Propolis Effect on Rodent Models of Streptozotocin - Induced Diabetic Nephropathy

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Abstract: Diabetic nephropathy (DN) is a frequent complication in patients with long-standing type I diabetes mellitus (DM1). The current study was aimed at identifying quantitatively and qualitatively renal histopathological abnormalities that occur with streptozotocin-induced diabetes in rats and their possible reversal after the establishment of good metabolic control with propolis. Fifty healthy adult male wistar rats were used, divided into 2 groups. Group I: [10 rats Control group] and Group II: [streptozotocin-induced diabetic rats], received a single intra-peritoneal injection of 60mg/kg. Diabetes appeared after 1-3 days after STZ injection. The second experimental group was divided into 4 subgroups (10 rats each). Group II-a: Non-treated diabetic [positive control] rats, Group II-b: Diabetic rats treated daily with Humulin insulin 5 IU/kg/day. Group II-c: Diabetic rats treated daily with same insulin dose + propolis 0.3 g/kg. Group II-d: treated similar to group IIc but with double dose of propolis 0.6 g/kg. The animals were monitored for six weeks. After scarification, kidneys were excised and fixed by immersion in 10% neutral buffered formalin. The kidneys were processed for paraffin embedding and sections of 5μ thickness were produced and stained with H &E, PAS and anti-laminin immunohistochemical staining. The morphometric data obtained were analyzed using one way ANOVA test within SPSS Graph Pad software. Examination of the diabetic non-treated group revealed significant increase in blood glucose level accompanied with qualitative & quantitative morphometric changes in the renal cortices with enlargement of the renal corpuscular volume, significant increase in mesangial volume, number of intra-corpuscular nuclei and vacuolated tubules. On the other hand, Bowman's spaces showed significant decrease while the thickness of glomerular basal laminae and the intensity of anti-laminin stain were insignificant as compared to the control group. Insulin administration to diabetic rats controlled the hyperglycemia and lowered blood glucose level but quantitative & qualitative findings revealed more evident glomerulosclerotic changes and degenerative changes. This could be attributed to the concept of metabolic memory where the diabetic vascular stresses persist after glucose normalization due to modification of mitochondrial functions. Propolis administration reflected effective protection and improved all histo-pathological findings. Propolis induced good glycaemic control leading to a reduction in GFR reflecting reduction of hyper-filtration, so it delays onset of diabetic nephropathy and even correct it after it starts. This could be contributed to its antioxidant & immune-modulatory effect.

Introduction

Insulin-dependent diabetes mellitus (IDDM) or type 1 diabetes is characterized by a clinical disorder of sugar, fat and proteins metabolism, caused by absence of insulin to promote sufficient glucose output from the liver [35].

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity [43]. The problem is expected to grow, as the prevalence of diabetes is expected to increase from 285 million patients at present to 438 million patients in the year 2030 [32].

Diabetic nephropathy (DN) is a frequent complication in patients with long-standing type 1 diabetes mellitus (DM1) [34]. It has become a worldwide epidemic accounting one third of end-stage renal disease and 50% of cases requiring dialysis or transplantation [31]. With increasing global prevalence, nephropathy will have a major societal impact because of the enormous financial burden of renal replacement therapy [32].

Experimental type 1 diabetes induced with streptozotocin in rats display many of the features seen in human subjects with uncontrolled diabetes mellitus due to its selective pancreatic β-cell cytotoxicity caused by DNA alkylation and nitric oxide (NO) generation [6].

Glycemic control via the use of exogenous insulin injections in diabetic patients is incomplete, resulting in multiple long-term complications, such as retinopathy, neuropathy, vasculopathy and nephropathy [30]. The development of additional

protective therapeutic interventions is a major priority [19].

Propolis is a sticky, resinous material that honey bees collect from various plants, and mix with wax and other secretions [15]. It is known to have abundant bioactive constituents and a variety of biological activities [44]. Recently, the antidiabetic effect of propolis was thoroughly investigated using different biochemical markers to clarify its effect on blood glucose, lipid metabolism, insulin sensitivity and antioxidant potential [1, 5, 44].

The current study was aimed at identifying quantitatively and qualitatively renal histo-pathological abnormalities that occur with the evolution of streptozotocin-induced diabetes in rats and to appreciate their possible reversal after the establishment of good metabolic control with propolis.

2. Material and methods

This work was conducted at the Faculty of Medicine, University of Alexandria and Dammam.

Chemicals

Streptozotocin (STZ) was purchased from Sigma-Aldrich (USA) and 60 mg/kg was injected as a single dose intraperitoneal (IP) [41]. For this purpose, 100 mg of STZ was dissolved in 5 ml distilled water to reach final concentration of 20 mg/ml just before use [26].

Insulin was prepared (0.5 IU/0.1 ml) by dissolving 0.5 ml of Humulin insulin (100 IU/ml) in 9.5 ml normal saline. Insulin dose (5 IU/kg/day) was administered to rats through IP injection [13].

Propolis was obtained from Dosic Import and Export Co., Ltd China, of best purity (70%) and was supplied in powder. The two selected doses of propolis (0.3 g/kg and 0.6 g/kg/day) were administered orally by oro-gastric feeding. Daily, fresh ground propolis was weighed and dissolved in distilled water to reach final concentration of 200 mg/1ml [13].

Animals

Male wistar rats (n = 50) weighing 150-200 g were used for this study. All rats had free access to standard laboratory food pellets and water. Rats were divided into two groups as follows:

1. Group I: A control group (*n* = 10) Rats **[negative control].** Rats were injected daily with normal saline intra-peritoneal (IP) and received water (1ml) through an orogastric metallic needle [2].

2. Group II: An experimental group (*n* = 40) [streptozotocin-induced diabetic Rats]. Rats were injected with STZ 60 mg/kg, IP to induce diabetes [41]. Diabetes appears after 1-3 days of STZ injection, Rats with glycosuria detected by urine strips (Medi_Test Combi 10) as dark green color (blood glucose range of $\geq$ 200 mg/dl) were

selected and used for the experiment [2]. Rats were considered diabetic if blood glucose concentration increased to 200 mg/dl or more. Diabetic rats were kept for 6 weeks for the development of the pathology of experimental diabetic nephropathy [24].

The experimental group was divided into 4 subgroups:

- Group II-a: (*n* = 10) Non-treated diabetic [positive control] rats.
- Group II-b: (*n* = 10) Diabetic rats treated daily with Humulin insulin (Eli Lilly, USA) 5 IU/kg/day [37].
- Group II-c: (*n* = 10) Diabetic rats treated daily with insulin 5 IU/kg/day + hypoglycemic dose of propolis 0.3 g/kg as recorded by Wang and Li [41] in aqueous solution using an orogastric metallic needle.
- Group II-d: (*n* = 10) Diabetic rats treated daily with insulin 5 IU/kg/day + double the hypoglycemic dose of propolis 0. 6 g/kg.

Blood sampling and organ collection:

At the end of STZ exposure for 6 weeks and insulin, propolis treatment regimen for different experimental groups of rats, the treatment was withheld for the following day and food for 12hrs before sample collection. All rats were lightly anaesthetized with ketamine 50 mg/kg (Alfasan International, Wo-erden, The Netherlands) injected IP [18]. After sedation, each rat was fixed on the rat dissecting table, and midline thoracic incision was made to the lower abdominal cavity through which the greater vessels were easily exposed. Blood was directly collected from rat's aorta by means of a vaccutainer tubes**.** Kidneys were dissected out, washed in normal saline and fixed in 10% neutral buffered formalin. After histological processing, paraffin blocks were prepared and 5μ sections were stained using: haematoxylin & eosin, PAS [8] and anti-laminin immunohistochemical stain. The slides were then examined under the light microscope.

Quantitative morphometric analysis of renal cortices:

The images were captured using a 10X object in H&E stain**,** and 40X object in anti-laminin stained sections with numerical aperture of a high resolution of (16-bit digital camera (1280X1024 pixels). Images were viewed and recorded using Olympus microscope – equipped with Spot digital camera, using computer program MATLAB software (image J, THE MATHWORKS, inc., USA).

a) H&E stain

The means of renal corpuscular volumes, mesangial expansion, Bowman's spaces volumes, intra-corpuscular nuclei and vacuolated tubules were determined. The mean values of each measure were

based on the mean of pixel number except for counting the intra-corpuscular nuclei and the vacuolated tubules (Figure 1).

b) Anti-laminin immunohistochemical stain

The maximum, minimum and integrity of intensity color based on Gray-level acquisition, analysis of the data was carried out by reading 20 fixed areas in one image (X5 images). The image analysis identified individual image of five kidney groups revealing compactness of stains and calculating the relative density of laminin stains at fixed area**,** the integrity of intensity color based on Gray-level transition probabilities in digitilized images. Regarding stain intensity as measured by image analyzer, the intense positive stain from dark to light according to the gray value ranged from (0 to 256 which means dark to light). The mean values of each reaction were based on the mean of pixel number (Figure 1).

Statistical analysis:

The measured values were presented as mean ± standard deviation (±SD WRITTEN SD NOT S). Comparison among the mean values of all groups was done using one way ANOVA test. The level of significance was taken at the P value of < 0.05 A special computer program SPSS Graph Pad software was used for such assessment.

Quantitative results

Blood glucose level

The diabetic non-treated group had a significant increase in blood glucose level as compared to the negative control group. Insulin-treated group showed significant decrease as compared to diabetic non-treated group, while it was significantly increased as compared to the negative control. Simultaneous administration of one dose propolis with insulin showed insignificant increase in blood glucose level as compared to the negative control. Double dose propolis- treated group showed significant decrease in blood glucose as compared to all other groups (Table I).

Morphometric renal corpuscular measurements:

As compared to the negative control group, the Bowman's corpuscular volumes were higher in all treated groups but showed significant increase in insulin-treated diabetic rats.

As regard the mesangial expansion, compared to the negative control group, all the treated groups showed a significant increase in the mesangial volume. As compared to the positive control, insulin treated group was the only group depicted a significant increase in the mesangial volume.

Bowman's spaces of all treated groups showed a significant decrease in their sizes as compared to the control group.

Intracorpuscular nuclei included podocytes, mesangial & endothelial nuclei. The positive control (diabetic non-treated) group as well as insulin-treated diabetic rats showed a significant increase in the nuclear count as compared to the negative control group. Administration of one dose of propolis lowered the count to nearly control level while doubling this dose reflected non-significant increase to the negative or positive control.

Using anti-laminin immunohistochemical staining technique, the measured values of the intensity of the stain of the glomerular basal laminae reflected no significant difference in all tested groups (Table I).

Table (I): Blood Glucose level & renal corpuscular morphometric comparison of the different studied groups.

	Group I	Group IIa	Group IIb	Group IIc	Group IId
Blood Glucose Level **(mg/dl)**					
Min.	120.0	348.5	152.0	122.0	98.5
Max.	168.9	620.5	210.6	189.5	162.2
Mean	143.9[a]	509.3[b]	182.0[c]	156.2[a]	127.1[d]
S.D.	23.6	34.9	24.6	31.6	21.5
FP	29.80.001*				
Bowman's corpuscular volume (pixel)					
Min.	98.86	90.55	111.43	92.36	76.58
Max.	211.48	190.88	288.18	192.78	256.16
Mean	131.25[a]	151.45[a]	208.96[c]	146.73[a]	155.68[a]
S.D.	21.32	26.36	48.55	28.07	40.78
FP	49.890.001*				
Mesangial expansion (pixel)					
Min.	56.8	83.63	105.69	83.82	61.55
Max.	123.85	186.81	287.26	186.68	242.17
Mean	100.35[a]	140.31[b]	198.47[c]	134.65[b]	141.99[b]
S.D.	17.44	27.88	48.00	27.74	39.14
FP	52.650.0001*				
Bowman's space (pixel)					
Min.	3.64	0.07	0	4.25	4.43
Max.	101.88	52.22	28.88	25.78	33.62
Mean	30.91[a]	11.14[b]	10.49[b]	12.09[b]	13.69[b]
S.D.	16.03	11.73	8.06	5.79	7.73
FP	27.980.001*				
Number of intra-corpuscular nuclei					
Min.	21	12	21	14	25
Max.	46	156	60	40	52
Mean	30.59[a]	37.59[b]	37.06[b]	29.74[a]	34.46[ab]
S.D.	6.40	29.95	10.03	7.60	8.36
FP	7.250.0214*				
Intensity of anti-laminin reaction of glomerular basal lamina (pixel/area)					
Min.	181.52	174.63	153.54	174.65	169.71
Max.	209.27	204.31	203.05	206.30	207.12
Mean	194.49	189.00	182.77	190.65	185.18
S.D.	9.33	9.93	13.43	8.23	10.92
FP	2.8680.322 N.S.				

The same small letters indicate that there was no significant difference while the different letters indicate that there was a significant difference.

Morphometric tubular measurements:

As compared to the negative control group, all the treated groups showed a significant increase in the number of vacuolated tubules with no significant difference between diabetic and insulin treated rats. Propolis treated groups reflected a significant decrease in the vacuolated tubular count as compared to diabetic and insulin treated rats.

Using anti-laminin immunohistochemical staining technique, the measured values of the intensity of the stain of tubular basal laminae reflected no significant difference in all tested groups (Table II).

Table (II): Tubular morphometric comparison of the different studied groups

	Group I	Group IIa	Group IIb	Group IIc	Group IId
Numbers of vacuolated tubules					
Min.		35	25	13	20
Max.	0.0	46	48	36	23
Mean		40.50[a]	36.50[a]	24.50[b]	21.50[b]
S.D.		7.78	16.26	16.26	2.12
F			25.65		
P			0.001*		
Intensity of anti-laminin reaction of tubular basal lamina (pixel/area)					
Min.	69.24	62.19	75.30	83.23	85.30
Max.	117.22	128.17	108.30	126.23	128.23
Mean	90.89	96.15	90.40	107.82	108.31
S.D.	13.21	15.60	12.10	14.04	13.42
F			3.08		
P			0.105 N.S.		

The same small letters indicate that there was no significant difference while the different letters indicate that there was a significant difference.

Qualitative Results
H&E Stain

Examination of kidneys collected from negative control rats revealed classical structure and architecture of cortical tissue with well defined renal corpuscles, proximal and distal convoluted tubules (Fig. 1). Examination of kidneys of group IIa diabetic rats (positive control) revealed glomerular sclerosis, glomerular enlargement, mesangial hypercellularity and narrowed Bowman's spaces. Degenerated vacuolated proximal and distal tubules with pyknotic nuclei were also seen. Absent brush borders were encountered in most degenerated proximal tubules (Fig. 2). Examination of group IIb renal cortices of insulin treated diabetic rats revealed more severe degeneration as group IIa with apparent obliteration of Bowman's space in some glomeruli (Fig. 3). Examination of group IIc& IId renal cortices of diabetic rats that received one or two doses of propolis respectively revealed mild glomerular sclerotic changes with few degenerated tubules (Fig. 4,5). Preservation of Bowman's spaces was more pronounced in group IId (Fig. 5).

PAS stain

Examination of negative control rats' renal cortices showed positive PAS reaction in the basement membrane of renal corpuscles, proximal and distal convoluted tubules as well as brush borders of PCT (Fig.6a). Examination of kidneys of group IIa & IIb revealed strong positive reaction in the degenerated tubules (Fig.6b, c). Examination of group IIc renal cortices of diabetic rats revealed slight increase in PAS reaction of the degenerated tubules (Fig.6d). Group IId revealed nearly control image (Fig.6e). No apparent change was noticed in the reaction of the basal laminae of the renal corpuscles or convoluted tubules or brush borders in all examined groups (Fig.6).

Laminin immuno-histochemical stain

Examination of negative control rats' renal cortices stained by laminin antibody revealed positive reaction in the basal laminae of renal corpuscles and convoluted tubules (Fig.7a). Examination of group IIa, IIb & IIc revealed stronger reaction in glomerulo-sclerotic renal corpuscles (Fig.7b, c, d). Group IId revealed nearly negative control image (Fig.7d).
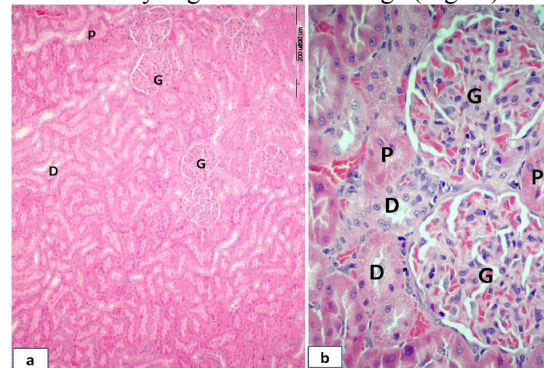

Figure 1: Control rats' renal cortices revealing classical structure with well defined renal corpuscles (G), proximal (P), and distal (D) convoluted tubules. H&E stain Mic Mag a-X 100, b-X 400
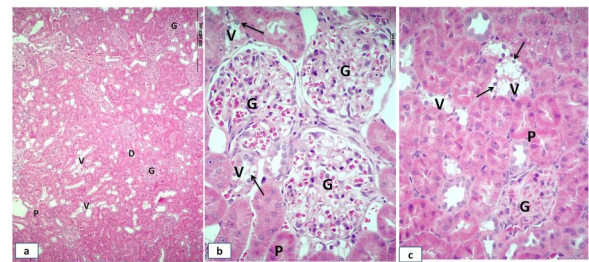

Figure 2: Renal cortices of group IIa diabetic rats showing glomeruloscelerosis (G) with enlargement of renal corpuscles, expansion of the glomeruli and narrowing of Bowman's spaces. Many proximal (P) and distal (D) convolutd tubules reflected classical structures while others show excessive vacuolation (V) and pyknotic nuclei (arrow). H&E stain Mic Mag a-X 100, b&c-X 400
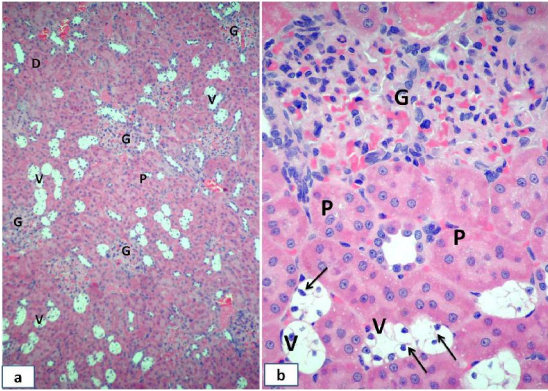
Figure 3: Renal cortices of group IIb insulin-treated diabetic rats showing glomerulosclerosis (G) with apparent obliteration of Bowman's spaces and excessively vacuolated tubules (V) with pyknotic nuclei (arrow). P; PCT, D; DCT.
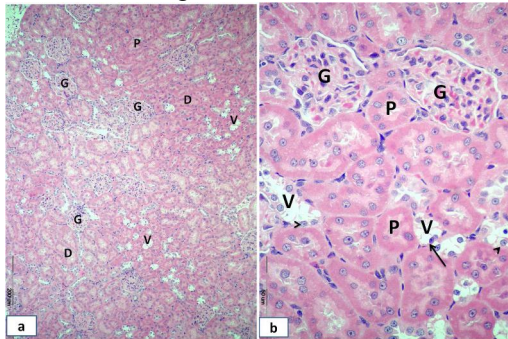H&E stain Mic Mag a-X 100, b-X 400

Figure 4: Renal cortices of diabetic rats group IIc received single dose of proposil with insulin showing apparently normal renal corpuscles (G) with preserved Bowman's spaces. Proximal (p) and distal (D) convoluted tubules depict classical structures. Few vacuolated tubules (V) are also seen. Pyknotic nuclei were rarely encountered (arrow).
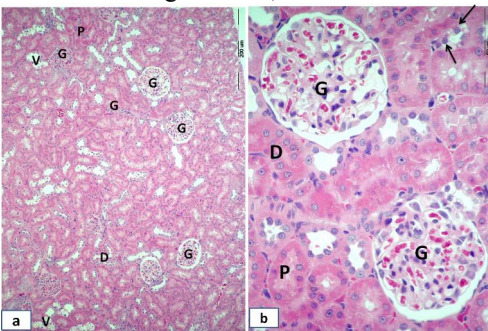H&E stain Mic Mag a-X 100, b-X 400

Figure 5: Renal cortices of diabetic rats group IId received two doses of proposil with insulin depicting classical structure of renal corpuscles (G), Proximal (p) and distal (D) convoluted tubules. Vacuolated tubules (V) were rarely encountered with normal looking nuclei (arrow).
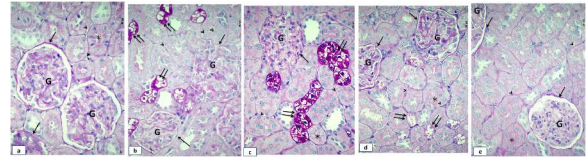H&E stain Mic Mag a-X 100, b-X 400

Figure 6: Rats' renal cortices showed positive PAS reaction in the basement membrane (arrow) of renal corpuscles (G), proximal and distal convoluted tubules (arrow head) as well as brush borders of PCT (*). Note the strong positive reaction in the degenerated tubules (double arrows) in group IIa& IIb and nearly normal reaction in it in group II d.
a; control group, b; group IIa, c; group IIb, d; group IIc, e; group IId.
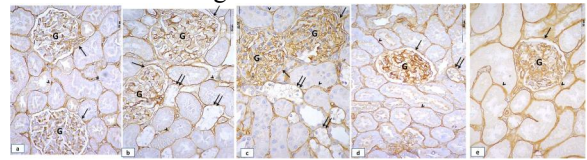 PAS stain Mic Mag X 400

Figure 7: Rats' renal cortices showed positive reaction in the basal laminae (arrow) of renal corpuscles (G), convoluted tubules (arrow head) vacuolated tubules (double arrows).
a; control group, b; group IIa, c; group IIb, d; group IIc, e; group IId.
 Laminin antibody stain Mic Mag X 400

DISCUSSION

Diabetic nephropathy (DN) is the most serious, life limited complication of both types of diabetes mellitus [39]. It is a major cause of end-stage renal disease [31]. The development of glomerulopathy in diabetic patients has always been an important field of research [3].

In the current study, examination of the diabetic non-treated group revealed significant increase in blood glucose level accompanied with quantitative morphometric changes in the renal cortices with enlargement of the renal corpuscular volume, significant increase in mesangial volume, number of intra-corpuscular nuclei and vacuolated tubules. On the other hand, Bowman's spaces showed significant decrease while the thickness of glomerular basal laminae and the intensity of anti-laminin stain were insignificant as compared to the control group. These results were further supported by qualitative histo-pathological finding of glomerular sclerosis, mesangial hypercellularity, degenerated vacuolated tubules and narrowed Bowman's spaces.

Similar results have been made by several previous studies, indicating that such changes occur as a complication of short-term diabetes. On the contrary, in other researches, long-term diabetes caused increased glomerular basement membrane thickness [11, 23, 28].

Diabetic nephropathy is typically defined as a progressive deterioration in kidney function, initially represented by augmented glomerular filtration rate (GFR), glomerular hypertrophy, and urinary leakage of albumin [27]. There are several hypotheses for the pathogenic mechanisms that result in the progression of diabetes-induced renal dysfunction. In early diabetic nephropathy, glomerular pressure increases, causing an increased GFR. Glomerular hypertension and hyperfiltration may play a crucial role in the subsequent development of overt diabetic nephropathy [28].

One of the accused mechanisms is excessive production of reactive oxygen species (ROS). During hyperglycemia, the increased level of various reducing sugars in the blood enhances the production of ROS and triggers tissue damage.

Mouzannar et al 2011 [25] proved that brief and non-toxic exposures to ROS induced transduction of insulin resistance and transformation signaling in stem cells leading to diabetes. Lambeth 2007 [17] considered ROS as chemically reactive reagents with damaging effect to biomolecules including DNA, protein, and lipid. Lipid peroxidation of cellular membranes as well as DNA damage could explain the tubular degeneration with loss of brush border of PCT and pyknosis of the nuclei seen in this study.

On the other hand, ROS may also explain the mesangial expansion reported in the current work as it activates intracellular signaling pathways leading to the activation of redox-sensitive transcription factors. This in turn leads to change in the expression of genes encoding extracellular matrix proteins and the protease systems responsible for their turnover [23].

Several growth factors have been implicated in the pathogenesis of DN, through complex intra-renal systems. Transforming growth factor beta, vascular endothelial growth factor, growth hormone, insulin-like growth factor, epidermal growth factor and platelet derived growth factors are among those best known and investigated. These factors were proved by many researches to affect not only mesangial cells but also both endothelial cells and podocytes and this could explain the increased morphometric counting of intra-corpuscular nuclei seen in diabetic rats in the present study [7, 14, 33].

Moreover, enhanced expression of glucose transporter 1 and rapamycin as well as the connective tissue growth factor that mediates downstream increased fibronectin are also included in mesangial matrix expansion pathogenesis and glomerular hypertrophy in diabetes [5, 42].

In the current study, insulin administration to diabetic rats controlled the hyperglycemia and lowered blood glucose level but quantitative as well as qualitative findings revealed more evident glomerulosclerotic changes and degenerative changes. This in agreement with Richter et al 2011[30] who stated that the use of exogenous insulin injections in diabetic patients is incomplete, resulting in multiple long-term complications.

Ceriello and co-workers 2009 [5] established the concept of a "metabolic memory", that the diabetic vascular stresses persist after glucose normalization. This has been supported both in the laboratory and in the clinic and in both type I and type II diabetes. They concluded that modification of mitochondrial respiratory chain proteins can lead to excess ROS, which can lead to a catastrophic cycle of mitochondrial DNA damage as well as functional respiratory chain decline, which further triggers ROS generation and cellular injury. This aids in maintaining the activation of the pathways involved in the pathogenesis of diabetic complications via a metabolic memory which is now independent of glucose levels. Tonna et al 2010 [38] indicated that biochemical mechanisms such as advanced glycation and molecular pathways involving epigenetic events might have a role in the pathogenesis of metabolic memory.

Moreover, Buller et al 2011 [4] proved that increased renal insulin-like growth factor IGF-1 and increased nitrous oxide production during the very early stages of STZ-induced diabetic nephropathy are associated with renal hypertrophy and hyperfiltration in diabetic rats. On the other hand, natural insulin is produced as C peptide proinsulin. Proinsulin connecting peptide (C-peptide) is proved to have many functions as: activation of protein kinase C, Na+,K+- ATP-ase, nitric oxide synthase, MAP and ERK 1/2 kinases, improvement of nerve conduction velocity and interactions with exogenous and endogenous insulin so modulating the pathogenesis of complications in diabetes mellitus [40]. Additionally, administration of C-peptide to STZ-diabetic rats for 8 weeks resulted in the inhibition of diabetes-induced expansion of the mesangial matrix through inhibition of diabetes-induced excessive formation of mesangial type IV collagen [36].

In the current work, PAS stained sections showed intense reaction of the vacuolated degenerated tubules especially in diabetic non-treated rats and insulin-treated group. This could be explained by the work of Lewis et al 2006 [20] who established that, hyperglycaemia through several pathways that are interconnected with each other, can lead to the increased synthesis of sorbitol and hexosamines, glycosylation of proteins and synthesis of "advanced glycosylation end products" (AGE) as well as the alteration in synthesis of hyaluronan which is a ubiquitous connective tissue

glycosaminoglycan component of most extracellular matrices that is suggested to be involved in the diabetic nephropathy. .

Induction of inflammatory processes through receptor binding of AGEs or oxidative stress mediates changes of the structure of the extracellular matrix. Subsequent fibrosis and the extension of extracellular structures impair capillary blood flow and in particular reduce capillary density, then facilitate microvascular complications [12].

In the present study, examination of propolis treated-diabetic rats reflected effective protection and improved quantitative morphometric measures and qualitative histo-pathological findings. Fuliang el al 2005 [10] concluded that propolis can control blood glucose and modulate the metabolism of glucose and blood lipid, leading to decreased outputs of lipid peroxidation and scavenge the free radicals in rats with diabetes mellitus. Moreover, Okutanand et al 2005 [29] added that caffeic acid phenethyl ester, an active component of propolis, has several biological and pharmacological properties, including antioxidant, anti-inflammatory, anti-carcinogenic, antiviral, and immunomdulatory activities.

In 2009 El-Sayed el al [9] supported the antidiabetic and hypolipidemic effects of propolis in STZ-induced rat model. Oxygen radical scavengints activity of propolis and its potent antioxidant potential was recorded as the main protective mechanism in all these studies. Abo-Salem et al 2009 [1] supported this hypotheses by the increase in renal antioxidant enzymes including glutathione, serum superoxide dismutase and catalase and the marked decrease in malonaldehyde (MDA) in diabetic rats under propolis administration. These parameters were more tested and proved in the work of Zhu et al 2010 [45] who correlated the decrease in nitric synthetase with decreased MDA. Additionally, Kang et al 2010 [15] proved that propolis inhibits the expression of G6Pase. In 2011, Li et al [21] proved that propolis can control blood glucose, modulate lipid metabolism, and improve the insulin sensitivity in T2DM rats.

Propolis induced good glycaemic control leading to a reduction in GFR reflecting reduction of hyperfiltration, so it delays onset of diabetic nephropathy and even correct it after it starts. However, in this work, there was no significant difference between single or double dose propolis protective roles. The most evident difference was seen in PAS stained sections where double protective dose reflected markedly decreased positive reaction of the remaining vacuolated tubules.

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