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Original Research Article

Black Seed Oil-induced Amelioration of Renal Dysfunction in a Rat Model of Diabetes Mellitus and Periodontitis

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ABSTRACT

This study explored the effects of administering black seed (*Nigella sativa*- NS) oil on the function and structure of the kidneys in rats with induced diabetes mellitus (DM) and/or periodontitis (PD). A total of 48 Wistar rats were separated into eight groups of six, with Group I serving as the control and Group II receiving NS oil along with their standard diet. Diabetes was induced in Group III, and NS oil was given to Group IV after diabetes had developed. PD was induced in Group V, and NS oil was administered after the disease was established in Group VI. Group VII had both DM and PD without treatment, while Group VIII received NS oil after the induction of DM and PD. Diabetes was induced using streptozotocin (STZ), while PD was induced using 3/0 silk sutures. Blood samples were collected through heart puncture, and the kidney was examined using histopathology. The levels of urea and creatinine were significantly increased ($p<0.01$) in the untreated diabetes mellitus and diabetes mellitus plus periodontitis groups compared to the untreated control group. Conversely, diabetes and periodontitis caused a significant ($p<0.01$) decrease in serum electrolyte levels compared to the control group. Both diabetes mellitus and periodontitis disrupted the normal kidney structure in the untreated group, but the administration of NS oil significantly reduced these effects in the treatment groups. The study's biochemical and histological findings indicate the potential beneficial effects of *Nigella sativa*oil and its bioactive components in combating kidney damage caused by diabetes and periodontitis.

Keywords Diabetes, Periodontitis, *Nigella sativa*, Kidney

Introduction

Periodontitis, a complicated infection caused by a variety of microorganisms in the subgingival plaque, results from an ecological imbalance of oral microbes. This persistent infectious disease damages the alveolar bone and periodontal membrane, which support the teeth.[1]Severeperiodontitis affects about 10% of the world's population[2] and can negatively impact the body's equilibrium and contribute to chronic disease emergence.[3]

Recent research has shed light on the link between periodontitis and systemic illnesses. Pathogenic mechanisms of periodontal disease (PD) have shown a correlation with conditions beyond the oral cavity, including diabetes, stroke, autoimmune disorders, rheumatoid arthritis, atherosclerosis, and coronary heart disease.[4,5,6,7,8] Additionally, the relationship between PD and kidney disease has been studied.[9,10]

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Periodontitis can exacerbate and promote the progression of chronic kidney disease through direct or indirect processes such as the impact of periodontal pathogens, antigens, endotoxins, and chronic inflammatory load produced by released cytokines.[11-15]

When the pancreas fails to create enough insulin or when the body has difficulties utilizing the insulin that is produced, diabetes mellitus (DM), a chronic illness, can arise. Insulin is the hormone that regulates blood sugar. Hyperglycemia, a symptom of this metabolic and endocrine condition, results from either inadequate insulin synthesis by the pancreas (DM type 1) or incorrect insulin response by the pancreas (DM type 2), and it is defined by elevated blood glucose levels.[16,17] Diabetes mellitus (DM) is a condition that has serious side effects. It is a significant global cause of disease and mortality. According to statistics, 2.8% of people worldwide have diabetes, and by 2025, that number is expected to rise to more than 5.4%.[18]Oxidative stress, cardiac problems, renal failure, neuro-degeneration, and immunological dysfunction are all caused by oxidative stress, which is directly linked to the formation of free radicals in diabetes mellitus.[19]

Strong evidence exists to back up the idea that diabetes and periodontitis are interdependent, with diabetes increasing the likelihood of developing periodontitis and periodontal inflammation negatively impacting glycemic control.[20,21]Diabetes individuals experience periodontitis three times more frequently than non-diabetics do.[22] Diabetes and periodontitis have both been linked to kidney damage.[15,23]A serious socioeconomic and healthcare issue, kidney dysfunction, particularly Chronic Kidney Disease (CKD), affects almost 7 billion people globally. In the world, 8724 out of every 100,000 individuals have kidney disease, an amount that is ten times greater than cancer patients and surpasses diabetes by 80%; it is anticipated that by

2040, kidney disease will be the fifth leading cause of death globally. Regardless of the initial nephropathy, once a critical mass of nephrons have been lost, kidney disease generally progresses towards more advanced stages, which are characterized by increased morbidity and mortality.[23,24] Diabetes is the leading cause of CKD, followed by hypertension and glomerular diseases. The impact of CKD complications and significant comorbidities, as well as the management of end-stage renal disease, together constitute the burden of kidney disease.[25] Renal failure and numerous histological alterations in various organs are both caused by untreated diabetes mellitus, and the incidence of these conditions is rising.[26]

The body's waste is expelled by the kidneys as urine. Before returning blood to the heart, they aid in filtering it. The kidneys carry out a variety of vital tasks, such as maintaining fluid balance throughout the body, regulating and filtering blood minerals, filtering waste products from food, medications, and toxic substances, and producing hormones that support the production of red blood cells, foster bone health, and control blood pressure. Renal function is regularly assessed using electrolytes, creatinine, urea, and uric acid tests.[24]

Since many years, medicinal plants have been an essential component in the treatment of many ailments, outperforming orthodox medicine in terms of accessibility and safety.[27,28,29,30] By squelching free radicals, a variety of antioxidant-rich plants improve kidney failure.[31] Among these, *Nigella sativa* L., popularly known as black cumin or black seed, has a number of highly promising health effects. It is widely regarded as a practical choice for battling nephrotoxicants due to its actions in preventing oxidative stress and the apoptotic cascade, as well as alleviating kidney damage biomarkers and histological features.[32,33,34] Several scientists are working to validate NSS's supposedly useful role in treating renal failure due to the large quantity of phytochemicals in it.

Black seed, also known as *Nigella sativa*, contains a variety of bioactive substances, including tannins, fixed oil, alkaloids, protein, and essential oil.[35] Broad-spectrum biological actions of *Nigella sativa*include antioxidant, hepatoprotective, nephro-protective, and anti-diabetic characteristics.[36,38-41] Thymoquinone, the monoterpenes p-cymene and apinene1, nigellidine 2, nigellimine 3, and a saponin are only a few of the compounds found in black seed oil.[37,42,40]According to a study by Daryabeygi-Khotbehsara *et al*.[43], thymol, thymohydroquinone, dithymoquinone, nigellone, alpha-hederin, flavonoids, and fatty acids are additional ingredients found in black seed (NS), which contribute to its therapeutic effects. These various chemicals work synergistically to enhance the medicinal benefits of NS. Additionally, both human and animal studies have indicated that NS does not have any harmful side effects or toxicological impacts, as reported by Yimer *et al*.[44]Unfortunately, understanding of the effect of black cumin administration on alterations in renal functioning is still inadequate, notably in rat model of diabetes mellitus and periodontitis. Thus, this study evaluated whether treating rats with black seed oil for diabetes mellitus and periodontitis separately or combined, improved their renal impairment.

Materials and Methods

Sample collection

The *Nigella sativa*oil was made by Hemani International KEPZ Karachi, Pakistan, and was provided by Shifaa Pharmacy in Lagos.

Chemicals and reagents

The kits for the urea, creatinine, and serum electrolytes assays were made by Randox Laboratories in the UK. In addition to other chemicals and regulators, the following substances were used: heparin injection, streptozotocin, formaldehyde, distilled water, formic acid, urethane, saline and chloralhydrate. These were all analytical-grade materials.

Experimental animals

The study utilized 48 mature male Wistar rats, weighing between 140-160 g. The animals were acclimatized for two weeks at the faculty animal home before the commencement of the experiment. They were housed in wire-mesh cages in a room maintained at a temperature of approximately 29°C +/- 2°C, with a 12-hour light and dark cycle. Throughout the experiment, the animals had free access to water and food pellets. The experiment protocols were developed in compliance with the Handbook for the Care and Use of Experimental Animals.

Ethical approval

The Faculty of Medicine of the University of Lagos' animal care and use research ethics committee gave its clearance for this work with reference number (CMUL/ACUREC/02/20/71).

Experimental design

A total of forty eight (48) Wistar rats were divided into eight groups of six each (Table 1). Rats from Group I were used as the Control group; they had unrestricted access to typical rat food and were not subjected to any treatment. NS oil was given to the rats in group II in addition to their standard rat chow and water. In Group III animals, diabetes was induced without treatment. When diabetes was developed (DB + NS0), Group IV rats received NS oil (1 ml/kg) intraperitoneally. Untreated periodontitis was induced in Group V. 1 ml/kg of NS oil was given intraperitoneally after periodontitis was established in group VI rats. Group VIII rats received 1 ml/kg of NS oil after intraperitoneal induction of diabetes and periodontitis (DB + PD+NS1). Group VII rats underwent induction.

Diabetes induction

Laboratory animals were fasted the night before to the induction of diabetes by streptozotocin. The rats received a single intraperitoneal injection of streptozotocin (STZ) (50 mg/kg body weight) in a freshly buffered (0.1 m citrate, pH 4.5) solution. Tail vein blood was taken 72 hours after STZ injection, and blood glucose levels were determined using an extremely sensitive glucometer.

Table 1: Experimental Design

Groups	Name	Induction	Treatment
1	Normal Control	Normal chow + water	No treatment
2	Treatment	Normal chow + water	Oral *N. sativa* oil admin.
3	Diabetic	Injection of STZ	No treatment
4	Diabetic+ Treatment	Injection of STZ	Oral *N. sativa* oil admin.
5	Periodontitis	Ligature-induced	No treatment
6	Periodontitis+ Treatment	Ligature-induced	Oral *N. sativa* oil admin.
7	Diabetes+ Periodontitis	Injection of STZ and ligature induction of periodontitis	No treatment
8	Diabetes+ Periodontitis + Treatment	Injection of STZ and ligature induction of periodontitis	Oral *N. sativa* oil admin.

Periodontitis induction
Each rat was subjected to ligature-induced periodontitis by having a 3/0 silk suture put subgingivally on its incisor while under general anesthesia (Chlorahydrate 30mg/kg of body weight).[45]The ligature served as a gingival irritant for 21 days, encouraging plaque development and the subsequent onset of periodontal disease. When the ligatures were applied, the animals were observed for 21 days, and daily ligature checks were performed.

*Nigella sativa*Administration
For 21 days, the oil was administered intraperitoneally to the treatment groups at a rate of 1 milliliter per kilogram of the test animals.[46]Before being dosed, the animals were famished.Each set of animals was killed after the projected exposure and treatment timeframes had passed. After being starved for a night and being put to death by cervical dislocation, rats were slaughtered.[47]Blood was obtained using a cardiovascular puncture and promptly spun at 3000 rpm for 10 minutes to create serum that was utilized to measure the kidney function.

Biochemical assays
According to Weatherburn[48]'s approach, urea in the sample was converted into a colored complex by the urease enzyme, and the resulting color was evaluated at 600 nm.
Bartels and Bohmer[49]'s method was used to quantify creatinine. In this method, creatinine in the sample reacted with picrates in an alkaline solution to generate a colored complex at 500 nm.
Electrolytes like sodium, potassium, chloride, and bicarbonate were assessed. Lithium-heparinized vials were used for the collection of blood samples. Thereafter, using commercially available Roche and Cobas kits and a Roche/Hitachi 904 automated analyzer (Roche Professional Diagnostics, Rotkreuz, Switzerland), plasma samples were examined for sodium, potassium, chloride, and bicarbonate ions.[50]

Histological Assessment of the Kidney
After fixation, tissues were divided into tiny (1 cm3) slices using a clean, sharp knife. Hematoxylin and Eosin (H&E) staining was performed after tissue sections were cut at 5-7 m and paraffin method was used to treat them.[51]Using an automated image analysis system, image, slides were inspected under a microscope at a magnification of 100X to determine the thickness (m) of the endometrium and myometrium. In the kidney tubules, vacuolar and degenerative alterations were also seen.

Statistical analysis
The ANOVA and SNK posthoc tests were used to examine the data. A 0.05 significance threshold was chosen. Graphpad Prism 9 was used for data analysis.

Results and Discussion

Effects of Nigella sativa Oil on Serum Urea and Creatinine Level
The result of the effect of *Nigella sativa* Oil on serum urea and creatinine in the experimental rats is presented in Figure 1 and 2 respectively. Intraperitoneal induction of DM alone resulted in a significant ($p<0.0001$) increase urea compared to the untreated control group. The chart below also shows a significant ($p<0.0001$) elevation in urea in rats induced with DM+PD when compared to the untreated control group. Similarly, periodontitis induction in rats resulted in a mild elevation of urea compared to the control group. However, administration of *Nigella sativa* Oil to rats induced with DM, PD and DM+ PD caused a significant reduction in urea when compared to the untreated DM and PD groups. There was a significant ($p<0.01$) elevation of reatinine in the untreated Diabetes mellitus and Diabetes mellitus + Periodontitis groups respectively compared with the untreated control group. In the Periodontitis group, no significant difference was seen compared with the control. Moreso, creatinine level was shown to be significantly reduced in the treated Diabetes mellitus and Periodontitis groups compared with the untreated Diabetes mellitus and Periodontitis groups.

The current study's objective was to investigate the effects of black seed oil administration on the structure and function of the kidney in rats that had either one or both of diabetes mellitus and periodontitis induced. The findings revealed that the untreated Diabetes mellitus group's urea level was substantially higher than that of the control group (Figure 1). Usman*et al*.[52], as well as Baragob[53], validate these findings. Mild urea elevation was seen in the periodontitis group, but it was not statistically different from the control group. Vasconcelos*et al*.[54]and Souza *et al*.[55]reported comparable results. When compared to the control group, the untreated Diabetes mellitus group's creatinine level was shown to be considerably higher (Figure 2). These findings concur with those of Usman*et al*.[52], Baragob[53], Egbune *et al*.[56]A little increase in creatinine was seen in the periodontitis group, but it was not statistically different from the control group. Vasconcelos *et al*.[54] and Souza *et al*.[55]reported comparable results.

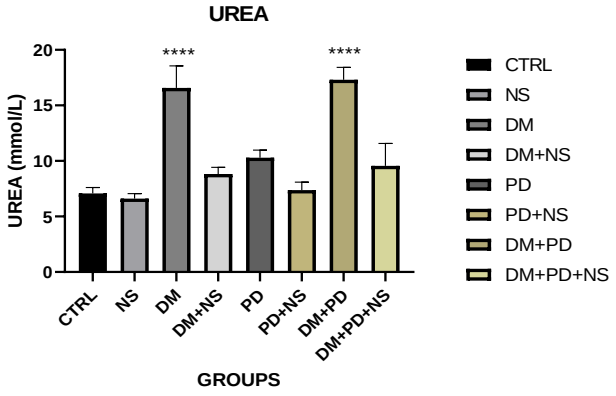

Figure 1: Effects of NS oil on urea across the groups. Values are Mean±SEM(n=5). **=Significantly higher ($p<0.01$) in DM group vs control and also in periodontitis vs control. CTRL=Control, NS=*Nigella sativa*, DM= Diabetes mellitus, DM+NS=Diabetes mellitus+*Nigella sativa*, PD=Periodontitis, PD+NS= Periodontitis+ *Nigella sativa*, DM+PD= Diabetes mellitus+ Periodontitis, DM+PD+NS= Diabetes mellitus+ Periodontitis+*Nigella sativa*.

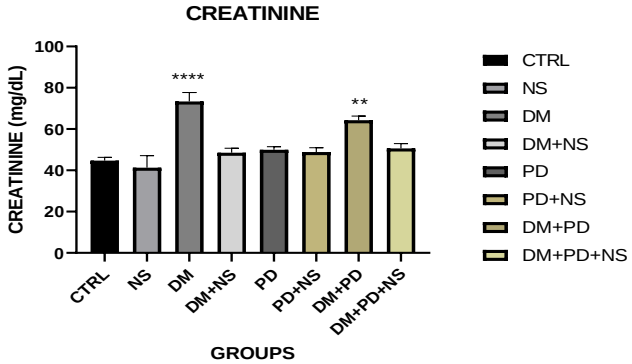

Figure 2: Effects of NS oil on creatinine across the groups. Values are Mean ± SEM (n=5) *= Significantly higher ($p<0.05$) in DM group vs Control. **=Significantly higher ($p<0.01$) in DM+PD group vs Control. ****= significantly higher ($p<0.001$) in PD+DB vs control. CTRL=Control, NS=*Nigella sativa*, DM= Diabetes mellitus, DM+NS=Diabetes mellitus+*Nigella sativa*, PD=Periodontitis, PD+NS= Periodontitis+ *Nigella sativa*, DM+PD= Diabetes mellitus+ Periodontitis, DM+PD+NS= Diabetes mellitus+ Periodontitis+*Nigella sativa*

Table 2: Effects of *Nigella sativa* Oil on Electrolytes in Rats with Diabetes Mellitus and Periodontitis.

GROUPS	Na^+mmol/L	K^+mmol/L	Cl^-mmol/L	HCO_3^-mmol/L
CTRL	131.4 ± 2.821	4.322 ± 0.3474	98.04 ± 1.132	26.76 ± 1.471
NS	134.2 ± 1.934	4.512 ± 0.1355	100.1 ± 2.803	27.00 ± 0.9984
DM	118.8 ± 3.484 *	3.652 ± 0.2301**	78.76 ± 2.652**	17.66 ± 0.8483****
DM+NS	127.0 ± 2.236	4.130 ± 0.1661	93.02 ± 2.128	25.12 ± 1.567
PD	127.8 ± 1.562	4.014± 0.2597	97.62 ± 3.408	25.13 ± 1.491
PD + NS	129.8 ± 1.934	4.244 ± 0.4026	98.10 ± 1.240	21.84 ± 0.5836
DM+PD	116.0 ± 1.581**	3.578± 0.2545***	74.12 ± 7.101***	15.84 ± 0.7928****
DM+PD+NS	126.6 ± 2.227	3.952 ± 0.1967	90.62 ± 2.824	23.46 ± 1.642

Values are Mean ± SEM (n=5) *p<0.05 vs Control, **p<0.01vs Control ***p<0.001 ****p<0.001vs Control.
CTRL=Control, NS=*Nigella sativa*,DM= Diabetes mellitus, DM+NS=Diabetes mellitus+*Nigella sativa*, PD=Periodontitis, PD+NS= Periodontitis+ *Nigella sativa*, DM+PD= Diabetes mellitus+ Periodontitis, DM+PD+NS= Diabetes mellitus+ Periodontitis+*Nigella sativa*

The renal system has two bean-shaped organs called kidneys. They aid the body in excreting waste through urine. Before returning blood to the heart, they aid in filtering it. The kidneys carry out a variety of vital tasks, such as controlling and filtering blood minerals, removing waste products from food, drugs, and hazardous substances, and producing hormones that support the production of red blood cells, enhance bone health, and control blood pressure.[55] As a byproduct of protein metabolism, urea is created in the liver and transported to the kidneys for excretion. The majority of renal illnesses result in insufficient urea excretion, which raises urea levels in the blood. One of the most often used clinical markers for evaluating renal function is the amount of urea in the blood.[57] On the other hand,creatinine is a breakdown product of creatine, a crucial part of muscle. The muscle mass, which fluctuates very little, is what determines how much creatinine is produced. The glomerular filtration rate and creatinine levels in the blood are inversely correlated. Creatinine is only eliminated by the kidneys. Because renal impairment is virtually the only cause of high creatinine, the blood creatinine level offers a sensitive diagnostic of kidney function.[55]

Kidney injury can be identified biochemically using techniques such detecting serum concentrations of urea, creatinine, and electrolytes, which also have a good predictive capacity. Elevated levels of these indicators have been linked to kidney disease and damage in both animal and human research, and it has been determined that these increases are caused by the kidneys' inability to regulate fluid and electrolyte hemostasis.[58,59,60] In the current investigation, it was discovered that both periodontitis and diabetes mellitus alone can harm the kidneys, and that the combined effects of the two diseases are more pronounced. Due to various modifications in tubular cellular structure and endothelial dysfunction brought on by diabetes mellitus and periodontitis, renal tubular degeneration (as confirmed by histopathological results) and an increase in serum urea and creatinine levels was observed in the rats not receiving black seed extract. Many studies have demonstrated a close correlation between diabetes mellitus and the generation of free radicals, which leads to oxidative stress and renal failure.[19]Rats with obesity and periodontitis had significantly higher serum levels of urea and creatinine, according to Chen *et al*.[61] Similar to this, Zeng *et al*.[62] found that rats with periodontitis had higher serum creatinine levels. Human investigations have indicated that periodontitis alone can result in large increases in these indices and a loss of kidney function, similar to what has been seen in animal research.[13,63]Untreated diabetes mellitus causes multiplehistopathological changes in variousorgans and the incidence of diabetic nephropathy and renal dysfunction has been reported.[26,64.] Furthermore, there is abundant proof that periodontitis and kidney disease are related.[23]

The abnormalities in serum urea and creatinine values caused by diabetes and periodontitis were, however, markedly reduced and returned to normal following intraperitoneal delivery of black seed oil. A similar outcome was obtained by Okoye and Igwilo[39] Al-Seenia*et al*.[37], Elkhateeb *et al*.[65], Parsegian *et al*.[66]Thymoquinone and nigellidine, together with other bioactive substances, present in NS, that support the immune system, reduce the risk of cellular damage by activating the body's antioxidant defense mechanism. Researchers' interest in NS screening for novel bioactive chemicals has increased because of its potential medicinal benefits. Thymoquinone, one of the key bioactive molecules that were revealed to have a protective effect against diabetes, is primarily responsible for the therapeutic actions of NS.[43,42,44]

Effects of Nigella sativa Oil on Electrolytes in Rats with Diabetes Mellitus and Periodontitis

The effects of *Nigella sativa*oil on electrolytes in rats with diabetes mellitus and periodontitis is presented in Table 2. It shows significant ($p<0.05$) reduction in sodium level in the untreated diabetic group compared with the untreated control and intraperitoneal administration of *Nigella sativa*oil increased the level of sodium in treated group compared with untreated DM group. Moreso, significant ($p<0.01$) reduction in sodium level was observed in untreated Diabetes+ Periodontitis group compared to the untreated control group. Administration of*Nigella sativa*oil cause mild increase of sodium in treated PD+DM group compared with untreated DM+PD group. However, in Periodontitis group, mild reduction of sodium level was seen with no significant difference when compared with the untreated control group. Also, mild elevation of sodium was seen in treated PD group compared to untreated PD. More so significant ($p<0.01$) reduction of potassium level was seen in untreated DM group compared with the untreated control group. Also, significant ($p<0.001$) reduction was seen in untreated DM+PD group compared with untreated control group. PD group shows mild reduction compared with untreated control group. However, in all treated PD, DM and PD+DM shows mild increase compared with untreated groups.

In chloride level, significant ($p<0.01$) decrease in chloride ion level in the serum of DM induced rats was seen compared with the untreated control group and mild increase in chloride level in rats treated with *Nigella sativa*oil compared with untreated DM group. Similarly, mild reduction was seen in PD group compared with the untreated control group and the administration of *Nigella sativa*oil mildly increase Chloride ion level in treated PD group compared with untreated group. More so, Chloride level was significantly ($p<0.001$) reduced in untreated DM+PD group compared with the untreated control group. However, the administration of *Nigella sativa*oil cause increase of GSH level in treated PD+DM group compared with untreated group. In bicarbonate level, a significant ($p<0.01$) decrease of Bicarbonate ion level in the serum of DM induced rats was seen compared to the control. Also, a similar pattern was observed in rats Induced with PD+DM, which shows ($p<0.01$) significant reduction in Bicarbonate ion level. More so, there was no significant decrease in serum bicarbonate in rats Induced with PD. However, rats treated with Black seed oil shows mild increase of bicarbonate level in DM, PD and PD+DM compared to the untreated groups.

Thus, the results of this study indicate that untreated diabetes mellitus was associated with hypochloremia, hypokalemia, and hyponatremia. These findings are consistent with those of Woyesa*et al*.[67], Khan *et al*.[68],

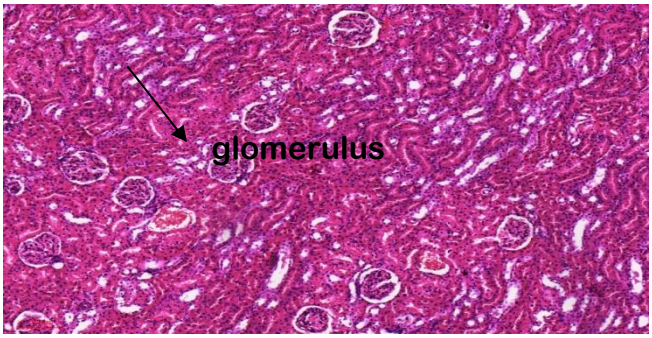

Plate 1: Photomicrograph of kidney section of rat from control group displaying normal glomeruli, tubules and interstitium (H&E, x 200).

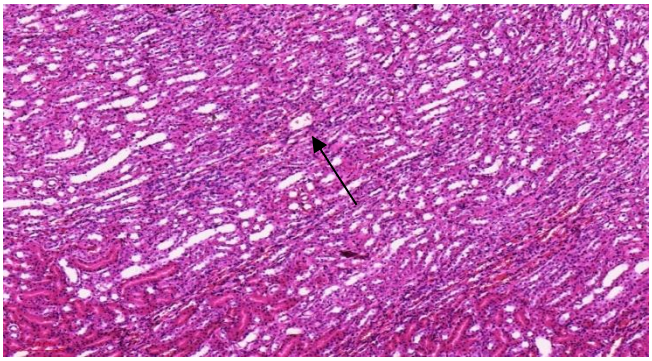

Plate 2: Photomicrograph of kidney section of rat from *Nigella sativa*group displayed normal glomeruli, tubules and interstitium (H&E, X200).

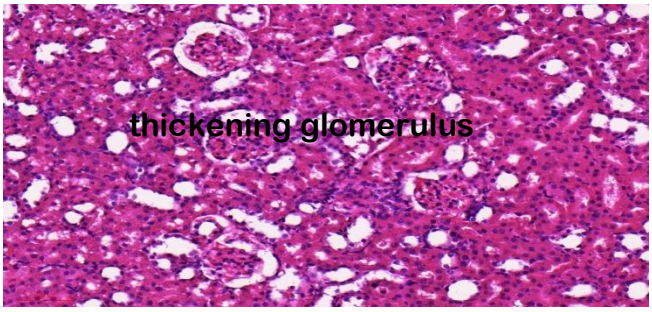

Plate 3: Photomicrograph of kidney section of rat from Diabetic group shows thickening glomeruli basement membrane, mesangial expansion (H&E, x 200).

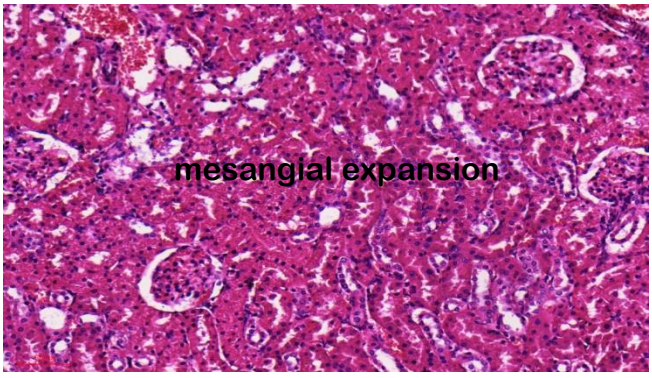

Plate 4: Photomicrograph of kidney section of rat from Diabetes and treatment groupshowing thickened glomeruli basement membrane, mesangial expansion (H&E, X200).

Alatawi and Alshubaily[69]. This could be correlated to cell membrane damage leading to disturbances in Na^+ and K^+ pumping and disorders in membrane permeability.[36]Two of the electrolytes, or minerals that the body needs in relatively substantial amounts, are sodium and potassium. As electrolytes are dissolved in bodily fluids like blood, they acquire an electric charge. The fluid surrounding cells and the blood contain the majority of them. Sodium plays a crucial part in maintaining the body's fluid balance and in maintaining appropriate neuron and muscle function. The main ways that the body loses salt are through sweat and urine and through food and liquids. A blood potassium level that is very high (hyperkalemia) or excessively low (hypokalemia) can have detrimental effects, such as an irregular heartbeat or even the heart stopping (cardiac arrest).[37]The substantial amount of potassium that is kept in reserve by cells can be used by the body to assist keep the level of potassium in blood steady. By regulating the amount of sodium and potassium expelled in the urine, healthy kidneys keep the levels of those substances stable in the body.[70]Consequently, the aberrant serum electrolyte values seen in rats with periodontitis and diabetes mellitus indicate that these two diseases caused renal failure, which was treated by black seed oil. This outcome is consistent with those of comparable research. Due to its bioactive components, black seed oil has been shown in this study to improve electrolyte imbalance. This agrees with the reports of Laranjeira*et al*.[71] and Chaturvedi *et al*.[72] This study further showed that NS extract can reduce kidney histological damage.

Effects of Nigella sativa Oil on Histology of the Kidney in Rats with Diabetes Mellitus and Periodontitis.

The Effects of *Nigella sativa*Oil on Histology of the Kidney in Rats with Diabetes Mellitus and Periodontitis is presented in Plates 1 – 8 for the various experimental groups respectively.

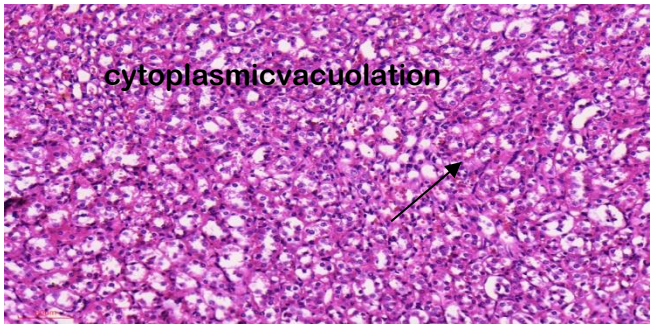

Plate 5: Photomicrograph of kidney section of rat from periodontits group showing cytoplasmic vacuolation of tubular epithelial cells in the cortex, loss of brunt borders and exfoliation of tubular epithelial cells (H&E, x 200).

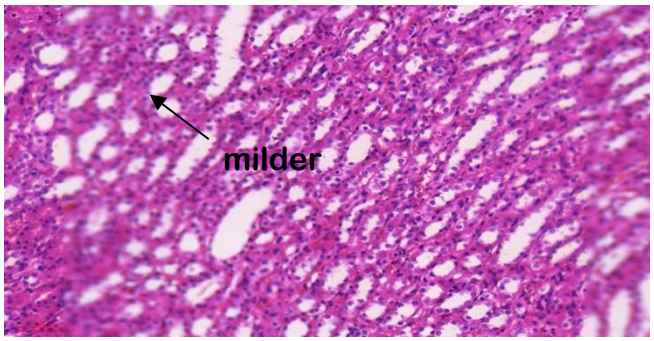

Plate 6: Photomicrograph of kidney section of rat from periodontitis and treatment shows milder cytoplasmic vacuolation of tubular epithelial cells in the cortex, loss of brunt borders and no exfoliation of tubular epithelial cells seen (H&E, x 200).

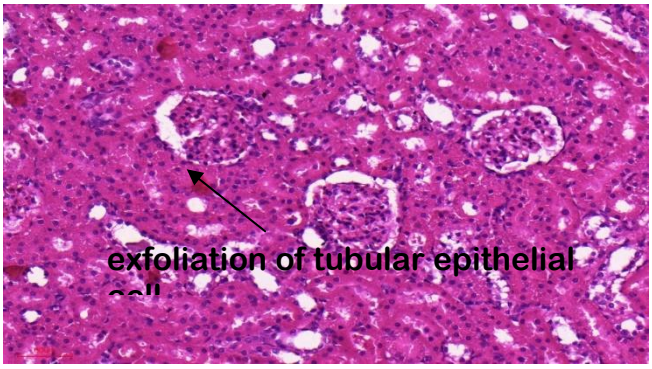

Plate 9: Photomicrograph of kidney section of rat from periodontitis and diabetes group showing cytoplasmic vacuolation of tubular epithelial cells in the cortex, loss of brunt borders, exfoliation of tubular epithelial cell thickening glomeruli basement membrane, mesangial expansion (H&E, x 200).

Plate 8: Photomicrograph of kidney section of rat from periodontitis +diabetes and treatment group showed no exfoliation of tubular epithelial cells, vacuolation seen, milder loss of brush border minimal mesangial expansion, reduced number of glomeruli showing mesangial expansion (H&E, x 200).

Conclusion

The impact of *Nigella sativa* oil and its bioactive components on renal dysfunction caused by periodontitis and diabetes was assessed through histological analysis of kidney tissues, as well as by measuring serum levels of urea, creatinine, and electrolytes. Based on the results obtained, it can be inferred that *Nigella sativa*oil provided effective protection to rat kidneys against the harmful effects of induced diabetes and periodontitis.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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