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## RESEARCH ARTICLE

# A STEREOLOGICAL STUDY OF THE EFFECTS OF THYMOQUINONE ON THE RENAL GLOMERULUS NUMBER IN EXPERIMENTAL DIABETIC RATS

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*Kawa Kamal AZEEZ's master thesis
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**ABSTRACT**

This investigation has been conducted to study the protective effect of Thymoquinone against Streptozotocin induced diabetes in rats. The rats were allocated into four groups each group contain seven animals; Control, Thymoquinone, Streptozotocin, Streptozotocin + thymoquinone. Control group was injecteda single dose by intra-peritoneal injection of saline (0.5ml/kg), Thymoquinone group provided thymoquinone (4mg/kg) with drinking water, Streptozotocin group was injecteda single doseby intra-peritoneal of streptozotocin (40 mg/kg) and thymoquinone was provided (4mg/kg) with drinking water three days after streptozotocin was injected a single dose intra-peritoneal injection of streptozotocin (40 mg/kg)to the streptozotocin+ thymoquinone group. Three days after the administration of streptozotocin, the development of diabetes in two experimental groups was confirmed by measuring the glucose levels in the blood. Eight days after the beginning of the experiment the rats were anesthetized then right kidney was taken for process was followed for light microscopic research. The kidney volume and glomerular number were calculated for each group using physical disector. A significant increase of the kidney volume in streptozotocin group andstreptozotocin + thymoquinone. A significant decreased glomerular number in streptozotocin group.



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## 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 affects 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).Early diabetes mellitus and acute experimental diabetes have been shown to improve renal function and glomerular function (Mogensen and Andersen, 1973; Hostetter, 1981). The number of glomeruli in a kidney can change as a result of disease and aging (Heptinstall, 1983). A decrease in the number of glomeruli causes various diseases (John *et al*., 1992)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).So as to make experimental diabetes mellitus in animal, STZ and Alloxan are widely utilized medicines. STZ 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

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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 STZ 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 *Nigella sativa* seeds is Thymoquinone (TQ). It has strong antioxidant characteristics. The organs are protected by TQ 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).

## MATERIALS AND METHODS

### Animals

We obtained ethical approval for our study from Van Yuzuncu Yil University Animal Research Local Ethic Committee in the session held on 9/8/2016 under number TYL-2016-5369. 28 healthy female wistar albino rats, weighing 180–220 g and averaging 20 weeks old were utilized in this study. The rats were allocated into four experimental groups each group contain 7 animals: Control, TQ, STZ, STZ+TQ. Control grup was injecteda single dose intra-peritoneal(i.p)of saline 0.5 ml/kg, TQ group was givedTQ for eight days (with drinking water, 4mg/kg/day), STZ grup was injected a single dose of STZ (i.p, 40 mg/kg) (Sigma). STZ+TQ group were given TQ (with drinking water, 4mg/kg body weight) two days after a single dose of STZ injection (i.p, 40 mg/kg). Diabetes was induced in two groups by a single dose of STZ (i.p, 40 mg/kg, freshly dissolved in 5.5 mmol/l citrate buffer, pH 4.5). Three days after STZ was injected, blood glucose levels of STZ and STZ+TQ grupswas measured. 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. The initial and final body weight changes of the various groups were recorded. The rats were perfused after being anesthetized with ketamine 50mg/kg and xylazine 4mg/kg.

### Histology

Following perfusion, right kidneys wereremovedand fixed in 10% neutral buffered formal in and washing the kidney were immersed in Bouin'ssolutions for repeat fixation. Right kid neys were dehydrated through a gradedalcoholseries, cleared in xylene and infiltrated with parafin for embeding. Serial sections of each kidney were cut in a vertical plane at 5µm using a rotary microtome. Selected sectionsweremounted on slides, hydrated through descending concentrations of alcoholseries and stained with hematoxyl in and eosin (H&E). Photos were taken by using a light microscope with a digital color camera attachment.

### Stereology

We used the physical fractionator and disector counting methodfor the total number of glomeruli. Thephysical dissector consists of two paralel cross-sectional planeseparatedby a certaindistance "t". Onecountstheparticlesthatarevisible on thereferencesection, but not thelook-upsection by unbiased counting frame. Toachievesystematicrandomsampling for stereology, the first section was chosen randomly while the next section was taken after cutting every 30 section. About nine to 11 sections were taken. The values obtained were multiplied by the sampling rate (Sterio, 1984)

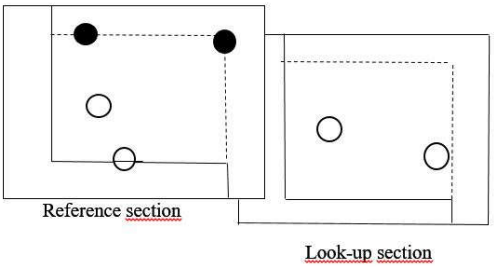


**Figure 1.Unbiasedcountingframe.Theblackparticleseen in thereferencesectionbut not seen inthelook-upsectionwascounted. Thus, a disectorparticle ($Q^-$)=2**

The total number of glomeruli in the kidney estimated using the formula:

$$N = N_v \, x \, V_{ref}$$

$N_v$ is thenumericaldensity of theparticleof interest (glomeruli/unitvolume) and $V_{ref}$is the total (reference) volume of the object.

We used Cavalieri's principle for total kidney volume calculation. With the Cavalieri's principle, the kidney was divided into parallelslices at equal intervals with systematic Random sampling from beginning to end. Some random slices were taken and divided into spaced sections equally (t). The total area of the received sections were calculated using the point counting grade (Fig.2). The total surface area obtained from all theslices was multiplied by the slice thickness to calculate the volume. The values obtained were multiplied by the sampling rate.We can formulate it like this:

$$V_{ref} = t \, x \, \Sigma a$$

''t'' issectionthickness and ''Σa'' is total surfacearea in sections (Howard and Reed, 1998).

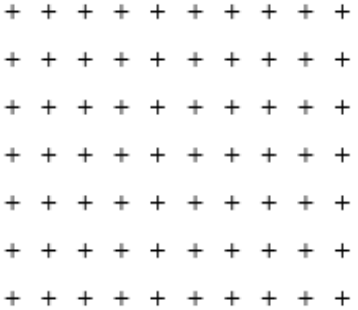

**Figure 2. Thepointcountinggrade**

### Statistical analysis

Statistical Analyses were performed using Microsoft SPSS Version 13.0 for statistical analyses. Kruskal-Wallis test was performed to compare groups.

## RESULTS

### Rat's blood glucose levels

After injectedstreptozotocin 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.

### Total number of glomeruli

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 (P=0,01).

### Total kidney Volume

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

**Table 1. Total number of the glomeruli**

| Glomeruli | Groups | Median | Mean | St. Dev. | Min. | Max. | p.* |
|---|---|---|---|---|---|---|---|
| | 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 | |

**Table 2. The total volume of groups**

| Volume ($mm^3$) | Groups | Median | Mean | St. Dev. | Min. | Max. | p. |
|---|---|---|---|---|---|---|---|
| | Control | 479,520 | 504,977 b | 887,444 | 367,740 | 638,280 | 0,01 |
| | TQ | 526,500 | 533,674b | 126,6499 | 401,760 | 758,160 | |
| | STZ | 701,460 | 698,966a | 256,473 | 659,340 | 725,760 | |
| | STZ+TQ | 742,770 | 740,340 a | 255,323 | 694,980 | 772,740 | |

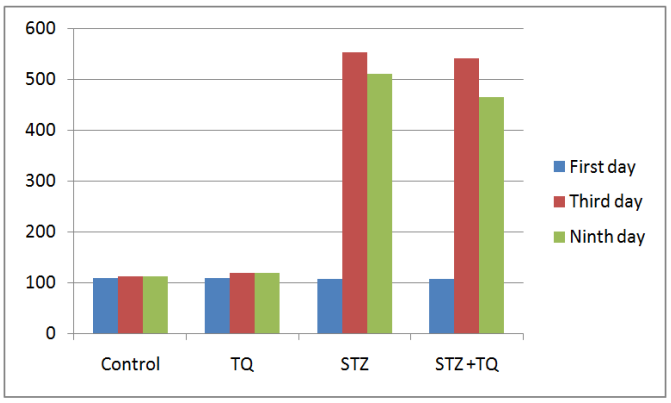


**Figure 3. Effect of TQ on blood glucose in STZ-induced diabetic rats (mg/dl)**

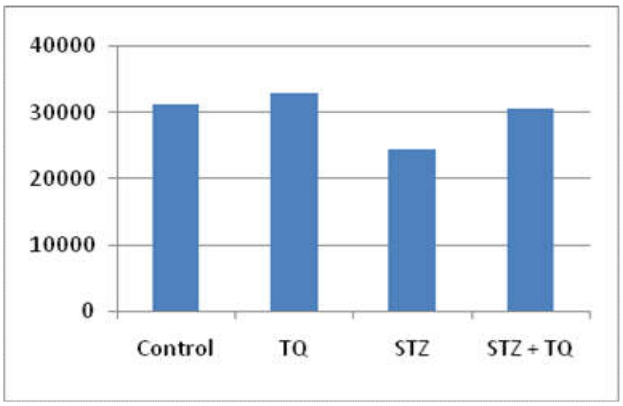


**Figure 4. Total number of glomeruli**

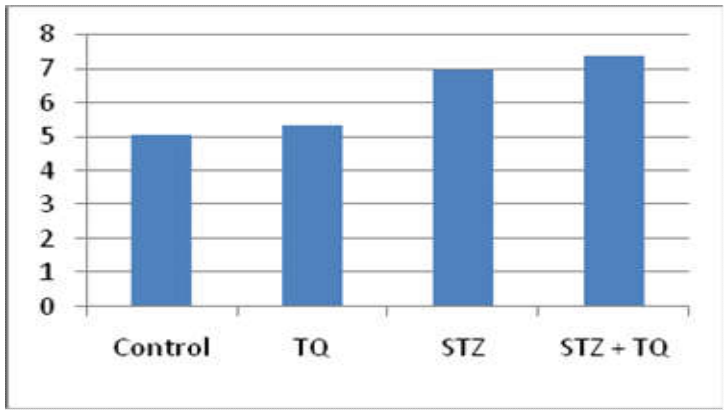


**Figure 5. Kidney volume ($mm^3$)x$10^2$**

detected (P=0,01). The coefficient variation (CV) and coefficient error (CE) valueswere calculated. CV values of grups; Kontrol 0,002, TQ 0,009, STZ 0,012, STZ+TQ 0,006 and CE values of grups; Kontrol 0,05, TQ 0,06, STZ 0,06, STZ+TQ 0,05.

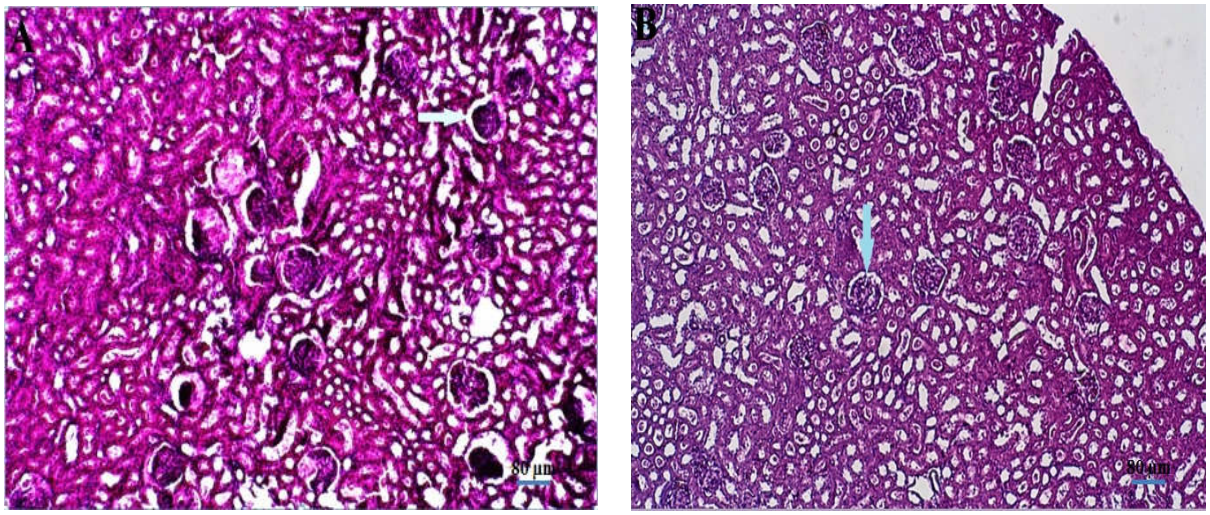


**Figure 6. Microscopical section of kidney showing glomeruli (A:Control group) (B:TQ group) (scale bar= 80µm)H&E x10**

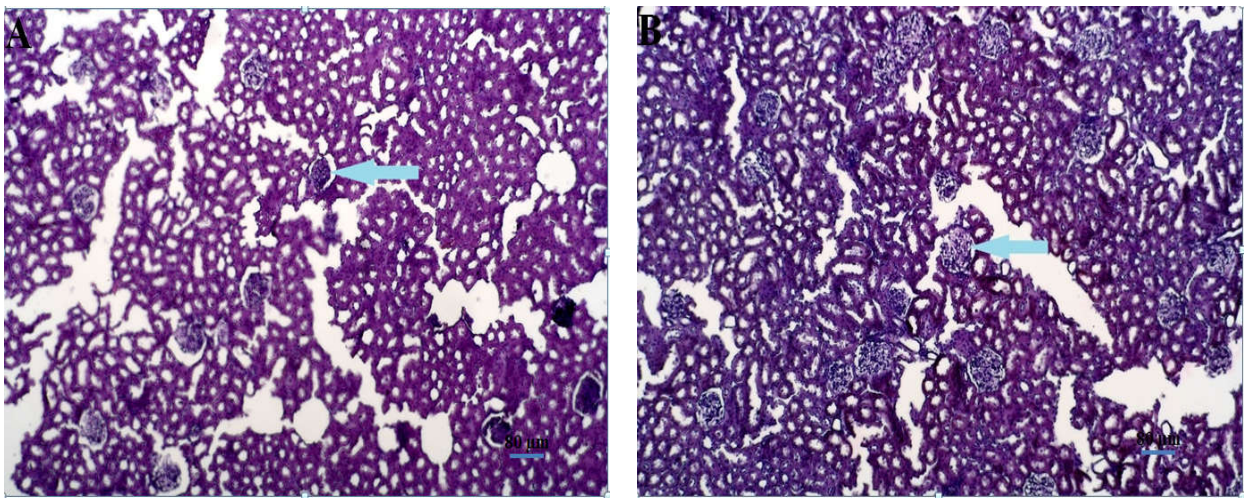


**Figure 7. Microscopical section of kidney showing glomeruli (A:STZ group) (B:TQ+STZ group) (scale bar= 80µm) H&E x10**

## 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 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 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 TQ is accounted for (Saravanan *et al*., 2016; Sayed, 1980; Kouidhi *et al*., 2011; Ragheb *et al*., 2009; Alkharfy *et al*., 2011). Early diabetes mellitus and acute experimental diabetes have been shown to improve renal function and glomerular function (Mogensen and Andersen, 1973; Hostetter, 1981). The number of glomeruli in a kidney can change as a result of disease and aging (Heptinstall, 1983). A decrease in the number of glomeruli causes various diseases (John *et al*., 1992). In conclusion, the present study demonstrated that in the 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, TQ group indicated an increase in glomerular number in comparison to control group. Moreover, in the treatment of TQ+STZ group were decreased in comparison to control group while the total number of glomeruli were increased in the comparison to STZ group. The result showed that pretreatment with TQ against STZ cause increasing kidney volume and glomerular number decreased accordingly, but in in treated group show protection of glomeruli against STZ damaging. Finally TQ can be utilize as a protective drug against diabetes in kidney damage.

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