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Original Research Article

EFFECT OF NIGELLA SATIVA SEED ON STREPTOZOTOCIN INDUCED DIABETIC RENO TOXICITY: HISTOLOGICAL OBSERVATIONS

Shaik Hussain Saheb *[1], Desai S D [2], Kusal K Das [3], Haseena S [4].

[*1] Ph.D Scholar, Department of Anatomy, Shri B M Patil Medical College Hospital & Research Centre, Bijapur and Assistant Professor in Anatomy, JJM Medical College, Davangere, Karnataka India.

[2] Principal, Shridevi Institute of Medical Sciences and Research Hospital, Tumkur, Karnataka, India.

[3] Professor, Department of Physiology, Shri B M Patil Medical College Hospital & Research Centre, Bijapur, Karnataka, India.

[4] Ph.D Scholar, Department of Physiology, Shri B M Patil Medical College Hospital & Research Centre, Bijapur and Lecturer in Physiology, JJM Medical College, Davangere, Karnataka India.

ABSTRACT

Background: Diabetic nephropathy has long been recognized as the leading cause of end-stage renal disease, but the efficacy of available strategies for the prevention of Diabetic nephropathy remains poor. The aim of this study was to investigate the renoprotective effects of Nigella sativa seed and thymoquinone against streptozotocine induced renotoxicity.

Materials and Methods: 24 rats were selected for this study and divided in to 4 groups each contains 6 rats, one group served as normal control, one group served as Streptozotocin (50mg/kg BW) induced diabetic control rats, one group served as diabetic rats treated with the nigella sativa seed powder(300mg/Kg body weight), one group served as diabetic rats treated with the thymoquinone(4mg/ Kg body weight).

Results: Histopathological observations of present study showed that nigella sativa seed and thymoquinone treatment has proved its nephroprotective effect in streptozotocin induced diabetic rats.

Conclusion: The results of present study concluding that Nigella sativa seed powder and thymoquinone having renoprotective effect in streptozotocine induced diabetic rats.

KEY WORDS: Kidney, Nephropathy, Nigella Sativa, Antioxidant, Thymoquinone.

Address for Correspondence: Shaik Hussain Saheb, Assistant Professor in Anatomy, JJM Medical College, Davangere, Karnataka India. Mobile no.: +91-9242056660 **E-Mail:** anatomyshs@gmail.com

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INTRODUCTION

The most common endocrine disease in the world is diabetes mellitus, characterized by the state of hyperglycemia, defect in the insulin secretion, insulin action or even both are causing the diabetes mellitus [1]. Diabetes patients are prone to some long-term complications like nephropathy, retinopathy and neuropathy [2]. Out of these Diabetic nephropathy is one of the most serious complications of diabetes and the most common cause of end-stage renal failure. Diabetic nephropathy is characterized by

changes in both glomerular and tubular structure and function. The pathogenesis of diabetic nephropathy includes genetic, hemodynamic and metabolic factors, and oxidative stress as well as renal hypertrophy, but the exact mechanism is not clear [3]. The previous studies have focused on alterations in the glomerulus, including abnormalities in glomerular permeability and capillary pressure, glomerular hyperplasia or hypertrophy and increases in mesangial volume [4]. To prevent and treat diabetic nephropathy, current methods using agents such as angiotensin converting enzyme inhibitors, angiotensin-receptor blockers and antihypertensive drugs have been tried in clinical practice [5]. Unfortunately, currently available medical interventions are unable to effectively reverse or even delay the progression of diabetic nephropathy [6].

Nigella sativa is an annual herb of the Ranunculaceae family, which grows in countries bordering the Mediterranean sea and Indian sub-continent. This widely distributed plant is native to Arab countries and other parts of the Mediterranean region [7]. For thousands of years, this plant has been used in many Asian, Middle Eastern and Far Eastern Countries as a spice and food preservative as well as a protective and health remedy in traditional folk medicine for the treatment of numerous disorders [8-10]. The active constituents of black cumin have been identified as thymoquinone, dithymoqu-inone, thymohydroquinone, and thymol. Several studies have shown the various therapeutic actions of black cumin. It has activity against diabetes [11,12] radical scavenging activity [13,14,] prevents lipid peroxidation and increases the antioxidant defense system [15]. Renal dysfunction is a common complication in diabetes mellitus that is involved in oxidative stress changes [16,17]. The amelioration of renal hemodynamic and function changes in diabetics has been elucidated by supplementation with antioxidants. Black cumin was believed to be responsible for restoration in renal dysfunction in nephrotoxic rats through the antioxidant effect [18-21]. The present study is focused on renoprotective effect of nigella sativa seed against streptozotocin induced diabetic rats through histopathological observations.

MATERIALS AND METHODS

This work was conducted as part of Ph.D work under department of Anatomy, Shri BM patil Medical College, BLDE University, Bijapur. University ethical committee and Institution Animal Ethical committee are approved the work according to CPCSEA (Govt of India) Rules. 18 rats were selected for this study and divided in to 3 groups each contains 6 rats, one group served as normal control, one group served as Streptozotocin (50mg/kg BW) induced diabetic control rats and one group served as diabetic rats treated with the nigella sativa seed powder(300mg/Kg body weight). The treatment was continued for 45 days, at 46th day morning the all the groups were sacrificed and renal tissue collected in 10% formalin and proceeded for further tissue processing.

Plant material: Nigella sativa seeds were grinded in to fine powder with piston and mortal with help of Bapuji pharmacy college, Davangere. Nigella sativa powder administrated orally according to study of M. Murugesan [22].

Streptozotocine - Induced diabetes: The rats were given Streptozotocine intraperitoneal injection 50mg/kg body weight, Streptozotocine dissolved in ice-cold citrate buffer (PH 4.5). The diabetes was confirmed by measuring glucose by Code free Glucometer, the glucose level above 180mg/dl considered as diabetes, glucose levels were checked at regular periodical timings.

RESULTS

Histopathology of Kidney:

Normal control rats (group I): Section studied under H&E stain showed normal renal parenchymal tissue which is composed of glomeruli and tubules separated by small amount of intestinal connective tissue containing peritubular capillaries. Each glomerulus is spherical collection of interconnected capillaries within Bowman's space lined by flattened parietal cells. The outer aspects of the glomerular capillaries are covered by a layer of visceral epithelial cells (podocytes). The capillary tufts are supported by the mesangial cells. Tubules appear normal, Glomeruli appear

normal in morphology and in cellularity, interstitium appears normal, vessel appear normal (Figure 1).

Diabetic control rats: Section studied under H&E stain showed In most of the glomeruli, there were deposits of hyaline material in the mesengium of the lobules of the glomerulus. In many of the glomeruli, the hyaline deposits were diffusely and evenly spread throughout the glomerulus. There was a diffuse infiltration of the glomerular tuft with eosinophilic material and also heavy focal deposition. The diffuse infiltrate appeared to be in the basement membranes of the capillaries, and the capillary bed had been obliterated in places. In place, this deposition caused complete hyalinization of many glomeruli. In many of the glomeruli, lesions characteristic of diabetic glomerulosclerosis were present. It consisted of round, practically acellular, hyalinized nodule in the glomerular tuft. There was a considerable deposit of hyaline material thickening in Bowman's capsule. Both afferent and efferent arterioles appeared as narrow ovals and also showed quite marked strongly eosinophilic hyaline thickening. The nodules were scattered among some non-affected normal glomeruli which is a characteristic of diabetes mellitus. In most of the sections, areas of lymphocytic infiltrate were seen in the interstitium (Figure 2-5).

Diabetic rats treated with nigella sativa seed powder: The group that was treated with nigella sativa seed powder showed features of normal glomerulus, very less number of inflammatory cells, normal basement membrane and capillaries, decrease in the hyaline deposit. Overall the treated diabetic rats histology appears near to normal histological architecture of kidney(Figure 6).

Fig. 1: Normal control rats kidney H&E section showing normal glomeruli and tubules.

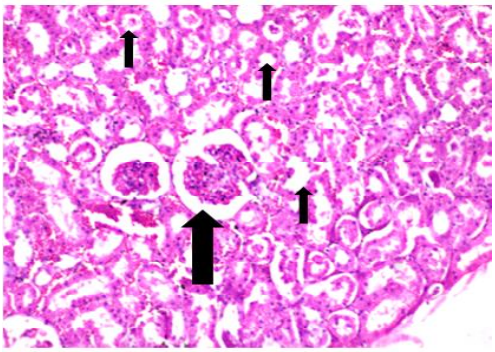


Fig. 2: Diabetic control rats Kidney H&E section showing hyalinization of Glomerulus and tubules'↑.

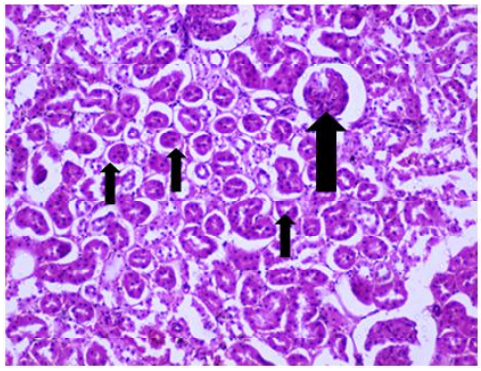


Fig. 3: Diabetic control rats Kidney H&E section showing ticked wall of vessel.

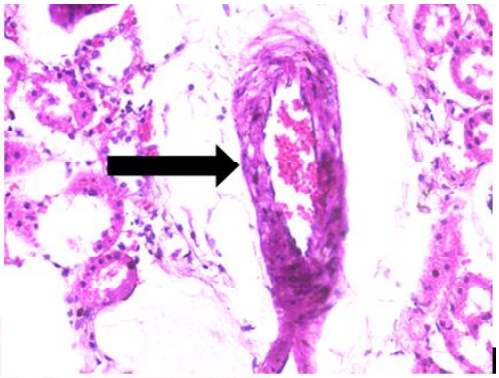


Fig. 4: Diabetic control rat kidney H&E(10X) section showing lymphocyte infiltration.

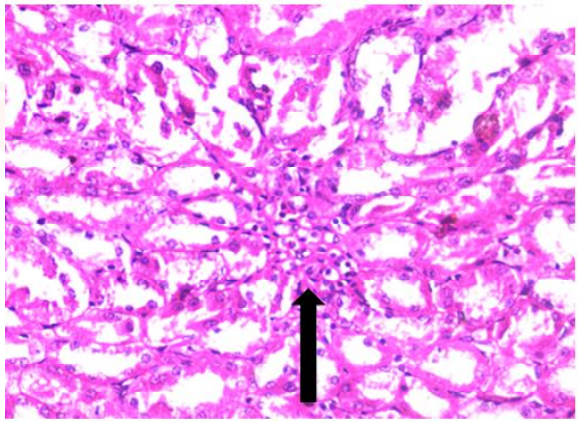


Fig. 5: Diabetic control rat Kidney H&E(20X) section showing hyalinization of glomerulus and tubules.

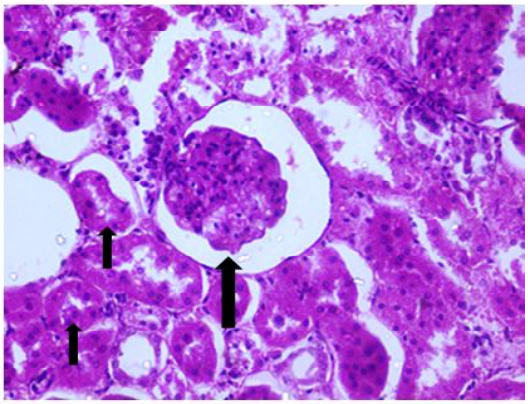
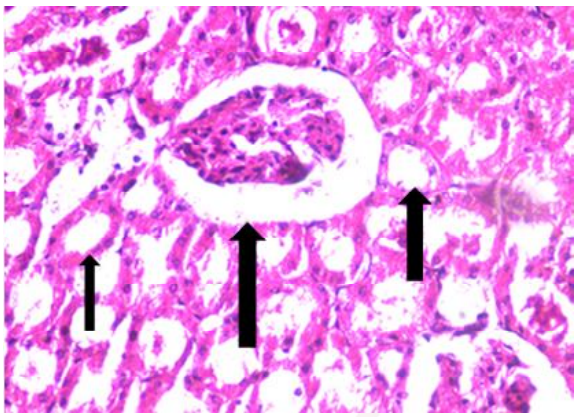


Fig. 6: Diabetic rats treated with nigella sativa seed powder kidney section showing the glomerulus and tubules with normal architecture.



DISCUSSION

The H&E sections of normal control rats showed classical features of normal renal parenchymal tissue. Our study observations are in agreement with study of Le et al., 2004 [23]. The section of streptozotocine induced diabetic rats renal sections showed in most of the glomeruli, there were deposits of hyaline material in the mesengium of the lobules of the glomerulus. There was a diffuse infiltration of the glomerular tuft with eosinophilic material and also heavy focal deposition. The diffuse infiltrate appeared to be in the basement membranes of the capillaries, and the capillary bed had been obliterated in places. In many of the glomeruli, lesions characteristic of diabetic glomerulosclerosis were present. The nodules were scattered among some non-affected normal glomeruli which is a characteristic of diabetes mellitus. In most of the sections, areas of lymphocytic infiltrate were seen in the interstitium. In study of S. L. TEOH et al [24] observed the kidneys of diabetic rats showed damage to the glomerulus, thickened basement membrane and edematous proximal convoluted tubule with increase in mucopolysaccharide deposits. In Sugano M et al study also showed similar findings in diabetic rats [25].

The groups that were treated with nigella sativa seed powder showed features of normal glomerulus, very less number of inflammatory cells, normal basement membrane and capillaries, decrease in the hyaline deposit. Overall the treated diabetic rats histology appears near to normal histological architecture of kidney. The beneficial effect of nigella sativa seed may be due to antioxident effect of nigella sativa and its major bioactive component thymoquinone. In study of Abdul Karim Salim Mahood [26] also found similar results in streptozotocine diabetic rats treated with nigella sativa showed features of healing that are normal glomerulus, absence of inflammatory cells, normal basement membrane and capillaries, decrease in the mucopolysaccharide and hyaline deposit, respectively. The tissue necrosis was also observed to decrease. In study of Kanter M [27] treatment of thymoquinone reduced the glomerular size, thickening of capsular, glomerular and tubular basement membranes, increased amounts of mesangial matrix and tubular dilatation and renal function as compared with diabetics untreated. Kanter M conclude that thymoquinone therapy causes renal morphologic and functional improvement after streptozotocine induced diabetes in rats. The study of Ola Omran [28] also found similar results and concluded that thymoquinone has protective effects on experimental diabetic nephropathy.

The all above mentioned previous studies concluded that the reparing ebility of diabetic nephropathy by nigella sativa seed and thymoquinone is due to their antioxidant properties. Thus we can conclude that nigella sativa has ability to repair the diabetic changes in kidney through its antioxidant property because of its major bioactive component thymoquinone.

Conflicts of Interests: **None**

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