Histopathological effects of the prednisolone and vitamin E in the different organs (liver, lung, heart and testis) of the rabbits

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التأثيرات النسيجية لكل من البردنسلون وفيتامين هاء في الأعضاء المختلفة (كبد ,رئة, قلب, والخصية) في الأرانب

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فرع الأدوية والسموم- كلية الطب- جامعة واسط

المستخلص

الهدف من الدراسة هو تحديد التأثيرات النسيجية لكل من البردنسلون وفيتامين هاء في الأعضاء من أجهزة الجسم المختلفة في الأرانب.حيث أجريت الدراسة النسيجية بعد فترة التجربة 21 يوم باستخدام التقطيع النسيجي والتصبيغ بصبغة الهيماتوكسلين-ايوسين. اذ تم تقسيم 20 أرنب ذكر ناضج إلى أربعة مجاميع:

الأولى مجموعة سيطرة وجرعت ماء مقطر 4 مل/ كغم / يوم / فمويا.المجموعة الثانية أعطيت البردنسلون 4 ملغم/كغم / يوم/ فمويا.المجموعة الثالثة أعطيت فيتامين هاء 400 وحدة دولية /كغم /يوم/ فمويا والمجموعة الرابعة أعطيت كل من البردنسلون وفيتامين هاء بنفس الجرعة أعلاه فمويا. بعد العلاج وفي اليوم الثاني والعشرون قتلت الحيوانات واخذ الكبد والقلب والرئة والخصية والتي تمثل الجهاز الهضمي والتنفسي والوعائي والتكاثري واجري التقطيع النسيجي لها.حيث أظهرت النتائج التأثير المفيد لفيتامين هاء في التخفيف من التأثيرات الجانبية والضرر الحاصل من علاج البردنسلون في الصورة النسيجية للأعضاء .

Abstract

The aim of this study was to investigate the histopathological effects of prednisolone and vitamin E on the different organs of the different system in the experimental animal.

Materials and methods: histopathological study was carried out in 21 days, using a hematoxylin- eosin method, twenty adult males rabbits were randomly divided into four groups: Grop-1: control group C. (n = 5) received Distilled water 4 ml /kg b.w./day/orally

Group-2: first treated T1. (n=5) with 4mg/kg b.w/day/orally prednisolone.

Group-3: second treated T2.(n=5) with 400 IU/kg b.w/day/orally/ vitamin E.

Group-4: third treated T3. (n=5) with prednisolone and vitamin E orally.

At day 22th, the animals were sacrificed and samples from Heart, liver, lung and testis). were obtained for Histopathological study.

Results: the results showed that vitamin E appeared to ameliorate the adverse effects of prednisolone on the histopathological picture of the different organs.

Conclusion: vitamin E is effective in reducing damage in prednisolone – Treated rabbits.

Introduction

Prednisolone is one of the glucocorticoids used as effective anti-inflammatory and immunosuppressive agents (1, 2). Various manifestations of allergic reaction caused by steroids have been reported (3).

Aghaiafari *et al*, (4) suggested that repeated doses of antenatal corticosteroids may have beneficial effects in terms of lung function but may have adverse effects on brain function and fetal growth.

Gucuyener *et al*, (5) recorded that the treatment with high dose methyl prednisolone and clarithromycin led to rapid clinical improvement of mucoplasma pneumonia. Treatment with steroids may have beneficial effects on cardiac function (6). Therapy with prednisolone may be potentially useful in sever intestinal disease (7). Glucocorticoids inhibit the reproductive functions and the anatomical sites (8).

Talu *et al*, (9) recorded that direct intratunical instillation of bupivacaine and methyl prednisolone around the testis reduces the postoperative pain, scrotal swelling and peritesticular fibrosis.

On the other hand, Alpha-Tocopherol (Vitamin E) which is lipid-soluble acts mainly within cell membranes is the first line of defense against lipid peroxidation and it is important for normal function of the immune cells (10, 11).

Although Durmus *et al*, (12) showed that in vitro studies of vitamin E has antioxidant, anti-inflammatory, anticoagulant and antifibroblastic effects and decrease collagen production. Recently, the Cambridge Heart Antioxidant Study (CHAOS) reported strong protection by high vitamin E doses against the risk of fatal and nonfatal myocardial infarction (13, 14).

While Subbaiah *et al*, **(**15) investigated the effect of vitamin E on pro/anti oxidant status in the liver, brain and heart of Newcastle disease virus infected chickens, they

demonstrated that antioxidant defense mechanism is impaired after the induction of the disease and suggest that vitamin E treatment will ameliorate the antioxidant status in the infected animals. Also Birkner *et al,* **(**16) showed a beneficial influence of methionine and vitamin E supplementation on liver statuses development.

While Stanford *et al,* (17) reported that Vitamin E in goats or feedlot cattle did not reduce the extent or severity of lung lesion.

Suchankova *et al,* (18) recorded that vitamin E with dose 400 mg/kg/day for 10 day in rats of asthma had no major effect on airway inflammation. The vital role of vitamin E in reproduction was first investigated 80 years ago, some have investigated the role of supplementary vitamin E in improving pregnancy outcomes, and some conclude that vitamin E supplementation, either alone or in combination with other supplements, can be beneficial during pregnancy (19) .

Vitamin E supplementation has become a common procedure to promote growth and health and improve the qualitative characteristics of farm animal. It has been demonstrated to be efficient strategy for improving their reproductive function (20).

Oda and EL-Maddawy,(21) reported that treatment with vitamin E and selenium combination improved the reduction in the reproductive organ weights, sperm characteristic , DLM- induced oxidative damage of testis and histopathological alterations of reproductive organs.

Materials and methods

Twenty healthy, adult males, local rabbits were used for the study, with weight range (1400 - 1500 gm). The rabbits were housed in a well- ventilated animal house and caged separately, at a tempreture of 30-35 C and exposed to 11 to 12 h. of day light. They received food and water ad libitum.

Rabbits were acclimatized for 7 days before the beginning of the study. Then randomly divided into equal four groups (each group consist of five rabbits) and handled as follows:

1- Group C: control group**:** rabbits were maintained on food and water ad libitum with oral administration of distilled water 4 ml/kg b.w./ day.

2- Group T1: first treated**:** rabbits of this group were treated with 4mg/ kg b.w./ day/ orally/ prednisolone (Micro labs limited-India- B.No.PDSY0051).
3- Group T2: second treated**:** rabbits of this group were treated with 400 IU/ kg b.w/ day/ orally / vitamin E (Strides Arcolab Limited-India-B.No.011202).
4- Group T3: third treated**:** rabbits of this group were treated with prednisolone and vitamin E orally with doses similar to above treatments.

Treatment was administered orally by gavage for 21 days. After treatment in the 22ed day, specimens from (heart, liver, lung and testis) were taken immediately after scarifying animals.

Histological section were prepared and stained in the clinical Laboratory of AL- Karama-Teaching hospital/ Kut/ Iraq.

according to Bancroft and Stevens(22), the tissue was keep in a 10 % neutral formalin's solution for fixation, after that washed under running tap water , then dehydrated through ascending grades of alcohol, cleared Xylen and embedded and blocked in paraffin . Sections 4-5 *um* thickness was taken, stained with hematoxylin - eosin and was examined under the microscope.

Results

Reported from the all histological sections of the different organs of the different system in the experimental animal, that prednisolone with the doses 4mg/kg b.w/day/orally for 21 days will cause damage and necrosis to the different tissues.

While vitamin E with the doses 400 IU/kg b.w/day/orally for 21 days doesn't cause any damage to the different organs in the male rabbits and the tissue appeared look like that of control group.

On the other hand, the vitamin E when used with prednisolone with the doses similar to above, will cause improvement in the histopathological changes and decreases the damage which may results from the side effects of the prednisolone.

1-Heart tissue

Histological sections obtained from male rabbits treated with prednisolone (T_1) showed many pathological changes included cardiac damage degeneration, necrosis and fibrosis with infiltration of neutrophil cells. While, T_3 histological section showed mild damage and inflammatory changes. On the other hand, histological sections obtained from control and T_2 demonstrated normal appearance of cardiac muscle and wall of artery without observation any damage or fibrosis. (Figs. 1,2,3,4).

2-Lung tissue

As shown in Figs (5,6,7,8). T_1 male rabbits lung demonstrated degenerated and destructed with mild exudation in the alveolar wall with increase number of neutrophils. Compared with normal observations in control and T_2 male rabbits pulmonary tissue.(T_2 group appeared look like that of control).

While histological sections obtained from T_3 showed mild pathological changes progress to improved status and showed present number of neutrophil cells.

3-Liver tissue

Liver section from T_1 male rabbit showed degeneration with mild damage and detected engorgements of hepatic central vein with observation of mild cholestasis.

While the liver from control, T_2 and T_3 groups showed normal liver tissue, proliferative hepatocytes, normal hepatic sinusoid and central vein, with reported the present a number of inflammatory cells in the liver of T_3 groups. (Figs. 9,10,11,12).

4-Testis tissue

As shown in Figs (13,14,15,16). Histological section, of testis obtained from T_1 male rabbit treated with prednisolone shows a significant reduction, degeneration and necrosis of spermatogenic cells, revealed the presence of degenerative and necrotic cells. Degenerated seminiferous tubules, spermatocytes with the absent of spermatids.

While the Histological section, of testis obtained from control, T_2, and T_3 group demonstrated normal appearance, The sections showed normal: seminiferous tubules, sertoli cells,spermatogoneum, spermatocytes and spermatids.

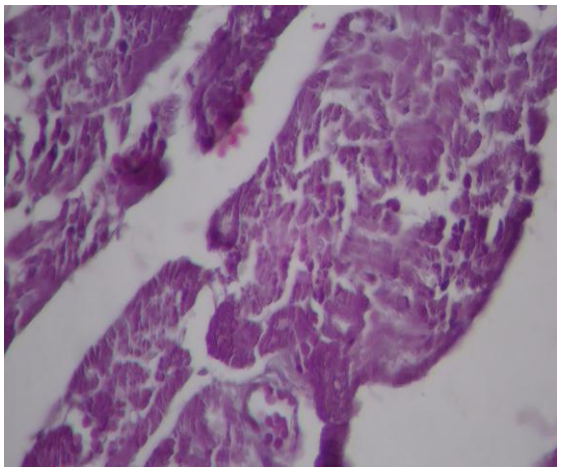

Figure (1): Section of heart from control male rabbit showed normal heart tissue (H&E,X100).

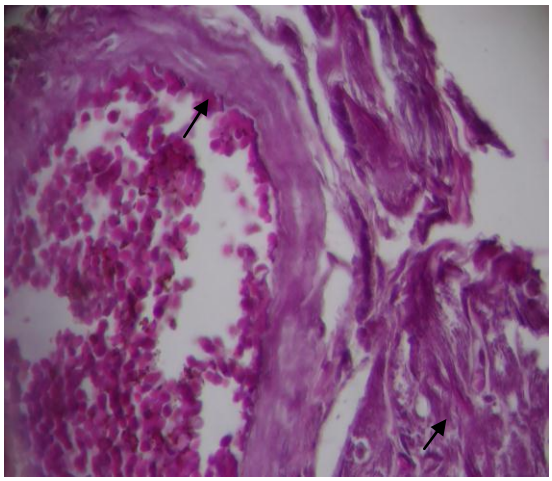

Figure (2): Section of heart from T1 male rabbit showed cardiac damage and fibrosis with infiltration of neutrophil cell (H&E,X100).

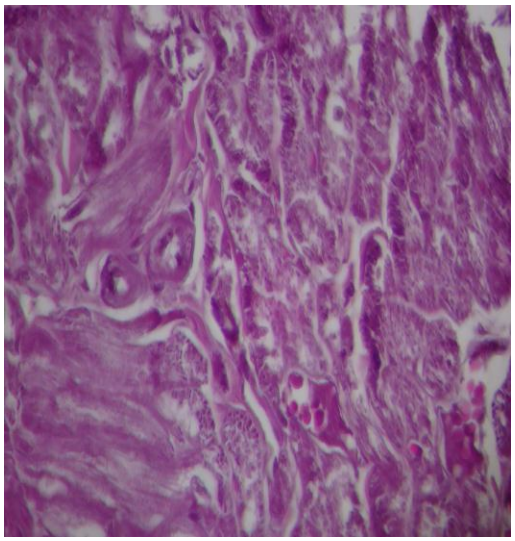

Figure (3): Section of heart from T2 male rabbit showed normal heart tissue; cardiac muscle &wall of the artery .(H&E,X100).

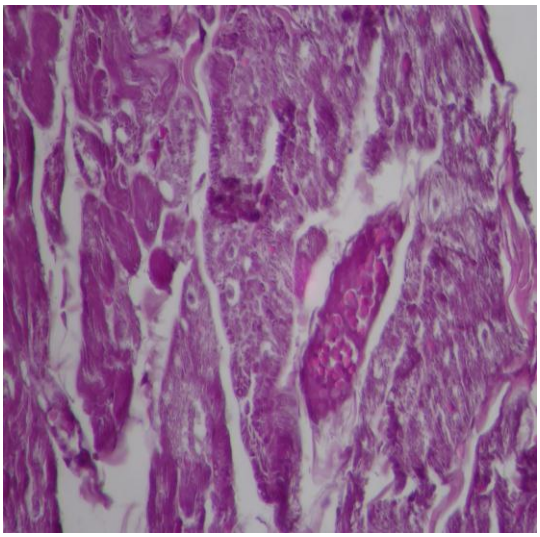

Figure (4): Section of heart from T3 male rabbit showed normal heart tissue; cardiac muscle with centrally located nuclei (H&E,X100).

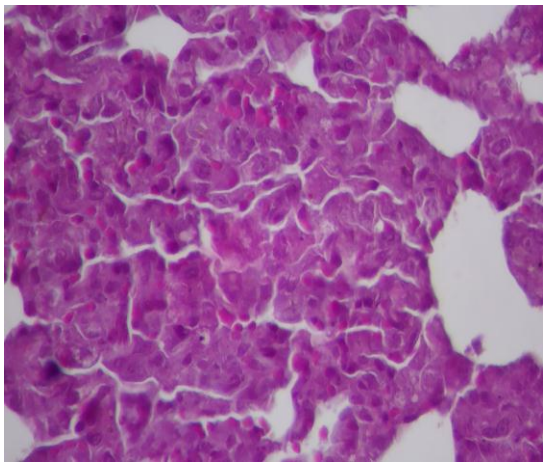

Figure (5): Section of lung from control male rabbit showed normal pulmonary tissue (H&E,X100)

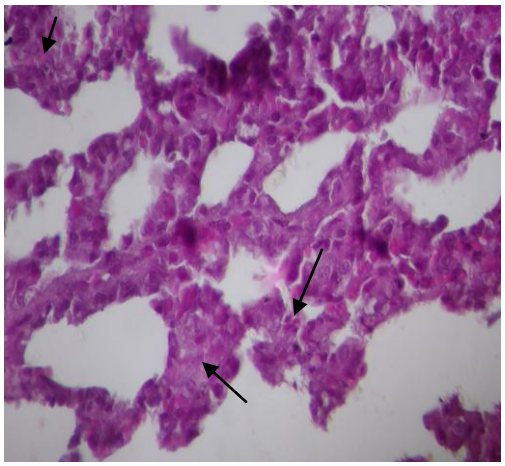

Figure (6): Section of lung from TI male rabbit showed Mild exudation (EX) in the alveolar wall, with increase number of neutrophils (H&E,X100).

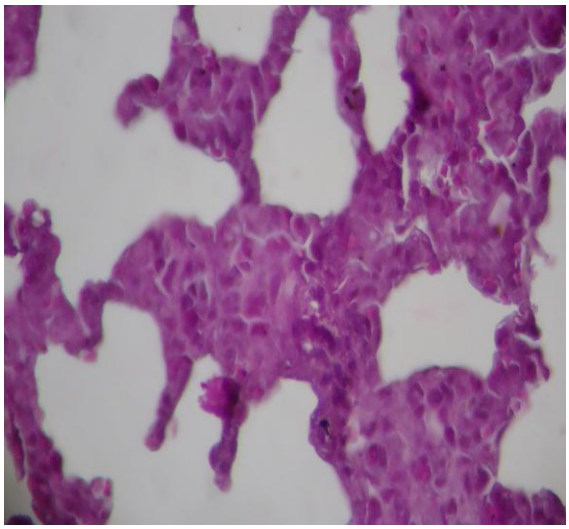

Figure (7): Section of lung from T2 male rabbit showed normal pulmonary tissue (H&E,X100).

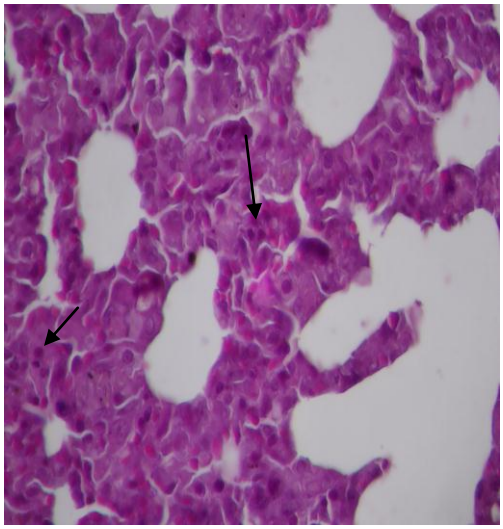

Figure (8): Section of lung from T3 male rabbit showed present number of neutrophil cell (H&E,X100).

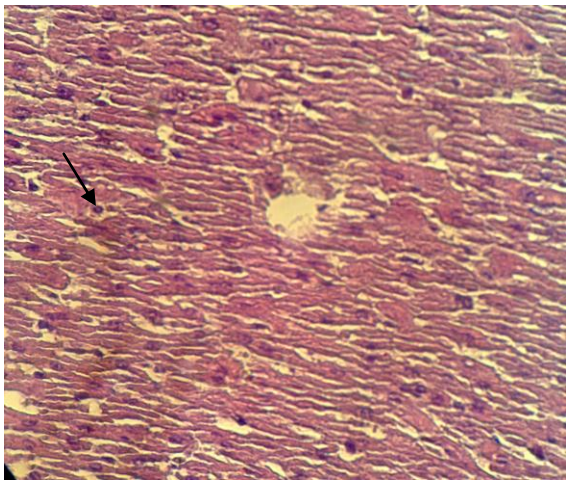

Figure (9): Section of liver from control male rabbit showed normal liver tissue, 1-hepatic sinusoid & central vein-2 (H&E,X100)

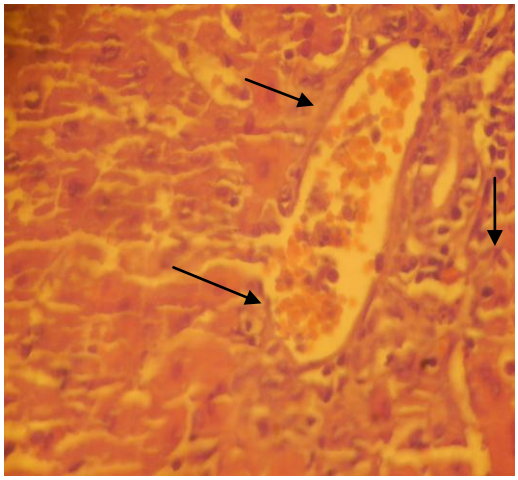

Figure (10): Section of liver from TI male rabbit showed engorgements of hepatic central vein(1) with evidence of mild cholestasis(2)&hepatic degeneration(3) (H&E,X100).

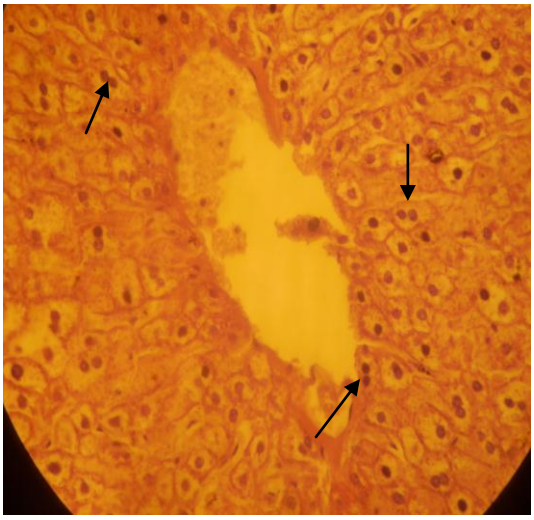

Figure (11): Section of liver from T2 male rabbit showed normal liver tissue& proliferative hepatocytes (H&E,X100).

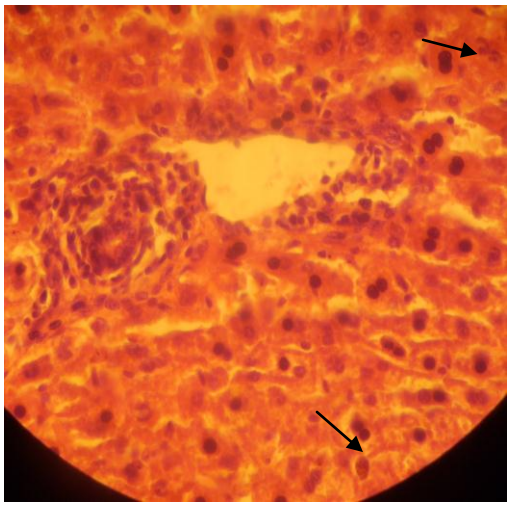

Figure (12): Section of liver from T3 male rabbit showed present number of inflammatory cells (H&E,X100).

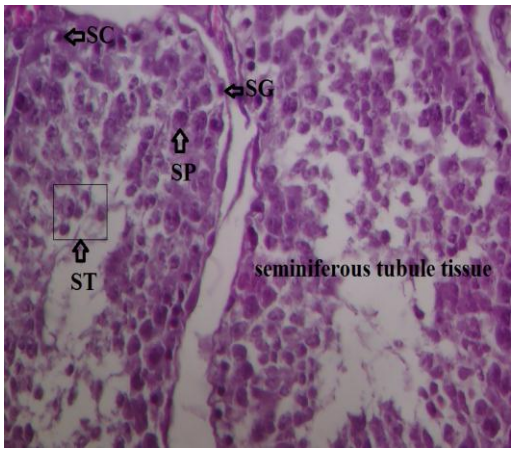

Figure (13): Section of testis from control male rabbit showed normal seminiferous tubules,: sertoli cell (SC) is present, spermatogoneum (SG), spermatocytes (ST) and spermatids (SP). (H&E,X100)

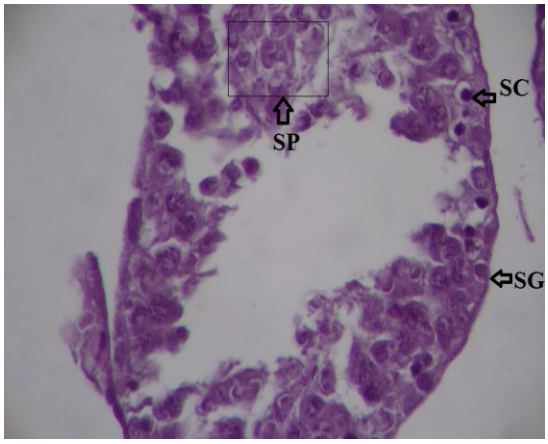

Figure (14): Section of Testis from T1 male rabbit showed degenerated seminiferous tubules, there are sertoli cells (SC), spermatogoneum (SG, spermatocytes (SP) but the spermatids are absent (H&E,X100)

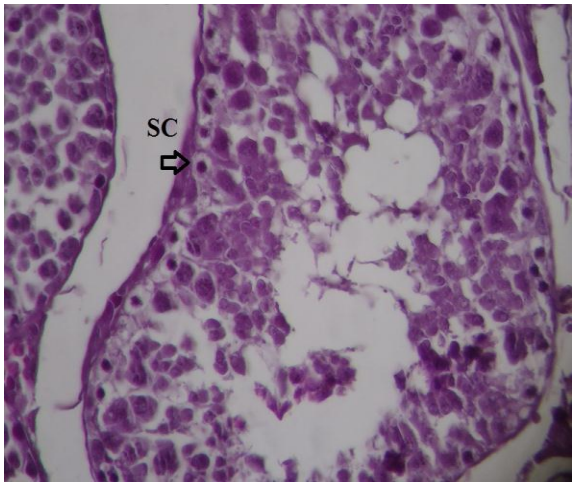

Figure (15): Section of Testis from T2 male rabbit showed normal seminiferous tubules tissue with mature and active sertoli cells (SC), notice that the nuclei of the sertoli cells are centrally located and surrounding by huge cytoplasm. (H&E, X100).

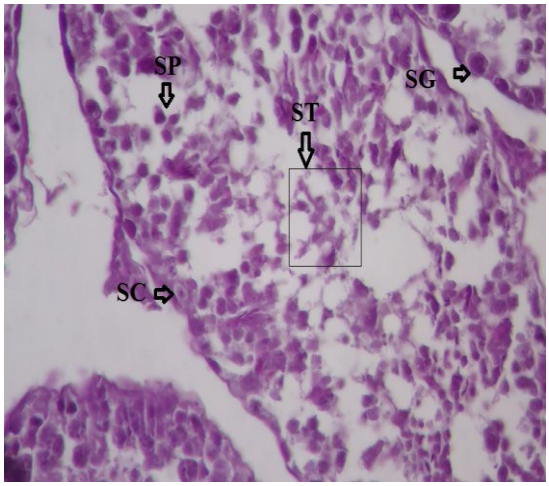

Figure (16): Section of testis from T3 male rabbit showed normal seminiferous tubules; sertoli cell (SC) is present, spermatogoneum (SG), spermatocytes (ST) and spermatids (SP). (H&E, X100).

Discussion

Our results report the significant effect of the prednisolone to causes damage and necrosis in the histological sections of the different organs. Our result was in agreement with results of Varas *et al*., Cerisier *et al*., Katoh *et al*., and Deviche *et al.* (23-26).

Increased risk of acute myocardial infarction with oral corticosteroid use with a greater risk observed among user of high corticosteroid dose (23).

Cerisier *et al*., (24) reported two new cases of angina and/or myocardial infarction and one sudden death after an infusion of a bolus of high dose steroids. Although Katoh *et al*., (25) recorded that high doses of methyl prednisolone caused increase in the number of neutrophils in broncho alveolar. Deviche *et al*., (26) showed that glucocorticoid inhibit of reproductive function. That may be due to the effect of corticosteroid hypersensitivity which may occur due to the corticosteroid itself or to the preservatives and stabilizers that are components of the preparation (3). Or maybe the effect in the reproductive organ due to that glucocorticoids directly suppress leydig cell steroidogenesis by decreasing gonadotropin stimulation of cAMP production and the activity of 17 alpha hydroxylase (27) . While the results in the present study clearly demonstrate that oral administration of vitamin E for 21 days is effective in reducing damage of organs from different systems in the rabbits treated with prednisolone.

Our result was in agreement with Hahn *et al*., Bai *et al*., Assem and Yousri., Kartal *et al* ., Mohammad *et al*., Ben *et al*., Sen *et al*. (28-34).

Oral administrated of alpha tocopherol dramatically suppressed primary tumor growth and reduce the incidence of lung metastases (28). Combination vitamin E with low dose of dexamethasone could effectively to inhibit inflammation and expression of myosin light chain kinase protein in acute lung injury (29). Combination pentoxifyllin and vitamin E significantly a meliorates the reduction of hemoglobin level during treatment with peginterferon and ribavirin in chronic hepatitis-c Egyptians' patients (30).

Kartal *et al*., (31) reported the protective effect of vitamin E against hypercholesterolemic atherosclerosis is not produced by another antioxidant. Supplementation of selenium and vitamin E may improve semen quality and have beneficial and protective effects, especially on sperm motility (32).

Ben *et al*.,(33)showed the significant protection of vitamins pretreated sperimatozoa against the cytotoxic effects induced by dimethoate (organophosphorous compounds).

Supplementation with vitamin E reduced testicular reactive oxygen species and restored normal testicular function in cadmium exposed rats (34). The vitamin E reducing damage and necrosis in the different tissue due to protecting cells functions from oxidative stress induced toxicity and transformation (35). Antioxidants are important to inhibiting oxidative mechanisms that lead to various degenerative diseases (36).

Or may be due to that vitamin E protect critical cellular structures against the damage from oxygen free radicals and reactive products of lipid peroxidation (37). While the increased in the plasma testerone because the vitamin E played key roles in the steroidogenic process that stimulates testerone synthesis (10).

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