HEMATOLOGICAL AND HISTOPATHOLOGICAL CHANGES INDUCED BY CADMIUM CHLORIDE TOXICITY IN SHEEP, WITH USING ALPHA LIPOIC ACID AS ANTIOXIDANT.

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ABSTRACT

Cadmium (Cd) is a heavy toxic metal, with harmful effects on animals and public health. Recently the risk of cadmium toxicity is substantially regarded. Therefore the present study was performed to clarify the effects of acute cadmium toxicity in sheep with using alpha lipoic acid (ALA) as an antioxidant agent. Fifteen male lambs 5 - 7 months old were divided equally in to three groups, they were supplied with ordinary diet and provided with water *ad-lib*, The first group (I) was administered a single dose of $CdCl_2$ 3 mg/kg.bw subcutaneously (S/C), the second group(II) was injected with the same dose of $CdCl_2$ and via by the same route, and then simultaneously administered an alpha lipoic acid 50 mg/kg.B.W intramuscularly, the later drug was repeated after 12 hours via the same route also. The third group (III) was left as control and given normal saline (S/C).

Resulted revealed a significant decrease ($P<0.05$) in the Hb, MCHC and platelets were recorded, whereas significant elevation ($P<0.05$) of PCV value was observed, in addition non significant variations of RBCs count and MCV of the first group were noticed. The results also indicated a significant ($P<0.05$) increase in WBC (neutrophilia) with significant ($P<0.05$) lymphocytopinia in the first group while Insignificant variations in hematological parameters in the second group as compared with the control group**.**

The histopathological study of these animals showed infiltration of inflammatory cells, congestion, hemorrhage, vacuolation and necrosis in different internal organs of animals in the first group while showed slight histopathological changes were detected in the second group. That indicated the ALA has important role as antioxidant.

INTRODUCTION

Cadmium (Cd) was discovered in 1817 by German chemist Strohmeyer . It is usually found naturally integrated together with zinc, so that obtained as a by-product of zinc production (1).

The cadmium has indirectly ways to caused oxidative stress and damage of cells and vital organic tissue structures (2). It has a long biological half-life (15–30 years primarily) because the metal poor excretion and excessively accumulated in the blood, kidneys, liver, and other organs (3).

The high pollution of soil and water with cadmium lead to increase its concentrations inside animal tissues, fishes, plants and vegetables. Therefore, foods are considered the primary source of exposure for the population (4,5).

The *ALA* is as essential antioxidant compound used for treatment of heavy metal intoxication (6). It has many biological functions as its efficiency equivalent to antioxidant vitamins (C, E) and coenzyme Q 10 (7).

In Iraq, Al- Basra province- showed high pollution with heavy metals especially cadmium, mercury, lead and arsenic were reported (7,8). Therefore this study was designed to clarify the hematological and histopathological changes induced by $CdCl_2$ toxicity, with trial to use the alpha lipoic acid as a therapeutic antioxidant agent in sheep.

MATERIALS AND METHODS

Experimental animals and design of study

Fifteen males lambs aged from 5-7 months, weight from 17-20 kg, apparently healthy were used in the experiment; they were administered Albendazol (7.5 mg/kg) orally. Lambs were provided twice daily with green fodder (alfa alfa) and concentrate (ordinary diet), water was provided ad-lib. They were kept 20 days for adaptation.

Lambs were allocated equally in to three groups (5 lambs in each group)

First group (I): lambs were administrated subcutaneously (S/C) a single dose of cadmium chloride (sub lethal dose) 3 mg/kg (9).

Second group (II): the lambs also injected (S/C) a single dose of cadmium chloride 3 mg/kg and at the same time administered Alpha lipoic acid 50 mg/kg intramuscularly followed by the same dose(50 mg/kg) and route (I/M) repeated 12 hours (10).

Third group: was left as control group, administered S/C normal saline (milliliters were equivalent to cadmium chloride solution).

Blood was collected aseptically from jugular vein by 10 ml disposable syringe in EDTA test tubes for hematological tests, post administration of $CdCl_2$ and ALA (after appearance of toxicity signs).

TRBCs count, Hb, PCV, Platelets count, MCV, MCHC, TWBCs Count and differential white blood cells were determined according (11)

Histopathological examination:

The specimens of $1cm^3$ were taken from internal organs such as liver, spleen and kidneys were stained according Luna (12).

Statistical analysis:

Variance of analysis was employed, and by using SPSS program version 20, t-student test was used for comparison at level of $p \leq 0.05$

RESULTS

Hematological study

Non significant increase in red blood cells count (RBCs) which was observed in the animals of the first group as compared with the second and control groups. However significant ($P<0.05$) increase of Packed cell volume in first group was recorded, beside that the hemoglobin concentration (Hb) and the platelets count were significantly ($P<0.05$) decreased in first group, In addition no significant variation in mean corpuscular volume (MCV), whereas significant ($P<0.05$) decrease of mean corpuscular hemoglobin concentration (MCHC) in first group when compared with second and control groups (table 1), this indicated type of anemia was normocytic hypochromic. There were non significant variation in the values of hemogram parameters between second and control group (table 1).

Table 1 Hemogram parameters in acute cadmium toxicity.

Groups / Parameters	I G1	II G2	III Control G
RBC $\times 10^6$ µl	11.97 ± 0.24	11.19 ± 0.435	11.14 ± 0.487
PCV %	42 ± 0.68 [a]	34.6 ± 0.733 [b]	34.1 ± 0.345 [b]
HB g/ dl	10.6 ± 0.47 [a]	11.5 ± 0.33 [b]	11.6 ± 0.14 [b]
Platelets count X 10^3	289.4 ± 15.3 [a]	390.6 ± 7.42 [b]	395.4 ± 12.7 [b]
MCV fl	35.3 ± 1.08 [a]	31.8 ± 2.2 [b]	30.5 ± 1.2 [b]
MCHC gm/dl	25 ± 3.6 [a]	33.1± 1.53 [b]	33.7 ± 2.6 [b]

*Different small letters horizontally referred to significant differences between groups

Significant (P<0.05) increase of the total WBCs in the first group was recorded, this was due to an increase in the number of the neutrophil, whereas a significant (P<0.05) decrease in the number of lymphocyte also observed in the same group. However no significant alterations in total and differential leukocytic count were recorded among second group as compared with the control (Table 2).

Table 2 Total and differential WBCs of acute stage.

Group / Parameter	I G1	II G2	III Control G
Total WBCs X 10^3	12.48 ± 0.43 [a]	10.38 ± 0.33 [b]	9. 18 ± 0.39 [b]
Neutrophils %	56.2 ± 2.63 [a]	37.8 ± 3.95 [b]	31.9 ± 2.2 [b]
Lymphocyte %	36.4 ± 3.09 [a]	54.8 ± 4.57 [b]	57 ± 2.08 [b]
Basophile %	0.6 ± 0.24	0.4 ± 0.28	0.5 ± 0.31
Eosinophils%	3 ± 0. 43	4 ± 0.54	3.4 ± 0.60
Monocyte%	3.4 ± 0.6	3.0 ± 0.77	3.2 ± 0.35

*Different small letters horizontally referred to significant difference between groups

Histopathological study

The histopathological studies of the liver section in the first group showed disruption of hepatocytes and expansion of sinusoids, congestion in central vein as well as infiltration of mononuclear inflammatory cells around portal vein with present of mild fibrosis surrounded the portal regions (Fig 1). While in the second group the result showed dilation of sinusoids with present of mononuclear inflammatory cells in the congested central vein (Fig 2).

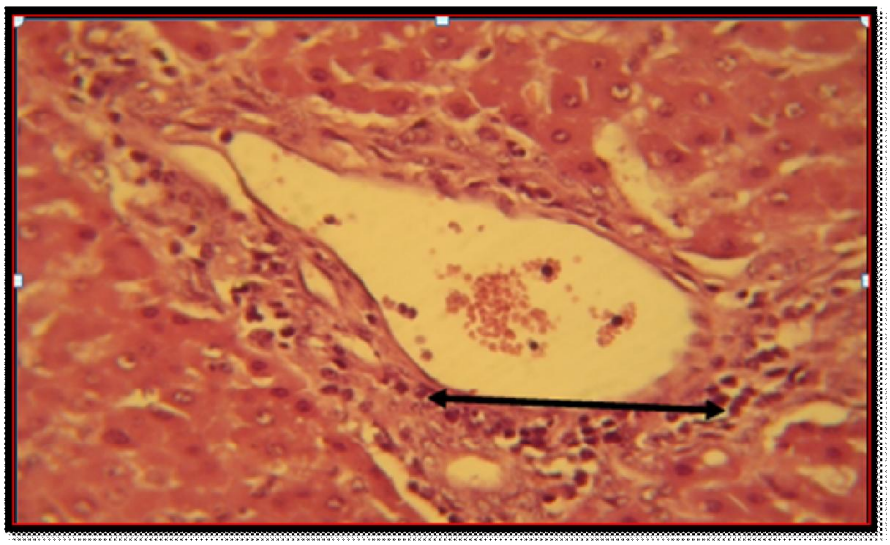
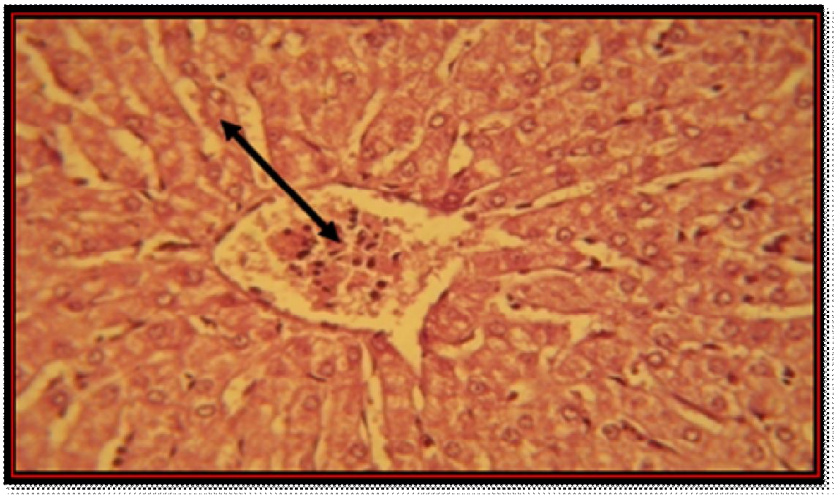


Fig (1): Histopathological section of liver of I (acute phase) showed infiltration of mononuclear inflammatory cells around portal vein with the presence of mild fibrosis around portal region (H&E stain. 40X).



Fig(2): Histopathological section of liver of II (acute phase) showed dilated of sinusoids with the presence of mononuclear inflammatory cells in the congested central vein (H&E stain. 40X).

Also the histopathological section of kidney in the first group showed atrophy of glomeruli with infiltration of mononuclear inflammatory cells in the interstitial tissue between renal tubules Fig (4-3). While the histopathological changes of the kidney in second group showed marked vacuolation of some renal tubules, revealed less sever effect by cadmium chloride toxicity when compared with first group (1-3).

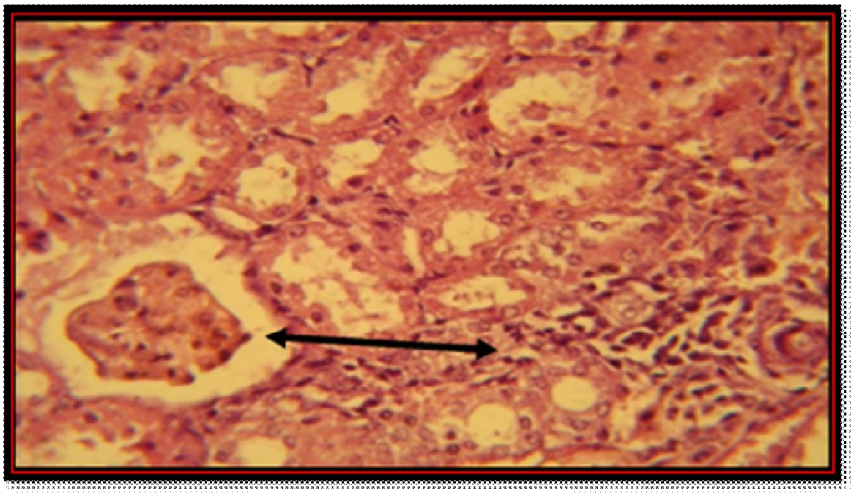
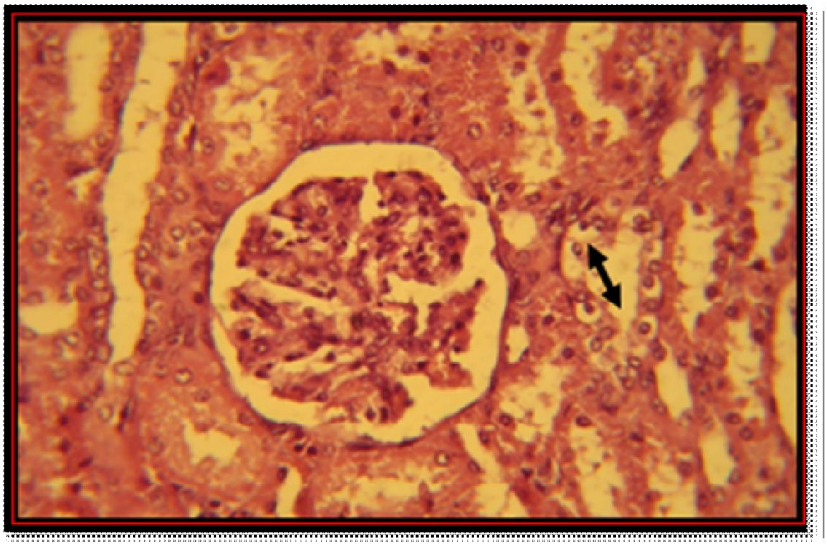


Fig (3): Histopathological section of kidney of I (acute phase) showed atrophied glomerulus with infiltration of mononuclear inflammatory cells in the interstitial tissue between renal tubules (H&E stain, 40X).



Figure(4): Histopathological section of kidney of II (acute phase) showed marked vacuolation of some renal tubules. (H&E stain, 40X).

The histopathological changes of the spleen in the first group revealed depletion and atrophy of white pulps as well as proliferation of red pulps and fibrin deposition (Fig 5). While mild atrophy of white pulp and mild severity changes in the second group were recorded (Fig 6).

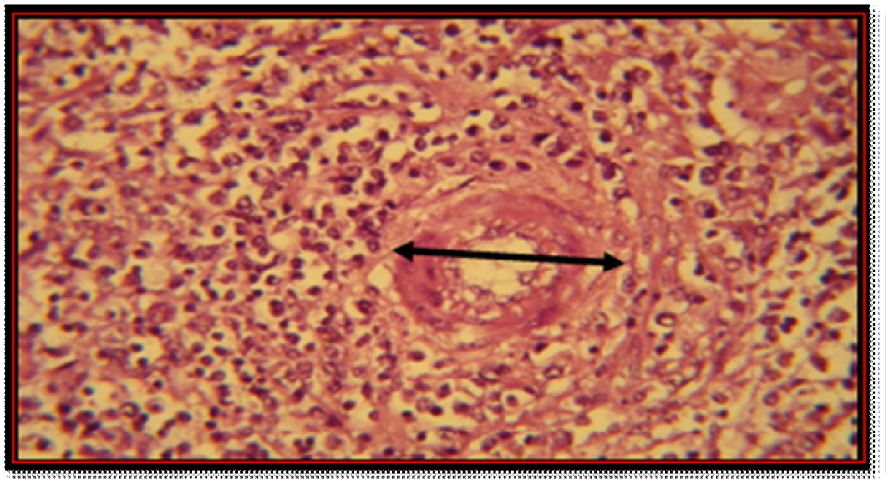


Fig (5): Histopathological section of spleen of I (acute phase) showed atrophy of white pulp with fibrin deposition (H&E stain, 40X).

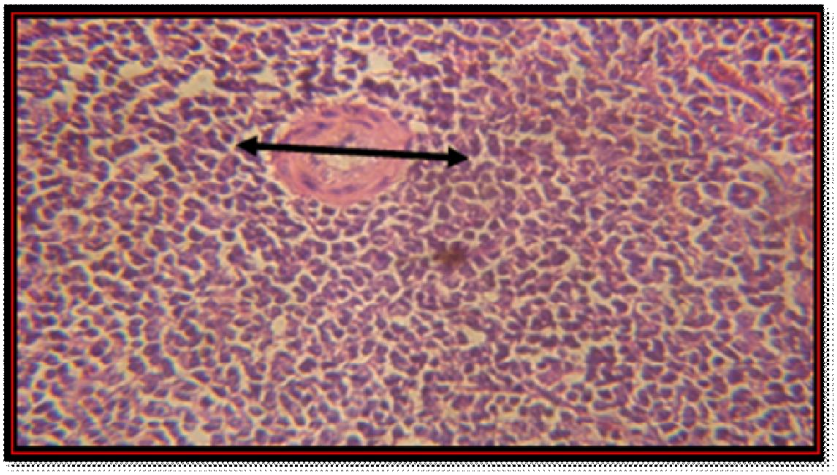


Fig (6): Histopathological section of spleen of (II acute phase) showed mild atrophy of white pulp. (H&E stain, 40X).

DISCUSSION

Cadmium (Cd) is a heavy toxic metal, with harmful effects on both animals and public health. The slight increase in the RBCs count and Packed cell volume in the first group belonged to dehydration, also the increased in RBCs count (relative polycythemia) contributed in an increase of packed cell volume without increase in the actual size of the erythron (13).

Morage (14) mentioned that relative polycytheamia occurred due to dehydration because the decrease in water intake lead to reduce body water content , and the RBC can't escape from circulation , causing an increase their concentration and hence the PCV will be increased.

Significantly ($P<0.05$) Hb, MCHC and platelets count were decreased in the first group which were, similar to findings reported previously by (15) , These changes were attributed to decrease the amount of the iron which is an essential component for hemoglobin and myglobin synthesis (16,11) they referred that iron plays an important role in synthesis of some enzymes, these enzymes have functions or roles in energy and prostaglandine production as well as free radicals detoxification, consequently the iron will be decreased in levels due to consumption in these processes, in addition to the decrease of platelet may be due to their consumption them in repairing of damaged tissues or in the disorganization of hemostatic mechanism that enhanced by disseminating intravascular coaggulopthy (14). Beside that thrombocytopenia occurred due to effects of some drugs and poisoning leading to decrease number of platelet due to depression of bone marrow or enlargement in the size of the spleen.

Moreover no deference observed between second and control groups in blood parameters were recorded: RBCs count, PCV, Hb, MCV, MCHC and platelets count these results indicated that alpha lipoic acid had a role in prevention the hematological changes which belonged to cadmium chloride toxicity, as the ALA is a potent antioxidant , It lowered the consuming of the iron in synthesis of the essential enzymes which have roles in detoxification and regeneration of antioxidant vitamins such as(vitamin C and vitamin E), beside it s ability of chelating metals, consequently prevent the $CdCl_2$ effect these results were agreement with others (17,18,19).

Recently (20) reported that ALA essential for functions of many enzymes which take part in mitochondrial oxidative metabolism, also the ALA has deferent biochemical functions: acting as biological antioxidants, regenerate of other antioxidant in body organisms such as vitamin C and E, metal chelating and modulator of the signaling transduction by several pathways.

The significant ($P<0.05$) increase of the total WBCs count due to significant ($P<0.05$) increase of neutrophil in the first group , this might be occurred due to stress , the stress causes releasing of cortisol and the later in turn causing increase liberation of mature neutrophils from bone marrow stores and depressing the migration of neutrophils from blood into tissues, in the same instance, the necrotic lesion which occurred due to the $CdCl_2$ administration in different tissue also lead to a stimulation the leukocyte and release the endogenous, the endogenous will cause increase the numbers of neutrophils and decrease the lymphocyte (21,14). (11,16) suggested the pain, trauma, stress and high body temperature lead to release the endogenous and caused netrophilia. A significant ($P<0.05$) decrease in the lymphocyte percentage in the first group as compared with others was observed, the decrement in the percentage of lymphocyte might be occurred due to the cadmium toxicity as causes inhibition in the lymphocyte proliferation this result was similar to findings by others (22). (23) found strong negative correlation between amount of the cadmium and lymphocytes proliferation in vivo, moreover the effect of cadmium toxicity on immune system were studied in different animal species (rats, sheep and fish), it was found the little amount of cadmium slightly stimulated the lymphocyte proliferation while the high concentration caused inhibition of lymphocyte proliferation (24).

The total and differential leukocytic count in the second group showed no significant variation as compared with control group, these results might be due to effect of ALA lipoic acid as antioxidant, It maintains the body function through protection the body tissue from the damage by $CdCl_2$, in addition Its had roles as metal chelating and immune stimulator, (25) reported that ALA can recruit both arms of humoral and cell mediated immunity through direct effect and indirect effect.

(25) and (26) reported that ALA maintains the number and function of the lymphocyte and increase interferon levels which is a potent stimulator responsible for recruitment of macrophages and other immune cells.

The histopathological changes might be due accumulation of cadmium within hepatocytes causing disruption in the histoarchitecture of the liver tissues, these findings were similar to previous studies (9.27,28).

The histopathological changes of the liver section in second group were lesser in severity than in the first group, this might be revealed the protective effect of ALA against the heavy metals because of its ability for neutralization of the free radicals these results agreed with (29,30) .

The free radicals and some of cadmium were excreted through the kidney and accumulation of large quantity of cadmium metallothionine complex cause damage to glumeruli and renal tubules these results were agreed with (27) who stated that renal histopathological changes occurred because of it is considered one of the target tissues for cadmium toxicity and the reactive oxygen species causes damages of protein. Less severity changes in renal sections in second group might be due to the ALA has role to protect the renal tissues via inhibitory effect on free radicals or scavenge them, (30) showed that large doses of alpha lipoic acid can be protect the rats against cisplatin-induced renal damage.

The cadmium chloride toxicity was responsible for the production of reactive oxygen species resulting in degeneration and apoptosis of lymphoid cells of spleen and later reducing T- and B- cells number within the lymphoid tissues these result in agreement with (27) who found that cadmium chloride suppressed the immune response in sheep spleen cells *in vitro* by causing exposed cells to undergo apoptosis.

In other hand mild histopathological changes in spleen tissues of second group were detected, might be belonged to protective and ameliorative effect of alpha lipoic acid against the immuno-toxicity of cadmium chloride on the lymphoid tissue by reducing the production of free radicals, in addition the alpha lipoic acid increases glutathione synthesis which has regulatory effect on immune system by activation of T-cell (25).

التغيرات الدموية و النسيجية لسمية كلوريد الكادميوم المحدثة تجريبيا في الاغنام و استخدام حامض الفا لايبوك كماده مضادة للاكسده

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الخلاصة

يعد الكادميوم من المعادن الثقيلة التي له تأثير سمي على حياة الإنسان و الحيوان و الصحة العامة . و بسبب زيادة خطر التلوث البيئي الحاصل بهذا العنصر نتيجة للتطور الصناعي الحاصل لذلك اجريت هذه الدراسه لمعرفة تأثيرات السمية للكادميوم للطور الحاد في الأغنام و استخدام مادة الالفا ليبوك اسد كماده علاجيه مضادة للاكسده. . اجريت هذه الدراسة على 15 حمل تراوحت أعمارها من 5-7 أشهر قسمت الى ثلاث مجاميع كل مجموعه 5 حملان : (I) عوملت بالكادميوم كلوريد 3 ملغم/كغم تحت الجلد جرعه واحده. (II) عوملت بالكادميوم كلوريد 3 ملغم/كغم تحت الجلد جرعه واحده و نفس الوقت جرعت حامض الفا لايبوك 50ملغم/كغم عضلي على جرعتين صباحا و مساء (III) جعلت كمجموعة سيطرة و أعطيت المحلول الملحي المتعادل.

أظهرت نتائج التحليل الإحصائي في ألمجموعه الأولى وجود انخفاض معنوي في نسبة خضاب الدم، و معدل تركيز خضاب الدم و الصفيحات الدمويه و كذلك وجود زيادة معنوية في حجم كريات الدم المرصوصة مع عدم وجود فرق معنوي في معدل كريات الدم الحمر و كان نوع فقر الدم ذي كريات سوية الحجم قلية الصباغ. بينما لم تسجل أي فروق معنوية في ألمجموعه الثانية عند المقارنة مع مجموعة السيطرة. كما أظهرت نتائج الدراسة و جود زيادة معنوية ($P<0.05$) في عدد كريات الدم البيضاء نتيجة لزيادة الخلايا ألعدله و وجود انخفاض معنوي في نسبة الخلايا اللمفيه في ألمجموعه الأولى مع عدم وجود أي فروق معنوية في ألمجموعه الثانية عند المقارنة مع مجموعة السيطرة.

كما أظهرت نتائج الفحص النسجي المرضي للحيوانات في المجموعة الاولى وجود ارتشاح في الخلايا الالتهابية نزف في الأعضاء الداخلية المختلفة واحتقان شديد في ألاوعيه الدموية وتنخر وتفجي واسع في مختلف الأعضاء بينما كانت الآفات اقل حده في المجموعة الثانية.

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