The short – term nephropathological complications in experimentally induced diabetes mellitus in female domestic rabbits*

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Abstract:

This study was designed to assess the pathological changes in kidneys after twenty days from induction of diabetes mellitus in female rabbits.

Sixteen female rabbits were used in this experiment and divided into two equal groups: diabetic group (Df) and control group (Cf). Animals were housed in cages under 12/12 h light/dark cycle at 25±2 °C& 60% relative humidity with standard granulated food & water available *ad libitium.* The animals were left one month for adaptation. Diabetes mellitus was induced by i.v injection of alloxan monohydrate at dose rate 100 mg/kg dissolved in 1 ml of normal saline, while the control group was injected with 1 ml of normal saline. Blood was collected after three days to check fasting serum glucose. The procedure of alloxan injection and blood collection were made on empty stomach. The laboratory tests including serum glucose estimation at days (0, 3, 10 and 20) days. After taking blood samples four rabbits were sacrificed by decapitation. The kidneys were removed for histopathological study by using H/E stains. We found the following results according to the periods of the experiments: Serum glucose level was elevated, by checking after 3 days of injection of alloxan, and still elevated to the end of the experiment. The histopathological results after 10 days of induction of diabetes revealed that there were edema, hypertrophy and hypercellularity of glomeruli without any cast or inflammation, while there were necrosis as well as glomerular swelling after 20 days of induction of diabetes.

Introduction:

Diabetes mellitus is a serious public health disease & its prevalence has reached astronomical numbers. Experts forecast that in the year 2025, 9% of the adult population will suffer from diabetes, increasing by 1.6% from the 1995 estimates (1). It is a major public health problem worldwide (2). Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (3).

Clinical studies in subjects with type 1 and type 2 diabetes clearly link hyperglycemia to vascular complications, including diabetic nephropathy (4 , 5). Hyperglycemia is responsible for the development and progression of diabetic nephropathy through metabolic derangements, including increased oxidative stress, activation of protein kinase C-mitogen-activated protein kinases, and accumulation of advanced glycation end products, as well as such hemodynamic factors as systemic hypertension and increased intraglomerular pressure (6). Hypertrophy is observed in both the glomerular basement membrane and capillaries of diabetics and may contribute to end-stage renal damage(7,8).

Materials and Methods:

Sixteen female rabbits (White Newzealand Rabbits) purchased from the local market, weighing 1.2-2.0 kg were used in the experiment. Animals were housed in cages with dimensions (130×100×70) under 12/12 h light/dark cycle at 25±2 c & 60% relative humidity with standard granulated food, & water available *ad libitium.* The animals were divided into two equal groups: Diabetic group (Df), includes (8) rabbits and control groups (Cf) includes (8) rabbits

Animals were left one month for adaptation. The animals were given Clopidol 0.06 mg/kg with feed as a prophylactic drug against coccidiosis during adaptation period.

Induction of Diabetes Mellitus:

Diabetes mellitus had induced in overnight fasting rabbits by a single injection of alloxan (alloxan monohydrate) at dose 100 mg /kg into marginal ear vein .Each 100 mg of alloxan had diluted in 1 ml of 0.9% normal saline(11).

Immediately, after alloxan injection, 10 ml of 20% glucose I.V

& 5 ml of 20% glucose I.P was given to the rabbits in order to overcome sudden decrease in blood glucose level (hypoglycemia).The rabbits were prevented from feeding for 12 h and the drenching water replaced by 5% glucose for 24h.The procedure of administration and blood collection made under sedation of animals by using kitamin 44 mg /kg and xylazine 5 mg/kg. The control groups were I.V injected with 1 ml of 0.9 % of normal saline(12).

Blood Collection:

The blood was collected according to the following equation: Total blood volume (TBV) =6% of body weight. Maximum blood collection =20%of total blood volume every two weeks.

- Animal weight in kg×o.o6×o.o2×1000= (….) ml (9).

The blood collected at the following periods: Zero day (before the injection of alloxan), three days, ten days and twenty days after injection of alloxan.

Site of Blood Collection:

The blood was collected from marginal ear vein with empty stomach. The collected blood was centrifuged to obtain the serum. The serum used immediately for checking fasting serum glucose (FSG. The blood collected in test tubes, contain sodium fluoride (NaF)(10).

Estimation of Fasting Serum Glucose:

After 3 days of alloxan injection, the animals were fasting overnight and bled for checking the hyperglycemia .Fasting serum blood glucose (FSG) was measured by using special kit prepared by (SPINREACT, S.A.Ctra.Santa Coloma, 7E-17176SANT ESTEVE DE BAS (GI) SPIN), then the (FSG) concentrations were checked after 3 days and then every 10 days .

Sacrificing of animals and histopathological procedures:

Four rabbits (two from diabetic group and tow from control group were sacrificed each (10) days intervals. Tissue samples were taken from the kidney, fixed in 10 % buffered formalin, embedded in paraffin, cut at 5 µm, stained with hematoxylin and

eosin, PAS stains, Osmium tetroxide and examined by light microscopy. (13 and 14).

Weighting the internal organs after sacrificing of animals:

Immediately after sacrificing of animals, the right and left kidneys were taken for recording the weights.

Statistical Analysis:

The data were expressed as mean ±standard deviation (SD) and analyzed using analysis of variance (ANOVA). Least significant difference (LSD) was used to test for differences among means for ANOVA indicated a significant ($P<0.05$), using computerized SPSS(15).

RESULTS:

Fasting Serum Glucose Concentrations (FSG):

On day zero, the results revealed no significant differences($P<0.05$) between the groups (Df and Cf) and had an average value of (93.56±8.20) mg/dl. On day ten of alloxan injection, the concentration had increased to (297.25±8.13) mg/dl in diabetic group, while still within normal ranges(94.99±8.36) mg/dl in control group. On day twenty of alloxan injection, the concentration of fasting serum glucose had elevated to (298.66±37.23) mg/dl in diabetic group, while continue within normal range (93.88±36.38) mg/dl in control group, so there were significant differences between diabetic and control groups ($P<0.05$), while the difference between diabetic group in the different periods(ten and twenty) were non-significant($P<0.05$). Table(1).

- **Table(1): represent the fasting serum glucose concentrations(mg/dl) in diabetic and control groups in different periods.**

	Fasting serum glucose concentrations	
Groups	Df Group	Cf Group

Periods		
Day Ten	297.25±8.13 bc	94.99±8.36 a
Day twenty	298.66±37.23 bc	(93.88±36.38 a

- The small letter (a) refers to non- significant differences between the control group in the different periods(ten and twenty)(P<0.05).
- The small letter (b) refers to non-significant differences between diabetic group in the different periods(P<0.05).
- The small letter (c) refers to significant differences between diabetic and control group in the different periods(P<0.05).

Weights of kidneys:

Right kidney: on day ten of alloxan injection the weights of right kidneys were (3.90±1.28)gm, while on day twenty of alloxan injection the weights were (3.12±0.19)gm. The weights of right kidney of control group after ten and twenty days were (3.31±1.07) and (3.34±0.26) gm respectively, according to that there were no significant differences(P<0.05) between diabetic and control groups, and between the diabetic group themselves. Table(2).

Left kidney: on day ten of alloxan injection the weights of left kidneys were (3.69±1.01)gm, while on day twenty of alloxan injection the weights were (3.57±0.14)gm. The weights of left kidney of control group after ten and twenty days were (3.24±1.12) and (3.21±0.24)gm respectively, according to that there were no significant differences(P<0.05) between diabetic and control group, and between the diabetic group themselves. Table(2).

- **Table(2): represent the average values of right and left kidney weights (gm) in different periods:**

	Right kidney weights		Left kidney weights	
Groups / Periods	Df Group	Cf Group	Df Group	Cf Group
Day Ten	3.90±1.28 a	3.31±1.07 a	3.69±1.01 a	3.57±0.14 a
Day twenty	3.12±0.19 a	3.34±0.26 a	3.57±0.14 a	3.21±0.24 a

- The small letter (a) refers to non-significant differences between all groups and periods of experiment.

Results of histopathological changes of Kidneys:

The results revealed that after ten days of alloxan injection, diabetes led to edema, hypertrophy and hypercellularity of glomeruli, without any cast formation or inflammation, while after twenty days of alloxan injection the results showed glomerular necrosis(the glomeruli appear under light microscope deeply eosinophilic in color) as well as glomerular swelling.

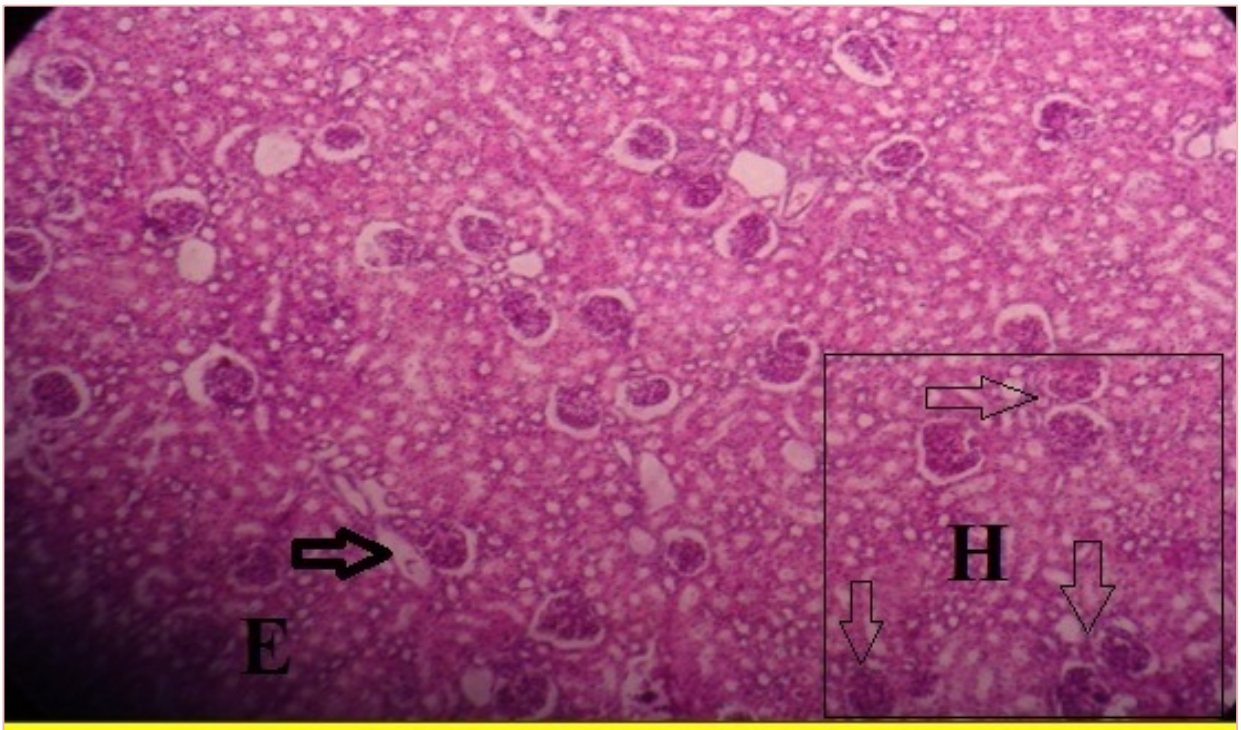
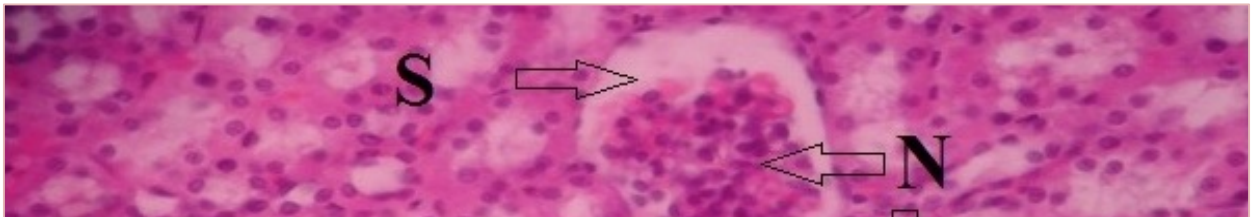


Figure-1.....Kidney.....100 X.....H & E

Day Ten -Diabetic Female

1. Edema (E).
2. Hypertrophy and hypercellularity of glomeruli without any cast or inflammation (H).



Discussion:

The study revealed a significant difference in serum glucose concentrations between the periods of the experiment as there was progressive increase in the serum glucose levels; this is similar to the results of (16) and (17).

The histopathological results revealed that on day ten and twenty the changes include: edema, glomerular hypertrophy and hypercellularity. Our results were in accordance with other, who mentioned that under conditions of sustained hyperglycemia, the glomerular cells are affected by various mechanisms. These changes lead to altered structure and function in the glomerulus (18). In the kidney, the first observed functional change is an abnormally increased glomerular filtration rate (19). This change develops before any major histological change in the glomerular structure (20), then the changes in renal structure will be nephromegaly and glomerular enlargement (21). (22) Showed that the diabetic animals demonstrate low weight gain, renal hypertrophy assessed by the left kidney weight and kidney/body weight ratio as compared with control. Glomerulus from an untreated db/db mouse at 18 weeks of age, showing hypertrophy and mesangial matrix expansion(23). Diabetes is accompanied by

oxidative stress, which plays a crucial role in the development of diabetic complications(24 and 25). The histopathological changes in the heart of streptozotocin-induced diabetic rabbits showed haemorrhage and cardiomyopathy which could be attributed to the hyperglycemia, which by the formation of oxygen free radicals induces degenerative changes in the tissues along with cardiomyopathy and nephropathy (26).

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التعقيدات المرضية الكلوية الحادة الناجمة عن داء السكر المستحث تجريبياً في إناث الأرانب المحلية*

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الخلاصة

أجريت هذه الدراسة لتقييم التغييرات المرضية التي تظهر في الكلى بعد عشرين يوماً من إحداث **الإصابة بداء السكّري**. استعملت في هذه التجربة ستة عشر أرنباً أنثى وقسمت إلى مجموعتين متساويتين:المجموعة المستحث بها داء السكر تجريبياً التي تضمّنت (8) أرانب ورمز إليها (Df) ،**ومجموعة** السيطرة التي تضمّنت (8) أرانب ورمز إليها (Cf). ربيت حيوانات الدراسة في أقفاص ذات أبعاد قياسيه وفي ظروف جيده من أضاءه ودرجة حرارة ورطوبة نسبية وكان الغذاء والماء متوفرين طول فترة الدراسة. **تركت** الحيوانات شهراً واحداً للتكيّف. تم استحداث داء السكر عن طريق الحقن الوريدي لمادة الالوكسان و بجرعة 100 **ملغم** / كيلوغرام كانت قد أذيبت في مللتر واحد من الملح الطبيعي الفسلجي، بينما حقنت مجموعة السيطرة بمللتر واحد من الملح الفسلجي فقط. جمع الدم من الحيوانات بعد ثلاثة أيامٍ من **حقن مادة الالوكسان** لغرض تقييم مستوى سكر المصل وقد أجريت عملية حقن مادة الالوكسان ومن ثم عملية سحب الدم بعد تجويع الحيوانات عدة ساعات في الفترات (3 و10 و20) يوماً بعد الحقن. بعد أخذ عينات الدمّ تمت التضحية بأربعة أرانب وأخذت منها الكلى لغرض عمل التقطيع النسيجي باستعمال صبغات الهيماتوكسلين والايوسين ،**وقد** أظهرت الدراسة النتائج الاتيه طبقا لفترات التجربة: اظهر مستوى سكر مصل الدم ارتفاع بعد الأيام الثلاثة **الأولى من استحداث داء السكّري في المجموعة المعاملة**. وقد لوحظ على الحيوانات المصابة العلامات

السريرية المميزة للمرض والتي تضمنت العطش وكثرة التبول وكثرة الأكل. **كما أوضحت نتائج الفحص** النسيجي المرضي وجود وذمة، تضخّم كبيبي في الكلية وبدون ارتشاح للخلايا الالتهابية بعد عشرة أيام من استحداث داء السكر، وورم كبيبي ونخر وتضخم مع وذمة بع عشرين يوماً من الاستحداث التجريبي لداء **السكر**.

*البحث مستل من أطروحة الماجستير للباحث الأول