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Effects of Black Seed Oil on Oxidative Stress Parameters and Gingival Expression of Inducible Nitric Oxide Synthase in Diabetes and Periodontitis-Induced Rats

Abdulwasiu A. Busari[1], Osuvwe Clement Orororo[2]*, Ugbome A. Opute[1], Israel O. Efejene[3], Kabir Badmos[1], Cynthia C. Obadiah[1], Warith A. Akinshipo[1] and Egoamaka O. Egbune[4]

[1]Department of Pharmacology, Therapeutics & Toxicology, College of Medicine, University of Lagos, Lagos State
[2]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State, Nigeria
[3]Deparment of Pharmacology, College of Medical and Health Sciences, Novena University, Ogume, Delta State, Nigeria
[4]Department of Biochemistry, Delta State University, Abraka, Delta State, Nigeria

*Corresponding author Email: osuvwec@yahoo.com Tel: +234 (0) 806 230 6783

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ABSTRACT: Using diabetic and periodontitis-induced rats, the effects of black seed oil on oxidative stress parameters and gingival expression of inducible nitric oxide synthase were examined in this study. Eight groups of forty eight were created. Rats from Group I were used as the Control group; they had unrestricted access to typical rat diet and were not given any medications. 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. Following the establishment of diabetes and periodontitis in Group VII rats without therapy, Group VIII rats were administered with 1 ml/kg NS oil (DB+PD+NS). To evaluate the expression of inducible nitric oxide synthase (iNOS), immunohistochemical analysis was done together with tests for superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and lipid peroxidation (MDA). With an increase in the levels of lipid peroxidation, diabetes and periodontitis induced a substantial reduction ($P<0.05$) in the activity and level of SOD, CAT, and GSH. Rats treated with black seed oil, however, exhibit increased levels of SOD, CAT, and GSH along with a commensurate decrease in MDA when compared to untreated animals in the DM, PD, and PD+DM groups. The administration of Nigella sativa oil significantly reduced the periodontal inflammation and elevated iNOS expression that were also brought on by diabetes and periodontitis. The findings of this study demonstrated that the administration of black seed oil as an adjuvant in the treatment of diabetic and periodontitis-affected rats effectively suppressed iNOS expression and decreased oxidative stress.

Keywords: Diabetes, Periodontitis, *Nigella sativa*, iNOS

Introduction

A chronic disease known as diabetes mellitus (DM) can arise when the pancreas fails to produce enough insulin or when the body has difficulties utilizing the insulin that is produced. As a result of absolute or relative insulin insufficiency, it is characterized by abnormalities in the metabolism of glucose, proteins, and lipids as well as organ system malfunction (Uloko *et al*., 2018). According to reports, there were over 451 million people with diabetes-related complications in 2017, and by 2045, that number is expected to rise to 693 million (Cho *et al*., 2018).

An inflammatory condition known as periodontitis damages the connective tissue's ability to adhere to the teeth and the bone that supports them (Kinane *et al*., 2017). Periodontitis symptoms include resorption of alveolar bone, apical migration of the junctional epithelium, loss of collagen fibers and adhesion to the cemental surface, and deepening periodontal pockets. If the condition is not addressed, it worsens and leads to bone degeneration, which eventually results in tooth loss (Albandar 2002; Gasner and Schure, 2020). Humans have suffered from infections of the gingiva and periodontal ligament from the beginning of recorded history. Paleopathological studies have shown that early people in many different societies around the world suffered from periodontal problems, specifically bone loss (Eid Abdelmagyd *et al*., 2019).

Diabetes mellitus (DM) metabolic dysregulation linked with hyperglycemia and dyslipidaemia has common chronic inflammatory processes identified in periodontitis impacting the tooth-supporting tissues. There is strong evidence that diabetes mellitus increases the likelihood of developing periodontitis by causing a pro-oxidant profile that is hyper-inflammatory and amplifies periodontal damage. In patients with periodontitis and DM, the bidirectional link between the two disease entities has negative implications on glycaemic management. A known risk factor for the development of periodontal disease is uncontrolled diabetes mellitus (DM), which is characterized by hyperglycemia and insulin resistance. On the other hand, established chronic inflammation like that observed in periodontitis may cause insulin resistance, the onset of DM, and associated consequences (Soory, 2012). Systemic problems like diabetes, autoimmune diseases, and cardiovascular concerns have all been related to periodontitis (Cullinan and Seymour, 2013; Gulati *et al*., 2013; Gurav, 2014).

The inflamed periodontium is said to have higher levels of inducible nitric oxide synthase (iNOS), an inflammatory mediator. Immunocompetent cells produce iNOS, which is involved in controlling inflammatory responses (Soskic, 2011). Since it is a critical component of the host response during the inflammatory response, nitric oxide (NO), which is produced from l-arginine by iNOS, is crucial to the infection process (Malachowa and Deleo, 2018; Nikolajevi, *et al*., 2019; Pautz *et al*., 2010). Peroxynitrite, which is formed when NO is converted to it, releases hydroxyl radicals that can harm endothelium cells. Moreover, it's thought that too much NO encourages periodontitis' inflammatory response (Herrera *et al*. 2011). Furthermore, interleukin (IL)-6, tumor necrosis factor-alpha (TNF-), and nuclear factor kappa B (NF-B) levels may increase in local or systemic chronic inflammatory conditions brought on by periodontitis (Nishikawa *et al*., 2012; Tawfik *et al*., 2019). Although elevated levels of inflammatory markers are thought to be significant risk factors for diabetes, elevated inflammatory cytokine levels brought on by periodontal disease may enhance the vulnerability to diabetes.

Reactive oxygen species (ROS) are created when iNOS gets uncoupled (Heusch *et al*., 2010). On the other hand, abnormal iNOS induction appears to be involved in the pathophysiology of a number of human disorders (Jaiswal *et al*., 2013). Oxidative stress is generated by the imbalance of free radicals and the body's antioxidant defenses (Guergouri *et al*., 2017; Orororo *et al*., 2018a; Orororo *et al*., 2018b). Lipoprotein peroxidation, which is characterized by MDA generation, will result from a failure to protect against free radicals. MDA levels have been linked to an increase in a number of oxidative stress-related illnesses (Singh *et al*., 2021; Orororo *et al*., 2023a). According to reports, oxidative stress and inflammation play a role in the events that lead to the development and occurrence of diabetes and periodontitis (Giacco and Brownlee, 2010; Pitocco *et al*., 2013; Marchesan *et al*., 2020).

In addition to mechanical scaling and root planing, devices for prolonged medication release, mouthwashes, irrigations, and restricted drug delivery systems are frequently employed to provide therapeutic agents in the treatment of periodontal disorders (Eid Abdelmagyd *et al*., 2019; Fischer *et al*., 2020). Current chemotherapeutic agents are highly efficient in treating periodontal disease, but they come with a number of unfavorable side effects, including taste changes, tooth and tongue discolouration, antimicrobial resistance, and increasing compound prices (Heta *et al*. 2018; Zhao *et al*. 2020). Hence, the use of natural and herbal remedies for periodontal treatment has drawn more attention recently and may have significant advantages, particularly for populations with lower socioeconomic level worldwide (Yimer *et al*., 2019; de Moraes *et al*., 2020).

Due to their promising results and uncommon side effects, the use of therapeutic herbs in the treatment of various ailments has received increasing interest on a global scale (Esiekpe *et al*., 2020; Mekhemar *et al*., 2020; Orororo *et al*., 2017; Al-Attass *et al*., 2016). More than two-thirds of the world's population, mostly in developing countries, rely on using traditional medicines and natural treatments for their primary health care and disease treatment, according to the World Health Organization (WHO). The WHO has therefore urged developing nations to incorporate the use of therapeutic plants as an additional resource to improve the efficacy of health care systems (Al-Attass *et al*., 2016; WHO, 2013). Nigella sativa (NS), frequently referred to as a "wonder herb," is one of the herbal medicines with scientific support (Yimer *et al*., 2019; Orororo *et al*., 2023b). Due to its rich nutritional content and active ingredients, studies have confirmed the health benefits of NS, including its antioxidant, antimicrobial, antifungal, anticonvulsant, immunomodulatory, anti-inflammatory, antihyperlipidemic, antitussive, anticancer, analgesic, antipyretic, antiviral, contraceptive and antibacterial effects (Ardiana *et al*., 2020; Yimer *et al*., 2019). Alkaloids, proteins, saponins, fixed and essential oils, and

other substances make up seeds. Thymoquinone (TQ), one of the pharmacologically active components isolated from NS to date, has received the greatest attention as an active and therapeutic component (Yimer *et al*., 2019; Ahmad *et al*., 2020).

Although studies on the expression of iNOS in periodontitis with or without diabetes have been conducted, more research is necessary to fully understand the pathogenic role of iNOS in the inflammatory response that leads to periodontal damage in diabetes. Moreover, no study has looked at the impact of black seed in rats having periodontitis and diabetes at the same time. This study examined the effects of black seed oil on oxidative stress parameters and gingival expression of inducible nitric oxide synthase in diabetes and periodontitis-induced rats in light of the claim that oxidative stress and tissue destruction due to ROSs, such as NO, in diabetes and periodontitis may be suppressed by using anti-oxidants.

Materials and Methods

Chemicals and reagents: Streptozotocin, trichloroacetic acid, formaldehyde, acetonitrile, methanol, Saline, distilled water Heparin injection, iNOS antibody and detection kit (abbexa), urethane, chloralhydrate, formic acid, Nigella sativa oil, and sodium chloride were products of Chemic Laboratory, India. 2,-thiobarbituric acid, adrenaline, acetic acid, Dichromate and Ellman's reagent were products of BDH Chemical Company, England.

Experimental animals: Forty eight (48) mature male Wistar rats weighing between 140 and 160 g were used in the experiment. The animals were maintained at the faculty animal home for two weeks prior to the experiment's start to allow for acclimatization. The wire-mesh cages housing the Wistar rats were maintained in one space. Throughout the trial, the animals had unrestricted access to water and food pellets at a temperature of about 29 °C plus or minus 2 °C. They had a 12-h cycle of light and dark. The Handbook for the Care and Use of Experimental Animals was followed for establishing the experiment protocols. The animal care and use research ethics committee for the Faculty of Medicine at the University of Lagos approved this work with reference number. CMUL/ACUREC/02/20/71.

Experimental design: Forty eight (48) Wistar rats were divided into eight groups of six each. Rats from Group I were utilized as the Control group; they were not subjected to any conditioning and had unlimited access to standard rat diet. The rats in group II received NS oil in addition to their regular rat food and water. Diabetes was untreatedly induced in Group III rats. Group IV rats were administered 1 ml/kg of NS oil intraperitoneally in order to produce diabetes (DB + NS0). In Group V, untreated periodontitis was induced. 1 ml/kg of NS oil was intraperitoneally delivered into group VI rats after they had periodontitis. Following intraperitoneal induction of diabetes and periodontitis (DB + PD+NS1), group VIII rats received 1 ml/kg of NS oil. In group VII rats, diabetes and periodontitis were induced without therapy. Table 1 shows the experimental design adopted for this study.

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 (DM)	Injection of STZ	No treatment
4	Diabetic+ Treatment	Injection of STZ	Oral *N. sativa* oil admin.
5	Periodontitis (PD)	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.

Diabetes and periodontitis induction: The previous night, laboratory animals were fasted in preparation for streptozotocin's induction of diabetes. Streptozotocin (STZ), 50 mg/kg body weight, was administered intraperitoneally to the rats in a freshly buffered solution (0.1 m citrate, pH 4.5). An incredibly sensitive glucometer was used to measure blood glucose levels in tail vein blood that was drawn 72 h after STZ injection.

By placing a 3/0 silk suture subgingivally on each rat's incisor while it was under general anesthesia (Chlorahydrate 30 mg/kg of body weight), ligature-induced periodontitis was inflicted upon each animal (Hatipoglu *et al*., 2015). Inducing plaque formation and the subsequent onset of periodontal disease, the ligature acted as a gingival irritant for 21 days. Following the ligatures' placement, the animals were monitored for 21 days while daily ligature inspections were made.

Nigella sativa administration and sample collection: The treatment groups received the oil intraperitoneally for 21 days at a rate of 1 millilitre per kilogram of the test animals (Mohamed *et al*., 2010). The animals were starving when they were dosed.

After the anticipated exposure and treatment times, each group of animals was put to death. Rats were slain after being fasted for a night and being killed by cervical dislocation (Morakinyo *et al*., 2014). Cardiovascular puncture was used to take blood, which was then spun at 3000 rpm for 10 minutes to produce serum for biochemical testing.

Determination of superoxide dismutase (SOD) activity: According to Sun and Zigma (1978), the ability of superoxide dismutase to prevent the auto-oxidation of epinephrine was measured by an increase in absorbance at 480 nm. The reaction was started using a reaction mixture (3 ml) that contains 2.95 ml of 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of liver homogenate, and 0.03 ml of epinephrine in 0.005 N HCl. 2.95 ml of buffer, 0.03 ml of substrate (epinephrine), and 0.02 ml of water were all present in the reference cuvette. By monitoring the change in absorbance at 480 nm for 5 min, enzyme activity was calculated.

Catalase activity determination: Sinha *et al*. (1972)'s method was used to measure catalase activity. It was measured colorimetrically at 620 nm and expressed as moles of H_2O_2 consumed per minute per milligram of protein at 250 °C. 1.0 ml of pH 7.0, 0.01 ml of phosphate buffer, 0.10 ml of tissue homogenate, and 0.40 ml of 2 M H_2O_2 made up the reaction mixture (1.5 ml). By adding 2.0 ml of the dichromate-acetic acid reagent - 5% potassium dichromate and glacial acetic acid - in a 1:3 ratio, the process was stopped.

Reduced glutathione determination: The method outlined by Sedlak and Lindsay in 1968 was used to measure the reduced glutathione (GSH) level in serum as non-protein sulfhydryl. 10% TCA was centrifuged after being added to the homogenate. The Ellmans reagent (19.8 mg of 5,5-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 mg of phosphate buffer were added to 1.0 ml of supernatant (0.2M, pH 8.0). At 412 nm, the absorbance was measured.

Lipid peroxidation measurement: Using the Buege and Aust (1978) technique, the lipid peroxidation index malondialdehyde (MDA) was measured. The reaction mixture of tricarboxylic acid, thiobarbituric acid, and hydrochloric acid was heated at 100 °C for 15 min and then allowed to cool. Centrifuging was used to remove flocculent debris for 10 min at 3000 rpm. After removing the supernatant, the absorbance was measured at 532 nm in comparison to a blank. The molar extinction coefficient for the MDATBA- complex, which is 1.56 105 M-1CM-1, was used to determine MDA.

Determination of inducible nitric oxide synthase expression: immunohistochemistry: The Anatomic and Molecular Pathology Laboratory of the College of Medicine of the University of Lagos, Idi-Araba, Lagos, performed an immunohistochemical study on the rat tissues. The rats were provoked for the immunohistochemical examination by administering 1.25 g/kg of urethane dissolved in 5ml of saline. The rats' lower jaws were removed, placed in 10% formaldehyde solution intraperitoneally, and then their heads were reattached. The samples were first decalcified in a 10% formic acid solution. Samples of around 5 mm in thickness were collected from the mandibles to fill the suture area of the incisors after the decalcification procedure, which lasted for about 2 days. These were recorded on cassettes. The samples whose tissue follow-up process was finished were embedded in blocks of paraffin. Microtome samples of 5 m thickness were cut from paraffin blocks ready for immunohistochemical analysis. To lessen background staining from endogenous peroxide, the tissue samples were deparaffinized, rehydrated, and then rinsed with a buffer solution. After that, the slides spent 10 min being incubated in Ultravision Hydrogen Peroxide Block. The buffer solution was used to wash the slides. After washing, Ultravision Protein Block was applied and allowed to prevent background staining for a further five minutes of incubation. After blowing the samples, the primary antibody (Inducible Nitric Oxide) was added, and it was allowed to sit for 10 min. The HRP Polymer Quanto was then applied, and the slides were allowed to sit for 10 minutes while the buffer solution was used to wash them. The samples were once more rinsed with the buffer solution, distilled water, and buffer. Before applying the mixture to the tissue samples on the slides, 1 drop of DAB Quanto Chromogen and 1 ml of DAB Quanto Substrate were swirled together. The tissue samples were then incubated for 5 minutes before being rinsed with distilled water. In the connective tissue beneath the junctional epithelium, the number of cells that stained positively with iNOS was counted. Each patient had a similar location selected, and a 0.1 mm^2 area was marked and examined. On this region, the iNOS-positive cells were noted and counted. The harmed cells were not included in the investigation. The reader conducted the evaluations without being aware of the subject's characteristics or the marker's stain.

Immunohistochemistry scoring: Using immunoreactivity score, percentages were allotted to the number of cells stained as: 0 - 5% (Negative), 6 - 25% (1), 26 - 50% (2), 51 - 100 % (3)

Then intensity was scored in a three-tiered scale of 0-2 with: 0 – Negative, 1 – Weak, 2 – Strong immunocytoplasmic stain.

Percentage score and intensity score were added together and using arbitrary cut-off of 2, all cases with immunoreactivity score <2 were considered negative while those ≥ 2 were positive.

Results

Effects of black seed oil on oxidative stress parameters in diabetes and periodontitis-induced rats: The effects of *Nigella sativa* oil on oxidative stress parameters in rats induced with diabetes and periodontitis is presented in Tables 2 (SOD and CAT) and 3 (GSH and MDA).

There was a significant ($p<0.001$) decrease of SOD in the serum of DM induced rats compared to the control. Similarly there was significant ($p<0.05$) decrease in serum SOD in rats Induced with PD. More so, a similar pattern was observed in rats Induced with PD+DM, which shows ($p<0.05$) significant reduction. However, rats treated with Black seed oil show elevation of SOD in DM, PD AND PD+DM compared to untreated groups.

As regards Catalase (CAT) activity, a significant ($p<0.05$) decrease of CAT in the serum of DM induced rats was seen compared to the control. Similarly there was significant ($p<0.001$) decrease in serum CAT in rats Induced with PD. Also a similar pattern was observed in rats Induced with PD+DM, which shows ($p<0.05$) significant reduction in CAT. However, rats treated with Black seed oil show increase of CAT in DM, PD AND PD+DM compared to the untreated groups.

Table 3 shows a significant ($p<0.01$) decrease in GSH level in the serum of DM induced rats compared with the untreated control group and an increase in GSH level in rats treated with *Nigella Sativa* oil compared with untreated DM group. Similarly, significant ($p<0.05$) reduction was seen in PD group compared with the untreated control group and the administration of *Nigella sativa* oil ameliorated this effect by increasing GSH level in treated PD group compared with untreated groups. More so, GSH 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 MDA, mild elevation of MDA was observed in untreated DM, PD and DM+PD groups compared with untreated control group. However administration of *Nigella sativa* oil shows mild reduction of MDA in treated PD, DM and PD+DM compared with untreated groups.

Table 2: Effects of *Nigella sativa* oil on Superoxide Dismutase (SOD) and Catalase (CAT) activities in rats induced with diabetes and periodontitis

	Parameters	
Groups	**SOD**	**CAT**
Control	5.914 ± 0.5195	23.47 ± 1.112
Positive Control (NS)	7.916 ± 0.1662****	30.75 ± 3.010*
DM	4.632 ± 0.1734***	16.22 ± 1.223*
DM + NS	6.468 ± 0.1805	27.30 ± 1.064
PD	4.716 ± 0.2219*	15.63 ± 1.016**
PD + NS	6.262 ± 0.0984	27.00 ± 0.5281
DM + PD	4.756 ± 0.446*	17.24 ± 16.22
DM + PD + NS	5.98 ± 0.1726	23.38 ± 0.6710

Results are mean ± SEM (n=5) *p<0.05 vs control, ***p<0.001 vs control, ****p<0.0001, *p>0.05 vs control. One way ANOVA followed by Tukey's *post hoc* multiple comparison tests.

Table 3: Effects of *Nigella sativa* oil on Glutathione (GSH) activity and Lipid Peroxidation Levels in rats induced with diabetes and periodontitis

Groups	**Parameter**	
	GSH	**MDA**
Control	96.29 ± 5.181	2.25 ± 0.54
Positive Control (NS)	116.0 ± 1.964***	2.03 ± 0.50
DM	64.38 ± 2.283	2.25 ± 1.67
DM + NS	99.53 ± 2.935	1.91 ± 0.30
PD	77.59 ± 2.238*	2.34 ± 0.36
PD + NS	109.8 ± 9.372	1.98 ± 0.23
DM + PD	59.57 ± 5.221***	2.53 ± 0.56
DM + PD + NS	97.73 ± 3.877	1.67 ± 0.86

Results are mean ± SEM (n=5) *p<0.05 vs control, ***p<0.001 vs control, ****p<0.0001, *p>0.05 vs control. One way ANOVA followed by Tukey's *post hoc* multiple comparison tests.

Effects of black seed oil on gingival expression of inducible nitric oxide synthase in diabetes and periodontitis-induced rats: The effects of *Nigella sativa* oil on gingival expression of inducible nitric oxide synthase in rats induced with diabetes and periodontitis is shown by the immunuhistochemistry result presented in Table 4 and

in plates 1 and 2. Out of the 40 cases stained for iNOS; Groups 3,4,5,6,7 and 8 gave significant results for the immunohistochemistry.

Table 4: Immunohistochemistry result showing periodontal inflammation

S/N	Groups	Results
1	Diabetes mellitus induced group	Periodontal inflammation was noted.
2	Diabetes mellitus + Nigella sativa oil treatment group	Reduced level of inflammation was noted in the treatment group.
3	Periodontitis induced group	Expressed level of iNOS was noted to be high to the peak.
4	Periodontitis + Nigella sativa oil treatment group	Expression level of iNOS was reduced, activity of inflammatory cells was reduced indicating that Nigella sativa oil assisted in healing.
5	Diabetes mellitus + Periodontitis induced group	Periodontal inflammation was noted but was not high compared to periodontitis induced group.
6	Diabetes mellitus + Periodontitis + Nigella sativa oil treatment group	There was a reduction in the level of expression of iNOS compared to the Diabetes mellitus + Periodontitis induced group.

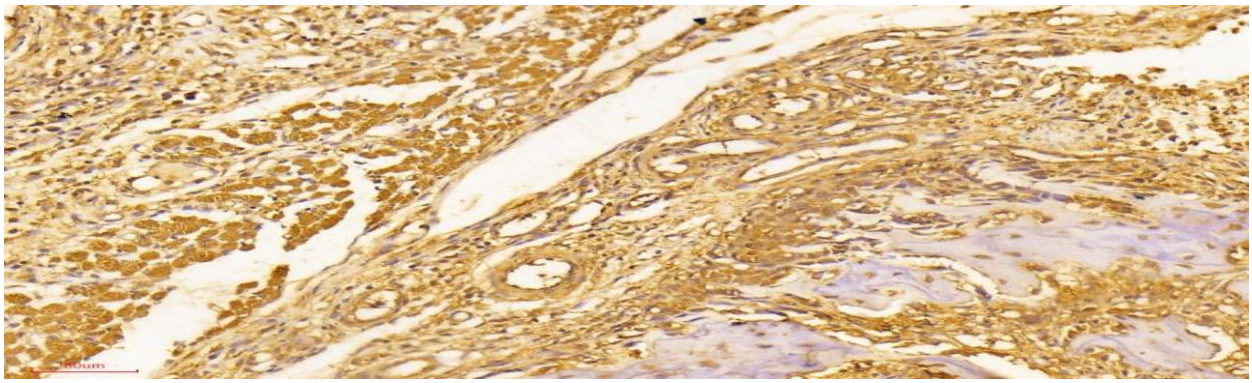

Plate 1: Photomicrographs of sections from gingival of periodontitis rat showing few inflammatory cells with positive immunostaining for iNOS, other cells appear flattened with tapering ends. (iNOS immunohistochemical staining with Mayer's haematoxylin counter stain scale bar = 50 mm)

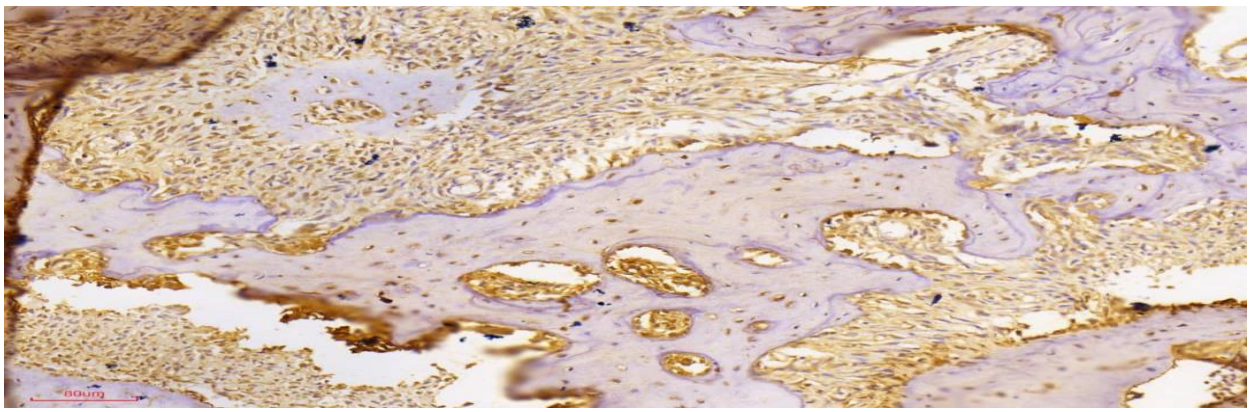

Plate 2: Photomicrograph of jaw section from control rat gingiva showing no (negative) immunostaining for iNOS within the stromal cells underlying the gingival epithelium (E). (iNOS immunohistochemical staining with Mayer's haematoxylin counter stain scale bar = 50 mm).

Discussion

In diabetes and periodontitis-induced rats, the effects of black seed oil were examined in terms of oxidative stress parameters and gingival expression of inducible nitric oxide synthase. Hyperglycemia is a result of the

systemic and metabolic abnormalities that diabetes causes. In addition to creating free radicals that weaken the body's antioxidant system, it interferes with the body's normal lipid, protein, and glucose metabolism (Alimohammadi *et al*., 2013). On the other hand, periodontitis is a dental infection linked to the microbiome that is brought on by Gram-negative bacteria and results in connective tissue deterioration around the teeth that extends over the gingival (Al-Bayaty *et al*., 2013). Natural diabetes and periodontal disease treatments are preferred to synthetic ones due to their low cost and safety (Baragob, 2015).

Tables 2 and 3 demonstrated that diabetes and periodontitis significantly reduced the levels and activities of SOD, CAT, and GSH while raising the levels of lipid peroxidation. This makes sense given that oxidative stress has been found to be induced in cells by diabetes and periodontitis. Reactive oxygen species (ROS) and oxidative stress have been linked to the events that occur during the development and incidence of diabetes and periodontitis (Marchesan *et al*., 2020). The body possesses a variety of extremely effective antioxidant defense mechanisms to reduce the amount of free radicals present there. These enzyme systems (SOD, CAT, and GSH) form the initial line of defense against free radicals (Evans and Halliwell, 2001; Prabhakar *et al*., 2005; Acharya and Ghaskadbi, 2010).

Black seed oil treatment for rats with periodontitis and diabetes results in an increase in SOD, CAT, and GSH levels, as well as a reduction in MDA, as compared to control group (Tables 2 and 3). This is undoubtedly related to the Nigella sativa oil and its ingredients' reputedly potent antioxidant abilities. Thymoquinone and its derivatives, including dithymoquinone, thymohydroquinone, and thymol, p-cymene, carvacrol, and -terpinene, are the bioactive components of NS and have been proven to have therapeutic effects for a variety of ailments (Fauzi *et al*., 2018). The ability of NS to lower the formation of reactive oxygen species (ROS) and malondialdehyde (MDA) has been demonstrated (Sahak *et al*., 2016). NS has also been found to have a high concentration of thymoquinone, a compound that can neutralize free radicals. Even more, NS has the power to raise levels of antioxidant enzymes as glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) (Kazemi, 2014). According to earlier studies, patients who took NS supplements produced much less MDA, NO, and SOD than controls (Namazi *et al*., 2015). In contrast to the results of this investigation, other studies (Hadi *et al*., 2016; Kaatabi *et al*., 2015) did not detect any appreciable changes in the MDA, GPx, or total antioxidant capacity (TAC) upon treatment of oxidative stress with NS.

This study also demonstrated how the combination of diabetes and periodontitis led to increased iNOS expression and periodontal inflammation, both of which could be significantly decreased by taking Nigella sativa oil. Elevated iNOS expression in diabetic and periodontitis-affected rats may possibly have contributed to the rise in oxidative stress, which was seen as a significant decrease in GSH, CAT, and GSH with a large increase in MDA. Vital physiological activities, as well as, pathological activities are controlled by the biological mediator nitric oxide (NO) (Rodeberg *et al*. 1995). NOS produces superoxide anion (O^2), peroxynitrite, and NO, and iNOS has been connected to a number of clinical diseases that are associated to inflammation (Fujimoto *et al.* 2005; Zhang *et al*., 2007). Primarily, post-transcriptional and transcriptional mechanisms control how iNOS is expressed. Different cells or animals have different mechanisms for iNOS induction. Oxidative stress has been shown to activate NF-kB and stimulate iNOS production in a variety of cell types, suggesting that activation of the transcription factors NF-kB and STAT-1 is necessary for most cells in order for iNOS to be produced (Kleinert *et al.*, 2003).

In keeping with the outcome of this study, it has been observed that iNOS level is higher in the presence of periodontal disease than in healthy persons (Pan *et al*., 2010). According to studies by Shibata *et al.* (2001) and Hatipolu *et al.* (2016), NOS activity is higher in neutrophils from people who have been diagnosed with localized aggressive periodontitis than it is in neutrophils from healthy people. Since black seed oil has strong antioxidant properties, it was able to drastically reduce gingival iNOS expression in the current investigation. Muià *et al*. (2006) who published a research evaluating the impact of pyrrolidine dithiocarbamate in experimental periodontitis reported similar results. They stated that periodontitis caused an increase in iNOS levels in the gingival tissues. Similar to this, Popkov *et al.* (2005) demonstrated that in an experimental rat diabetic model, mexidol's antioxidant abilities cause iNOS levels to drop. Like their study, the current study discovered that black seed oil treatment reduced gingival iNOS expression in diabetic and periodontitis-affected rats.

According to research, the etiology of periodontal disease is heavily influenced by inflammation and the formation of reactive oxygen species (ROS) (Marchesan *et al*., 2020). NS and TQ have been considered as anti-inflammatory and antioxidant mediators with therapeutic benefits in a number of studies (Shaterzadeh-Yazdi *et al*., 2018; Mahmoud and Abdelrazek, 2019; Varela-Lopez *et al*., 2015). According to earlier research, TQ causes an antioxidant effect by neutralizing a variety of free radicals, and it is just as good at neutralizing superoxide anions as superoxide dismutase is at neutralizing them (Nader *et al*., 2010; Kassab *et al*., 2017). TQ has also demonstrated observable anti-inflammatory properties in experimental studies (Yazdi *et al*., 2019). By the lowering of iNOS, it lowers nitric oxide (NO) levels. The capacity of TQ to suppress eicosanoid synthesis may be connected to its potential role in the combined anti-inflammatory and antioxidant effect. Experimental

results have demonstrated a considerable decrease of lipid peroxidation and eicosanoid production by TQ and NS extracts (Mostofa *et al.*, 2017).

Conclusion

The findings of this study demonstrated that the administration of black seed oil as an adjuvant in the treatment of diabetic and periodontitis-affected rats effectively suppressed iNOS expression and decreased oxidative stress. According to the limits of the current investigation, black seed oil is thought to reduce oxidative stress, which in turn reduces gingival inflammation and tissue deterioration. Based on the findings, the administration of black seed oil as an adjuvant therapy can prevent diabetes-related oxidative stress and periodontal tissue deterioration.

List of Abbreviations

iNOS - Inducible Nitric Oxide Synthase
SOD - Superoxide dismutase
CAT - Catalase
GSH - Glutathione

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data utilized in analysis for this project are available for presentation upon request.

Competing interests

The authors of this manuscript have no fnancial or non-financial conflicts of interest to disclose.

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Author contributions

All authors contributed to conception, design, analysis, and/or interpretation of the data. AAB, WAA and UAO conceived and designed the analysis. UAO, CCO and IOE collected the data. OCO and EOE analyzed the data. OCO and EOE wrote and edited the manuscript under the supervision of and with guidance from AAB and WAA. All authors have read and approved the manuscript.

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