The effect of different doses from hydrocortisone on the liver tissue in the male rat

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Abstract

The experiment was done in the Animal House of -Department of Biology-College of Education for Pure Sciences -University of Karbala from 20/12/2013- 20/2/2014 ,The study involved the use of (32) Sprague-Dawley rats which were divided into two groups, the first group included 16 rats and this last group was divided to four groups the first three groups were injected intraperitonial injection by double concentration of hydrocortisone (1.5,3, 4.5) mg / kg and volume 1 milliliter from weight of the body for 30 days and the the fourth group was injected by normal Saline 0.9% in the same volumes that used ,The second included 16 rats and this last group was divided to four groups the first three groups were injected intraperitonial injection by double concentration of hydrocortisone (1.5,3, 4.5) mg / kg and volume 1 milliliter from weight of the body for 30 days and the fourth group was injected by normal Saline 0.9% as control in the same volumes that used they were injected for (60) days.

The results of the study confirmed the existence of significant increase ($P \leq 0.01$, $P \leq 0.05$) in the liver enzyme when injected intraperitonial injection by double concentration of hydrocortisone (1.5,3, 4.5) mg / kg and volume 1 milliliter from weight of the body ALT for 30, 60 day was showed (50.6± 5.92, 58.25 ± 10.24) U/L, (41.50 ± 2.8, 49.25± 1.89, 51 ± 2.44) U/L Respectively When we compared with the control group (32.5± 8.58, 34.25 ± 4.5) U/L Respectively the existence of significant increase ($P \leq 0.01$, $P \leq 0.05$) in the liver enzyme AST for 30, 60 day (121 ± 45.5, 150.5± 21.1),(94.7± 18.5, 136.5± 12.4, 200.7 ± 44.9) U/L Respectively when we compared with the control group (93.5 ± 11.38, 85.5± 7.5) U/L Respectively the cross-section of liver tissue notice Infiltration of inflammatory cells Figure: (4),(5), Necrosis of hepatocytes Figures: (3),(4), (5), (6),(7) , Congestion of blood Figures: (4),(5),(7) and appearance of fatty vesicles inside cytoplasm Figure: (4),(7) . When we observe the sections of liver tissue of rats during the two periods (30, 60) day .

تأثير جرع مختلفة من الهايدروكورتزون على نسيج الكبد في ذكور الجرذ

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الكلمات المفتاحية : الهايدروكورتزون ، نسيج الكبد ، انزيمات الكبد

الخلاصة

تضمنت الدراسة استعمال الجرذان البيضاء (ذكور) بواقع (32) جرذ من نوع Sprague-Dawley وقد قسمت الى مجموعتين المجموعة الاولى تضمنت (16) جرذ وبواقع اربع مجاميع حقنت داخل الخلب بالجرع (1.5،3،4.5) ملغم /كغم وحجم ا مليلتر من وزن الجسم واعتبرت المجموعة الرابعة كمجموعة سيطرة حقنت بالمحلول الفسيولوجي بتركيز 0.9% وبنفس الحجوم المستخدمة ولمدة (30) يوما والمجموعة الثانية تضمنت (16)جرذا وبواقع اربع مجاميع حقنت داخل الخلب بالجرع (1.5،3،4.5) ملغم /كغم وحجم ا مليلتر من وزن الجسم واعتبرت المجموعة الرابعة كمجموعة سيطرة حقنت بالمحلول الفسيولوجي بتركيز 0.9% وبنفس الحجوم المستخدمة حقنت لمدة (60) يوما .

اظهرت نتائج الدراسة وجود ارتفاع معنوي ($P<0.01, P<0.05$) في فعالية انزيمي الكبد (AST , ALT) عند حقنها بالجرع (1.5،4.5،3) ملغم /كغم وحجم ا مليلتر كانت النتائج انزيم ALT ولمدة 30 و60 يوما (50.62± 5.92 ، 58.2 ±10.2)، (41.5±2.8 ، 49.2 ± 1.8 ،51 ± 2.4) على التوالي وبالمقارنة مع مجموعة السيطرة (32.5± 8.5 ، 34.2 ± 4.5) على التوالي وكانت النتائج انزيم AST ولمدة 30 و60 يوما (121 ± 45.5 ،150.5 ± 21.1)، (94.7 ±18.5 ،136.5 ±12.4 ،200.7± 44.9) على التوالي و بالمقارنة مع مجموعة السيطرة (93.5±11.3 ، 85.5 ± 7.5) على التوالي اما المقاطع النسيجية للكبد اظهرت حدوث ارتشاح خلايا التهابية كما في الصور (4) (5) وحدوث تنخر الخلايا الكبدية كما في الصورة (3) (4) (5) (6) (7) وظهور احتقان دموي كما في الصور (4) (5) (7) وظهور حويصلات دهنية داخل الخلايا الكبدية كما في الصور (4) (7) وللفترتين 30 ، 60 يوما .

Introduction

Corticosteroids are chemical compounds of hormonal nature derived from cholesterol. Their biological power and actions depend on their chemical structure. Due to the remarkable anti-inflammatory and immunoregulatory effects of the corticosteroids, they have been employed as first step in the management of different diseases, and sometimes they are the only possible drug to use in daily medical practice. Despite their clinical efficacy, they can induce multiple severe adverse effects[(1)].

Hydrocortisone is a rapid and short-acting glucocorticoid that is used for therapy of adrenal insufficiency and in treatment of allergic and inflammatory conditions. Hydrocortisone has the same chemical structure as cortisol and thus most closely resembles the human adrenal hormone.[(2)]

Increased synthesis of hepatic enzymes due to hydrocortisone is preceded by an increase in the rate of synthesis of nuclear RNA. Growth hormone stimulates RNA synthesis in both intact and adrenalectomized rats, but induces the rapid turnover enzymes (tyrosine transaminase and tryptophan pyrrolase) only in the presence of functional adrenals. It therefore seems that glucocorticoids initiate both a generalized increase in synthesis of RNA and a selective induction of specific enzymes.[(3)]

Consuming cortisone, particularly if the cortisol taking a long term, can result in the development of fatty liver disease. The cortisol can disrupt metabolic pathways of the body and result in fat becoming deposited in the liver. Elevated cortisol levels can also lead to high levels of triglycerides in the blood.[(4)]

Some of studies was showed Varying degrees of hepatocellular degeneration and necrosis were found in animals treated with cortisone in different doses and long term. [(5)]

The study was aimed to know the effect of hydrocortisone drug on histological changes of the liver in Sprague-Dawley rats male. The study included the effectiveness of liver enzyme (aminotransferase alanine, aminotransferase aspartate) (AST, ALT) and histological changes of the liver tissue during (30) and 60 days to determine the toxic effects of the drug during these periods of time.

Materials and Methods:

The experiment design

The study involved the use of (32) albino rats which were divided into two groups, the first group included 16 rats, they were injected for 30 days and the second included 16 rats, they were injected for (60) days, the last group was divided into four groups, each group included four rats the first three groups of the last group were injected by double concentration of hydrocortisone (1.5, 3, 4.5) mg/kg [(6)] intraperitonial injection once daily. The experiment was done in the Animal House of -Department of Biology-College of Education for Pure Sciences -University of Karbala from 20/12/2013-20/2/2014, The animals were Put under control in the air-conditioned room temperature under 25ºC and the cycle light was split into 14-hour light and 10 hours of darkness in the cages and left for a week for Localization to the conditions of the experiment, The animals were weighed at the beginning of The experiment and recorded the weights of each group were then given water and feed laboratory quantities of abundant and continuous feed was obtained from local plants, Statistical Analysis was adopted the Complete Randomized design CRD, Means were compared by using L.S.D at (0.05, 0.01) Probability level. [(7)]

Determination of serum aspartate transaminase (AST), alanine transaminase (ALT):

Three milliliters of blood were pulled from the heart of animals after a 30 and 60 days of injections and placed in plastic tubes non-container on Heparin and then placed in the centrifuge for (15) minutes at (3000) RPM , then the serum was pulled and isolated in the tubes then biochemical parameters were measured for the treatment and control groups.

Solution	**Test tube**	**tube(Blank)**
Sample(serum)	0.1	------
R1 Buffer solution (AST,ALT)	0.5	0.5
Mixed well and the contents of the tubes were incubated at a temperature of 37ºC for 30 minutes.		
R2 2,4- dinitrophenyl hydrazine	0.5	0.5
Mixed well and the contents of the tubes were incubated at a temperature of (20-25)º C for 20 minutes.		
R3 Solution NaOH	0.5	0.5

Note All sizes calculated by microliters …

The effectiveness level of enzyme AST, ALT was measured in blood serum by using Kit (Randox) England . The method is based on estimating the amount of Pyruvate and Oxaloacetate that liberated by interaction with a 2, 4- dinitrophenyl hydrazine [8].

The contents of the tubes were mixed well and then the absorbance were measured at wavelength (540) nm, The results recorded as present in the instruction sheet in the kit The relationship between the absorbance and the effectiveness of the enzyme has been drawn in units of U / L this unit represents the amount of enzyme that causes the liberation one micromoles of Pyruvate within one minute in reaction conditions .normal values for AST serum up to 45-98 U/L and normal values for ALT serum up to 17-40 U/L

Preparation of Histological Section:

Histological sections were attended by [9] and included the following:

1. Fixation: After the killing of animals and extract the organs (the liver) were cut into small pieces and placed in a fixed solution (10% formalin) and then after 24 hours the solution was renewed.

2. Dehydration: The organs placed in the concentration 70% of ethanol till the next day and then the samples passed in progressive concentrations of ethanol (80, 90, 100 and 100) % for 1.5-2 hours at each concentration.

3 .Clearing: the samples were cleared by using Xylene twice one hour for each time.

4. Infiltration: The Samples placed in the paraffin wax melted at a temperature 60º C for twice one hour to first wax and then placed in the second wax till the next day.

5. Embedding :Samples embedded in special templates container on the molten paraffin wax and left to freeze to be cut.

6. Sectioning :Histologic sections sequential attended by thickness 6 micrometers using Rotary microtome and the samples were fixed on glass slides by using adhesive material (albumin's Meyer) then the slides were put at a room temperature for the next day to dry.

7 .Staining: the sections were stained by hematoxylin and eosin stain, the slides were placed in xylene twice for 30 minutes each time and then passed in concentrations regressive of ethanol (100-95 - 90-80 - 70%) for two minutes for each one of them , and the sections were placed in the dye hematoxylin for 15 minutes and then dipped once in acid alcohol and washed sections with fresh tap water for 5 minutes and put in a dye eosin for 7 minutes then passed upward concentrations of ethanol (70-80 0-9-95-100) % for two minutes per concentration then the sections placed by xylene twice for 15 minutes each time.

8. Mounting: Canada balsam was put on the histological sections and covered by cover slid and then diagnosed to observe histological changes.

Result:

Table: (1) effect of different doses of hydrocortisone on liver enzyme (AST, ALT) in rats for (30) days of treatment

Does (mg/kg)	AST Mean ± SD	ALT Mean ± SD
Control	93.5 ± 11.38	32.5± 8.58
1.5	93.5± 33.83	35.25± 10.99
3	121 ± 45.5*	50.6 ± 5.92*
4.5	150.5± 21.1**	58.25 ± 10.24**

Value are mean ± S.D.

(P ≥ 0.05) no significant difference

*(P ≤ 0.05) significant difference

** (P ≤ 0.01) highly significant difference

Table :(2) effect of different doses of hydrocortisone on liver enzyme (AST, ALT) in rats for (60) days of treatment

Does (mg/kg)	AST Mean ± SD	ALT Mean ± SD
Control	85.5± 7.5	34.25 ± 4.5
1.5	94.7± 18.5*	41.50 ± 2.8*
3	136.5 ± 12.4**	49.25 ± 1.89**
4.5	200.7 ± 44.9**	51 ± 2.44**

Value are mean ± S.D.

*(P ≤ 0.05) significant difference

** (P ≤ 0.01) highly significant difference

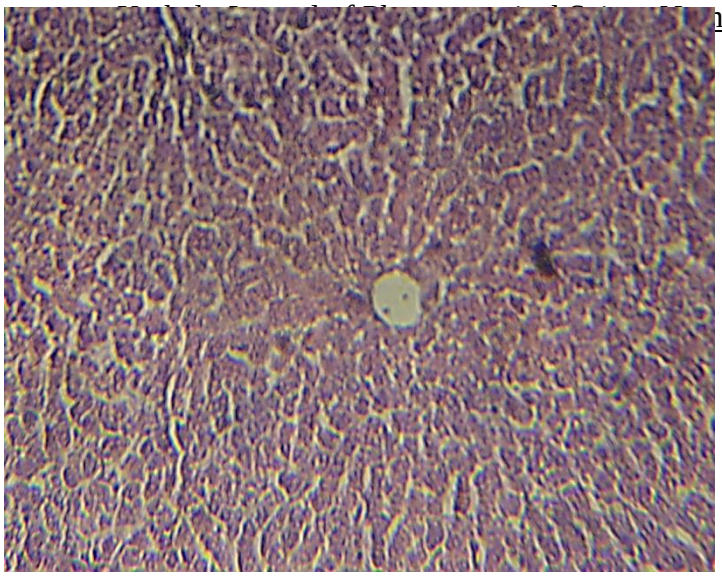

Figure: (1) cross-section in the liver tissue of the control group notice normal of hepatocytes and there is no Infiltration of inflammatory cells and Congestion of blood (400X H&E)

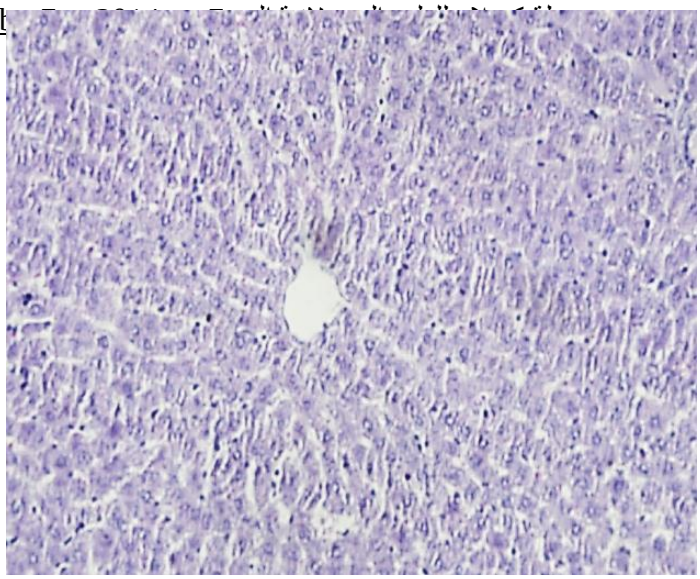

Figure: (2) the cross-section of liver tissue(dose1.5mg/kg) for 30 days notice no changes in the liver tissue (200X H&E)

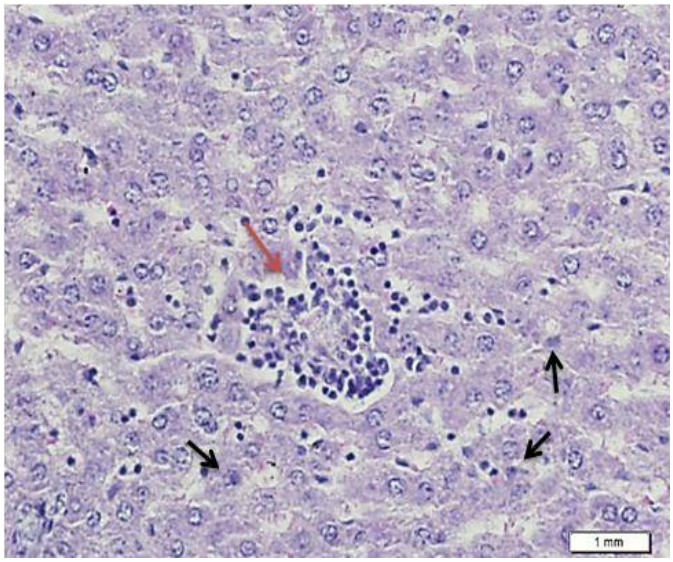

Figure: (3) the cross-section of liver tissue(dose3 mg/kg) for 30 days notice Infiltration of inflammatory cells ↘ and necrosis of hepatocytes ↘ (400X H&E)

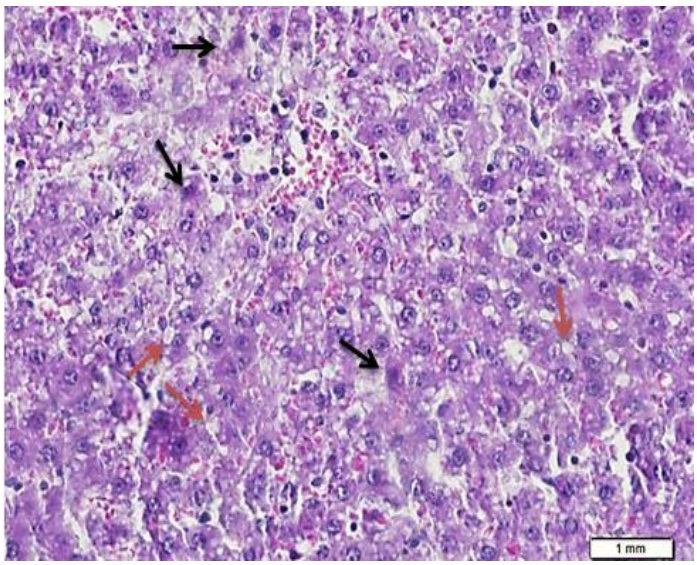

Figure: (4) the cross-section of liver tissue(dose4.5 mg/kg) for 30 days notice Necrosis of hepatocytes ↘ and the appearance of small vesicles of fatty inside cytoplasm ↘ (400X H&E)

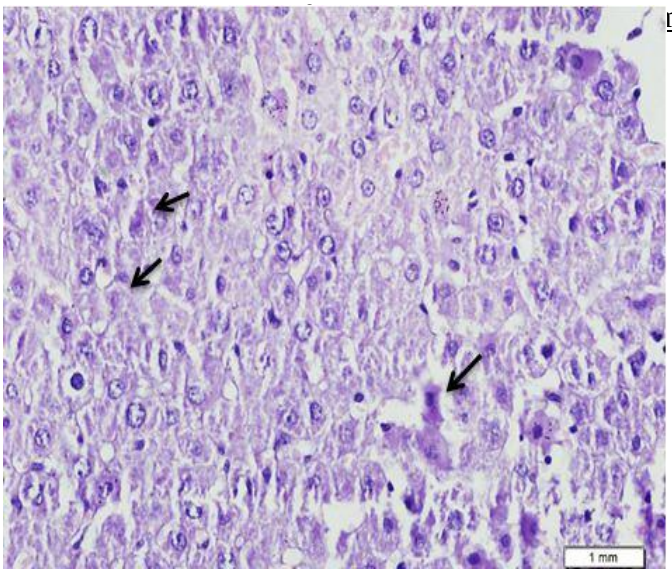

Figure: (5) cross-section in the liver tissue dose (1.5mg/kg) for 60 days, notice necrosis of hepatocytes (200X H&E)

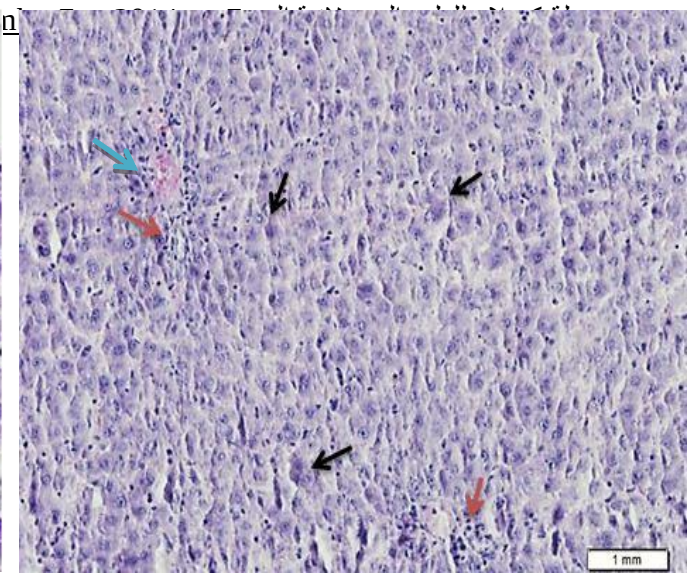

Figure: (6) cross-section in the liver tissue dose (3mg/kg) for 60 days, notice necrosis of hepatocytes Infiltration of inflammatory cells and Congestion of blood (H&E100x)

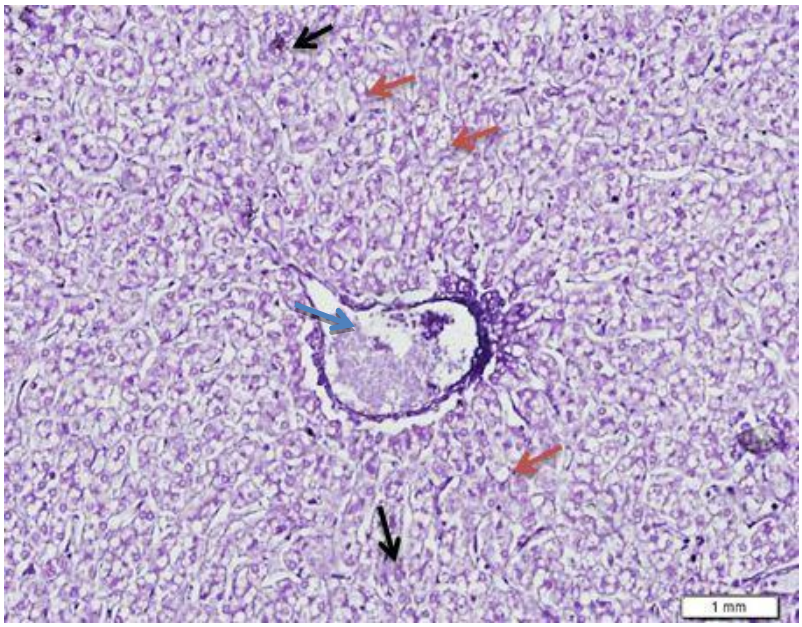

Figure: (7) cross-section in the liver tissue dose (4.5 mg/kg) for 60 days, notice necrosis of hepatocytes and the appearance of fatty vesicles inside cytoplasm Congestion of blood (200X H&E)

In the above tables (1), (2) note an increase in liver enzymes ALT, AST significantly increased $p<0.05$, $p<0.01$ by treatment with double dose in the groups when compared with the control group. There are no significant different $P<0.05$ in the (AST, ALT) in the first dose (1.5mg/kg) for period (30) day. While there are highest significant differences $P < 0.01$ for the second and third dose (3, 4.5) mg/kg of (AST, ALT) in the period (60) day and there are significant increase $P<0.05$ in the liver enzyme (AST, ALT) for the dose (3mg/kg) in the period (30) day and there are a highest significant increase $P<0.01$ in the liver enzyme (AST, ALT) for the dose (4.5mg/kg) in the period (30) day. There are significant increase $P<0.05$ in the liver enzyme (AST, ALT) for the dose (1.5mg/kg) in the period (60) day. the cross-section of liver tissue notice Infiltration of inflammatory cells Figures:(4),(5), Necrosis of hepatocytes Figures: (3),(4), (5), (6),(7) , Congestion of blood Figure: (4),(5),(7) . When we Observe the sections of liver tissue of rats during the two periods (30, 60) days of treatment, concluded that the increased by dose and duration of injection increased influence of the liver tissue, and this shows that the hydrocortisone has accumulative toxic effect on the liver tissue over time.

Discussion:

The present study aimed to assess the level of liver enzymes (AST, ALT) in serum of control and experimental animals injected by drug hydrocortisone In periods (30,60) day for the purpose of knowing the effect of the drug on the levels of these enzymes , which reflects the extent of the effect in the liver cells as a result of treatment by drug, Tables:(1), (2) showed an increase in liver enzymes ALT, AST significantly increased ($p<0.05$, $p<0.01$) compared with the control group the drug hydrocortisone raise the overall level of AST, ALT in blood serum , AST were exist in all cells. The enzyme ALT were exist in different concentrations and the liver contained the highest concentration from it and a small amount was exist in the heart and skeletal muscle [(9)] the percentage distribution of AST is variable from animal to another and from one tissue to another in the same animal. The AST existed in serum and all tissues this makes it contributes to the diagnosis of many diseases because the increase in serum which reflects the severity of the disease and the deficit of hepatocellular and tissue damage [(10)].ALT diagnostic is importance for many diseases, because many of the diseases of liver leads to high level of ALT[(11)] it can be explained the rise in AST, ALT through the toxic effects of the hydrocortisone, which affect significantly on the liver, in addition, the liver play the significant role in metabolizing toxic compounds that enter the body.[(3)]

Hydrocortisone affects the oxidation of phospholipids in the cell membrane causing a crash of these cells because these cells do not have the ability to resist the effectiveness of free radicals and this lead to an imbalance in the permeability of the cell membrane fatty molecules accumulate inside the cell as well as the aggregation of the small fatty vesicles inside the cytoplasm of liver cells may be due to crash of cell membranes as a result of the occurrence of oxidative stress induced by free radicals , collected fatty drops inside liver cells that resulted from the disorder and imbalance in liver function and lead to a change in the construction of lipoproteins , which in turn led to accumulate the fat within liver cells [(12)] that the appearance of lesions degeneration vesicles may be caused by a malfunction in the sodium pumps of mitochondria , resulting in a decrease in the ATP energy production necessary for the synthesis of proteins and this led to a lack of protein

necessary for the safety of cellular membranes [13] The interpretation of the increase infiltration phagocytic cells Figure: (4),(5) in the liver tissue . That the injection of the hydrocortisone led to damage and necrosis in the liver cells , and this in turn led to the attraction of the monocyte and neutrophils and to the site of injury, especially macrophages, which produces Lysozymes enzymes to remove the tissue damaged may be attracted other cells that produce (IL-1β , IL-6 , TNF-α , IL-12 , IL-18) that contribute in the tissue damage and this can be explained by increased phagocytic cells because of stimulating the local immune system to reticuloendothelium . These histological changes approved a significant increase in the level of effectiveness of the enzymes (AST, ALT), which confirms the crash occurrence in liver cells and an imbalance in the functions [14]. Necrosis cell of liver cells due to hydrocortisone is the result of the inhibition the enzymes of oxidative stress in these cells [15] [16].

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