REPUBLIC OF TURKEY
YUZUNCU YIL UNIVERSITY
INSTITUTE OF HEALTH SCIENCE

A STEREOLOGICAL STUDY OF THE EFFECTS OF THYMOQUINONE ON THE RENAL GLOMERULUS NUMBER IN EXPERIMENTAL DIABETIC RATS INDUCED BY STREPTOZOTOCIN

Kawa Kamal AZEEZ

DEPARTMENT OF MEDICAL HISTOLOGY AND EMBRYOLOGY

(MEDICAL PROGRAM)

MASTER THESIS

SUPERVISOR

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II

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ABBREVIATIONS

STZ	:	Streptozotocin
TQ	:	Thymoquinone
DM	:	Diabetes mellitus
DNP	:	Diabetic nephropathy
IDDM	:	Insulin Dependent Diabetes Mellitus
IIDM	:	Insulin Independent Diabetes M ellitus
GFR	:	Glomerular filtration rate
GLUT2	:	Glucose transporter 2
RDS	:	Respiratory distress syndrome
LPS	:	Lipopolysaccharide
CE	:	Coefficient error
CV	:	Coefficient of variation
H&E	:	hematoxylin-eosin
CBMs	:	Capsular basement membranes
GBMs	:	Glomerular basement membranes
TBMs	:	Tubular basement membranes
NS	:	Nigella sativa
CS	:	Camellia sinensis

1. INTRODUCTION

Diabetes mellitus is the very popular lifestyle illness distinguished via high blood glucose level and glucose intolerance due to insulin insufficiency, reduced performances of insulin work or, both. It influence 5% of the world community and turn out the third human murderer after cancer and cardiovascular sickness (Taylor, 1999). Type 1 diabetes mellitus is due to cellular-mediated autoimmune demolition of pancreatic islet beta cells, driving to lack of insulin output. It generally beginning at childhood, but has ability to take place at all ages. Type 2 diabetes mellitus is about 90% - 95% of all diabetes and had universal widespread evaluate of 2.8% in the year 2000 and is expected to be 4.4% in 2030 (Wild et al., 2004). Both Type 1 and Type 2 diabetes mellitus have complex pathophysiologies, including insulin resistance syndrome and hyperglycaemia, which are associated with abnormalities in reactive nitrogen species and fat species (Brownlee, 2001; Green et al., 2004). Streptozotocin (STZ) is surely wide vision antibiotic and cytotoxic chemical that is especially toxic to the pancreatic, insulin making beta cells in mammals (Szkudelski, 2001; Hayashi et al., 2006; Takeshita et al., 2006). It has been vastly utilized to stimulate diabetes in animal models particularly rats and mice (Brentjens and Saltz, 2001; Hayashi et al., 2006).

Diabetic nephropathy (DNP) is categorized via alteration in function as well as tubular and glomerular structure. The majority of investigation have concentrated upon changes in the glomerulus, such as irregularities in penetrability of glomerular and capillary pressure, hyperplasia of glomerular or hypertrophy and escalation in mesangial volume (Rohrbach et al., 1983; Ihm et al., 1992; Reddi et al., 2001).

It involves distinct alterations in the structure, comprising basement membranes thickening, renal hypertrophy, and progressive glomerular accumulation of extracellular matrix components (Wolf and Ziyadeh, 1999).

The basement membrane Thickening is a broadly acknowledged distinctive of small blood vessel diabetic illness (Huang, 1980). In glomeruli and capillaries in kidneys of diabetic patients, marked basement membranes thickening is monitored. Furthermore, it is

believed this alteration might result in the kidneys premature degeneration (Steffes et al., 1979; Isogia et al., 1998).

Heterogeneous metabolic illness that categorized via the change in the protein metabolism, carbohydrate and lipid is called Diabetes mellitus. That cause selective declination of pancreatic β-cells thereby prevents excretion of insulin, insulin work or both. The upsurge in level of blood glucose, which might cause critical complications, is another issue related with diabetes. In this case, it could impact different systems of the body such as nerves, eyes, heart, kidneys, and blood vessels due to long-lasting hyperglycemia. This type of Diabetes is separated into two classes: Insulin Independent Diabetes M ellitus (IIDM) and Insulin Dependent Diabetes Mellitus (Alberti and Zimmet, 1998).

So as to make experimental diabetes mellitus in animal, Streptozotocin and Alloxan are widely utilized medicines. Streptozotocin and Alloxan diabetogenic factors damage the B cell of the pancreas. In addition, they have various cytotoxic activities. Intracellular construction of hydrogen peroxide, hydroxyl radicals and superoxide radicals are generated by reaction the Alloxan. The hydroxyl radicals contain trace quantities of reactive iron within secondary lysosomes. The lysosomal hydrolases seepage and further cellular degeneration is caused by the ensuing lysosomal membrane damage. The weakness excretion of insulin results in production of nitrite, energizing of islet guanylyl cyclase and collection of cGMP, and inhibition of islet mitochondrial aconitase activity occurred when Streptozotocin through the glucose transporter (GLUT2) come in to the B cell. The local liberation of nitric oxide from STZ within islets might mediate impact of STZ on β-cells. This might results in DNA alkylation and insulin tyrosine kinase receptor activity reduction. After that, the damaged DNA makes B cells go through the destruction via necrosis (Szkudelski, 2001).

The most significant component of the volatile oil from Negella sativa seeds is Thymoquinone (TQ). It has strong antioxidant characteristics. The organs are protected by Thymoquinone against oxidative impairment enthused via a range of free radical producing agents like doxorubicin evoked cardiotoxicity, carbon tetrachloride induced hepatotoxicity, nephropathy produced by cisplatin autoimmune and allergic encephalomyelitis and

stomach mucosal damage evoked via ischemia reperfusion (Nagi and Mansour, 2000; Al-Majed et al., 2006).

This experimental research was conducted for investigating the impacts of supplementing thymoquinone in contrast to streptozotosin-induced diabetes by modern stereological approached in rats achieved via a histological evaluation. Whether if oral dispensing of thymoquinone could protect against streptozotosin that cause diabetes mellitus.

2. GENERAL INFORMATION

2.1. Kidney

2.1.1. Embryology of kidney

In vertebrates, the excretory organs are collected fundamentally of tubules of the uriniferous, originally linked at their proximal ends with the coelomic cavity by ciliated funnels (the nephrostomes). In addition, it communicate with the exterior via duct system, which is opened into the cloaca in lower vertebrates. The ducts and the tubules are originated from mesodermal, additionally they advanced from the somite stalks (the nephrostomes). A single tubule of the uriniferous grows from the nephrotome in every single mesodermal segment In the most primitive vertebrates, like the Gymnophiona and cyclostomes (Balinsky, 1975; Carlson, 1981).

Before the advanvement of a uriniferous tubule, the nephrotome is a strand of cells linking the lateral plate mesoderm to somite. The cell is divided to the cavity, visceral and parietal layers, which might be termed the nephrocoele. This nephrocoele might be continuous for a while with definitive coelom and myocoele between the two sheets of the mesoderm lateral plate. While the association with the cavity of the lateral plate continues in the higher primitive kind of vertebrate excretory organs and becomes the nephrostome, The connection of the nephrocoele with the somite cavity is shortly demolished (Balinsky, 1975; Carlson, 1981).

In vertebrates, An necessary feature is that the renal tubules are related to groups of the (glomeruli fine blood vessels) via the walls of endothelial where blood plasma comprising excretory products is filtered into the uriniferous tubules or in to the coelom in the immediate neighborhood of the nephrostomes, so as the waste products of nitrogenous might be carried via the ducts and the excretory ducts and detached from the body. The blood mass of vessels is invaginated into the wall of the renal tubule, which enlarges to contain the glomerulus and becomes the Bowman’s capsule, or else the blood vessel from a bulge on the wall of the coelom. The structure, in this circumstance, denotes to the external

glomerulus. It also referred to glomus when numerous sections are combined together. It is thought that the section of the coelomic cavity where projects of glomus is itself originated from an enlargement of the nephrocoele, or some nephrocoeles (Balinsky, 1975; Carlson, 1981).

The pronephros, which grows from those of the mid-trunk region, is caused by most anterior nephrotomes. In addition, it is originated from the nephrotomes of the subsequent section of the trunk. Therefore, these three kinds of kidneys can be classified as posterior, middle and anterior regarding to their origin (Balinsky, 1975; Carlson, 1981).

From these three kinds, the most anterior is the pronephros. In addition, it is the first to be formed. This entirely vestigial, only seeming like a slurred-over structural condition recapitulation that is present in the most primitive adults of the vertebrate stock. In the embryo, appearance of pronephros is somewhat earlier than mesonephros does. Furthermore, it pronephros forms caudal to mesonephros. During early embryonic life, principal organ of excretion is mesonephros. However, same as the pronephros, mesonephros vanishes in the adult as well, apart from for segments of its duct system. This become related to reproductive organs in male. The mesonephros is the latest to appear and the greatest caudally situated of the excretory organs. When the mesonephros is regressing, late in embryonic life it becomes functional. Additionally, it permanently persists like the functional kidney of the adultlt (Balinsky, 1975; Carlson, 1981).

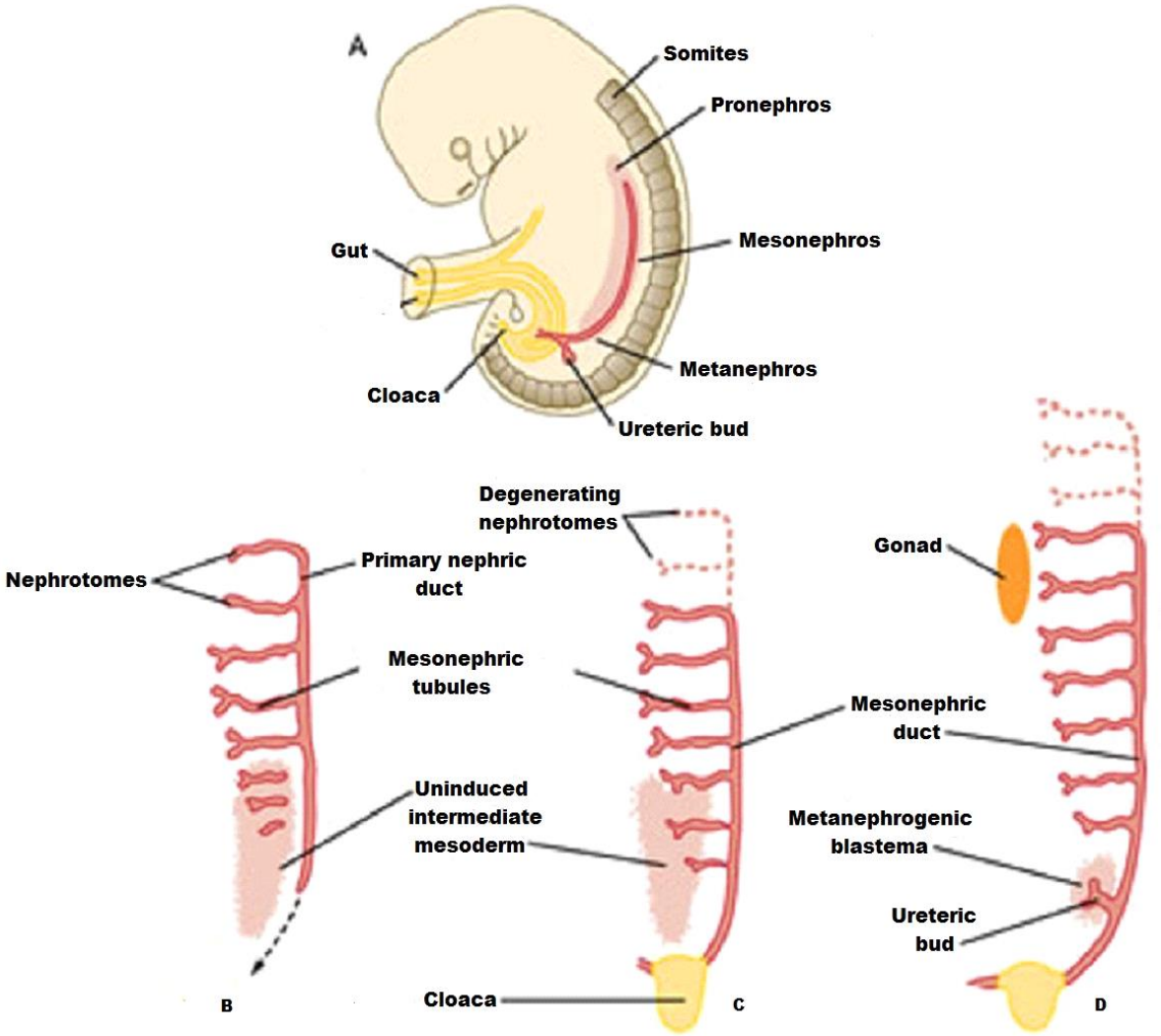

Figure 1. Graphical diagrams to demonstrate the communicatin of pronephros mesonephros, and metanephros at various stages of development. (A)Diagram showing the sub division of the intermediate mesoderm into pronephric, mesonephric, and metanephric segments. (B)Early stage showing the primary nephric duct extending toward the cloaca. (C)Establishment of the mesonephros. (D)Early appearance of the metanephros (Carlson, 1981).

2.1.2. Anatomy of kidney

It lie opposite to the wall of dorsal abdominal at the vertebrae T12 to L3 level. The middle of the kidney is approximately crosses Rib12. The left kidney is somewhat higher than the right owing to the distance employed by the great right lobe of the liver above it. Along with the ureter, renal artery and vein, urinary bladder, and the adrenal glands, the

kidneys are retroperitoneal. Although the right adrenal gland rests opposite to the surface of super medial of kidney, the left adrenal gland lies on the superior pole of that kidney (Seeley et al., 1995; Moore et al., 2002; Saladin, 2007).

Every single kidney in an adult is around 5-7 cm wide, 2.5 cm thick and 11 cm long. It is revealed there are that fat layer around renal capsule, which a layer connective tissue of fibrous the adipose capsule, and an outer fibrous membrane, the renal fascia. The kidney is protected by these structures, and attached to abdominal wall by fascia anchors. The ureter is attached at the medial hilum of every kidney and the nerves, lymph vessels and the blood leave enter and the kidney (Creager, 1992; Moore et al., 2002).

Collagen fibers provided from renal capsule, trough the lipid, to the renal fascia. The renal fascia is combine whith the peritoneum ventrally and whith the heavy fascia of the lumber muscles dorsally. Consequently the kidneys are hanging in site. Nevertheless, they falling around 3 cm when extend from a spine to a standing place, and beneath some state of affairs they become seperated and deflection even bring down, with pathological results. The renal parenchyma-the glandular tissue that produce urine look alike C-shaped in frontal part. It surround a medial space, the renal sinus, fiilled by blood and lymphatic vessels, nerves, and urine-collecting structures. Adipose tissue occuopy the residual area in the sinus and holds these structures in place (Creager, 1992; Moore et al., 2002; Saladin, 2007).

From renal capsule, the Collagen fibers are extend to the renal fascia. With the deep fascia of the lumber muscles dorsally and with the peritoneum ventrally, the renal fascia is attached. Therefore kidneys are suspended in position. However, as someone goes from a spine to a standing situation kidneys drift around 3 cm, and under several conditions kidneys become separated and drop even lower, with pathological outcomes. The renal parenchyma-the glandular tissue that produce urine look alike C-shaped in frontal part. It surround a medial space, the renal sinus, fiilled by blood and lymphatic vessels, nerves, and urine-collecting structures. In the sinus, the residual area is occuopied by adipose tissue and holds in place these structures (Moore et al., 2002; Saladin, 2007).

An outermost renal cortex around 1 cm thick and an innermore renal medulla covering the sinus are two zones of parenchyma. The cortex elongation is called renal columns extend to the sinus and distribute the medulla into 6-10 renal pyramids. With a expansive base opposing the cortex and a point blunt termed the renal papilla covering the sinus, every pyramid is conical. The overlying cortex and one pyramid and consists one lobe of the kidney. In a cup termed a minor calyx, the papilla of whole pyramid is nestled that gathered its urine. While the funnel formed by assembling of two or three major calyces , and the major calyces designed by combination of two or three minor calyces. Ureter is formed by tubular extention of the renal pelvis that urine down through it to the urinary bladder (Creager, 1992; Moore et al., 2002; Saladin, 2007).

2.1.3. Kidney Histology

2.1.3.1. Renal corpuscles

The glomerulus is the renal corpuscle comprises of a tuft of capillaries and bounded via a double layer of epithelial cells, which termed glomerular (Bowman's) capsule. Greatly adapted branching epithelial cells which called podocytes comprise the inner or visceral layer of the capsule. These cells completely invest the glomerular capillaries and adjacent to it. Parietal layer or the outer of the capsule comprise of simple squamous epithelium. For every nephron the renal corpuscle there is a preliminary section. Through the capillaries of the glomerulus, in renal corpuscles Blood is filtered. The filtrated blood enters the capsular (urinary) distance between visceral cell layers of the glomerular capsule and the parietal (Eroschenko and diFio, 2005; Junqueira and Carneiro, 2005).

Where arterial blood vessels that create the glomerulus exit and enter, every renal corpuscle has a vascular pole. The urinary pole is located on the opposite end of the renal corpuscle. At the urinary pole, filtrate that is generated by the glomerulus leaves every renal corpuscle and enters the convoluted renal tubule (Eroschenko and diFio, 2005; Junqueira and Carneiro, 2005).

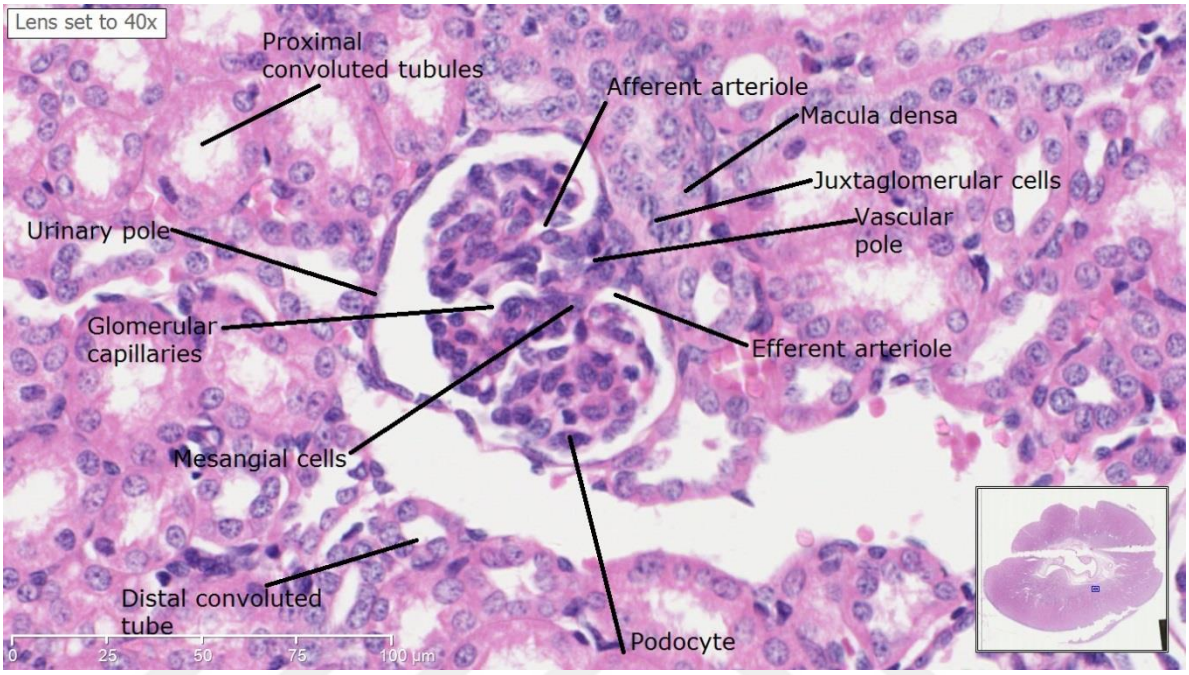

Figure 2. The kidney cortex: juxtaglomerular apparatus. Stain: hematoxylin & eosin (Eroschenko and diFio, 2005).

2.1.3.2. Proximal convoluted tubule

Proximal convoluted tubule is the glomerular capsules a prolongation which has several coils. Thick cuboidal lining cells are resting on the base membrane which has pyramidal shape. The apices are towards the lumen. A brush border with microvilli is displayed by proximal convoluted tubule. They are euchromatic and rounded central. The granules are shown by cytoplasm which is extremely eosinophilic. Because of interdigitating neighboring borders, the outline of cells are not marked appropriately. Alkaline phosphatase is included in the borders. This alkaline phosphatase located near the glomeruli. Several longitudinal stria, which are the pleated cell membranes, might be observed at the basal section. In a distended tubule, the cells might be flattened (Eroschenko and diFio, 2005; Junqueira and Carneiro, 2005).

2.1.3.3. Henle loop

It is a structure look alike U-shape compose of a thin ascending and descending limb, a thick ascending and descending limb. Structurally, the thick limbs quite the same as the distal convoluted tubule. In the outer medulla, the thick descending limb, with an outer width of around 60 mm, quikly constricts to about 12 mmm and stay as the thin descending limb. Because the wall contains squamous epithelial cells whose nuclei obtrude merely a litle into the lumen, the lumen of this section of the nephron is broad (Eroschenko and diFio, 2005; Junqueira and Carneiro, 2005).

2.1.3.4. Distal convoluted tubule

Subsequent describing a certain trajectory, the thick ascending limb of henle's loop enters the cortex. Later that, it becomes snaky and is termed the distal covoluted tubule. Same as the ascending limb, with simple cuboidal epithelium, this tubule is lined. The sructure of distal and proximal convoluted tubulesthat found in the cortex of the kidney vary. Because they have no brush border, no apical canaliculi, and smaller cells. Because the first one cells are flatter and smaller than those of the second one. the distal tubule have more nuclei than in the proximal tubule. Cells of the first one have detailed basal membrane that processed into iner layer and correlated mitochondrai sympomatic of their ion-transfering action (Eroschenko and diFio, 2005; Junqueira and Carneiro, 2005).

2.1.3.5. Collecting tubules and ducts

Collecting tubules, which combines each other for forming bigger, straight collecting ducts, broaden progressively as they moves to the tips of the medullary pyramids as urine moves from the distal convoluted tubules. The cuboidal epihtilium cell lined the iner layer of smaller collecting tubules and have a width about 40mm. As they infltrate heavy into the medulla, and cells growing in tallness till convert to columnar. The width of this duct reachs 200 mm close the apex of the medullary pyramids. Straight their whole

structure both collecting tubules and ducts are organized of cells that stain easely by the usual stians (Eroschenko and diFio, 2005; Junqueira and Carneiro, 2005).

2.1.4. Kidney physiology

2.1.4.1. Renal fuuction

The plasma portion of blood is processed and some substances are removed by the kidneys. Thus, kidneys execute a diversity of tasks. Firstly, and extremely significant, they have the essential role in the regulation of the inorganic-ion composition, water concentration as well as size of the internal environment. This is accomplished via the excretion only sufficient inorganic ions and water for keeping the quantities of these materials in the body comparatively constant. For instance, the kidneys will upsurge the quantity of the salt they excrete to regulate the intake when someone starts eating a lot of salt. Otherwise, the kidneys will excrete very little salt or almost none at all when there is no sufficient salt intake. Secondly, as fast as metabolic waste products are generated, kidneys excrete them into the urine. By this action, prevent accumulation of waste products, because which can be toxic in the body. By breakdown of protein to urea , nucleic acid to uric acid, and muscle creatine cnverted to creatinine. Lastly the last output of hemoglobin metabolism are examples of These metabolic wastes (Arthur et al., 2001; Robert et al., 2004; Stuart, 2006).

Thirdly, kidneys perform the excretion function of foreign chemicals like pesticides, drugs, and food additives, and their metabolites in the urine. Furthermore, gluconeogenesis is the fourth function of kidneys. The kidneys produce glucose from amino acid and other precursors and discharge them into the blood during extended fasting. At these times, the kidneys hav the capability to provide supply around 20% as much glucose as the liver does. Lastly, discharging at least three hormones such as renin, 1,25-dihydroxyvitamin D3 and erythropoietin, the kidneys perform as endocrine glands (Arthur et al., 2001; Robert et al., 2004; Stuart, 2006).

2.1.4.2. Glomerular filtration

Fenestrae are large pores (200 to 500 A in diameter) of endothelial cells of the glomerular capillaries. Therefore, the glomerular endothelium is thought to be fenestrated. Consequently, in comparison to the capillaries of skeletal muscles, the permeability of capillaries in glomeruli one handret to four handret time larger than capillaries of skeletal muscle for plasma, water, and dissolved solute,The stoma of glomerular capillaries are stable sufficiently tiny to inhibit to pathway of the platelets, white, and red blood cells into the filtration even though they are large. Filtrate has to pass through the basement membrane, the capillary pores and the inner layer of the glomerular capsule prior they can enter the interior of the glomerular capsule. Podocytes cell, which spaed to some extent like octopus with a bulbous cell body and numerous thick arms, composed the inner layer of the glomerular capsule. Every arm has thousands of cytoplasmic extensions called either foot processes or pedicels (Arthur et al., 2001; Robert et al., 2004; Stuart, 2006).

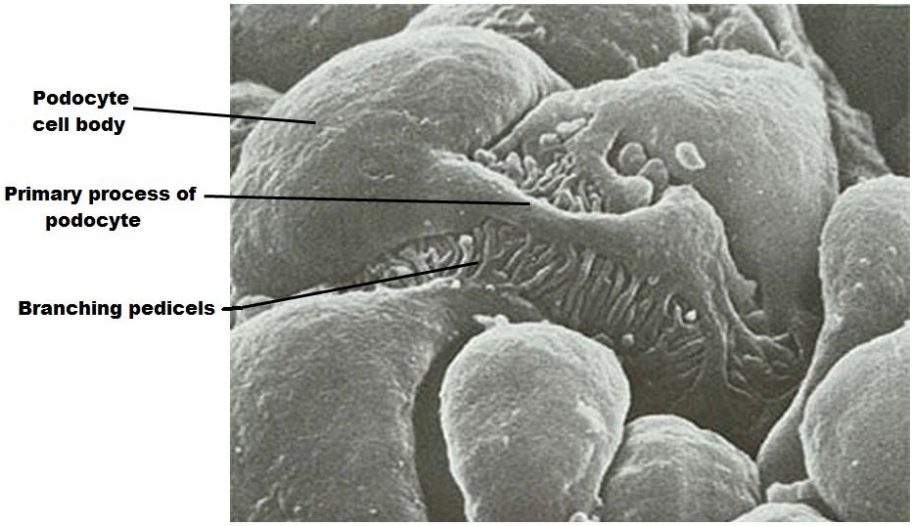

Figure 3. A scanning electron micrograph of the glomerular capillaries and capsule (Stuart, 2006).

As pedicels binding around the glomerular capillaries, it is interlace are like the digit of clasped hands. pathways through which cleared jot have to penetrate into the interior of the glomerular capsule is provided by the narrow slits between neighboring

pedicels. The fluid that enters the capsular space encompasses merely a tiny amount of plasma proteins even though the glomerular capillary pores are seemingly large enough for permitting the passage of proteins. This comparative prohibiting of plasma proteins from the filtrate is somewhat are caused by their negative charges. Specifically, in the basement membrane of the capillaries, the protein is hampered by the negatively charged glycoproteins. The negative charges and large size of plasma proteins might also limit their transition through the filtration slits between pedicels (Arthur et al., 2001; Robert et al., 2004; Stuart, 2006).

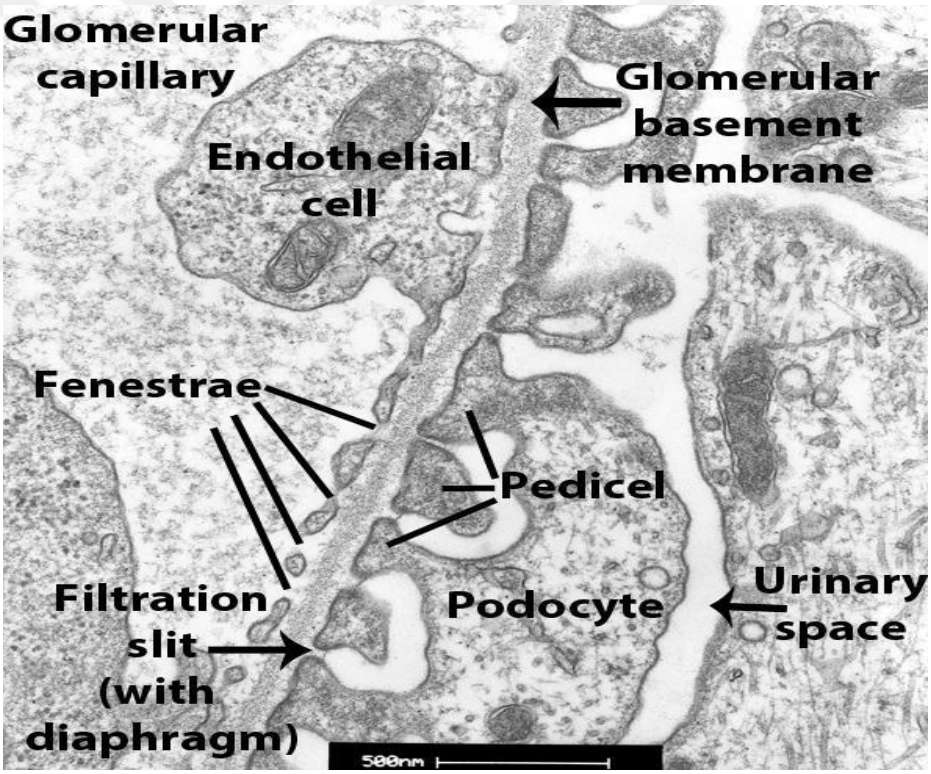

Figure 4. An electron micrograph of the filtration barrier (Stuart, 2006).

The glomerular filtration rate (GFR), in normal adults, is between 80 to 125 ml/min for women and 90 to 140 ml/min for men. Therefore, the amount of around 180 L/day of plasma is filtered at the glomerulus in a 24-hour time. The GFR decreases with age especially after age of 30. Nonetheless, the kidneys' excretory function, their ability for maintaining fluid, electrolyte, and acid base balance are not negatively impacted by this

decrease in GFR. The generation of an ultra-filtrate of the plasma at the glomerulus is the first stage in the creation of urine. The ultra-filtrate is fundamentally free of protein and is empty of cellular elements. The organic molecules (like glucose and amino acids) and salts concentration are same as in the ultrafiltrate and plasma. ultrafiltration is driven by Starling forces across the glomerular capillaries, and changes in these forces alter the GFR. Via a phenomenon termed auto-regulation renal plasma flow (RPF) and GFR are generally held within narrow range (Arthur et al., 2001; Robert et al., 2004; Stuart, 2006).

2.1.4.3. Regulation of glomerular filtration rate

The flowing blood rate to the glomerulus is affected by the Vasoconstriction or dilation of afferent arterioles, and as a result affects the rate of filtration of glomerular rate. Diameter alterations of the afferent arterioles are caused by both intrinsic regulatory mechanisms (those in the kidneys, also termed renal autoregulation) and extrinsic regulatory mechanisms (formed by innervations of sympathetic nerve). Stuart (2006) has mentioned that These systems are considered necessary to make sure that the GFR will be high enough to let the kidneys to the process of blood pressure regulation wastes elimination, although it is not so high like causing loss of water excessively.

2.1.4.4. Sympathetic nerve effects

Afferent arterioles constriction is stimulated by boosting in activity of sympathetic nerve, as happen during the exercise and reaction of fighr-or-fight. This assists diverting blood to the heart muscles and blood volume preservation. A comparable influence arises during the shock of cardiovascular as activity of sympathetic nerve stimulates vasoconstriction. The GFR decreasing and the consequential decreased formation of urine rate aid to recompense for the dropping blood pressure rapidly under these situations (Arthur et al., 2001; Robert et al., 2004; Stuart, 2006).

2.2. Diabetes Mellitus

Diabetes mellitus is an ordinary disease with elevated rates of early mortality and morbidity, leading to the disorders in neuropathic and vascular in a long-term, retinal, renal or in addition to the acute complications of metabolic. Approximately 2-5% of our people is estimated to have the type II diabetes incidence (NIDDM), which account for 80% of any type of diabetic, is. Particularly in countries where witnessed a significant change in the lifestyle, we are industrialized countries which lead to rising the incidence and occurrence of type 1 diabetes in developing countries (Bağrıaçık, 1997).

Ibn-i sina (980-1037) In the Middle Ages in Persia, illustrate the disease in a very comprehensive way from diabetes, describing it as increasing excessive appetite, reduce sexual performance, in addition that the sweet urine is. Like Aretaeus before him, in sinuses Ibn Sina also recognized diabetes in primary and secondary way.

In addition, Ibn Sina identified gangrene of diabetic used a mixture of, grass of cedar and some type of seed of turmeric for treating diabetes. In history for the first time "diabetes insipidus" has been described by Ibn Sina 17. , an anatomist scientist in the 19th century, Thomas Willis, first demonstrated that the diabetic patient's urine was sweet (Hatemi, 1996). Matthew Dobson In 1776 proved that the reason behind sweetened urine was attributable to some form of excess sugar in the diabetic patient's blood or in their urine. Paul Langerhans identified pancreatic cell types and Langerhans islets. The pancreas precursor is the pancreas-removed dogs in 1889, after which they showed all the diabetes signs and shortly, they had died. The reality that insulin existence and the function of endocrine that the pancreas plays in metabolism, have not been elucidated until 1921. scientists In 1921, Oskar Minkowski and Joseph von Mering replicated the carried out tests and they took this test one-step more showing that all the diabetes symptoms which have been left out by giving the prepared extracts from the Langerhans islets of healthy dogs pancreas to pancreas-removed dogs. The difference between diseases calls diabetes of Type 1 and Type 2 initially revealed by Sir Harold Percival Himaworth (Bağrıaçık, 1997).

2.2.1. Types of diabetes mellitus

2.2.1.1. Type 1 diabetes

Type 1 diabetes mellitus has been defined as a disease enlightened as beta cells of pancreatic produce inadequate amounts of insulin. This form of diabetes comprises a type of "immune-mediated", where the major responsibility for pancreatic beta cells losses is the T-cell mediated autoimmune response (Rother, 2007).

In young people who have a sudden onset the vital clinical characteristic of Type 1 DM is typically observed. The type 1 diabetes is typically seen as an acute disease for Patients who initially made a first application with a ketoacidosis tablature. In reality, it is the final appearance of a process that continues for many years, typically a long-term of 5-10 years. A person with characteristics of genetically diabetic might be caused by a variety of external factors (stress, physical stimuli and viruses). attack of Immune against cells of pancreatic beta starts. The attack of pathological appearance is called insulitis. Here, stimulated macrophages and T-lymphocytes attack beta cells (Yenigün, 1995).

Alteration of metabolic in Type 1 diabetes promotes lipogenesis, protein synthesis glycogen synthesis and glycolysis. Epinephrine reduces the glucose entry into the muscle in fat and muscle, and stimulates ketogenesis and glycogenolysis. gluconeogenesis stimulated by Cortisol, decreasing the use of muscle glucose. Proteolysis stimulates. lipolysis stimulated by Growth hormone and reduces the use of glucose in the muscle, promotes the metabolism of amino acid, and has an effect of insulin synergistic on synthesis of protein. Deficiency of Insulin disorders this effect. Increased anti-insulin hormones activation leads to the aggravation and appearance of changes in metabolic. In spite the fact that diabetes of Type 1 is the major deficit of deficiency in insulin, metabolic glaciations that hyperglycemia dominates following increasing plasma levels of anti-insulin hormones; osmotic dieresis, Hyperosmolarity. Acidosis and imbalance of Electrolyte occur with loss of fluid. Reduced rate of glomerular filtration with hypovolemia evolvement leads to reduce levels of electrolyte and glucose; which leads to boost in the cellular dehydration and hyperosmolarity, leading to an increase in the load of glucose in the organism. Acidosis

Cellular dehydration, primarily hyperosmolarity, have an effect on the system of central nerve (Bhatia and Wolfsdorf, 1991; Genuth, 1993).

2.2.1.2. Type 2 diabetes

The most and the widespread type of diabetes mellitus in the society is Type 2 diabetes. Although it is occurs with symptoms of classic such as loss of weight polyphagia, polyuria, polydipsia, pruritus, it frequently has a period of continuing asymptomatic (Altuntaş, 2001).

Typically, the first complaints commence in the age of 45 and above. complaints about chronic impediment such as polyphagia, polyuria and polydipsia, chronic complications such as neuropathy atherosclerotic disease of heart, nephropathy are generally chronic complications as they initially come to the clinic (Altuntaş, 2001).

Regardless of ketone bodies, hyperglycemia in urine and blood are low or absent, therapy of Insulin is frequently not essential. Ketoacidosis does not occur impulsively. kerasidosis coma develops rarely Only in unusual cases of hyperosmolarity and hyperglycemia. Ketoacidosis coma of Diabetic does not develop except there is a critical circumstance such as mesenteric artery embolism or harsh disease. The coma observed more often in these patients is the hyperglycemic hyperosmolar non-ketotic coma because of fluid withdrawal inadequately. It is acknowledged that three essential factors play a role in the diabetic hyperglycemia pathogenesis. These are increased producing glucose in the liver, resistance of insulin and impaired beta cell insulin secretion. Both factors resistance of insulin and impaired insulin secretion are controlled genetically in the of type 2 diabetes pathogenesis, and it is not until now obvious which one has a function in the weight primarily. Even though the family chronicle is nearly all, the disease has not yet established on a single layer genetically. Yet again, most forms of se 2 diabetes are related to overload genetically (Altuntaş, 2001).

Obesity and Type 2 diabetes is also extremely closely connected to each other. Type 2 diabetes develops without obesity even though rising insulin resistance of obesity leads to the rising in severity of hypeglycemia. Consequently, the discrepancy between creatures of type 2 diabetes in obese and non-obese is difference etiologically. Hence, while in obese Type 2 diabetes insulin resistance is significant, non-obese Type 2 diabetes also dominates in secretion of impaired insulin (Altuntaş, 2001).

Insulin resistance of uferic, main insomnia in insulin resistance syndrome (syndrome X), and happens prior to other findings. It is pursued by hypertension and hyperlipidemia obesity, atherosclerosis. There is a particular association between Hyperinsulinism and truncal obesity. The assumption of this hypothesis that hypertension development may contributed by hyperinsulinism by stimulating in the arterial wall proliferation, accelerating sodium renal tubular re absorption, enhancing activity of sympathetic nervous system, and eventually slowing the synthesis of lipoproteins in a very low-density from the liver and slowing them away from the environment. thus of the development of conditions of cerebrovascular, disease of coronary artery and atherosclerosis are common in these patients (Altuntaş, 2001).

Many assumptions have been presented regarding the occurrence cause of type 2 diabetes. It is acknowledged that central obesity (the type of obesity caused by surrounding organs with fat the in the abdominal region, not composed under the skin) inclines individuals to insulin resistance. Active fat mass hormone, composed in the abdominal region, A secretions of a group of hormone named adipokines and these hormones almost certainly interrupt tolerance of glucose. Roughly 55% of patients of Type 2 diabetic are obese (Eberhardt et al., 2005).

Other factors comprise inheritance (the disease is more common in family history in people of Type 2 diabetic) and old age (Approximately 20% of diabetic people are among the elderly in North America) and. Over the past decade, young people and children have been a victim of the type of type 2 diabetes. This boost is strongly related to an increasing number of child obes- tives in some countries in the past decade (Rosenbloom and Silverstein, 2003).

Lately, environmental pollution may have an effect to increase in the type 2 diabetes rate. There is a positive association between the Type 2 diabetes incidence and the bisphenol A amount in the polycarbonate plastics composition (Lang et al., 2008).

2.3. Gestational Diabetes

Gestational diabetes and type 2 diabetes are comparable in many respects. In this disease There is also a low insulin secretion level moderately and a reduce response in insulin. It is noticed in 2-5% of all pregnancies and might vanish following the birth. Gestational diabetes is a treatable situation nevertheless it should be observed cautiously during the entire pregnancy. Approximately 20-50% of women become type 2 diabetic in the late life period as the gestational diabetes develop in their pregnancy (Avery and Mead, 1959).

In spite of a temporary situation, untreated gestational diabetes can be perilous to both the life of mother and the fetus. Examples of the risks that happen to the baby are skeletal muscle malformation and deformity of the central nervous system, Macrosomia (high birth weight) and congenital heart. Production of fetal surfactant might be suppressed by Elevated levels of fetal insulin and (neonatal respiratory distress syndrome, also called Hyaline membrane disease, RDS), might be effected. This might lead to developmental disorders of various pulmonary that happen at birth (Avery and Mead, 1959).

Destruction of red cell might be the reason of Hyperbilirubinemia occurrence. In more serious cases, stillbirth might happen in consequence of inadequate feeding of placenta because of vein disorders that feeds the fetus. Reduced function of the placenta might necessitate birth artificial initiation. Birth might necessitate to be prepared by section of caesarean if there is a damage risk of correlated to the macrosomia or If there is a major distress of fetal (Avery and Mead, 1959).

In some ways, pregnancy may be affected by Gestational diabetes. continuing of Spontaneous abortions, Infections of Urinary is liable to increase; they are common are

pyelonephritis and pyelitis. This is a rising aspect in perinatal mortality. The more common is Preeclampsia. Adjusting tension and resting at bed are significant for the mothers with the aim of protecting the fetus. In 1/3 Polyhydramnios; happens in congenital birth and Causes prematurity and diabetic pregnancies. (Bağrıaçık, 1997).

2.4. Experimental Diabetes

For generating experimental diabetes, experimental animals like mice, rats, monkeys, dogs, rabbits, guinea pigs, hamsters, pigs and cats can be utilized. In experimental animals, this kind diabetes can be prompted via chemical agents, suddenly or by virus (Alarcon-Aguilar et al., 2000).

For this purpose, streptozotocin (STZ) and Alloxan are the most utilized chemical agents. Structurally, Alloxan monohydrate [2,4,5,6 (1H, 3H) -pyimidinetetrone] is a uric acid derivative. This agent has to be stored in powdery state at 2-8 ° C. it easily dissolves in water and the solution state should be stored at 4 ° C. It is stated that pancreatic beta cells are injuring selectively for causing insulin dependent diabetes (Dunn et al., 1944; Pushparaj et al., 2000).

2.5. Streptozotocin (STZ)

Streptozotocin (STZ), 2-Deoxy-2- (3-Methyl-3-Nitrozoureido) -D-Glucopyranose. STZ, the metabolite of Streptomyces Grisseus, has antibiotic, diabetic, antitumoral and carcinogenic effects. It is straightly toxic to pancreatic B cells. STZ that binds to the receptors of glycoprotein on the plasma membrane blocks glucose-stimulated secretion of insulin as it includes a glucose molecule in its structure. Nuclear DNA is one of STZ's key impact areas. The decay of STZ in the cell formed Reactive carbonium ions via causing alkylation in DNA bases. Via damaging B cells, the pancreas causes both insulin-independent and insulin-dependent diabetes. while single-dose intraperitoneal or intravenous injection of 100 mg / kg STZ to newborn rats, independent of insulin, single

dose (40-60 mg / kg) intravenous injection of STZ in adult rats is stated to result in insulin-independent diabete (Öntürk and Özbek, 2007).

STZ leads to fast and permanent necrosis of B cells. Its poisonousness is connected to the glucose molecule in the structure of pancreas-specific. It combines with the glucose receptor on the cell membrane and averts glucose-stimulated insulin release as the glucose molecule is contained in the structure. B Pancreatic cells comprise high amount of glucose transporter (GLUT2). While STZ is being transferred to the cells by GLUT2, Other glucose carriers do not recognize it. It was found that decrease in GLUT2 expression to avert the diabetic impact of STZ. Because the primary cause of B cell death, STZ has been confirmed to be the alkylation of DNA. DNA repair is followed when DNA damage induced by STZ. A nuclear enzyme and polyADP, is activated in excess of ribose syntheses during DNA repair. Consequently, cellular NAD + and ATP content decrease. The death of B cells could resulted from declining NAD + IN, an important element of oxidative metabolism. Therefore, insulin secretion and synthesis are repressed (Szkudelski, 2001).

The characteristic alteration in glucose concentrations and blood insulin are coexisted with the impact of STZ in B cells. A triphasic response happens in blood glucose after STZ has been administered. within the first 120 minutes blood sugar is elevated, this transitory of hyperglycemia is caused by the rapid damage of the glycogen in the liver and can be abolished or decreased when the animal is starved for around 18 hours prior the diabetic agent is applied. In hyperglycaemic period, plasma insulin levels are low. The consequence of epinephrine discharge is believed to be Heptatonic oxygen deposition. Within the first 24 hours following diabetic drug administration, the hypoglycaemia is typically accountable for deaths. in addition, a sweetened liquid administer to the animal is suggested. The death of B cells Hypoglycemia relies on the extreme discharge of insulin. During this period, plasma insulin amount were very high. The phase three begines in 10-12 hours, which is the time of hyperglycaemia. After that, the levels of Plasma insulin are declined and persist low for 30 days (Rajasekaran et al., 2005; Erbayraktar et al., 2007). Intraperitoneal injection of a freshly prepared STZ solution (stored in icy medium) in 20 mM sodium citrate buffer (pH: 4.5) intraperitoneally (as single dose) to 65 mg / kg induced Diabetes mellitus with streptozotocin in rats Diabetes (Öntürk and Özbek, 2007).

Another research exposed rats with 60 mg / kg of STZ dissolved in 0.1 M citrate buffer (pH: 4.5). In this study, measurements of fasting blood glucose after 72 hours was performed for determining of blood glucose level when the range more than 350 mg. After STZ application, water and feed intake has been released. The research stated that a single dose of 50 mg / kg STZ was applied to rats whose fasting of blood glucose level was higher than 250 mg / dL .Identified as diabetic (Öntürk and Özbek, 2007).

Measurement of fasting blood sugar level the auto-analyzer tool was applied for measuring blood sugar in the biochemistry laboratories. It is suitable to perform this measurement after blood is measured throughout the research since more blood is required for the auto-analyzer compared to other devices. Nevertheless, when the level of blood glucose is t measured during certain days. Selecting devices that operate with a drop of blood would be more suitable. The tail of the test animal, a drop of fresh blood from is engrossed into the strip of the measuring tool. The level blood sugar is read from the device after 15 or 20 seconds. The level of glucose of these devices is calculated via the "glucose-oxidase peroxidase" approach (Öntürk and Özbek, 2007).

2.6. Thymoquinone (TQ)

The major bioactive component of the volatile oil of the black seed (Nigella sativa, Ranunculaceae family) is Thymoquinone ($C_{10}H_{12}O_2$; molecular weight: 164.2). In addition, this substance has been utilized as antioxidant, anti- inflammatory and antineoplastic medicines for more than 2,000 years (Yi et al., 2008; Rogozhin et al., 2011). previous researches illustrated that thymoquinone exhibit inhibitory impacts on cell proliferation of several kiinds of cancer cell lines, such as human pancreatic adenocarcinoma, neoplastic keratinocytes ,ovarian adenocarcinoma, breast adenocarcinoma (Shoieb et al., 2003), lung carcinoma, colorectal cancer, human osteosarcoma, uterine sarcoma, fibrosarcoma, (Worthen et al., 1997; Halagali-Muhtasib et al., 2004). Inhibitory reasons on corneal neovascularization in the rat form (Erdurmus et al., 2007), is showed by Thymoquinone, whether it constrains tumor angiogenesis and destroys the growth of prostate tumor during tumor angiogenesis deterrence (Yi et al., 2008).

Al-Majed et al. (2006) revealed that thymoquinone is characterized by severe antioxidant possessions. It is active in protecting rats against transitory forebrain ischemia-encouraged impairment in the rat hippocampus. thymoquinone a promising agent is produced by this great defense in pathologies involving neurodegeneration for instance cerebral ischemia.

Hadi et al. (2016) expressed that Nigella sativa could decrease oxidative stress and improve inflammation in patients with rheumatoid arthritis. The investigation revealed that recommended that Nigella sativa might be a valuable supplement treatment in these patients.

It is stated that the mixture of tamoxifen/ thymoquinone had a superior impact compared to these drugs alone on cases with breast cancer. Thus, supplementing thymoquinone to tamoxifen might characterize a new healing modality for breast cancer management (Kabel et al., 2016).

It has been stated that thymoquinone has positive impact in curing and the preventing kidney stones or renal failure through different mechanisms for example anti-oxidative, anti-eicosanoid, anti-inflammatory and immunomodulatory impact. Thymoquinone has been expressed is the anticipated candidate in several cases however it appears that at least in part, mostly in kidney stones. In addition, the herbal melanin have a role however, it required to be examined further to confirm. In preventing and curing of renal illness in addition to nephrolithiasis and renal damages, N. sativa and its components are valuable (Hayatdavoudi et al., 2016). Lipopolysaccharide (LPS) induced depression, like actions in rats can be prevented by Thymoquinone (Hosseini et al., 2012).

According to Kapil et al. (2015), thymoquinone has activity against antibacterial Pseudomonas aeruginosa, Escherichia coli, Salmonella Typhimurium, Staphylococcus aureus, Shigella flexneri, Salmonella Enteritidis. It also inhibits Pseudomonas aeroginosa, Klebsiella pneumonia and Proteus mirabilis, Staphylococcus epidermidis.

For sickle cell disease treatment, numerous varicose drugs have been investigated drugs for example piracetam, hydroxyurea and calcium antagonists. The majority of these

drugs are perhaps poisonous and are not appropriate for lon-gterm treatment. Newly, Nigella sativa (NS) has been investigated to have calcium antagonist and antioxidant activities, each of w hich plays an important role in the management of the disease (Ibraheem et al., 2010).

2.7. Stereology Method

This science is recent, very active and quickly emerging. It is a subdivision of morphometry, which is utilized in taking three-dimensional data from two-dimensional sections from nearly any kinds of structure (metallurgical samples, biological structures, etc) (Altunkaynak et al.,2011, Sagheb and Moudi,2014). Furthermore, it is allocate of approach for gaining certain and effective information on morphometric from segments of an object of significance (Méndez et al., 2007).

Recognizing the structural inner three-dimensional arrangement action on the collapse of the structure section that indicate merely two-dimensional information, the stereology is the challenge (Mandarim-de-Lacerda, 2003).

Stereology approach depends upon applied statistics and mathematics with the assistance of a group of regulation, which permit various factors like constituents, dimensions and volumes is expected. Even though computing factors in histology like length, weight, and counting parts look simple, as these factors are assessed at the level of the microscopic, practice approaches are not simple and time effective. Direct measurement or utilization of ordinary techniques are not an option and microscopic techniques are comprise to confirm these parameters under microscopic conditions. Stereology approach supplies knowledge on shapes, images, and stereograms, and has pragmatic approach for identifying images and permit measurement of volumes and volume ratio, the area of samples, the number of particles per unit volume, particle size, unite volume. Therefore, for achieving dependable measureable information for a range of researchers in the field of histology this method is very significant (Sagheb and Moudi, 2014). In stereology

practicing, tools (grids, points, lines) are placed over surfaces cut through our specimens (histological or magnetic sections) (Altunkaynak and Ozbek, 2009).

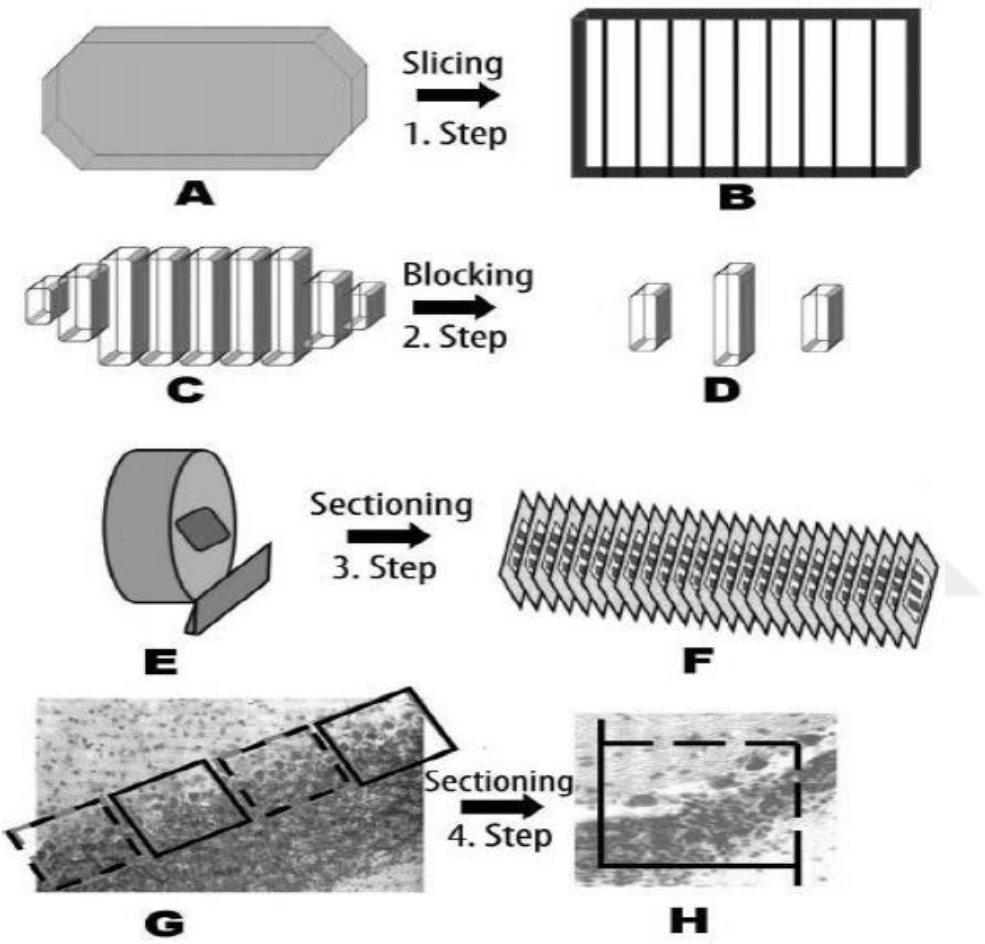

Figure 5. All possible steps of systematic sampling. Tissue slicing (A and B; step1), blocking (C and D; step 2), sectioning (E and F; step 3), microscopic evalution (G and H; 4) (Altunkaynak and Ozbek, 2009).

2.7.1. Disector

In the Dissector approach, the organ or tissue is be calculated by segment reception of numerous particles. In addition, particles takes orientation, direction and contraction. The expansion and contraction is assessed independently throughout the histological research. It is significant to be conscious throughout the management of distort for counting

all particles merely once. For accomplishing this, two consecutive segment have to be utilized, random sampling systematic selecting, and particle profiles counting, that noticeable in one segment while lacking in the other (Altunkaynak et al., 2012; Howard and Reed, 2005).

$$N=\Sigma\ \bar{Q}\times 1/asf\times 1/ssf$$

2.7.2. Fractionator

This is a sampling scheme joint with several kinds of measurement of stereological causing an impartial assess of the total amount of interest. An extrapolation is done for reaching at an approximation for the fractionators (Sterio, 1983; Gundersen, 1986; West et al., 1991; Altunkaynak et al., 2012). The design of physical dissector is the traditional projector of the dissector-Cavalieri kind. The blocks are not divided thoroughly in this design, for example merely a single segment pair for each block is normally required (Ddorph-petersen et al., 2001; Howard and Reed (2005).

Upon a direct particle count in a recognized predetermined fraction of the containing space the physical fractionators design is established. Firstly, the containing space is arbitrarily alienated into blocks. Secondly, blocks predetermined fraction is tested, after the tested blocks are partitioned comprehensively and once more a fragments predetermined fraction is tested (in successive pairs). Via impartial counting frames, a predetermined fraction of the space of the segment is sampled. Ultimately, via physical dissectors, particles are tested (Dorph-Petersen et al., 2001). A three- dimensional probe is required for guesstimating the number of objects in a three-dimensional region so as the particles leading edge is recognized. Pairs of thin sections is required to utilize thin section. While a numerical density is multiplied by the reference in physical dissector, the count up is concluded for the physical fractionator (Sterio, 1983; Gundersen, 1986;Howard and Reed 2005).

For fractionator designs, there are some particular necessity:

At each level, the testing fraction have to be identified. The shape and volume of the containing area are not related. Via multiplying the number of particles sampled by the reciprocal of the sampling fractions, the entire number of particles N is projected:

$$N = 1/bsf.1/ssf.1/asf. \Sigma \bar{Q}$$

As:

- bsf represents the sampling fraction of the block.
- ssf appear the fraction of sampling sectioned.
- asf symbolize the area of sampling fraction.
- $\Sigma \bar{Q}$ demonstrates the entire count of particles lastly tested by physical dissectors.

This number approximation is totally self-governing of distortion issue. Thus, for number approximation, the physical fractionator is the gold standard (Sterio, 1983; Gundersen, 1986).

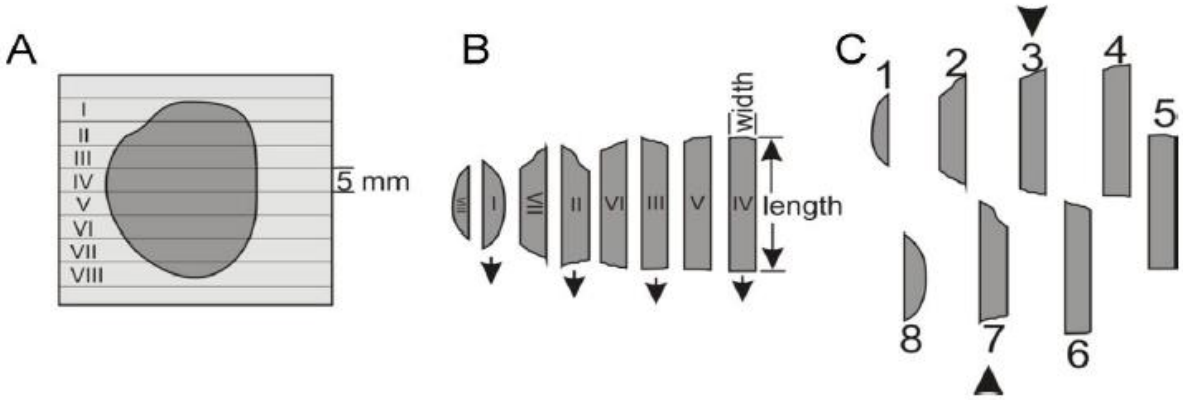

Figure 6. Smooth fractionator sampling (Tschanz et al., 2014).

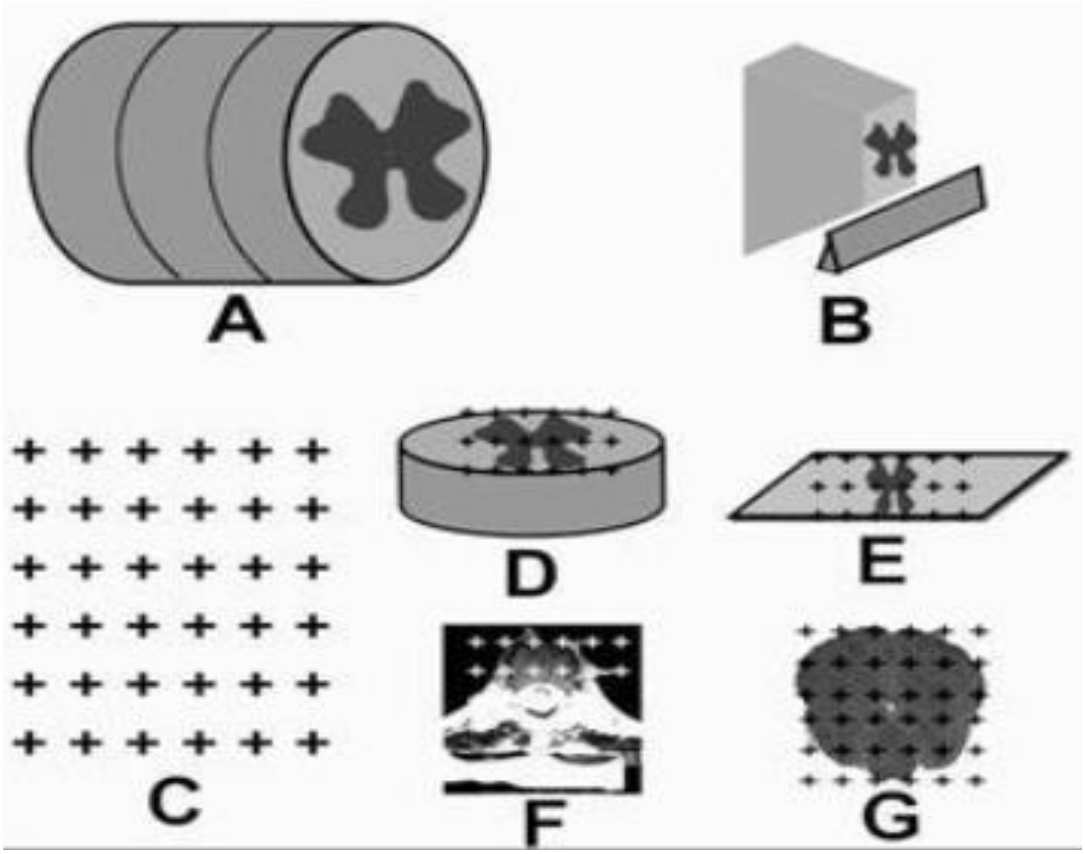

Figure 7. A simple procedure for the volume estimation using the Cavalieri's principle.. This method can be applied to physical slices of tissue (A,B,C,D), as well ashistological sections (E), MRI images (F) and microscopic areas (G) (Altunkaynak et al., 2011).

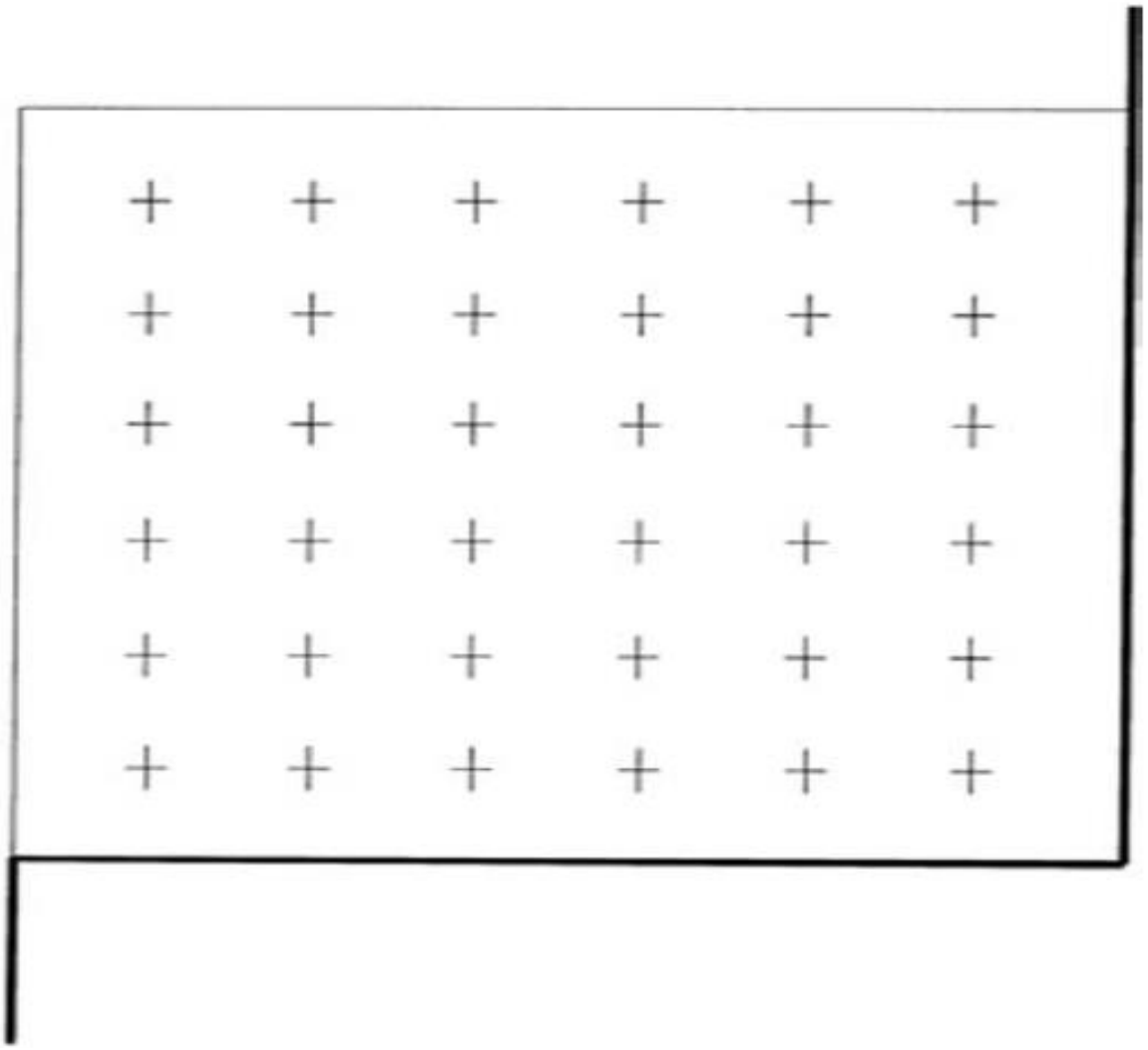

Figure 8. A test-system with 36 points. This system without lines allows fast counting and can be appropriated to majority of uses (Mandarim-de-Lacerda, 2003).

3. MATERIALS AND METHODS

3.1. Material

Twenty eight healthy female Wistar albino rats, weighing 180–220 g and averaging 20 weeks old were utilized in this study. They were housed in rat cages (Figure 9) under standard laboratory conditions as 25°C and a 12 h light/dark cycle. The rats were allotted into four experimental groups each group contain seven animals: A (Control), B (Thymoquinone), C (Streptozotocin) untreated, D Streptozotocin treated with thymoquinone. A received single intra-peritoneal normal saline 0.5 ml/kg, B (Thymoquinone) second group received 4mg /kg / day TQ for eight days is provided with drinking water, C and D groups received STZ (Sigma). Diabetes was induced in two groups by a single intra-peritoneal injection of STZ (40 mg/kg, freshly dissolved in 5.5 mmol/l citrate buffer, pH 4.5) Streptozotocin (STZ). Three days after STZ treatment, development of diabetes in two experimental groups was confirmed by measuring blood glucose levels in a tail vein blood samples. Rats with blood glucose levels of 250 mg/dl or higher were considered to be diabetic. Blood glucose levels in control animals remained normal for the duration of the study. Diabetes mellitus measurement was confirmed by eBsensor Glucometer (Taiwan) (Figure 10). The rats in TQ treated group (D) were given TQ (4 mg/kg body weight) is provided with drinking water starting two days after STZ injection. The initial and final body weight changes of the various groups were recorded. Rats were anaesthetized intra-peritonealy with ketamine 50mg/kg, and xylazine 4mg/kg , followed perfusion by 0.5 ml heparin, isotonic sodium chloride 30 seconds, followed for about 45 seconds by 10% neutral buffered formalin.

Final report of research project detailed above was approved by Yuzuncu Yil Univerasity Animal Research Local Ethic Committee in the session held on 9/8/2016 under number TYL-2016-5369.

Figure 9. Rats' cage

Figure 10. Glucometer

Figure 11. Perfusion process

3.2. Method

Following perfusion (Figure 11), the right kidneys were weighed and removed, after standing for that received 10% neutral buffered formalin for fixation followed and will be routine for 72 hour, then the rat's kineys were systematically and randomly sampled for stereological evaluation. The kineys were fixed in bouin's fixtive for 48 hour, after that washed by tap water for removing picric acid, Prior to embedding, the kidneys were dehydrated through a graded series of ethyl alcohol solutions 70%, 80%, 90%, 95% for 2h each, embedded in paraffin wax, and serially sectioned using a rotary microtome (Figure 13). Serial sections of 5µm thickness were mounted on glass slides by cutting tissue block, the first section was chosen randomly while the next section was taken after cutting every 30section. About nine to 11 sections were taken. As the second step of the stereological procedure; selected sections were stained with hematoxylin-eosin (H&E), and photographed on the computer monitor (PC) screen using a light microscope with a digital color camera attachment and dial indicator. The unbiased Camera (CM) was applied to the

light microscopic images for the stereological estimation of kidney volume, and number of glomeruli using the SHTEREOM 1.5 program. The CE and coefficient of variation (CV) were estimated according to Pakkenberg and Gundersen (1988) formula.

Vertical section stereology is valid on the same terms as standard stereological methods for isotropic random sections. The vertical axis direction is freely chosen, which makes the sampling procedure simple. Figure 12 shows vertical sections in an organ (Mandarim-de-Lacerda, 2003).

The glomerular counting was based on physical dissector (dissector-cavalieri combination) that photographed on the computer moniter (PC) screen with light microscopic (x4) magnification with (7cm) real linear. The volume area was estimated according to Pakkenberg and Gundersen (1988) formula:

$$\text{Volume}(\mu m^3) = t(\mu m) \times (a / p)(\mu m \times \mu m) \times \Sigma P$$

Volume: kidney volum

T = section thickness

(a/p) = represent of the point area

ΣP = the total number of points per section

This formula were using for measuring area

$$A = \Sigma p.\ (a / p)$$ (Kaplan et al., 2010)

This formula were using for calculating glomerulus

$$N = \Sigma p \times \bar{Q}^{-} \times k \times \frac{(a/p)}{a(frame)}$$

N = the total number of glomeruli, $\bar{Q}^{-}$ = average number of points in the sample space, Σp = Total number of dots, a / p = point area, k = section thickness, $a_{(frame)}$ = frame area.

The number of glomeruli was photographed on the computer monitor (PC) screen with light microscopic (x10) magnification. Then coefficient error for each animal (CE) was determined from the number of sections proficiency test. The number of animals in experiments to test proficiency coefficient of variation (CV) of the animals in the group considering making changes due to the biological variation of the application were determine whether sufficient in number. This way it will avoid an effective result without further processing. In addition, the value of the group was compared with statistics (Altunkaynak and Ozbek, 2009; Kaplan et al., 2010).

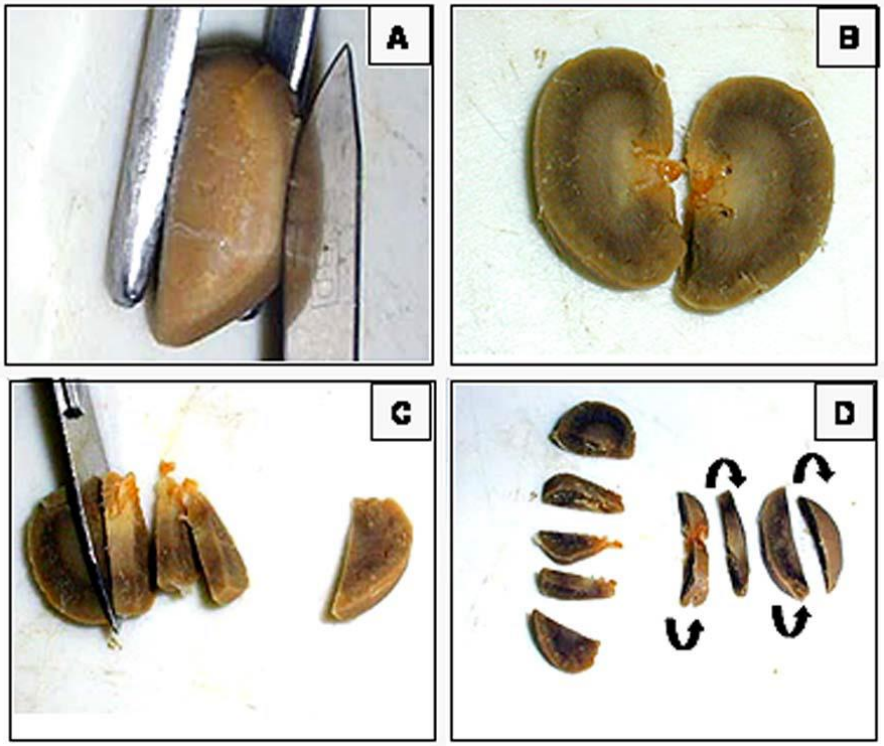

Figure 12. Application of the vertical section is method in rat kidney. For the kidney, select an arbitrary horizontal reference plane (e.g. the laboratory bench). Once a reference plane has been selected for a particular object it is then considered as fixed. Generate an isotropic orientation in the horizontal plane (A and B). Section the kidney with uniform random position along this orientation (C and D). The object is given an isotropic rotation on the horizontal plane (D) (Mandarim-de-Lacerda, 2003).

Figure 13. Tissue processing devices (fixation, blocking, cutting)

Bouin's fixative:

Picric acid	375 ml (71%)
Formalin 40%	125 ml (24%)
Acetic acid	25 ml (5%) (Crookham and Dapson,1991, Carson,2015)

10% Neutral Buffered Formalin:

40% formalin	100 ml
Distill water	900 ml
Sodium phosphate, monobasic, monohydrate	4 gr
Sodium phosphate, dibasic, anhydrous	6.5 gr

3.2.1. Histological Staining Process

Hematoxylin-eosin (H&E) stain

1.The slides put in oven 80 C^0 for about 30 minutes for keeping section and paraffin melted adhesion.

2. The slides were puted In three different xylene 5 minutes for each.

3. The slides were puted In three different alcohol 5 minutes for each.

4. The slides were left in tap water for about 30 seconds.

5. For staining the slides putted in hematoxylin stain for about 3-4 minutes.

6. Then the tissues were washed by tap water for 30 seconds.

7. In % 0.3 ammonia solution for about 5 seconds were kept

8. In tap water for about 30 seconds were washed.

9. In eosin stain for about 2-3 minutes were kept

10. In tap water for about 30 seconds were washed.

10. % 95 ethyl alcohol for about 5 minutes were kept.

11. Then the slides were kept in xylene 5 minutes.

12. The slides were mounted by mounting media and coverd with cover slides for examination.

3.3. Devices

1. Oven, Wiseven (Korea)

2. Tissue processing device, Leica asp 300 (Germany)

3. Blocking device, Shandon Histocenre 2 (England)

4. Monitor Sony Trinitron multiscan G520 (Japan)

5. Photo maker, Sony DSC-S 58 (Japan)

6. Refrigerator Beko 8460 T (Turkey)

7. Pure Water device, Kronsclinic (England)

8. Light Microscope, Zeiss Axioskop 40 (Gottingen/Germany)

9. Photo attachment, Zeiss axiocam (HRc) Carl Zeiss vision GmbH (Germany)

10. Water bath Thermo Shandon (England)

11. Microtome Leica RM 2125 RT (China)

12. Glucometer tool, eBsensor (Taiwan)

13. Paint pots

4. RESULTS

As showed in Table 1 and Figure 13 Mean body weights of rats in all groups 1,3,9 days during experimental period were recorded.

Table 1. Effect of TQ on body weight in STZ-induced diabetic rats (g).

Groups	First day	Third day	Ninth day
Control	174	177	175
TQ	178	181	179
STZ	176	166	161
STZ +TQ	190	165	164

There was not significant change in control and TQ groups' body weight. Animals in the STZ and STZ + TQ groups a significant weight loss was observed. As showed in table 1 and figure 14.

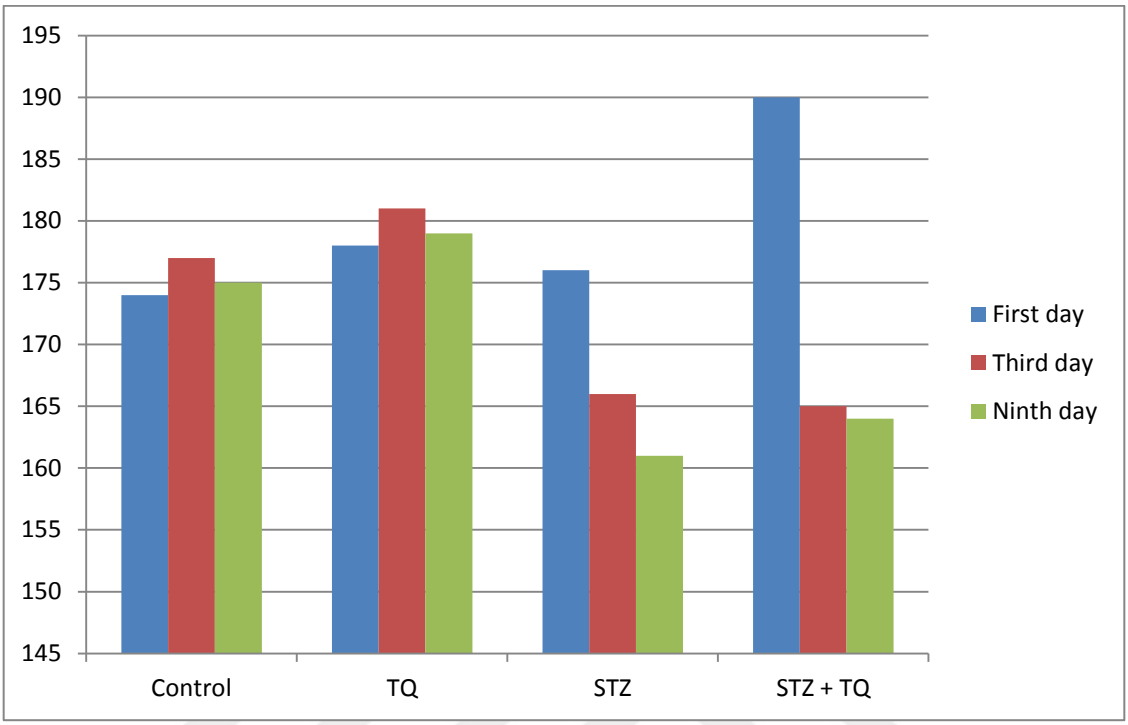

Figure 14. Effect of TQ on body weight in STZ-induced diabetic rats (g)

Before, 72 hour after injection of STZ and last day during experimental period blood glucose level of all groups were recorded.

Table 2. Effect of TQ on blood glucose in STZ-induced diabetic rats (mg/dl).

Groups	First day	Third day	Ninth day
Control	109	112	112
TQ	108	118	119
STZ	107	510	553
STZ +TQ	107	540	464

Effect of TQ on blood glucose in STZ-induced diabetic rats were summarized as showed in table 2 and figure 15. shows change in blood glucose levels of control and

diabetic rats during the experimental period. After STZ and STZ+TQ injections, the blood glucose levels increases gradually in STZ group but in STZ +TQ group show significant decrease while in Control and TQ group the blood glucose level remained normal during experimental period.

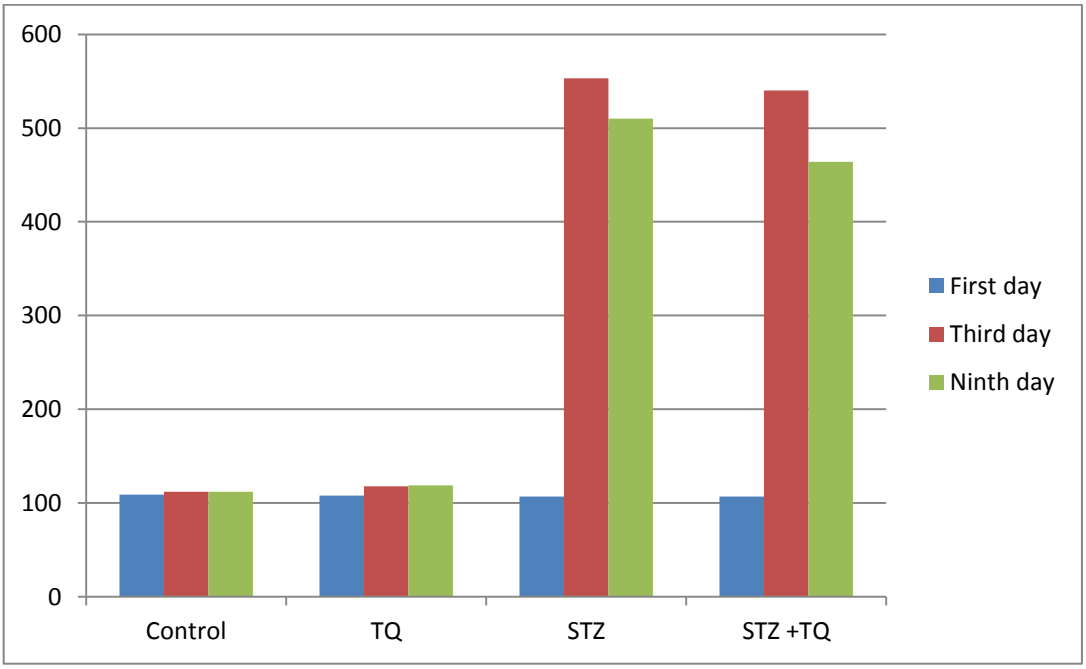

Figure 15. Effect of TQ on blood glucose in STZ-induced diabetic rats (mg/dl)

4.1. Stereological Results

Statistical Analyses were performed, using Microsoft SPSS Version 13.0 for statistical analyses. Kruskal-Wallis test was performed to compare groups, and it were applied to compare the control and treatment groups with respect to volumes of the kidney, and the total mean number of the glomeruli. All statistical values under 0.05 were considered significant.

Table 3. The total volume of four groups.

	Groups	Median	Mean	St. Dev.	Min.	Max.	p.*
Volume (cm3)	Control	479520	504977,1428 b	8874443,3387	36774000	63828000	0,01
	TQ	526500	533674,2857 b	12664994,2079	40176000	75816000	
	STZ	701460	698966,6666 a	2564734,2682	65934000	72576000	
	STZ+TQ	742770	740340 a	2553235,1243	69498000	77274000	

As showed in Table 3 and Figure 16, The results revealed that the total kidney volume for each of the of the groups (control and Thymoquinone (TQ)), there was no significant difference. Furthermore, When Thymoquinone (TQ) + Streptozotocin (STZ) and Streptozotocin (STZ) group was compared with control group, a significant increase was detected (P= 0,01).

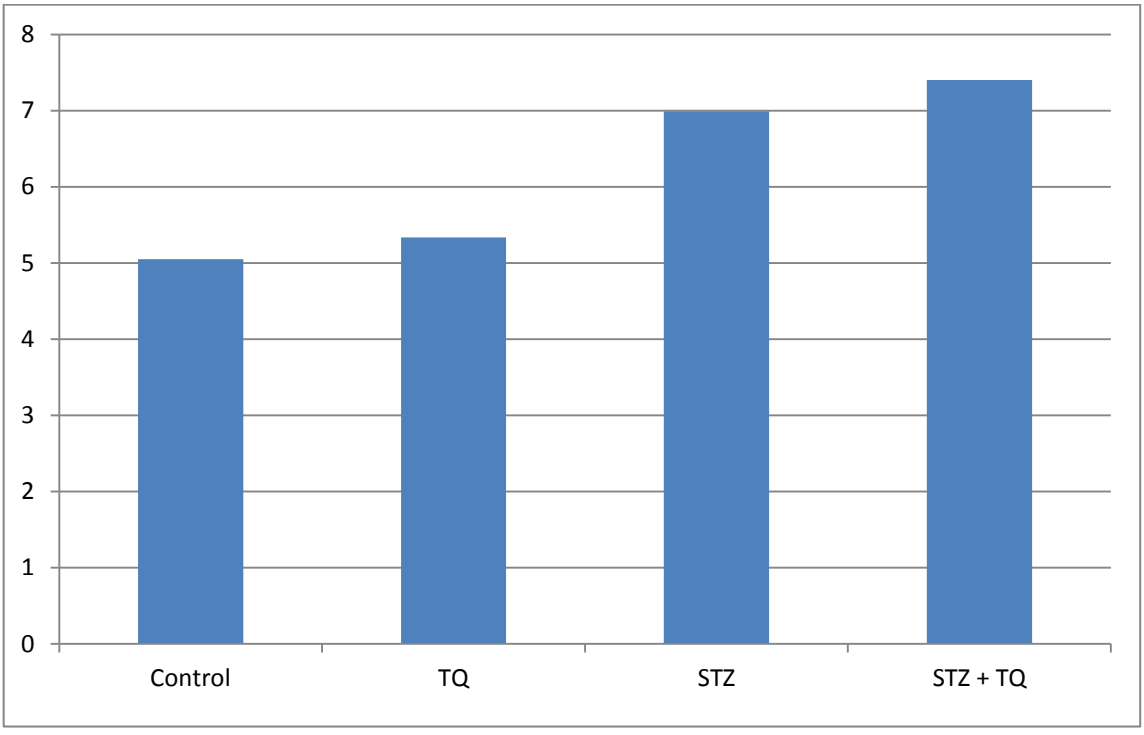

Figure 16. Kidney volume (cm^3)($\times 10^5$).

Table 4. Total number of the glomeruli.

	Groups	Median	Mean	St. Dev.	Min.	Max.	p.*
Glomeruli	Control	31307,00	31338,00 b	909,17	31208,00	31466,00	0,01
	TQ	32927,00	32992,00 a	3811,41	32534,00	33484,00	
	STZ	24309,50	24405,83 d	3360,69	24062,00	25007,00	
	STZ+TQ	30606,00	30566,67 c	2046,53	30211,00	30758,00	

In addition, in the Streptozotocin (STZ) group the total glomerular number were decreased when compared to control group. However, Thymoquinone (TQ) group indicated an increase in glomerular number in comparison to control group. Moreover, in the treatment of Thymoquinone (TQ) + Streptozotocin (STZ) group were decreased in comparison to control group while the total number of glomeruli were increased in the comparison to Streptozotocin (STZ) group (P= 0,01) as showed in the table 4 and Figure 17.

Table 5. The change ratio of the glomerulus number of all groups to control group.

Groups	Mean glomerular number	The change ratio %
Control	31338,00	-----
TQ	32992,00	% 105
STZ	24405,83	%78
STZ +TQ	30566,67	%97

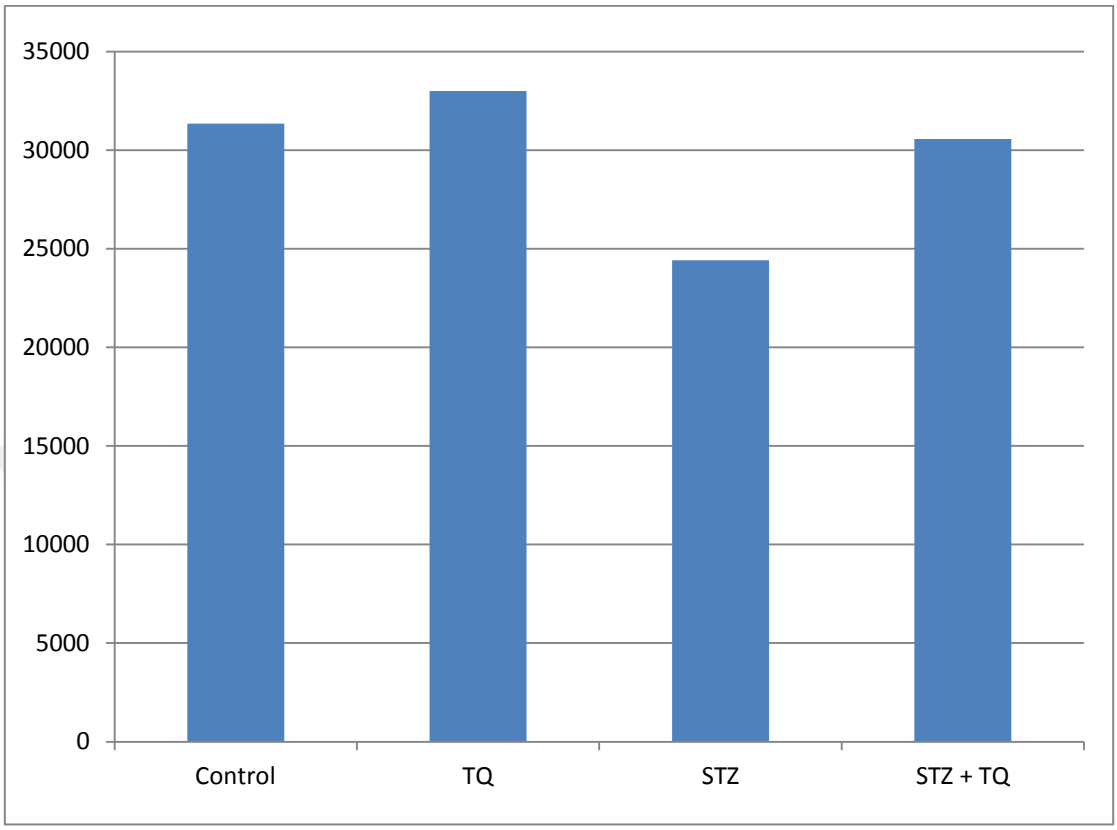

Figure 17. Glomerulus number.

Table 6. The CV of total glomerulus number counts of all groups and CE value.

Groups	Total glomeruli CV value	Total CE value
Control	0.002	0.05
TQ	0.009	0.06
STZ	0.012	0.06
STZ +TQ	0.006	0.05

The coefficient variation (CV) of total glomeruli counts and coefficient error (CE) were shown in table 4. If the CV is larger than the average, the distribution is widespread; if it is small, a significant proportion of the data is clustered close to mean.

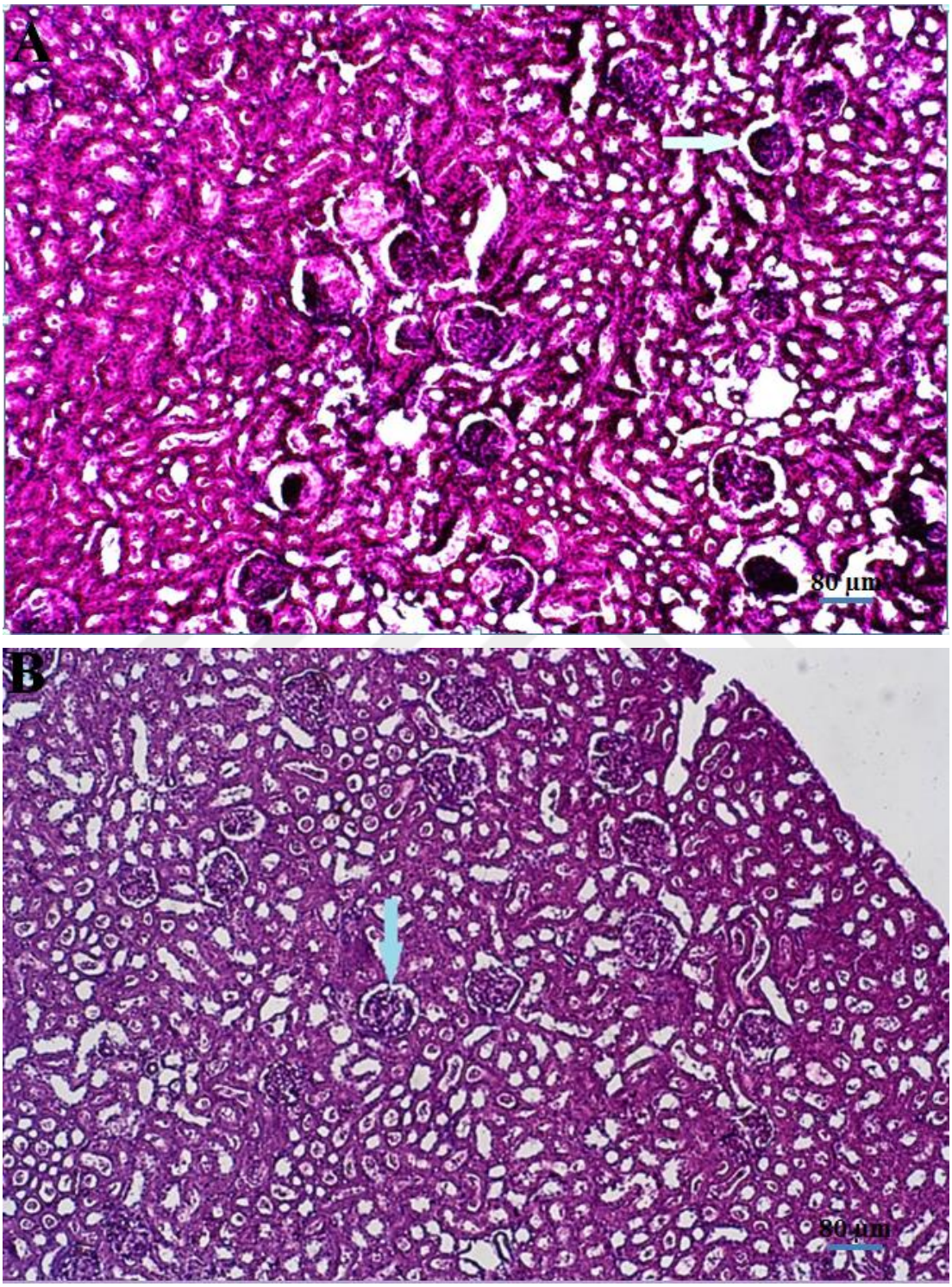

Figure 18. Microscopical section of kidney showing glomeruli (A;Control group) (B;TQ group) (scale bar= 80µm)H&E x10.

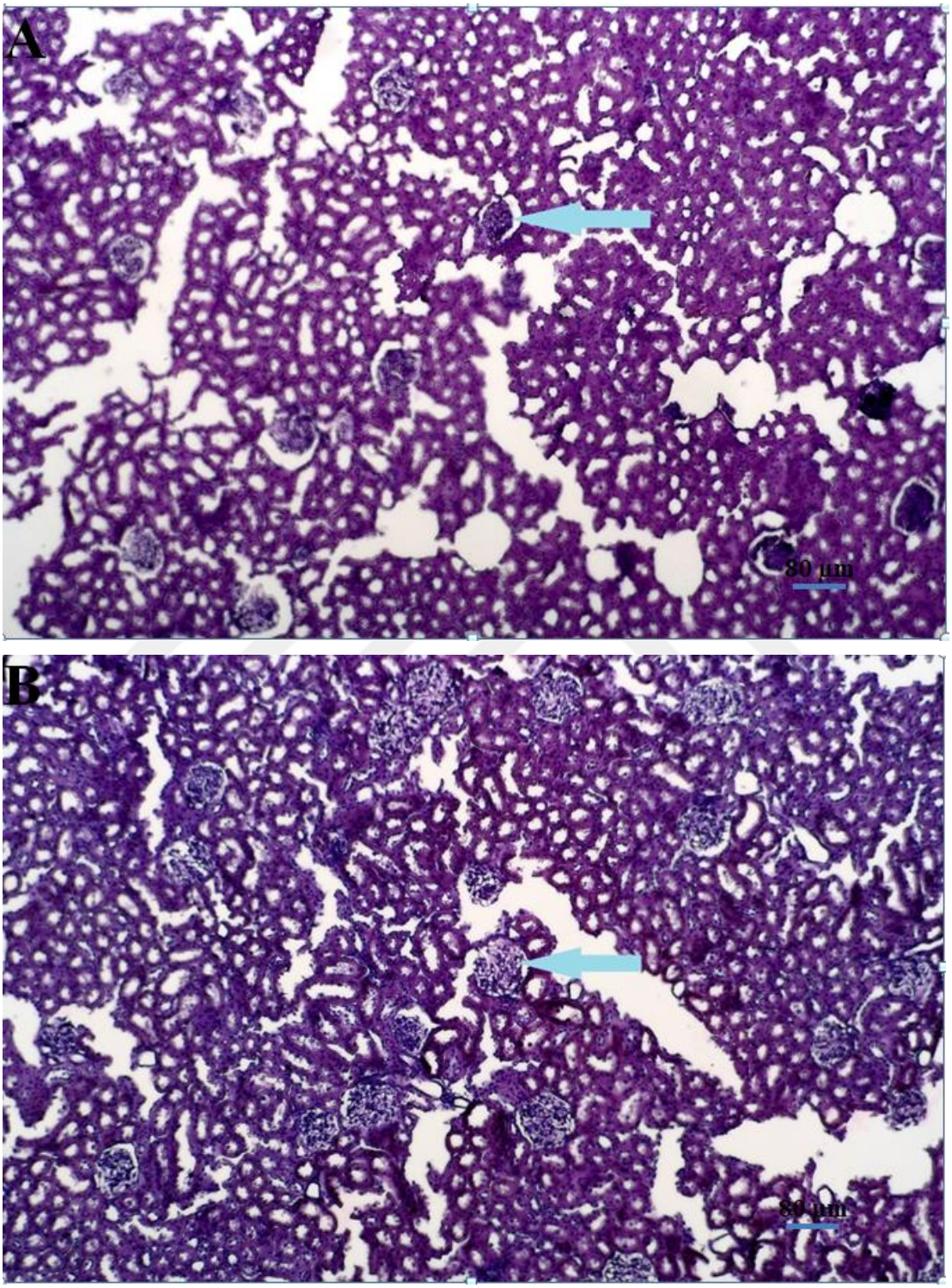

Figure 19. Microscopical section of kidney showing glomeruli (A;STZ group) (B; TQ+STZ group) (scale bar= 80µm) H&E x10.

5. DISCUSSION AND CONCLUSION

In controlling the composition and volume of body fluids, kidney have the subsequent vital roles controlling maintenance of blood volume ratio and pressure, Excretion, controlling the level of solutes in the blood, checking acidity and basicity of extracellular fluid, vitamin D synthesis and red blood cell synthesis regulation (Seeley et al., 1995; Saladin, 2007).

To remove poisonous waste products from the body and to maintain fluids, minerals, and electrolytes at physiological levels, kidneys are vital. The cells and microblood vessels of the kidney are elevated blood glucose is damaged (Suzuki et al., 2005).

For examining the pathogenesis of diabetic nephropathy, the streptozotocin (STZ) model of diabetes is normally utilized. like activation of protein kinase C is one of several pathological features (Carpenter et al., 2002).

The chief active constituent of Nigella sativa seeds which accountable for its medicinal effects is Thymoquinone (TQ). Additionally, it has a potential for treating of cancer. Anti- inflammatory, antioxidant, and anti-neoplastic effects both in vitro and in vivo are some pharmacological activities that Thymoquinone (TQ) is accounted for (Saravanan et al., 2016; Sayed 1980; Kouidhi et al., 2011; Ragheb et ai., 2009; Alkharfy et al.,2011).

As a result of disease and aging, the amount of glomeruli in a kidney and the amount of different cell kindes in a glomerulus can alter (Heptinstall, 1983).

Severe histopathological injury like atrophy dilatation, tubular damage, loss of brush border, and the deteriorations of hydropic epithelial cell are caused by renal reperfusion. Treatment with NS significantly attenuated the severity of reperfusion injury and considerably reduced tubulointerstitial injury score when its compared with the reperfusion group (Caskurlu et al., 2016).

A thickening of capsular basement membranes (CBMs): major expansion of the glomeruli, glomerular basement membranes (GBMs), and tubular basement membranes (TBMs); riased the size of mesangial matrix; and tubular redundancy were demonstrated in diabetic untreated rats. In untreated diabetic rats, the renal histology indicated increased mesangial development. In addition, thickening of CBMs, GBMs, and TBM are categorized via an escalation in PAS-positive space in comparison to control groups. The TQ treatment decreased the glomerular volume; thickening of GBMs, CBMs, and TBMs; escalated the qauntity of mesangial matrix; and tubular dilatation in contrast to untreated diabetics (Kanter, 2009).

Marked with H&E, normal glomeruli, renal tubules, and blood vessels have been indicated by the sections of first group. Inconstant number of glomeruli in the second group (diabetic) rats showed pivotal glomerulosclerosis, considered via glomerular basement membrane thickening and hyalinized deposits foci in the extracellular mesengium After 8 weeks of STZ application. Decomposition of several capillaries of the glomerular tufts was observed. Several clear tubular epithelial cells demonstrating undamaged membrane of the cell, vacuolated cytoplasm were monitored as well. after TQ administration (group C), some reversible alterations were observed such as decreased glomerular volume, thickening of capsular reduction, glomerular, and tubular basement membranes and mesangial matrix, decrease in the tubular dilatation, as well as formation of casts in comparison to untreated diabetic rats (group B) (Omran, 2014).

In encouraged diabetic rats, the impacts of the oil of N. sativa and glimepiride (Amaryl®) and its mixture on the treatment of lesions induced by STZ-] were investigated. The kidneys unveiled thickening of renal glomerular basement membrane and hyaline thickening of glomerular tufts In diabetic rat. renal glomerular basement membrane displayed calcification which demonstrated by basophilic constituent. The vacuolation and thickening of the wall of renal blood vessels with perivascular edema were observed. Cloudy swelling, hydropic deterioration and coagulated necrosis are uncovered by the renal tubules. Thickening of glomerular tufts demonstrated by pyknosis and karyolysis. The Other tubules either displayed contained hyaline and cellular casts or cystic dilation. As the kidneys and liver indicated hemorrhage and congestion, besides hydropic degeneration of

hepatic and renal cells in treated with N. sativa oil showed mild enhancement of the induced lesions in comparison to diabetic rat. Between the hepatic cells and renal tubules, several interstitial accumulations of round cells were monitored. Nevertheless, reasonable congestion in hepatic, renal, pulmonary, meningeal and pancreatic blood vessels demonstrated in diabetic rats treated with Amaryl. Conversely, co-treated by N. sativa oil and Amaryl unveiled normal histological and fine structure liver, kidneys, lungs, brain and pancreas (Refat, 2012).

The probable defensive impacts of thymoquinone (TQ) is investigated in the current examination a composite taken from Nigella sativa with strong anti-oxidant properties, against gentamicin (GM)-induced nephrotoxicity. From control rats, the Histopathological investigation of kidney specimens demonstrated normal renal glomeruli bounded by capsule and normal proximal, distal and convoluted tubules. Clear indications of glomerular and tubular necrosis, interstitial nephritis and desquamation of the tubular epithelial cells in the renal cortex is shown by Sections from rats treated with GM alone. Furthermore, the majority of tubules displayed vacuolated cytoplasm and dilatation of the tubular lumen with intraluminal blood stagnation in GM-treated rats. Stimulatingly, substantial development in glomeruli and renal tubules, showed by less vacuolation and higher conservation of tubular histology in comparison to the GM-treated group kidney specimens revealed by rats treated with TQ and GM (Sayed-Ahmed and Nagi, 2007).

Nigella sativa L (Ranunculacea) (NS) leaves and camellia sinensis (Theaceae) (CS) seeds have been shown to contain distinct bioactive components, this study aimed at the effects of a binary mixture of NS and CS water extracts on some diabetic nephropathy (DN) complications. diabetic kidney showed severe glomerular hypertrophy and interstitial inflammation. Although all administered treatments caused recovery in the pathological state of the kidney, the mixture of NS and CS extracts dosed at 100 mg/kg showed the highest efficiency as recovered the glomerular hypertrophy from the severe state to the mild and eliminated the observed interstitial inflammation as well as (Shokouhi et al., 2015).

In this research the TQ (10mg/kg) was orally applied for 10 days. The examination Kidney histopathological uncovered that TQ and control groups had normal structure of

glomeruli and renal tubules. On the other hand, MTX treated group displayed with atrophied glomeruli having widened capsular spaces. Furthermore, the majority of the tubules were widened and had intratubular cellular casts. It is revealed that administering MTX/TQ enhanced renal histology, with merely mild dilatation of renal tubules (El-Sheikh et al., 2015).

Considerably less glomeruli compared with Type I diabetic patients with mild or no glomerulopathy was shown in type 1 diabetic patients with severe diabetic glomerulopathy. A probable explanation is that Type 1 diabetic patients lose glomeruli in relation to the progression of diabetic glomerulopathy. Nonetheless, improvement of diabetic glomerulopathy is eased by a low amount of glomeruli (Bendtsen and Nyengaard, 1992).

Diabetic control rats that induced with Streptozotocin (50mg/kg BW), a group acts like diabetic rats administered with the powder of N. sativa seed (300mg/Kg body weight), a group acts as diabetic rats administered with the thymoquinone (4mg/ Kg body weight). Kidney demonstrated deposits of hyaline substances in the mesengium of the lobules of the glomerulus in Diabetic rats. The hyaline discharges were evenly and diffusely spread during the glomerulus in several of the glomeruli. The thymoquinone and N sativa treated group shows recovery of glomerular structure and illness close to the normal control animals histologically (Saheb et al., 2016).

Light and electron microscopic examination of tissues of rats rendered diabetic with a smaller dose of 45 mg/kg of bodyweight of streptozotocin were carried out in the present study. Kidney sections of diabetic animals showed thickening on the walls of nephrons filling their lumen thickening on the walls of nephrons filling their lumen and glomerulopathy. This work; undertaken to determine systematically the progression of early phase diabetic nephropathy in relation to various kidney-ralated parameters for a period of four months. various pathological lesions were observed in the renal tissue of diabetic rat. At the end of first month, two animals exhibited moderate distension of glomeruli while it was mild in three animals. Glomerular atrophy and mild loss of its cellularity were also seen in three animals. Glomerular symmetry was not normal in the experimental animals. One animal exhibited moderate fibrosis. Though,in the second and

third months, histopathological status of glomeruli remained almost same, in the fourth month, glomerular distension was found to be severe in one animal, while two animals exhibited it moderately. Mild to moderate fibrosis was also seen in experimental animals (Kiran et al., 2012).

In chronic renal disease not only the number of patent glomeruli but the total number of recognizable glomerular structures was reduced large numbers of glomeruli may disappear during the course of chronic renal disease it is suggested that the final histological pattern may not give as much information concerning the pathogenesis or severity of the disease as is commonly thought (Moritz and Hayman Jr, 1934).

Treatment of rats that takes 75 mg/kg body weight for 3 days consecutive days by N. sativa detected that treated group shows normal histological and function refinement of glomerular structure (Mahood and Karim, 2012).

According to the studies that performed on plants and its constituent Thymoquinone (TQ) helped in identification of plants with anticancer, scavenge numerous types of reactive oxygen that include superoxide and hydroxile radicals, to prevent hydroxyeicosatetraenoic acid and lipoxygenase synthesis and to display antioxidant activity by inhibiting the formation of free radicals (Bai et al., 2014; Aycan et al., 2014; Badary et al., 2003)

Although the purpose for these various effects is indistinct, TQ might cause hypoglycemia in 2 ways. First, it raises glucose usage by excess insulin secretion. Second, TQ might reduction hepatic gluconeogenesis (Fararh et al., 2005)

In conclusion, the present study demonstrated that in the Streptozotocin (STZ) group the total glomerular number were decreased when compared to control group. But our result do not compared to another studies, because there is no any articles about effect of thymoquinone on the glomerulus number by stereology method. However, Thymoquinone (TQ) group indicated an increase in glomerular number in comparison to control group. Moreover, in the treatment of Thymoquinone (TQ) + Streptozotocin (STZ) group were decreased in comparison to control group while the total number of glomeruli

were increased in the comparison to Streptozotocin (STZ) group. The result showed that pretreatment with Thymoquinone TQ against Streptozotocin (STZ) cause increasing kidney volume and glomerular number decreased accordingly, but in in treated group show protection of glomeruli against STZ dmaging. Finally thymoquinone can be utilize as a protective drug against diabetes.

SUMMARY

AZEEZ KA, A stereological study of the effects of thymoquinone on the renal glomerulus number in experimental diabetic rats induced by streptozotocin. Yuzuncu Yil University, Institute of Health Science, Department of Histology and Embryology, Master Thesis, Van-2017. This investigation has been conducted to study the protective effect of Thymoquinone (TQ) against Streptozotocin (STZ) induced diabete in rats. Thirty healthy male Wistar albino rats, weighing 180–220 g and averaging 20 weeks old were utilized in this study. The rats were allotted into four experimental groups each group contain seven animals: Control, Thymoquinone, Streptozotocin untreated, Streptozotocin treated with thymoquinone. Control group received single intra-peritoneal injection of saline (0.5ml / kg), Thymoquinone group were given TQ (4 mg/kg body weight) is provided with drinking water orally, Streptozotocin treated and Streptozotocin untreated groups received STZ. Diabetes was induced in two groups by a single intra-peritoneal injection of STZ (40 mg/kg, freshly dissolved in 5.5 mmol/l citrate buffer, pH 4.5). Three days after STZ treatment, development of diabetes in two experimental groups was confirmed by measuring blood glucose levels in a tail vein blood samples. Rats with blood glucose levels of 250 mg/dl or higher were considered to be diabetic. The rats in TQ treated groups were given TQ (4 mg/kg body weight) is provided with drinking water orally starting three days after STZ injection. Eight days after injection the rats were anesthetized then right kidney was taken for process was followed for light microscopic research. The photo was taken by using light microscopy. The kidney volume and glomerular number were calculated for each group using physical dissector. The data was evaluated statistically. A significant increase of the kidney volume and decreased glomerular number in STZ group while in treated group TQ+STZ were increased kidney volume and glomerular number comparing to STZ group. We conclude that TQ therapy causes renal morphologic improvement after STZinduced diabetes in rats. It was discussed in the light of literature that further preclinical research into the utility of TQ treatment may indicate its usefulness as a potential treatment in diabetes.

Key Words: Glomerulus number, kidney, rat, stereology, STZ, thymoquinon,

ÖZET

AZEEZ KA, Streptozotocin ile Oluşturulan Deneysel Diyabetli Sıçanlarda Böbrek Glomerül Sayısına Timokinonun Etkilerinin Stereolojik Yöntemlerle Araştırılması.Yüzüncü Yıl Üniversitesi Tıp Fakültesi, Histoloji ve Embriyoloji Anabilim Dalı, Yüksek Lisans Tezi, Van, 2017. Bu çalışma sıçanlarda Streptozotosin (STZ) enjeksiyonu ile oluşturulan diyabete karşı Timokinon'un (TQ) etkilerini incelemek için yapılmıştır. Bu maksatla ana grup olarak ağırlıkları 180 ile 220 g arasında değişen ve ortalama yaşları 20 hafta olan 28 adet Wistar albino sıçan kullanılmıştır. Sıçanlar, her birisi 7 birey içeren dört çalışma grubuna ayrılmıştır: kontrol, Timokinon, tedavi edilmeyen Streptozotosin, Timokinon ile tedavi edilen Streptozotosin. Kontrol grubu tek seferlik periton içi serum fizyolojik (0,5 ml / kg) enjeksiyonu, Timokinon ve tedavi edilmeyen Streptozotosin grupları ise STZ enjeksiyonuna maruz bırakılmıştır. Diyabet, ilgili bu iki gruba tek periton içi STZ enjeksiyonu ile (40 mg/kg, 5,5 mmol/l sitrat tamponunda taze çözdürülmüş olarak, ph 4,5) uygulanmıştır. STZ uygulamasından üç gün sonra iki deneysel grupta diyabet gelişimi kuyruk venöz damarlarından alınan kan numunelerindeki glikoz seviyelerinin ölçülmesi ile doğrulanmıştır. 250 mg/dl'den daha fazla glikoz içeren numunelerin alındığı sıçanlar diyabetik olarak değerlendirilmiştir. TQ uygulaması yapılan sıçanlarda TQ (4 mg/kg vücut ağırlığı), STZ enjeksiyonundan üç gün sonra içme suyuna karıştırılarak oral uygulanmıştır. Sıçanlar anesteziye alınmış ve sağ böbrekleri çıkarılarak bunlardan alınan dokular ışık mikroskobuyla incelenmiştir. İlgili fotoğraflar da ışık mikroskobu ile çekilmiştir. Görüntü tarayıcı kullanılarak glomerül sayıları hesaplanmıştır. Elde edilen verilen istatistiki olarak değerlendirilmiştir. Yalnızca STZ uygulanan grupta ciddi miktarda böbrek hacim artışı ve glomerül sayısında azalma tespit edilirken, STZ + TQ uygulanan grupta böbrek hacmi artışı ile beraber glomerül sayısında artış tespit edilmiştir. Neticede TQ tedavisinin STZ ile oluşturulmuş diyabetli sıçanların böbreklerinde morfolojik gelişme sağladığı sonucuna varılmıştır. TQ uygulamaları için yapılacak preklinik araştırmalarının, bu maddenin diyabet tedavisinde kullanılabilme potansiyelinin stereolojik açıdan önemi literatür ışığında tartışıldı.

Anahtar Kelimeler: Böbrek, glomrül sayısı, stereoloji, sıçan, STZ, timokinon,

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CURRICULUM VITAE

Kawa Kamal Azeez, he is from Erbil city in the North Iraq, also he was born on March 23, 1988, in Erbil. He was finished Primary, Secondary and High Schools in Erbil, after that he begin the undergraduate admission process at the University of Salahaddin-College of Science Department of biology during the years (2006-2010), and he was awarded a Bachelor's degree in biology in 2010. He got a work from 2010 to 2011 in Hayat company.While he was started working as an employer (Assistant biology) at the Ministry of Health for four years from 2011-2015 . Finally, he came to the Republic of Turkey to begin the postgraduate admission process at the Yuzuncu Yil University-Institute of Health Science, Faculty of Medicine-Histology amd Embryology Department during the years (2015-2017).

ATTACHMENTS

1. Ethics Committee Project Permission Form

T.C.
YÜZÜNCÜ YIL ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU

ARAŞTIRMA BAŞVURU ONAY BELGESİ

	Araştırmanın Adı	Streptozotocin ile oluşturulan deneysel diyabetli sıçanlarda optik sinir akson sayısına timokinonun etkilerinin sterelojik yöntemlerle araştırılması
	Araştırmanın Yürütücüsü	Prof. Dr. M. Çetin RAĞBETLİ
	Yardımcı Araştırıcılar	Yük. Lis. Öğr. Kawa Kamal AZEEZ
	Kurumu	Tıp Fakültesi
	Araştırmanın Tahmini Süresi	3 Ay
	Kullanılacak Hayvan Türü ve Sayısı	Sıçan 28 Adet
	Destekleyecek Kuruluş (lar)	
	Başvuru Tarihi	02.03.2016

KARAR BİLGİLERİ	**Karar No:2016/03** **Tarih:24.03.2016** Yüzüncü Yıl Üniversitesi Tıp Fakültesi öğretim üyesi/elemanı Prof. Dr. M. Çetin RAĞBETLİ sorumluluğunda yürütülmesi planlanan ve yukarıda başvuru bilgileri verilen Yüksek Lisans projesi gerekçe, amaç ve yöntemler dikkate alınarak ilgi başvuru belgeleri incelendi. Çalışmanın etik açıdan uygun olduğuna, projenin aşağıdaki hususlar dikkate alınarak yürütülmesine ve proje yürütücüsüne iletilmesine oy birliği / oy çokluğu ile karar verildi. 1) Projede herhangi bir değişiklik gerektiğinde kurulumuzdan onay alınması. 2) Projede çalışacağı bildirilen araştırıcılarda değişiklik olduğunda kurulumuzdan onay alınması. 3) Deney hayvanları üzerinde yapılacak girişimin başlangıç ve bitiş tarihlerinin bildirilmesi. 4) Çalışma süresinde tamamlanamaz ise ek süre talebinde bulunulması. 5) Çalışma tamamlandığında sonuç raporunun gönderilmesi.

ETİK KURUL ÜYELERİ

BAŞKAN
Prof. Dr. Semiha DEDE

ÜYELER

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***Bu form YÜHADYEK tarafından doldurulacaktır.**

2. Plagiarism Report

YÜZÜNCÜ YIL ÜNİVERSİTESİ
SAĞLIK BİLİMLERİ ENSTİTÜSÜ
LİSANSÜSTÜ TEZ ORİJİNALLİK RAPORU

Tarih: 22/06/2017

Tez Başlığı / Konusu: A stereological study of the effects of thymoquinone on the renal glomerulus number in experimental diabetic rats induced by streptozotocin.

Yukarıda başlığı/konusu belirlenen tez çalışmamın Kapak sayfası, Giriş, Ana bölümler ve Sonuç bölümlerinden oluşan toplam 60 sayfalık kısmına ilişkin, 22/06/2017 tarihinde şahsım/tez danışmanım tarafından Turnitin programintihal tespit programından aşağıda belirtilen filtreleme uygulanarak alınmış olan orijinallik raporuna göre, tezimin benzerlik oranı % 10 (on) dır.
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22/6/2017

KAWA KAMAL AZEEZ

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Programı: Tıbbi Histoloji ve Embriyoloji

Statüsü:Y.Lisans X Doktora☐

DANIŞMAN ONAYI
UYGUNDUR

Prof. Dr. Murat Çetin RAĞBETLİ
Tıbbi Histoloji ve Embriyoloji
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