$) on the differential leucocytes count.

Treatment	Lymocytes (%)	Monocytes (%)	Granular (%)
Control	30.4±0.481	1.00±0.253	68.6±0.427
240ppm	33.2±0.481[a]	1.6±0.253 [a]	65.66±0.427 [a]
320ppm	35.6±0.481 [a]	2.364±0.253[ab]	62.9±0.427 [ab]
400ppm	38.5±0.481 [ab]	2.364±0.253 [ab]	59.133±0.427 [ab]
LSD	**1.09**	**0.569**	**0.978**

LSD: Least Significant Difference (P<0.05)
N: 6 animals

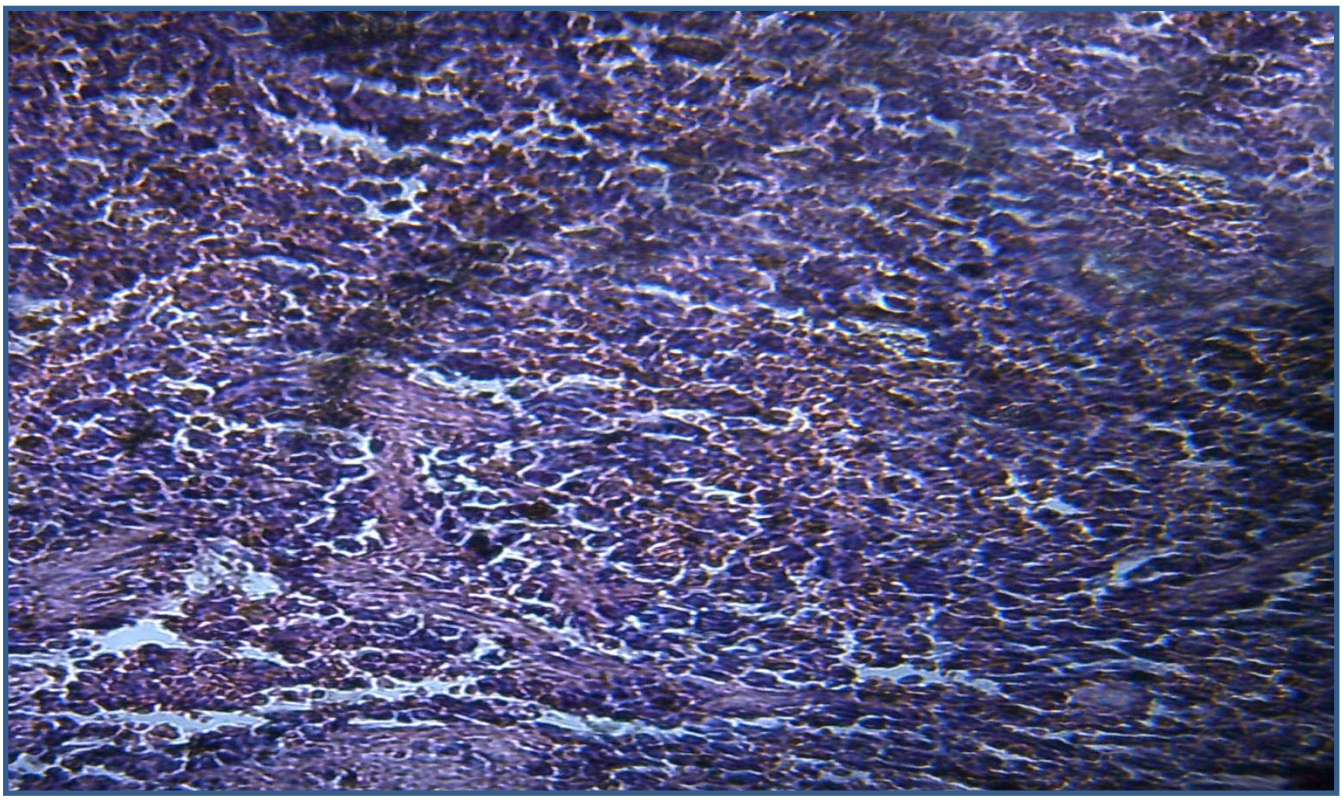

Figure NO (1) section from spleen tissue for control group (400x)

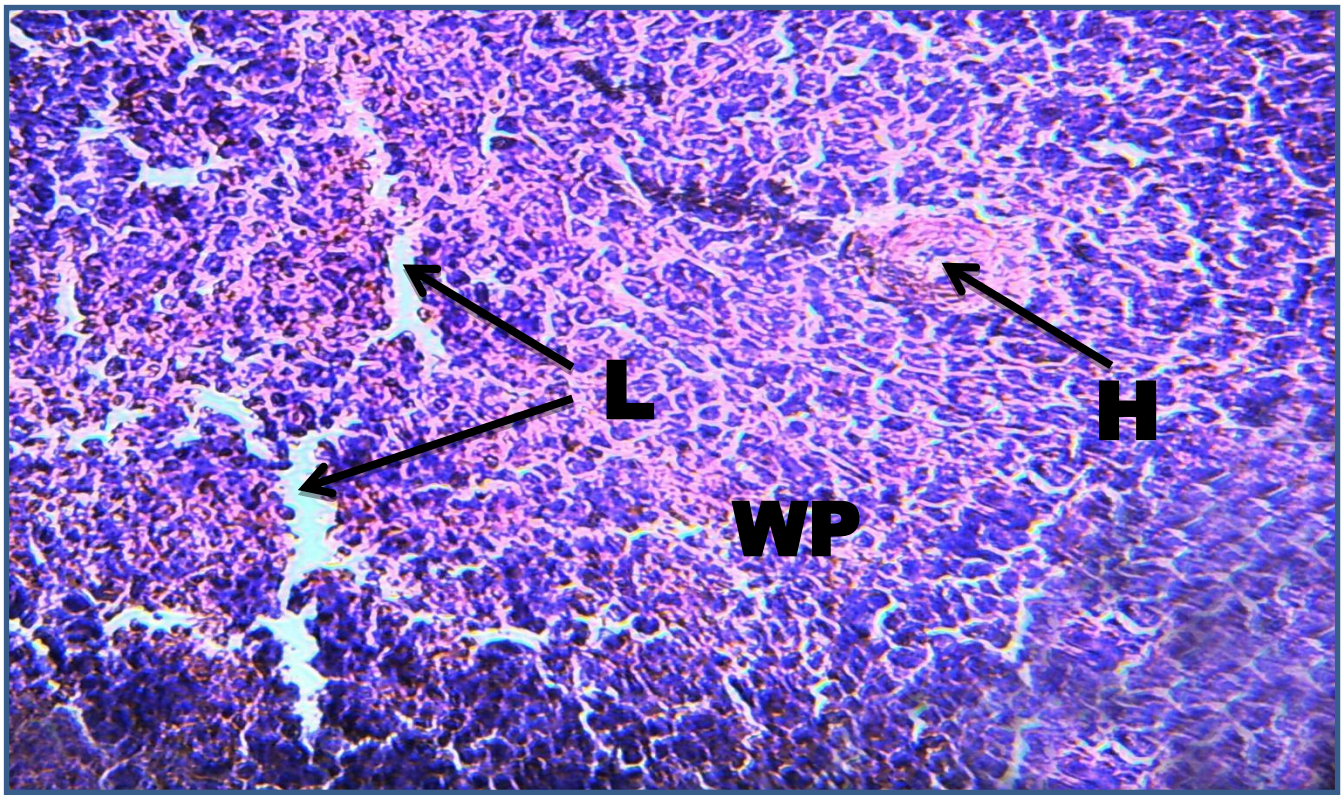

Figure NO(3) cross section from spleen for treated with (240) ppm for 45days showed alysis (L) , hemorrhage (H) , increase in white pulp (WP) (400x)

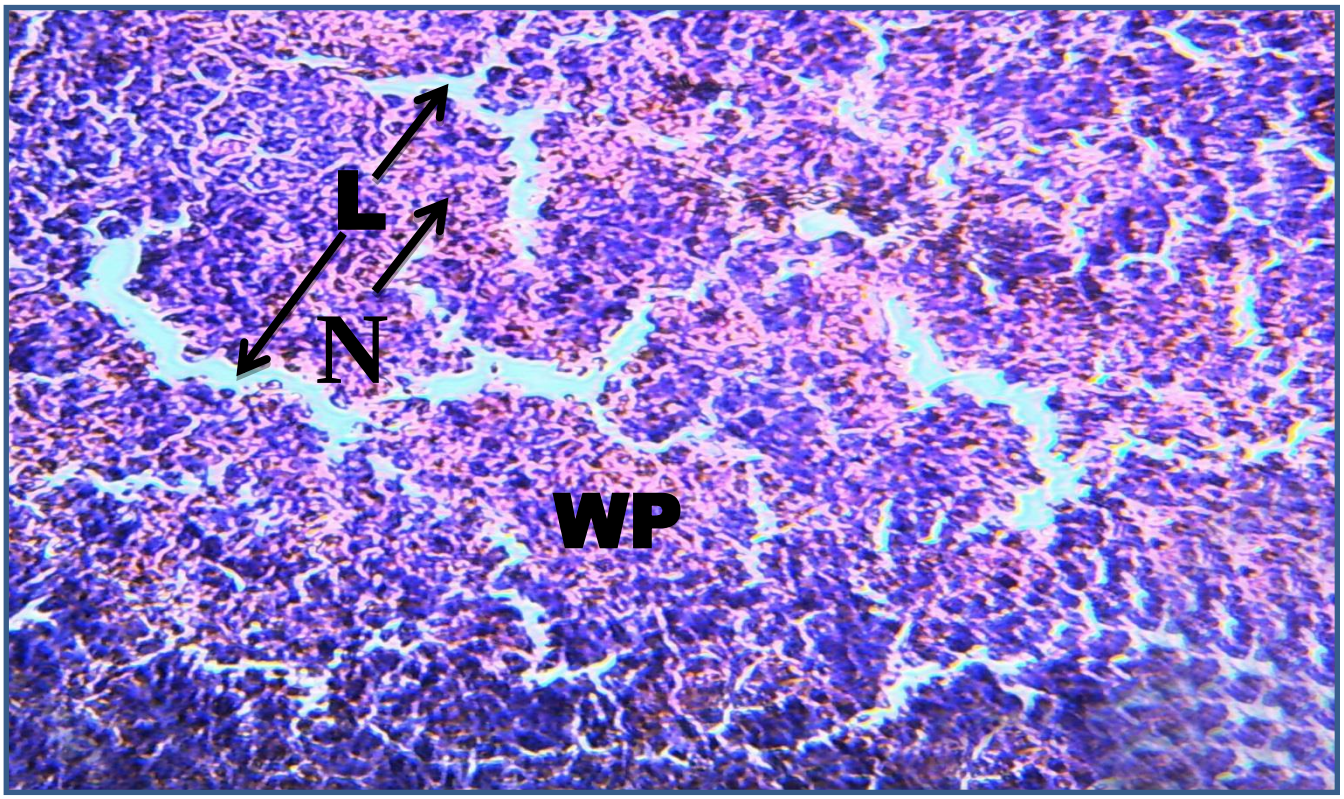

Figure NO (3)cross section from spleen tissue for treated with (320) ppm for 45 days showed alysis (L), necrosis (N) (400x)

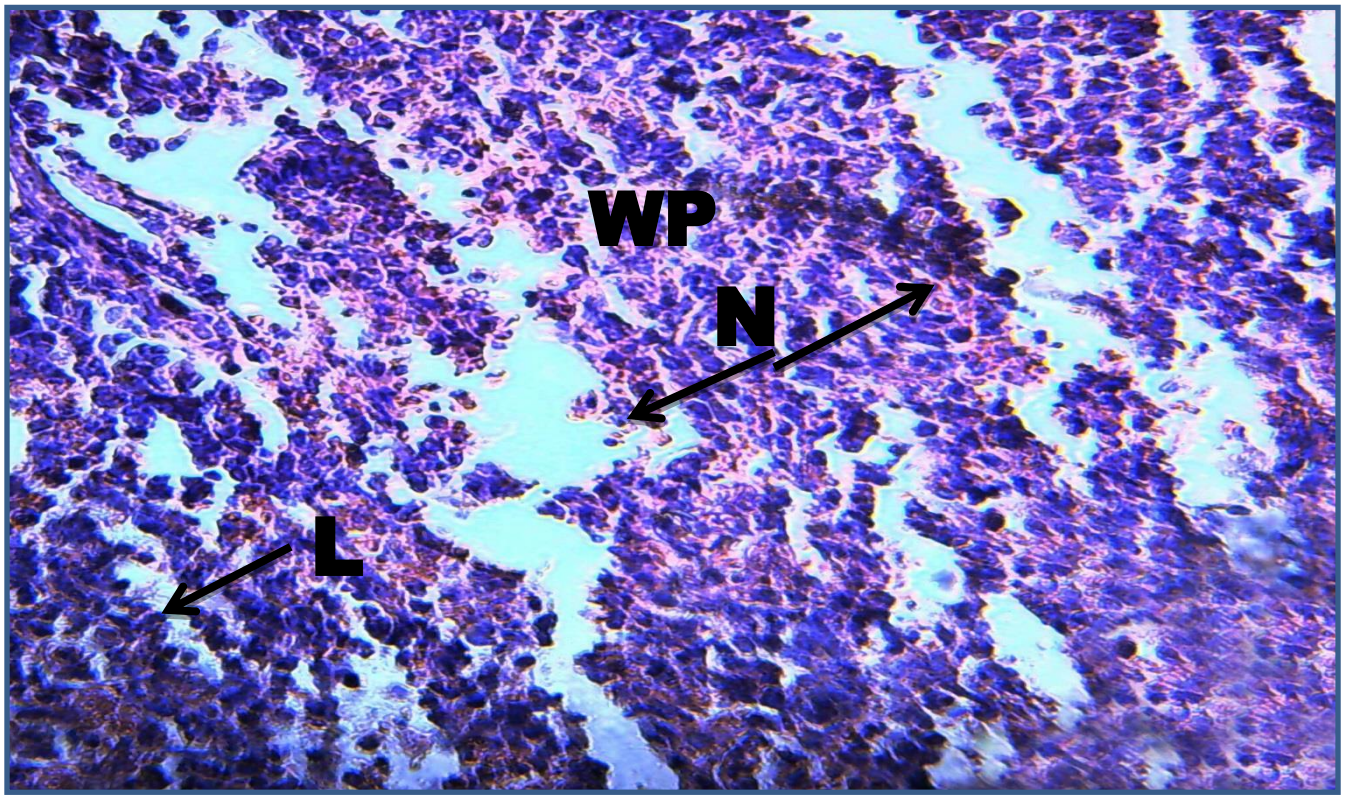

Figure NO (4) section from spleen tissue for treated with (400) ppm for 45 days showed alysis (L) and increase in white pulp (WP) (400x)

Discussion

In this study during the 45 days of (240,320,400) ppm observation of rats injected Aluminum chloride assessment of harmful effects of Aluminum ions was based on the analysis of blood parameters, Aluminum concentration showed a significant decrease ($P<0.05$) on the mean of blood cell count after 45 days,in the biological system aluminum Ions replace Iron and mangnisium Ions reduceFe+2binding to ferritin and disrub hem synthesis(Yakubu; 2017). The other cause of its inhibition of hemooxygenase, this enzyme necessary for hemoglobin formation series, stopped by the toxicity of Aluminum and increase the destruction of RBS and transformed to bilirubin (Kalaiselve *et al.,* 2015).

Toxicity of Aluminum increased free radical in target organs such as liver and inhibit of glutathione enzyme in liver. This important to maintain hemoglobin in red blood cell and increase removing of hydrogen peroxide (H2O2) and increase the lifetime of red blood cell (Buraimoh *et al*., 2011), reduction of some enzyme such as glutathione reductase, catalase, glucose-6-phosphate dehydrogenase lead to accumulate of toxins inside red cells (Kalaiselve *et al.,* 2015)

The effects of Aluminum treatment on blood hematocrit (HCT), hemoglobin (Hb), MCV, MCH. The results were showed that's value marked significant decrease ($P<0.05$). The reason of this protoporphyrin and it's necessary for hemoglobin formation, the presence of aminolivolinic acid in urine refers to the failure synthesis of hem caused by toxicity of Aluminum (Kawahara *et al.,* 2007). The other cause of this result caused by effects of Aluminum chloride on bone marrow, especially mothers cell of red blood cell. This lead to decrease in RBC count and hemoglobin and reflect on the hematocrit value (Mahieu *et al.,* 2000) and caused anemia, a decrease in HCT, MCV, MCH and Hb values has often been proven to be a serious indicator of Aluminum toxicity.

As for the estimation of erythrocyte sedimentation rate (ESR) we noted a significant elevated in ESR value, we suggest the cause of its increasing of infections in joints of laboratory animals as compared to control group. Similarly, the ESR value of mice was increased when mice treated with Aluminum .

The present study showed that the level (240,320,400) ppm of Aluminum chloride induce a highly significant ($P<0.05$) increase in white blood cell and differential leucocytes count especially lymphocyte and monocyte because of Aluminum chloride induce infections in target organs such as liver, brain, kidney, spleen and smooth muscle (Mukherjee & Bhattacharya;1975). This result reflected in the histolopathological study of the spleen, we noted increases in white pulp and occurs bleeding and degeneration in spleen tissue. The cause of this destruction because the action of free radicals and increase in lipid peroxidation this process initiate tumor in target organ . (Buraimoh *et al.,* 2012).

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