

## Pancreatic, hepatic, and renal histopathological changes in thymoquinone- treated streptozotocin-induced diabetic male rats.

Jabbar A.A. Al-Sa'aidi;<sup>1</sup> BS, MS, PhD, Prof. Physiol.; Hashim M.A. Kareem<sup>2</sup>; BS, MS, PhD, Asst. Prof. Histol.; Wijdan T.M. Al-Tameemi<sup>2</sup>; BS, MS, Asst. Prof. Histol.

<sup>1</sup>Dept. Physiol., College of Pharmacy, Al-Qadisiya University.

<sup>2</sup>Dept. Biology, College of Science, Al-Qadisiya University.

### Abstract

The present study has been conducted to investigate the potency of thymoquinone (TQ) in ameliorating the pathological changes of pancreas, liver, and kidneys in streptozotocin-induced diabetic male rats. Sixty five adult male rats (aged 56 days and weighted  $138 \pm 8.8$ g) were subjected to five equal groups (13 male rats each), non-diabetic control and four diabetic groups. Diabetes was induced by single injection with streptozotocin (60 mg/kg b.w., *i.p.*). Rats with 200 mg/dl of blood glucose were used as diabetic. Diabetic groups (DM, TQ50, TQ100, and DMI) were drenched with drinking water, TQ (50 mg/kg, bw), TQ (100 mg/kg, bw), or injected with insulin (4 IU/animal), respectively, for 6 weeks. Twenty four hours after the last day of experiment, male rats have been anaesthetized, sacrificed, and pancreatic, liver, and kidney tissues were removed and fixed in formalin (10%) for histopathological examination. Histological findings of thymoquinone treated (TQ50 and TQ100 groups) pancreases revealed normal cellularity of islets of Langerhans and normal exocrine tissue except few congestion, whereas those obtained from non treated diabetic rats (DM group) showed complete impairment of some islands and highly destructed of others. Normal hepatic architecture with the appearance of radiating shape around the central vein, has been shown in the section obtained from thymoquinone treated diabetic rats (TQ50 and TQ100 groups) except few congestion, obvious regeneration and mitotic division in the nuclei of hepatocytes. Sections obtained from non treated diabetic male rats (DM group) showed severe congestion, large thrombi in the hepatic tissue, and loss of hepatic architecture with severe hemorrhage, degeneration in hepatocytes, and dilation of sinusoids. Section obtained from kidneys of thymoquinone treated diabetic rats (TQ50 and TQ100 groups) reveals normal renal convoluted tubules with normal epithelium of the tubules and high cellularity of glomeruli. Whereas those obtained from non treated diabetic male rats (DM group) revealed dilation of renal convoluted tubules with necrosis in the epithelium of the tubules and severe hemorrhage in the renal tissue. It can be concluded that drenching of 100 mg/kg of TQ for 6 weeks has potent ameliorating and regenerative effect of pancreas, liver, and kidneys in experimentally-induced diabetic male rats.

**Key words:** Thymoquinone, *Nigella sativa*, diabetes mellitus, histopathology.

## Introduction

Plants have long been used to treat many ailments. Medicinal plants used to treat hyperglycemic conditions are of considerable interest for ethno-botanical community as they are recognized to contain valuable medicinal properties in different parts of the plant. The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents (1). Traditional plant medicines or herbal formulations might offer a natural key to unlock diabetic complications (2). Of the several indigenous plants used in the treatment of DM among middle east countries is *Nigella sativa*. The Holy Prophet Muhammad appreciated the black seed at many places by saying that it is a remedy for every illness except the death (3). Seeds of *Nigella sativa* contain a considerable amount of fixed and volatile oils (4), proteins, alkaloids and saponins (5,6,7). The oil and the seed constituents, in particular thymoquinone, have shown potential medicinal properties in traditional medicine (8). Most of the pharmacological activities are attributed to the presence of thymoquinone as an active component (9). Thymoquinone possess antioxidant effects through enhancing the oxidant scavenger system as well as its potent antiinflammatory mediators prostaglandins and leukotriens (8).

Meral *et al.*, (10) concluded that *N. sativa* might be used in diabetic patients to prevent lipid peroxidation, increase anti-oxidant defense system activity and also to prevent the liver damage. Hosseinzadeh *et al.* (11) also reported that thymoquinone and *N. sativa* oil may have protective effects on lipid peroxidation process in rat hippocampus. Diets with *N. sativa* fixed oils 4% or *N. sativa* essential oils 0.3% were found safe in rats as serological indices like liver and kidney functioning tests, serum protein profile, level of cardiac enzymes, electrolytes balance, red and white blood cells remained in the normal ranges (12).

Kanter *et al.* (13,14) investigated the effect of *N. sativa* on histopathology of pancreatic beta-cells, in streptozotocin-induced diabetic rats. They concluded that *N. sativa* treatment exerts a therapeutic protective effect in diabetes by decreasing oxidative stress and preserving pancreatic beta-cell integrity. It was also suggested that *N. sativa* may be clinically useful for protecting beta-cells against oxidative stress (15). The pharmacological actions of the seeds of *N. sativa* that have been reported include protection against nephrotoxicity and hepatotoxicity induced by either disease or chemicals (6,16,17).

## Materials and methods

**1. Experimental rats:** Mature male Sprague-Dawley rats have been used in the experiment, and were allowed one week to acclimatize to the animal house environment before beginning of experiment. Rats were fed on the standard chow and drinking water *ad libitum* throughout the experiment. Room temperature was maintained at  $23 \pm 2^{\circ}\text{C}$ , the light-dark cycle was on a 12:12 h with light on at 06:00 a.m and off at 06:00 p.m throughout the experimental period.

**2. Preparation of TQ suspension:** TQ suspension at a dose of 50mg/kg bw (10) was prepared by dissolving 5 mg of TQ powder in 1 ml of drinking water to be used as 5mg/ 100 g bw, so that each 100 g bw will need drenching 1 ml of TQ suspension. For eg: adult male rat of 150 g bw must be drenched 1.5 ml of TQ suspension which contain 7.5 mg of TQ powder (5 mg in 1ml/ 100g bw or 50 mg in 10 ml/ kg bw).

**3. Induction of diabetes in rats:** According to Mansford and Opie (18), diabetes has been induced in 52 male rats (weighted  $138 \pm 8.8$  g and aged 56 days) by injection of single dose of sitriptozotocin; SZT (Sigma Aldrich, England) (60 mg/kg b.w., i.p.). STZ was dissolved in 1 M of sodium citrate buffer (pH 4.5). STZ induces diabetes mellitus within 3-5 days by destroying the beta cells of Langerhans islets in the pancreas. The rats with plasma glucose  $> 200$  mg/dL were considered as DM rats and used for experiment (19).

**4. Experimental design:** Intact and STZ-induced male rats were classified into five equal groups (13 rats, each); intact control (C), diabetic control (DM), diabetic TQ treated (TQ50 and TQ100), and diabetic insulin treated (DMI) groups. Intact and diabetic control rats were injected with normal saline (100 $\mu$ l, *sc*) and drenched with drinking water daily for 42 days. Diabetic TQ treated rats were injected with normal saline (100 $\mu$ l, *s.c*) and drenched with TQ suspension (50 and 100 mg/ kg, b.w.) daily for 42 days. Diabetic insulin treated were injected with insulin (4 IU, *s.c*) and drenched with drinking water daily for 42. All overnight fasted rats were sacrificed after general anesthesia by combination of xylazine and ketamine (10mg and 90mg/kg, *i.p*, respectively). Liver, kidney and pancreatic tissue has been removed and fixed in formalin (10%) for histological examination.

**5. Histological study:** According to Luna (20), histological sections have been prepared from pancreas, liver, and kidneys, stained, and examined under light microscope.

## **Results**

In comparison with pancreatic sections obtained from control male rats (figure 1), thymoquinone treated (TQ50 and TQ100) groups revealed normal cellularity of islets of Langerhans and normal exocrine tissue except few congestion in it (figure 4 and 5), whereas those obtained from non treated and insulin treated diabetic male rats (DM and DMI groups) showed complete impairment of some islands and highly destructed of others (figure 2 and 3). Sections obtained from non treated diabetic male rats (DM group) showed severe congestion with thrombi in pancreatic tissue. Other sections showed necrosis in exocrine tissue of pancreas and there is absence of islet of Langerhans. Insulin treated diabetic rats (DMI group) revealed some congestion and the presence of thrombi in the exocrine tissue with the presence of atrophied islet of langerhans.

Normal hepatic architecture with the appearance of radiating shape around the central vein, has been shown in the section obtained from control male rats (figure 6). Also there is normal

hepatocytes with prominent nuclei and profuse cytoplasm. In comparison with control male rats, thymoquinone treated diabetic male rats (TQ50 and TQ100 groups) showed normal hepatic architecture which appear as radiating shape around central vein except few congestion and there is obvious regeneration process and mitotic division in the nuclei of hepatocytes (figures 9 and 10). Insulin treated diabetic male rats (DMI group) revealed normal hepatic architecture which appear as radiating shape around central vein, also there is thrombi in the mildly congested central vein, degeneration of some hepatocytes and loss of hepatic architecture (figure 8). Sections obtained from non treated diabetic male rats (DM group) showed sever congestion, large thrombi in the hepatic tissue, and loss of hepatic architecture with sever hemorrhage, degeneration in hepatocytes, and dilation of sinusoids (figure 7).

Figure (11) showed section obtained from kidney of control male rat, which revealed normal renal convoluted tubules with normal epithelial cells lining these tubules and high cellularity of glomeruli. Whereas kidney sections obtained from thymoquinone 50 mg treated diabetic male rats (TQ50 group) revealed dilation of renal convoluted tubules with normal and high cellularity of glomeruli and tubular basophilia in the epithelial cells of renal tubules (figures 14). Sections obtained from thymoquinone 100mg treated diabetic male rats (TQ100 group) revealed mild dilation of renal convoluted tubules with normal and high cellularity of glomeruli (figure 15). Sections obtained from insulin treated male rats (DMI group) showed mild dilation of renal convoluted tubules with normal and high cellularity of glomeruli with tubular basophilia in the epithelial cells of renal tubules (figure 13). Figure (12) showed section of kidney obtained from non treated diabetic male rat which revealed dilation of renal convoluted tubules with necrosis in the epithelial lining of these tubules, also there is sever hemorrhage in the renal tissue. Other sections showed obvious atrophy in the glomeruli.

## **Discussion**

Light microscopy investigation of the pancreas of control rats demonstrated normal pancreatic architecture. In contrast, sections of the pancreas from untreated diabetic rats (Group DM) revealed that the islets were relatively small, atrophied, and showed a reduction in the number of polygonal islet cells. Extensive fibrosis was noted in connective tissue areas surrounding blood vessels. Insulinitis was noted in several islets. In the diabetic groups treated with thymoquinone (Groups TQ50 and TQ100), light micrographs revealed lightly stained, small, round islets with a reduced number of polygonal cells compared with the control group. Sections of pancreases obtained from treated rats with thymoquinone (Groups TQ50 and TQ100) revealed islets of a relatively small size and irregular in shape compared with control.

In the present study, light micrographs revealed that streptozotocin altered islet morphology. Moreover, decreased secretory granules, and cytoplasmic vacuolation were noted. These results are in agreement with those reported previously (21,22). The increased lipid peroxidation resulted in disruption of important lipid-containing membranes, including the nuclear envelope, ER membranes, and vacuoles, leading to the observed dilation. Vacuolation and fragmentation of the mitochondria accompanied diabetes led to disruption of the antioxidative mechanism, reflecting a limited capacity of the mitochondria to overcome the oxidative stress (23).

The results of the present study are consistent with previous studies reported increased lipid peroxidation and decreased antioxidant enzymes in diabetes mellitus (13, 15). Kennedy and Baynes (24) suggested that this effect could be due to non-enzymatic glycosylation of proteins and enzymes. Schettler *et al.* (25) suggested that the reduced antioxidant capacity was due to increased oxygen metabolites, which cause a decrease in the antioxidant defense system. Morphologically, treatment with the thymoquinone indicated partial regeneration of the islet and b-cells, in agreement with the results reported by Kanter *et al.* (15). These protective effects may be attributed to the antioxidant properties of the thymoquinone, which inhibited lipid peroxidation.

Histologically, the islets appeared relatively small in size and irregular in shape. In addition, most of the secretory granules appeared empty and the cytoplasm appeared disrupted, with wide intercellular spaces. These findings could be explained by decreased SOD activity and the increase in MDA (26). It is appeared that thymoquinone ameliorated most of the toxic effects of STZ on pancreatic islets, with normal morphology observed in this group. Structurally, the b-cells retained their normal structure. This may reflect a compensatory mechanism to adapt to metabolic changes by dividing to supply the energy for the synthesis and secretion of insulin and to increase the production of SOD and other antioxidant enzymes to protect against oxidative stress in b-cells. This is agreement with the biochemical findings of non-significant changes in MDA or lipid peroxidation and restoration of normal SOD levels. The increased insulin level may be due to the amelioration of the structure of b-cells, including the mitochondria and endoplasmic reticulum.

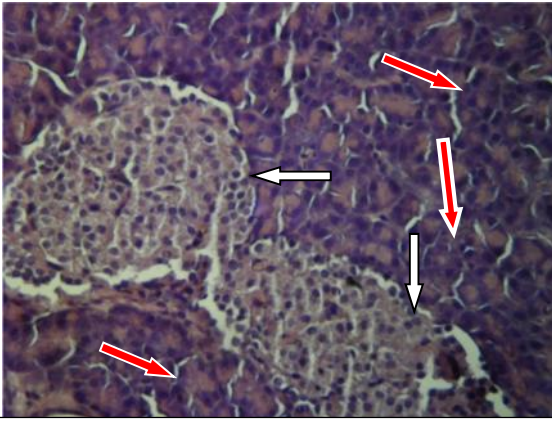
From the present findings, some questions have been answered about the possible mechanisms of thymoquinone in ameliorating the hyperglycemic effects and regeneration of islets of Langerhan's. So, the present study provided important information concerning the possible therapeutic use of thymoquinone improving diabetic effects of mammals, as the present study propose that drenching of thymoquinone, in a dose of 100 mg/kg bw for six weeks, to diabetic male rats (as a model of mammals) significantly improves the hyperglycemia and diabetes control.

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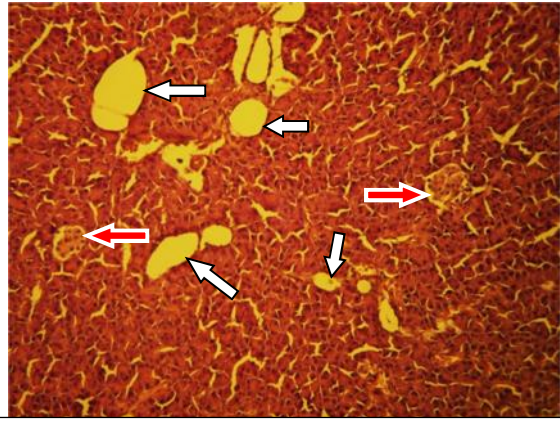
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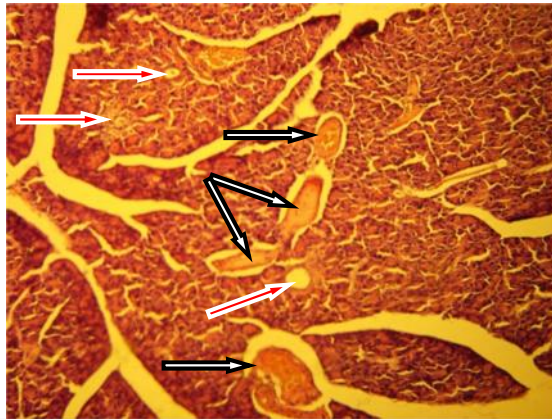




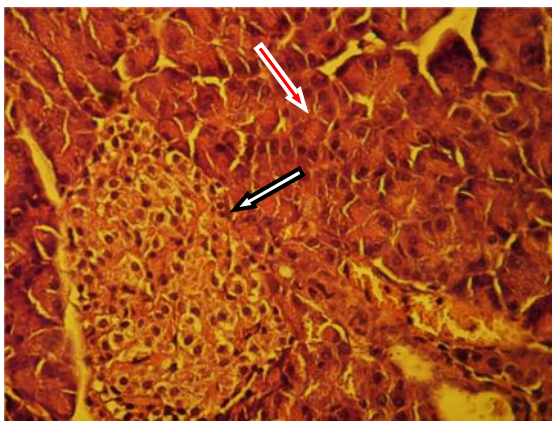
**Figure (1):** Pancreatic section obtained from control male rat shows normal, high cellularity of islets of Langerhans (white arrow) and normal exocrine tissue (red arrow) 200X H&E.



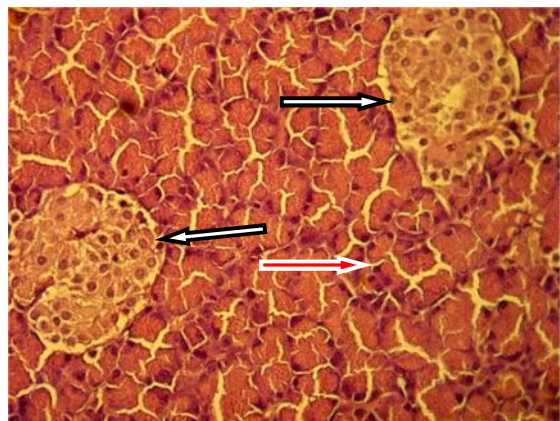
**Figure (2):** Pancreatic section obtained from non treated diabetic male rat shows either depletion (white arrows) or constriction (red arrows) of islets of Langerhans and the presence of congestion in the exocrine tissue 50X H&E.



**Figure (3):** Pancreatic section obtained from insulin treated diabetic male rat shows sever congestion and thrombi (white arrows) in exocrine tissue of pancreas, with presence of atrophied or depleted islets of Langerhans (red arrows). 50X H&E.

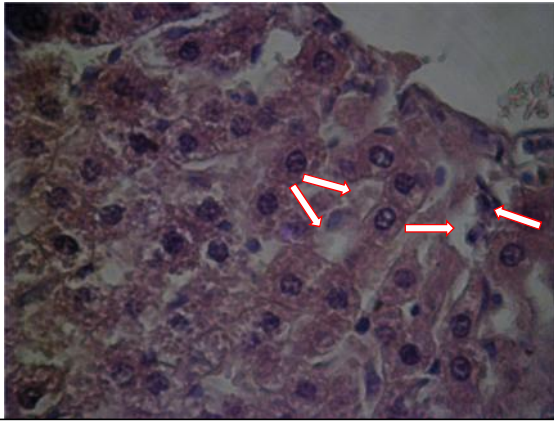


**Figure (4):** Pancreatic section obtained from thymoquinone (50mg/kg) treated diabetic male rat shows normal and high cellularity of islets of Langerhans (white arrow) and normal exocrine tissue except few congestion in it (red arrow) 200X H&E.

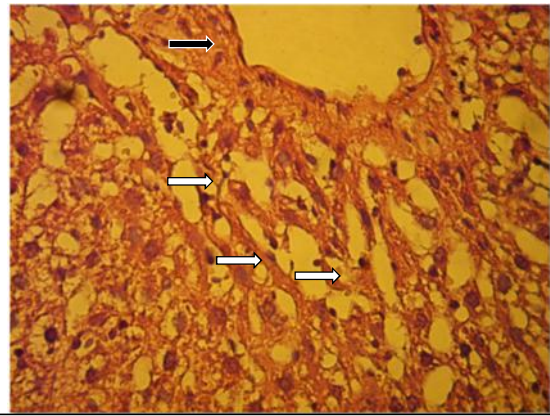


**Figure (5):** Pancreatic section obtained from thymoquinone (100mg/kg) treated diabetic male rat shows normal, developed and high cellularity of islets of Langerhans (white arrows) and normal exocrine tissue (red arrow) 200X H&E.

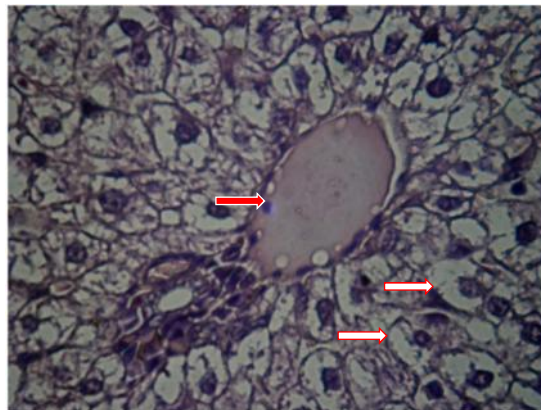




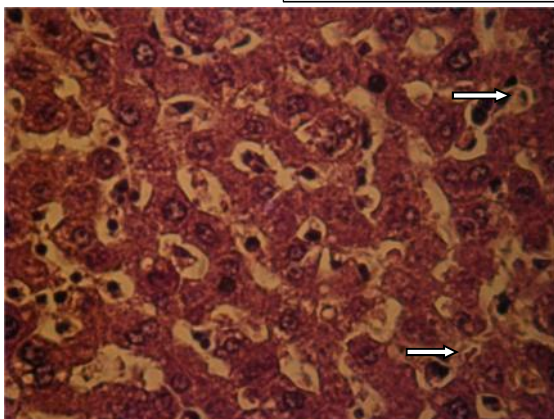
**Figure (6):** Liver section obtained from control male rat shows normal hepatic architecture which appear as radiating shape, also there is normal hepatocytes with prominent nuclei and profuse cytoplasm (white arrows), 200X H&E.



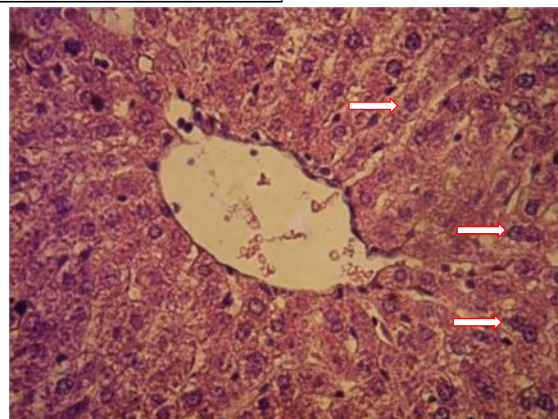
**Figure (7):** Liver section from non treated diabetic rat shows degeneration in hepatocytes (fatty degeneration) characterized by swelling of hepatocytes and the nuclei located at periphery of cells (white arrows). Also there is obvious dilation of sinusoids (black arrow) 200X H&E.



**Figure (8):** Liver section obtained from insulin treated diabetic male rat shows vacuolation of hepatocytes with fatty degeneration, hepatocytes appear with (signet-like shape) (white arrows). Also there is large thrombus in the central vein (red arrow), 200X H&E.

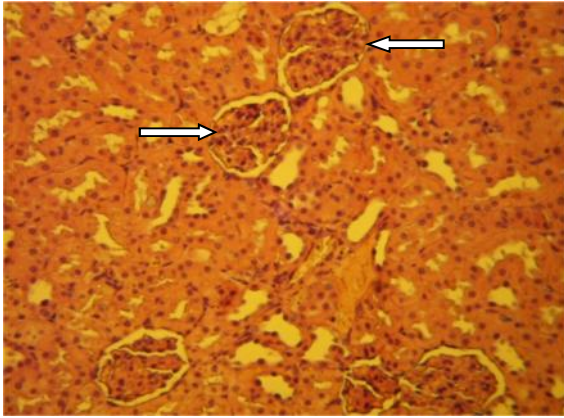


**Figure (9):** Liver section obtained from thymoquinone 50mg treated diabetic male rat shows obvious regeneration and mitotic division in the nuclei of hepatocytes (white arrows), 200X H&E.

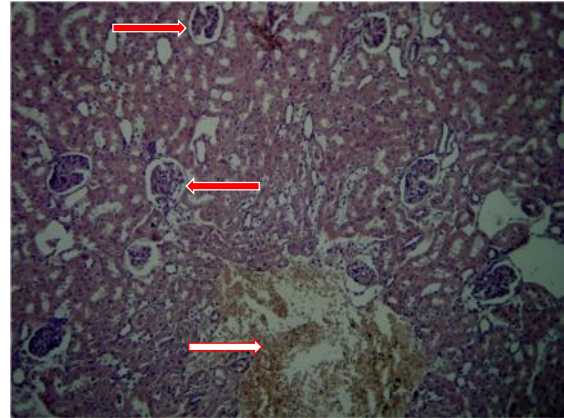


**Figure (10):** Liver section obtained from thymoquinone 100mg treated diabetic male rat shows normal hepatic architecture with obvious regeneration and mitotic division in the nuclei of hepatocytes (white arrows), 200X H&E.

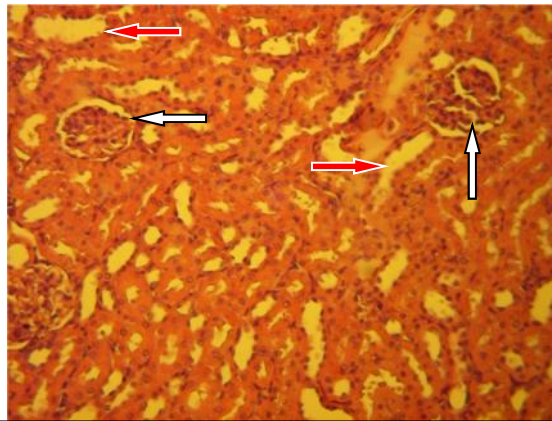




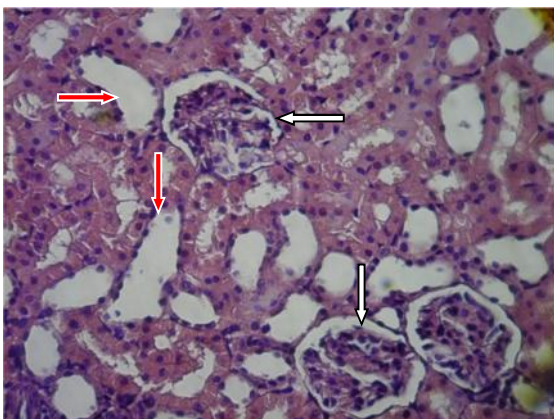
**Figure (11):** kidney section from control male rat shows normal renal convoluted tubules, normal epithelial cells lining these tubules, and normal and high cellularity of glomeruli (white arrow), 50X H&E.



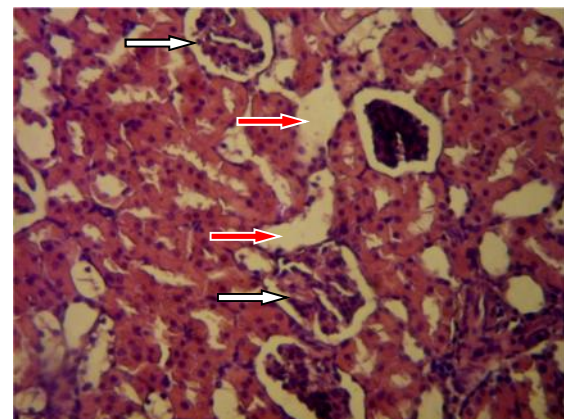
**Figure (12):** kidney section from non treated diabetic rat shows dilation of renal convoluted tubules (red arrows) with necrosis in the epithelial lining of these tubules, also there is sever hemorrhage in the renal tissue, 50X H&E.



**Figure (13):** kidney section obtained from insulin treated diabetic male rat shows mild dilation of renal convoluted tubules (red arrows) with normal and high cellularity of glomeruli (white arrows), 50X H&E.



**Figure (14):** kidney section from thimoquinone 50mg treated male rat shows dilation of renal convoluted tubules (red arrows), high cellularity of glomeruli (white arrows) and tubular basophilia in the epithelial cells lining the renal tubules, 50X H&E.



**Figure (15):** kidney section obtained from thimoquinone 100mg treated male rat shows mild dilation of renal convoluted tubules (red arrows) with normal and high cellularity of glomeruli (white arrows), 50X H&E.

## التغيرات النسجية- المرضية لبنكرياس وأكباد وكلى ذكور الجرذان المستحث فيها داء السكري والمعالجة بالثايموكوينون

الأستاذ الدكتور جبار عباس أحمد الساعدي<sup>1</sup> و الأستاذ المساعد الدكتور هاشم محمد عبد الكريم<sup>2</sup> والأستاذ المساعد وجدان ثامر مهدي التميمي<sup>2</sup>  
<sup>1</sup>قرع الفسلجة، كلية الصيدلة، جامعة القادسية، العراق.  
<sup>2</sup>قسم علوم الحياة، كلية العلوم، جامعة القادسية، العراق.

### الخلاصة

أجريت الدراسة الحالية بهدف تقييم فعالية الثايموكوينون في تلطيف التغيرات النسجية- المرضية لبنكرياس وأكباد وكلى ذكور الجرذان المستحث فيها داء السكري تجريبياً باستخدام عقار الستريبتوزوتوسين. أجريت الدراسة الحالية باستخدام 65 جرذاً ذكراً ناضجاً بعمر 56 يوماً ووزن  $138 \pm 8.8$  غرام. أستخدمت داء السكري في 52 جرذاً باستخدام حقنة مفردة من عقار الستريبتوزوتوسين (60 ملغم/كغم من وزن الجسم في البريتون). تم التأكد من حدوث داء السكري عن طريق قياس مستوى سكر الدم، إذ أن تجاوز التركيز لأكثر من 200 ملغم/100 مل يعد مصاباً بداء السكري. تم توزيع الجرذان السليمة والمصابة عشوائياً على خمس مجموعات متساوية العدد (13 جرذ لكل مجموعة)، ضمت الأولى حيوانات سليمة وعدت على أنها مجموعة سيطرة (C) وجرعت ماء الشرب وحقنت بالمحلول الفسلجي يومياً، وضمت المجموعات الأخرى حيوانات مصابة إذ تركت الثانية بدون علاج (DM) وجرعت ماء الشرب وحقنت بالمحلول الفسلجي يومياً، والثالثة (TQ50) جرعت معلق الثايموكوينون (بجرعة 50 ملغم/كغم من وزن الجسم) وحقنت بالمحلول الفسلجي يومياً، وجرعت الرابعة (TQ100) معلق الثايموكوينون (بجرعة 100 ملغم/كغم من وزن الجسم) وحقنت بالمحلول الفسلجي يومياً، وحقنت الخامسة (DMI) بهرمون الانسولين (بجرعة 4 وحدات دولية لكل حيوان) وحقنت بالمحلول الفسلجي يومياً. استمرت الدراسة لمدة 6 أسابيع. بعد مرور 24 ساعة على آخر يوم من التجربة، تمت التضحية بالحيوانات وأخذت منها نماذج من كل من البنكرياس والأكباد والكلى وثبتت في الفورمالين (10%) لغرض الدراسة النسجية- المرضية.

بينت النتائج تحسناً في معالم التغيرات المرضية لأنسجة البنكرياس في كلتا منطقتي الإفراز الخارجي والصمي (جزر لانكرهانس) لذكور الجرذان المصابة بداء السكري جراء المعالجة بالثايموكوينون، كما بينت الدراسة أن هذا التحسن كان إيجابياً مع تقدم مدة المعالجة، إذ أظهر الفحص النسيجي لمقاطع البنكرياس المأخوذة من من ذكور الجرذان المصابة بداء السكري والمعالجة بالثايموكوينون تغيرات مرضية طفيفة تمثلت بوجود بعض الاحتقان في حين سجلت نتائج المجموعة المصابة وغير المعالجة ضرراً كبيراً في بعض جزر لانكرهانس مع التحطم الكامل للبعض الآخر. أما أنسجة الكبد لذكور الجرذان المصابة والمعالجة بالثايموكوينون فقد أظهرت هي الأخرى مظهراً طبيعياً متمثلاً بوجود الشكل الشعاعي لترتيب حبال الخلايا الكبدية حول الوريد المركزي مع وجود بعض النزف والتتكس إلا أن الخلايا الكبدية تبدو في مراحل انقسام خيطي وافر، في حين عانت أكباد الحيوانات المصابة وغير المعالجة من احتقان شديد مع وجود خثر بين الخلايا الكبدية مع اختفاء الترتيب الطبيعي للحبال الكبدية مع تنكس في بعض الخلايا الكبدية وتوسع في الجيبانيات الكبدية. كما أشارت المقاطع المأخوذة من أنسجة كلى ذكور الجرذان المصابة بداء السكري والمعالجة بالثايموكوينون الى معالم طبيعية بالنفرونات الكلوية ويطانة نبيباتها بينما عانت كلى ذكور الجرذان المصابة وغير المعالجة من توسع في النبيبات الكلوية مع تنكس بطانتها مع وجود نزف شديد في أنسجة الكلية.

يستنتج من الدراسة الحالية أن تجريع ذكور الجرذان بمعلق الثايموكوينون (100 ملغم/كغم من وزن الجسم) له تأثير فعال في تحسين وزيادة تجديد أنسجة البنكرياس والكبد والكليتين لذكور الجرذان المصابة بداء السكري.