EFFECT OF ZINC ELEMENT AND DEXAMETHASONE ON SOME HEMATOLOGICAL BIOCHEMICAL TESTS IN RABBITS MALE

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ABSTRACT

This study was conducted to investigate the effect of zinc in dose 15mg/kg.bw daily taken by the mouth and dexamethasone 4mgIkg.Bw by injection for 30days on some hematological biochemical tests and some histological changes of liver spleen in male rabbits. Thirty rabbits were used that divided into 3 randomized groups (each group contain 10 male rabbits). Control group was taken normal food and water, Zinc group that gave zinc at dose of 15mg/kg.BW/daily/oral on 1, 2, 3, 4 weeks. Dexamethasone with zinc group : Employ dexamethasone 4mg/Kg.Bw . I.M dialy for 1 and 2 weeks for experiment and at 3, 4th weeks they gave zn 15mg/lKg.Bw day/orally. Blood samples were taken from the heart directly in 2 and 4weeks to examine packed cell volume (pcv), white blood cells (WBCs), Red blood cells (RBCs) with differential Leuckcyte count.separation blood collection to plasma and examine glucose mg/dl , cholesterol mg/dl. In histological tests, rabbits were killed and separate their organs tissue from the body to examine liver and spleen. The results revealed a decrease in level of RBCs, pcv after treatment with zinc 15, mg/Kg.Bw orally (zinc group) and increase in WBCs with differential leuckocyte count specially neutrophil cell, while biochemical tests show increase in glucose and cholesterol levels after treatment with dexamethasone 4mglkgBw. I/M seen increase in counts of RBCs , PCV, WBCs and differential lenkocyte count and decrease in glucose with cholesterol parameters, histological changes show change in liver after treatment by dexamethasone 4mglKg.Bw ,spleen tissue seen necrosis and pigmentation with hemorrhage after take dexamethasone 4mglkg in (dexamethasone + zinc group). Results also showed that zinc enhanced the immune system in at normal dose for limited time because of its effect on other mineral such as copper and causes anemia , while the dexamethasone is a drug used for antianflammatory but for a short time.

Keywords:Zinc, Dexamethasone, packed cell volume, Red blood cells, white blood cells.

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تاثير عنصر الزنك والديكساميثازون في بعض الفحوصات الدمية والكيموحيوية في ذكورالارانب

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مدرس

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المستخلص

نفذت هذه الدراسة للتحري عن تاثيراعطاء عنصرالزنك بجرعة 15ملغم/كغم يوميا عن طريق الفم وحقن دواءالديكساميثازون 4ملغم/كغم وزن الجسم بالعضلة لمدة30يوم على بعض الفحوصات الدمية والكيموحيوية وبعض التغيرات النسيجية لانسجة الكبدوالطحال في ذكور الارانب .استخدم 30ارنب قسموالى 3مجاميع وبصورة متساوية (10ارانب لكل مجموعة) مجموعة السيطرة واعطيت الماء والغذاء فقط ،مجموعة عنصر الزنك واعطيت عنصر الزنك بجرعة 15ملغم /كغم وزن الجسم عند الاسابيع 1و2و3و 4يوميابا تجريع فموي ومجموعة الديكساميثازون وعنصر الزنك عوملت هذه المجموعة بالديكساميثازون 4ملغم/كغم وزن الجسم حقنا بالعضلة وعندالاسبوعين 3و4و عوملت بالزنك 15ملغم /كغم وزن الجسم فمويا، تم اخذ عينات الدم من القلب مباشرةبالاسبوع ال2و4 من التجربة لاجراء فحوصات قياس نسبة حجم خلايا الدم المرصوص وعددكريات الدم الحمراء والبيض والعد التفريقي لخلايا الدم البيض وتم فصل بلازما الدم لااجراء فحوصات الكلوكوز والكولسترول اماعن الفحوصات النسيجية تم قتل الارانب وفصلت الاعضاء لاجراء فحوصات على انسجة الكبدوالطحال.لقدا,اوضحت النتائج حصول قلة باعداد كريات الدم الحمر وحجم خلايا الدم المرصوصة بعد المعاملة بعنصر الزنك بجرعة15ملغم/كغم وزن الجسم مع زيادة كريات الدم البيض والعدالتفريقي للبيض وخصوصا الخلايا العدلة مع زيادة في مستويات الكلوكوز والكولسترول و اظهرت زيادة باعدادكريات الدم الحمروالبيض وحجم خلاياالدم المرصوصة والعدالتفريقي للبيض مع قلة تركيز الكلوكوز والكولسترول بعدالمعاملة ب4ملغم/كغم وزن الجسم بالديكساميثازون كماظهرالفحص النسيجي تغيرات لنسيج الكبدالمعامل بالديكساميثازون 4ملغم /كغم مع حصول تنخر ونزف بنسيج الطحال عندالمجموعة المعاملة بالديكساميثازون والزنك. وقد تبين من هذه الدراسة ان الزنك هو عنصر جيد للجهاز المناعي ضمن الجرعة المقررة له ولفترة محددة لتاثيره على عنصر النحاس وحدوث حالة فقرالدم وان الديكساميثازون يستخدم كمضاد للالتهاب ولفترة قصيرة.

الكلمات المفتاحية: الزنك ، ديكساميثازون ، خلايا الدم المرصوصة، خلايا الدم الحمر، خلايا الدم البيض.

INTRODUCTION

Zinc is a trace mineral essential to all forms of life because of its fundamental role gene expression cellwith symbol [Zn] and atomic number 30(20), and chemically similar to magnesium , common oxidation state +2 ,the largest mineable on amounts are found in Australia , Asia ,United state (7) which is analloy of copper and zinc has been used in science at least 10^{th} century Bc in Judea & by 7^{th} century inancient Greece (16).Zn is an essential mineral perceived by the public today is being of exceptional biological and public health importance especially regarding prenatal and postnatal development (19).This mineral found in plants varies based on levels of the element in soil . when there is adequate in the soil, the food plants contains the most zinc are wheat (germ & brain) and Various seeds (sesame , poopy , alfalfa , celery, mustard)(14). Its also found in beans , nuts , almonds , whole granis pumpkin seeds , sunflower seeds and black currant (41) Zn content of selection food per common measure.In human ,Zn plays ubiquitous biological roles and interactswith a wide range of 2 igands (19) with role in metabolism of RNA,DNA asingale transduction and gene expression (7) the function of zinc on the body,the body contains 1.5-2.5gm of zn ,its acomponents of every living cell and best known for its participation in enzyme structure and function .zn excellent for cell replication ,fertility and reproduction hormones activity, sexual maturation, night vision, immune function, cell growth, gene expressions,protein metabolism,hemoglobin activity.(23)The low amounts of zinc in the body causes children and women to developmental health problem recent studies show the low zinc deficiency causes attention deficient hyperactivity disorder ADHD in children and depression in women(10)while the increasing zinc level with pharmacologic treatment according to studies will help to fix this problem(43 Dexamethasone **:** is atype of steroid medication (21). It is used in treatment of rheumatic problems , anumber of skin diseases , sever allergies , asthma , chronic obstructive lung disease , croup , brain swelling and along with antibiotics in tuberculosis (21). It may be taken by mouth , as an injection into amuscle , or intravenously (21). The effects of dexamethasone are frequently within a day and last for about 3 days (21). Dexame. Was first made in 1957(30).Its also used to treat inflammatory , autoimmune conditions such as rheumatoid arthritis and bronchospasm (39), diapathic thrombocytopenic purpora (decrease of platelet due to an immune problem , a respond to 40mg dialy for5 days (29).It is also given in small amount before or after some forms of dental surgery (33).Dexam . useful to counteract allergic anaphylactic shock if given in high doses and also presnt in certain eye drops particulary after eye surgery and nasal spry , In cardiac pacing used intravenously screw leads to minimize the inflammatory response of the myocardium and is often administrated before antibiotic in cases of bacterial meningitis (42).Dexa. Also used in cancer patient undergo chemotherapy to counteract certain side effects of antitumor treatment (22).Bortezomib plus dexamethasone as induction treatment prior to autologous steam cells transplantation in patient with newly diagnosed multiple myeloma (22).It maybe given to woman at risk of delivering prematurely to promote maturation of fetus Lungs (6), It is used to treatment of high altitude cerebral edema(HACE) as well as high altitude pulmonary edema (HAPE) (9) .In veterinary use combined with marbofloxacin and clotrimazole and is available under the name Aurison and treat difficult ear infection espically in dogs combined with trichlormethiazide to treat horses with swelling for distal limbs and general bruising (13).

MATERIAL AND METHODS:

In this study we stable 30 male rabbits in Animal houses stabling under normal condition air and heat .which divided into 3 groups:Control group : no treatment (which given food and water), Zinc group : which was given zinc 15 mg/kg.Bw(46) in 1,2,3,4 weeks orally.,Dexamethasone and zinc group : in this group treat with dexamethasone 4 mglkg.Bw(45) I.M in 1,2 week and in 3,4 week was given Zn15mg/kg.Bw orally .

Parameters :

Prepare EDTA tube for collect blood from heart directly to examine :

1-RBCs count : Calculate the RBCs count by equivilant :

$$\text{RBCs} = \frac{no.\ of\ RBCs}{80} \times 400 \times 200 \times 10\ cell/mm^3 \text{(4).}$$

2-WBCs count : calculate total WBCs count by equivilant :

$$\text{WBCs} = \frac{no.\ of\ WBCs}{4} \times 20 \times 10 \times cell/mm3^3 \text{(27).}$$

3- packed cell volume (PCV)%:Measurment the percentage of cells under the methods of reference(28).

4-Differential Leukocyte count%: Blood smear stained with Leishman's and examine the percentage of WBCs cells under 100 x. (8) After centrifigate the blood (that's collect from the heart directly), Take the plasma of blood to examine .

5-Glucose mg/dl : which was examine by kit that product from spain Linear company.

6-Cholesterol mg/dl : which was examine used kit by product from spain Linear company.

STATISTICAL ANALYSIS:

Statistical analysis was performed using SAS (Statistical analysis system – version 9.1) . Data ware subjected to analysis using Tow-way analysis of Varian (ANOVA) . Least significant difference (LSD) post hoc test was used (multiple comparison)to asses significant differences among means . $p < 0.05$ was considered statistically significant (34).

HISTOLOGICAL TISSUE

After killed the experimental animals, collect organ tissues from all animals body and fixitative in formalindehyde 10% with maintained in an artificial environment, underlying mechanistic chemistry of staining with eosin and hematoxylin stain commonly used light microscopical to examine histologylogy of tissues. Organ tissues that take from animals liver,adrenal gland ,spleen

RESULTS AND DISCUSSION

Table 1. The effect of zinc 15mg/kgand dexamethasone 4mg/kg on RBCs cell/mm^3

Groups	Week 2	Week 4
C	A7.78±4.89ab	A 7.78±8.60a
Z	A 7.44±4.00c	B 7.00±6.32b
D+z	A 8.54 ±5.09a	B 7.92±4.89a

*means with different small letters in the same column differ significantly (p< 0.05)

*means with different capital letters in the same raw differ significantly (p<0.05)

C=CONTROL Z=Zinc D+Z= dexamethasone +zinc.

Table 2. The effect of zinc15mg/kgand dexamethasone 4mg/kg on PCV%

Groups	Week 2	Week 4
C	A44.40±0.50a	A44.20±0.48a
Z	A43.80±0.86a	B37.40±0.50b
D+z	B 41.60±0.50b	A45.60±0.81b

*means with different small letters in the same column differ significantly (p< 0.05).

*means with different capital letters in the same raw differ significantly (p< 0.05). C=CONTROL Z=Zinc D+Z= dexamethasone +zinc

Table 3. The effect of zinc15mg/kg and dexamethasone 4mg/ kg on WBCs /mm^3

Groups	Week 2	Week 4
C	A77.40±4.00a	A77.60±2.44c
Z	B77.60±5.09a	A 82.80±8.00
D+z	B 88.80±3.74a	A 93.00±3.66b

*means with different small letters in the same column differ significantly (p< 0.05).

*means with different capital letters in the same raw differ significantly (p< 0.05). C=CONTROL Z=Zinc D+Z= dexamethasone +zinc-

Table 4. The effect of zinc 15mg/kg and dexamethasone 4mg/kg on differential leukocyte count%

Neutrophil %

Groups	Week 2	Week 4
C	A 80.40±0.24a	A 79.40±0.40b
Z	B 78.60±0.50b	A 85.00±0.94a
D+Z	B 87.40±0.87a	A 98.40±0.50a

Monocyte%

Groups	Week 2	Week 4
C	A 11.60±0.40a	A 10.80±0.48a
Z	A 10.40±0.50a	A 10.80±0.48a
D+Z	AB11.20±0.80a	AB10.80±0.48a

Lymphocyte %

Groups	Week 2	Week 4
C	A 70.60±0.40a	A 7.40±0.24a
Z	A 72.40±0.50a	A 71.60±0.50a
D+Z	A 71.60±0.67a	A 71.00±0.31a

Eosinophil %

Groups	Week 2	Week 4
C	A 1.00±0.00b	A 1.00±0.00b
Z	A 0.60±0.24b	A 1.00±0.00b
D+Z	A 1.60±0.24b	A 1.80±0.20a

* Means with capital letters in same raw differ significantly (p<0.05)

*means with small letters in same column differs significants (p<0.05).

C=CONTROL Z=Zinc D+Z= dexamethasone +zinc.

Table 5.The effect of zinc15mg/kg and dexamethasone 4mg/kg on concentration of glucose (mg/dl).

Groups	Week 2	week 4
C	A96.00±0.54b	A94.80±0.20a
z	A92.40±0.81a	C75.00±0.83b
D+z	D97.60±0.50b	D76.40±0.67b

*means with small letters in same column differ significant(p<0.05).

*means with different capital letters in the same raw differ significantly (p< 0.05).

C=CONTROL , Z=Zinc,D+Z= dexamethasone +zinc.

Table 6. The effect zinc 15mg/kg and dexamethasone 4mg/kg on cholesterol (mg/dl).

Groups	Week 2	week 4
C	A101.40±0.40a	A101.80±0.48b
Z	A99.60±0.97b	D77.20±0.48c
D+z	C109.60±0.87a	D101.80±2.15a

*means with different small letters in the same column differ significantly (p< 0.05).

*means with different capital letters in the same raw differ significantly (p<0.05)

C=CONTROL, Z=Zinc,D+Z= dexamethasone +zinc

DISCUSSION

RBCs cell/mm^3:- The results in table(1)showed asignificant (p<0.05) decrease of number RBCs in z group at 2-4weeks (7.44+_4.00) (7.00+_6.32)compared with control group(7.78+_4.89)(7.78+_8.60) because the use zinc mineral in dose 15 mg/kg for long time that disturb of essential elements example copper in the blood and cause copper utilization and anemia (decrease RBCs) (2)or that's effect on kidney and inhibit erythropoitien hormone (regulate erythropoiesis). In group (D+Z) the dexamethasone 4 mg/kg.Bw cause significant increase in the count of RBCs from 2 weeks (8.54+_-5.09) (p<0.05)compared with control and zinc group because corticosteriodes increase hemoglobin and red blood cells content of blood possible by retarding erythrophagocytosis also that's simulating bone marrow to produce red blood cells (3) . a reduction in RBCs numbershow in 4 week after treats by zinc 15 mg/kg.Bw from (7.92+_4.89)and a significant alteration(p<0.05)compared with control group this decreasment propablty occure by the use of zinc mineral which cause effect on bone marrow and inhibit production of blood cells or effect on another mineral and causes copper defeciency (24) .

Packed cell volume % : Table (2) revelaed that the level of pcv was stable across the weeks of control group and ranged from(44.20 +_ 45. 00) with no significant changes (p>0.05), while group Z showed a significant decreasing in the pcv level along with advanced age . the level of pcv in group (D+Z) showed asignificant (p<0.05) increase in the second week as compared with fourth week then the level decreased significantly (p<0.05) with advanced age because the packed cell volume affected by Red blood cells count so if the RBCs is decrease the pcv is low and the use of zinc in doses 15 mg/kg that effects on the production of Red blood cells with effect on other minerals such as copper cause cooper deficiency (24) while in (D+Z) the dexamethasone showed increase in level of pcv at 2 week with asignificant differences (p<0.05) (41.60+_0.05) because the effect of corticosteroids on the kidney, erythropoitien thats increase in production of erythropoiesis and increase Red blood cells produce with increase of hemoglobin and RBCs content(3) compared with a decrease in average of pcv at4 week after treatment by zinc 15mg/kgB.w and record a significant differences (p<0.05) because zinc cause decrease in production of RBCs (35) .

White blood cells (cell/mm^3) : Data in table (3) appear that WBCs increase significantly (p<0.05) in group Z from second week to fourth week (77.60-5.0)(82+_8.00) compared with control group the increase in WBCs may be due to that zinc mineral is necessary for increase cellular activity with WBCs function and it acts a catalyst in the immune system's killer response to foreign bodies (40).While in (D+Z) group show a significant correlation(p<0.05) (88.80+_3.74)compared with zinc and control groups, because that's treat with dexamethasone its known increase WBCs upon predominantly neutrophils polymorph nuclear leukocytes (PMN) the biological effect that contribute to the increase wbcs, in PMNS is the circulation are acuminator of the demargination of leukocyte from endovascular living about 61% of increase (5).concerning in week4 seen

asignificant differences. ($p<0.05$) (93.00-8.30)and increase in numbers of WBCs after give zinc 15mg/kg because the zinc stimulation bone marrow to induce blood cells into circulation .

Differential Leukocyte count % : Table (4) show asignificant alteration ($p<0.05$) in column of neutrophils cell that treated by zinc 15mg/kg/bwfrom week2(78.60+_0.50)) to week4 (85+_0.94)compared withcontrol may be due to the effect of zn on immune system, with increase cellular activity and release WBCs from bone marrow (44))while group(D+Z) dexamethasone 4mg/kg.Bw +zinc15mg/kg/bw show increase in neutrophile, monocyte cellsin week2 dexamethasone as asignificant variance ($p<0.05$) (87.40+_87) (98.40+_0.50) (11.20+_0.080) (10.80+_0.48)compared with another groups, may be due to the greatest effect of cortisone on demargination of neutrophile cells from endo vascular lining ,where the cell attached to the endothelial lining of blood vessels become deattached and are then free in circulation (31). Ahigh value in week4(D+Z)due to a zinc is known to be essential mineral for immune system especially on immune cells(31).

Glucose (mg/dl):Table (5) appear asignificant ($p<0.05$)reduction in value from second week to fourth week (92.40+_0.81)(75.00+_0.83)in z group because its improves insulin ability to bind to receptors on cell membraneand transport glucose into the cells to be used as energy and may be Zn required in pancreatic beta cells to process of insulin biosynthesis and maturation of insulin secretory granules (12) , ahigh concentration value appear asignificant changes ($p<0.05$) in group (D+Z) specially inweek2 thats treat with Dexamethasone (97.60+_0.50)compared with another 2 groups may be return corticosteroids that can increase blood glucose level s,by stimulate conversion of protein to carbohydrate through gluconeogenesis and promote the storage of carbohydrate as glycogen(47)the increase in urinary nitrogen after increase glucorticoids is the result of amino acid mobilization from protein and its subsequnts breakdown as asourse of carbon during gluconeogenesis ,its simulatethe process of hepatic gluconeogenesis resulting in elevated plasma glucose and promote deposition of liver glycogen(38) with reversible extrahepatic insulin resistance and increase the production (1) The average of concentration significant decrease ($p<0.05$) in week4 and after giving Zn15mg/kgto (76.40+_0.67)compared with another groups due to Zn stimulating pancrease to produce insulin with adding to the imbalances in the body (12) .

Cholesterol (mg/dl): Table (6) show adecrease in concentration of cholesterol in zinc group at week2 from (99.60+_0.97)to(77.20+_0.48) and week4 in group of dexamethasone +zinc(101.80+_2.33) compared with control group this may be due to Zn is chemical elements antioxidant effect of zn and protect cells in the body from the damage and causes a decrease in the level of cholesterol and lipids (25),its may be capable of reducing cellular injury that might have a component of site specific oxidative damage (48)another reason Zn is a cofactor for antioxidant enzyme such as superoxide dismutase enzyme(SOD)that's catalyze the breakdown of superoxide ion into oxgen and hydrogen peroxide (36) concerning with group (D+Z)that cause increase levels of cholesterol in weeek2 ($p<0.05$) (109.60+_0.87)compared with Zn group because corticosteroids established effect on lipid metabolism impairs cholesterol egress from lipoprotein depoted by reduction of early inflow of mononuclear cells , partial inhibition of cholesterol ester hydrolysis and C enhancement of cholesterol esterification (32) and cortisol induced rise in CAMP.

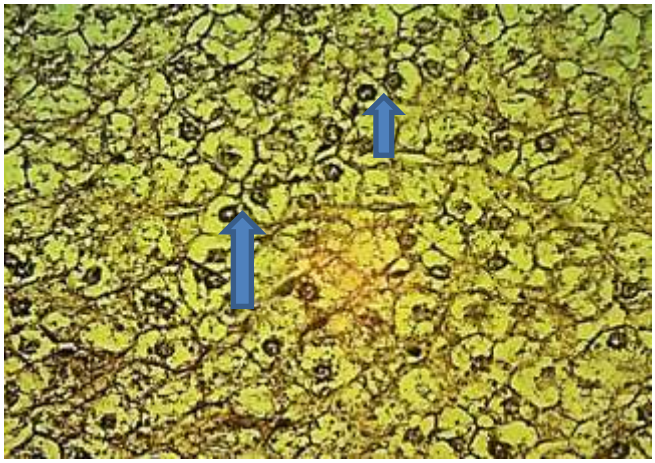


Figure 1. Histological section in liver (control group)show normal cells( )40X

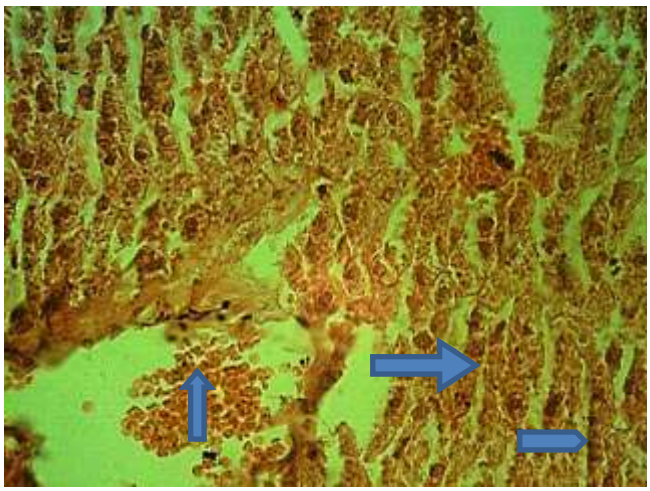
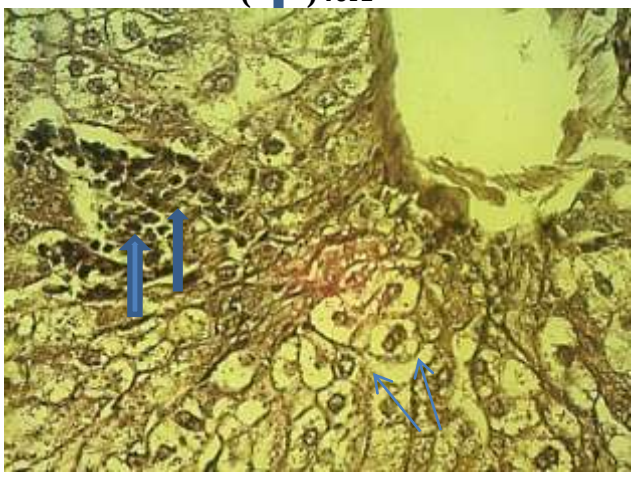
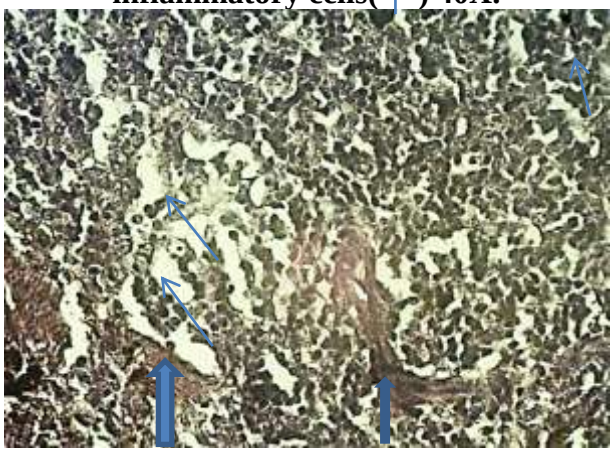


Figure 2. Histological section in liver (dexamethasone group).show coffer cells dilated and sinusite dilation with hepatocyte enlargement like cord (⟶)and increase of inflammatory cells with hemorrhage (↑)40X



`Figure3.Histological section in liver (zinc group)seen normal hepatocyte()with little inflammatory cells(↑) 40X.



Figure(4)Histological section in spleen (dexamethasone group)showed necrosis(↖), pigmentation with hemorrhage (↑)40X

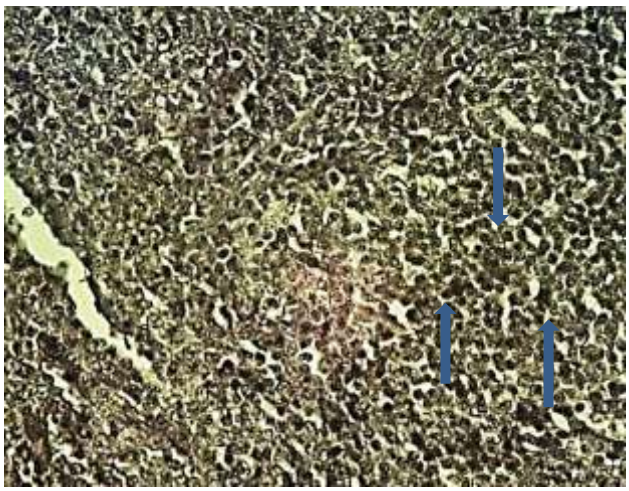


Figure 5. Histological section in spleen (controlgroup)show normal spleen tissue (↑) 40X.

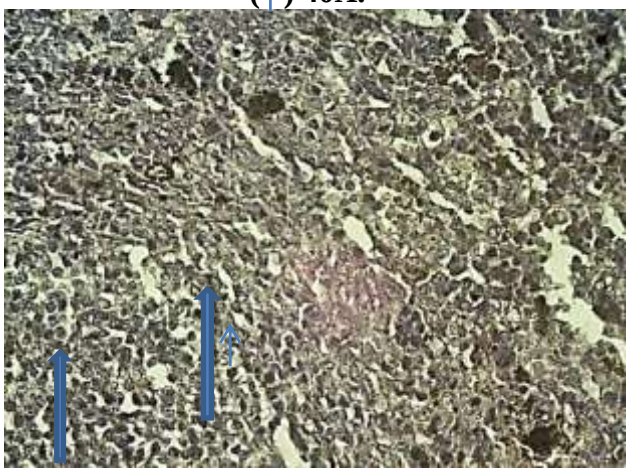


Figure 6. Histological section of spleen(zinc group)show normal cell (↑)40X.

Result of histology: In figure (1) show the section of liver staining with eosin and hematoxylin stain under its appear normal hepatocyte compared with figure (2) treatment with Dexamethosone , the coffer cell dilated and dilated of sinuosite and hepatocyte engargement like cords with increase in inflammatory cells and show hemorrhage because the dexa . stimulates gluconeogenesis in liver , using amino acid , locate , glycerol and propionate , its also involved in glyogenolysis (break down of glycogen stored in liver and muscle cell , which is necessary as it activates glycogen phosphorylase an enzyme needed to complete the whole process. Also cortisol partially shuts down the immune system when levels . are high or its makes the body more sensitive to effects of epinephrine and norepinephrine causing vasoconstriction and reduced blood flow in many psrts of the body . (15). While the figure (3) zinc group seen normal hepatocyte with little

inflammatory cells because the Zn play role in immune system and increase in white blood cells in the tissues (11) . figure (4) appear the effect of dexamethasone on spleen tissue , its leads to necrosis and pigmentation with Hemorrhage distruction of spleen tissue compared with figure 5,6 (control + zinc group) shown a normal in spleen tissue . This result because the spleen plays important roles in regard to Red blood cells (26) and cortisol in low levels and high levels effect on the spleen tissue and cause spleen disruption and increase in the number of the RBCs .

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