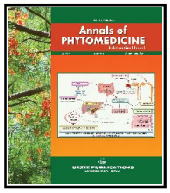
Annals of Phytomedicine 13(1): 558-566, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.1.58

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Ameliorative effect of spices on oxidative stress induced damage in glandular tissues in diabetic rats: A chronic study

T. J. Sheikh◆, D.V. Joshi*, B.J. Patel*, S.H. Raval*, Ranjan Rajeev and C.M. Modi*****

Department of Veterinary Pathology, College of Veterinary Science and A.H, Rewa-486001, Madhya Pradesh, India
** Department of Veterinary Pathology, College of Veterinary Science and A.H, Kamdhenu University, Dantiwada-385505, Gujarat, India*
*** Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A.H, Rewa-486001, Madhya Pradesh, India*
**** Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A.H, Kamdhenu University, Dantiwada-385505, Gujarat, India*

Article Info

Article history
Received 18 January 2024
Revised 6 March 2024
Accepted 7 March 2024
Published Online 30 June 2024

Keywords
Gland
Streptozotocin
Nicotinamide
Black cumin
Fenugreek
Histopathology

Abstract

Various medicinal properties are inherent in Indian spices. To evaluate the antioxidant activity and its impact on glandular tissues in streptozotocin-nicotinamide (STZ-NT)-induced diabetic rats, the extract was prepared from black cumin, fenugreek, garlic, and their combination. The experimental rats were divided into six different groups (normal, diabetic, diabetic + Black cumin, diabetic + Fenugreek, diabetic + Garlic, diabetic + Mixture). Induction of diabetes was carried out by intraperitoneal injection of STZ-NT (45 mg/kg -110 mg/kg), followed by treatment with extracts for 90 days. It was found that the level of lipid peroxidation was elevated, whereas superoxide dismutase was diminished in STZ-NT-induced diabetic control rats. The diminished activities of superoxide dismutase exhibited a significant increase when treated with extracts of garlic, fenugreek, black cumin, and their combination in descending order, as compared to the positive control rats. Microscopic tissue alterations revealed vacuolization in acini of the submandubular gland and parotid gland, as well as atrophy of the epithelium of seminal vesicle. The testes revealed vacuolization in the seminiferous tubule with degenerated pachytene spermatozoa in diabetic control rats. The aqueous extracts treatment of garlic, fenugreek, black cumin, and their mixture have shown a positive impact on superoxide dismutase activity, potentially mitigating the oxidative stress and tissue pathological changes induced by diabetes. This protective effect can be ascribed to the diverse array of bioactive compounds present in the spices.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by glycosuria, polydipsia, hyperglycemia, polyphagia and polyurea due to insufficient insulin secretion from beta cells of the pancreas. Essentially, DM is multiorgan affecting chronic disease. The chronic nature of diabetes mellitus results in the generation of an excessive amount of reactive oxygen species (ROS) and disruption of endogenous antioxidant enzyme activities (Kurek *et al.,* 2014; Knas *et al.,* 2006). This excessive generation of ROS induces damage, leading to permanent changes in DNA, RNA, proteins, lipids, and carbohydrates, thereby causing the loss of vital cell organelle function (Lushchak, 2014). This damage extends to critical systems such as the reproductive system, nervous system, kidneys, and salivary glands (Premkumar and Pabbidi, 2013; Cakatay, 2005).

Furthermore, diabetes mellitus (DM) poses a global health challenge, given its severe complications to nephritic tissue, cardiovascular, nervous and reproductive systems (Melendez-Ramirez *et al*., 2010; Atkinson and Maclaren, 1994).

Corresponding author: Dr. T.J. Sheikh
Assistant Professor, Department of Veterinary Pathology, College of Veterinary Science and A.H, Rewa-486001, Madhya Pradesh, India
E-mail: drsheikh2@yahoo.co.in
Tel.: +91-8770846169

Diabetes-induced salivary gland damage can modify salivary parameters, including pH, flow rate, salivary protein levels, and the occurrence of xerostomia. These modified parameters may reflect salivary gland damage (Ivanovski *et al*., 2012). The qualitative alterations in salivary gland structure may potentially contribute to the onset of diverse oral pathological conditions (Soysa *et al.,* 2006; Bell *et al.,* 2000; Rees, 2000).

The induction of a chronic type-2 diabetes model in rats can be achieved through the administration of streptozotocin-nicotinamide (STZ-NT) intraperitoneally, a common method to investigate diabetes-related effects on vital tissues (Morimoto *et al.,* 2005; Kuhn-Velten *et al.,* 1982). This STZ-NT induced diabetic model is linked to changes in the functions of the reproductive and salivary parameters (Orth *et al.,* 1979). Moreover, it is important to note that STZ does not induce any physiological or morphological changes in the reproductive organs of diabetic rats; rather these lesions are attributed to diabetes itself (Ballester *et al.,* 2004). Hence, this method for inducing diabetes is applicable to investigate diabetes-related lesions in target organs.

The primary approach to diabetes treatment involves managing hyperglycemia through insulin; however, insulin treatment is rather expensive and for the time being only. Numerous review on traditional medicinal plants/herbs/spices as an alternative treatment for diabetes available in the literature. So, in our current research, we have assessed the ameliorative effect of common Indian spices (Fenugreek, Black-cumin, garlic and their mixture) by evaluating changes in blood

glucose, lipid peroxidation (LPO), superoxide dismutase (SOD) levels, and histopathological alterations in the testicular gland, parotid gland, and submandibular gland in STZ-NT induced diabetic rats.

2. Materials and Methods

2.1 Experimental animals

Male rats weighing 180 ± 50 g were used in the present study. All animal-related procedures, including both oral and intra-peritoneal dosing, as well as postmortem activities, adhered strictly to the CPCSEA guidelines on the Care and Use of Laboratory Animals. The Institutional Animal Ethics Committee (IAEC) of Veterinary College, SDAU, Gujarat, India granted prior approval for this experiment under proposal No. VET COLL-13-2011. Sixty (60) healthy male rats were chosen for the experiment, undergoing a 7-day acclimatization period before the study initiation. During the 90-day study period, rats were kept in a controlled environment at 23-25°C with 30-70% humidity, following a 12/12 h light/dark cycle. They were given standard feed pellet diets and had bottled drinking water.

2.2 Experiment design

The study involved six groups of 10 rats each, and the entire experiment duration was 90 days.

Group I (Normal rats): Treated with vehicle (*i.e.,* Normal saline).

Group II (Controlled diabetic rats): STZ-NT @ 45-110 mg/kg bwt intra-peritoneal (I/P) once.

Group III: STZ-NT @ 45-110 mg/kg I/P once + 500 mg/kg bwt Black cumin oral daily.

Group IV: STZ-NT @ 45-110 mg/kg I/P once + 500 mg/kg bwt Fenugreek oral daily.

Group V: STZ-NT @ 45-110 mg/kg I/P once + 500 mg/kg bwt Garlic oral daily.

Group VI: STZ-NT @ 45-110 mg/kg I/P once + 500 mg/kg bwt mixture of Black cumin, Fenugreek and Garlic oral daily.

2.3 Induction of diabetes

Induction of diabetes in rats was accomplished through the administration of two different chemicals, *i.e.,* streptozotocin (STZ) and nicotinamide. Intraperitoneal (I/P) injections of nicotinamide @ 110 mg/kg bwt and streptozotocin @ 45 mg/kg body wt, were given in overnight fasted rats in a gap of 15 min. Hyperglycemia was confirmed by the elevated level of blood glucose, measured twice using a glucometer, first at 72 h and second on the 7th day of injection. Rat with fasting glucose above 200 mg/dl was taken in the present study (Pellegrino *et al*., 1998).

2.4 Aqueous extract of Black cumin (*Nigella sativa*) seed

Prepared a solution of black cumin by mixing 100 g of black cumin seed powder with 200 milliliters of distilled water. Filtered the resulting mixture and then lyophilized it. Obtained the stock solution by dissolving 600 milligrams of the lyophilized powder in 10 milliliters of distilled water (Kasim *et al*., 2012).

2.5 Aqueous extract of Fenugreek (*Trigonella foenum-graecum*) seed

Extract prepared by combining 1 liter of hot distilled water with 50 g of dried fenugreek, allowing it to steep for 5-8 h at room temperature. The 5% (W/V) concentration was used as a treatment (Farman *et al*., 2009).

2.6 Aqueous extract of Garlic (*Allium sativum*)

It was prepared by thoroughly mixing 600 mg of dried garlic powder with 6 milliliters of water. Centrifuge the solution at 20,000 revolutions per min (rpm) for 5 min at 4°C, and the supernatant was used for the study (Pedraza-Chaverrí *et al.,* 2004).

2.7 Hematology and oxidative stress analysis

2.7.1 Hematology

Blood were collected in Potassium-EDTA and a hemogram was performed through an automated hematology analyzer (Medonic CA 620/530 VET, Boule Medical AB, Sweden).

2.7.2 Lipid peroxidation

The assessment of RBC membrane damage was conducted by quantifying malondialdehyde (MDA) levels. The primary underlying mechanism in the formation of lipid peroxidation at pH 4 involves the reaction between thiobarbituric acid and MDA. The optical density (OD) value was recorded at 523 nm, and the quantification of LPO activity was expressed as nmol/mg protein, serving as an indicator of the extent of peroxidation (Rehman, 1984).

2.7.3 Superoxide dismutase (SOD)

Generation of superoxide by pyrogallol autoxidation and the inhibition of superoxide-mediated reduction of the tetrazolium dye MTT (3-(4-5 dimethyl thiazol 2-xl) 2, 5-diphenyltetrazolium bromide) to its formazan, measured at 570 nm. By the addition of dimethyl sulfoxide (DMSO) reaction was terminated, resulting into solubilize the formazan formed. The color evolved is expressed as SOD units (1 unit of SOD is the amount (µg) of haemoglobin required to inhibit the MTT reduction by 50%) (Madesh and Balasubramanian, 1998).

2.8 Tissue preparation for histopathological studies and hematoxylin eosin staining

On the 91st day of the experiment, all rats were sacrificed. For histopathological examination, collected tissues were immersed in neutral buffer formaldehyde (10%) solution for fixation. The formaldehyde-fixed samples were processed in alcohol and xylene followed by embedding in paraffin wax; blocks were prepared and sectioned at 5-6 micron through microtome machine. The sectioned tissues were subsequently stained by hematoxylin and eosin (H and E) procedure for the identification of pathological lesions, observed through light microscopy.

2.9 Statistical analysis

The data were presented as means ± standard error (SE). Statistical analysis was conducted using SPSS version 20. ANOVA, followed by Duncan's Multiple Range Test was used for multiple comparisons. Statistical differences were determined at the 5% level of significance.

3. Results

3.1 Effects of extracts on LPO, SOD and blood glucose

Streptozotocin (STZ) is a diabetogenic agent, that induces β-cell destruction in the pancreas, resulting in an elevation of blood glucose concentration. Fasting blood glucose level revealed a statistically significant ($p>0.05$) decrease in glycemia among rats in groups III, IV, V, and VI as compared to those in the diabetic control group (Group II). In the present study, the LPO level in diabetic control rats (Group II) was significantly ($p<0.05$) higher compared to other groups. In treatment groups from Group III to Group VI, there was a significant ($p<0.05$) reduction in malondialdehyde (MDA) value after 90 days. The enzymatic activity of superoxide dismutase (SOD) in diabetic positive control rats (Group II) exhibited a notable and statistically significant reduction ($p<0.05$) when juxtaposed with the corresponding activities in the remaining experimental groups, as shown in Table 1. SOD values showed gradual improvement in rats belonging to Groups III, IV, V and VI compared to untreated diabetic rats. Among all treatment groups, the garlic-treated animals (Group V) showed particularly promising results.

Table 1: Lipid peroxidase (LPO), super oxide dismutase (SOD) and glucose level in different groups at 90th days of experiment

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
LPO (nmol/ml of RBC)	4.80 ± 0.27^{d}	10.10 ± 0.35^{a}	6.10 ± 0.42^{bc}	7.10 ± 0.48^{b}	5.10 ± 0.30^{cd}	6.20 ± 0.46^{bc}
SOD (U)	13.10 ± 0.60^{a}	4.40 ± 0.13^{e}	8.50 ± 0.40^{c}	6.60 ± 0.56^{d}	12.20 ± 0.30^{a}	10.40 ± 0.50^{b}
GLUCOSE (mg/dl)	115.4 ± 3.00^{d}	313.6 ± 8.50^{a}	166.8 ± 4.60^{c}	196.6 ± 8.00^{b}	202.9 ± 3.40^{b}	201.4 ± 4.70^{b}

*Compare data column wise, superscript (abcd) differ in column wise is significant ($p>0.05$).

3.2 Effects of extracts on hematological parameters

Hematological alterations were assessed in STZ-NT induced diabetic and normal control rats as shown in Table 2. Upon completion of 90-day experiment, Group II exhibited significantly ($p>0.05$) lower levels of red blood cells (RBC) and hemoglobin (Hb) compared to the normal control animals (Group I). However, no significant difference ($p>0.05$) was discerned in the total leukocyte counts and the differential leukocyte count (DLC) within any of the experimental groups. Rats in aqueous-treated groups (Group III, IV, V and VI) exhibited non-significant improvement in any hematological parameters as compared to untreated diabetic rats (Group II).

Table 2: Hematological parameters in different groups at 90th days of experiment

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
RBC ($nx10^6$/cumm)	8.6 ± 0.11^{ab}	7.8 ± 0.15^{c}	8.2 ± 0.21^{bc}	8.7 ± 0.11^{a}	8.5 ± 0.17^{ab}	8.1 ± 0.21^{bc}
Hb (gm %)	14.7 ± 0.18^{a}	13.7 ± 0.34^{b}	14.2 ± 0.49^{ab}	14.9 ± 0.22^{a}	14.8 ± 0.38^{a}	13.6 ± 0.30^{b}
WBC ($nx10^3$/cumm)	16.5 ± 0.38	13.3 ± 0.71	15.4 ± 0.88	15.5 ± 1.51	16.6 ± 1.44	18.1 ± 1.87
Neutrophils (%)	26.9 ± 0.46	27.6 ± 0.69	28.2 ± 0.74	27.0 ± 1.02	28.0 ± 0.82	26.8 ± 0.75
Lymphocyte (%)	65.8 ± 0.61	65.6 ± 0.95	65.2 ± 0.76	66.6 ± 0.68	65.3 ± 0.80	65.5 ± 0.82
Eosinophils (%)	5.2 ± 0.39	4.4 ± 0.37	4.1 ± 0.51	4.1 ± 0.35	4.5 ± 0.50	5.1 ± 0.55
Monocytes (%)	21.1 ± 0.31	2.4 ± 0.30	2.45 ± 0.38	2.3 ± 0.31	2.3 ± 0.45	2.6 ± 0.56

*Compare data column wise, superscript (^{abcd}) differ in column wise is significant.

For pathological lesions, there was not any gross lesion in glandular tissue *viz*. testes, parotid glands, sub-mandibular gland and tracheal gland. Microscopically, in STZ-NT-induced diabetic rats, testicular lesions revealed the presence of amorphous interstitial connective tissue between seminiferous tubules. Additionally degenerated pachytene along with vacuolization (lipid space) in certain seminiferous tubules in diabetic rats (Figure1 and 2) were observed.

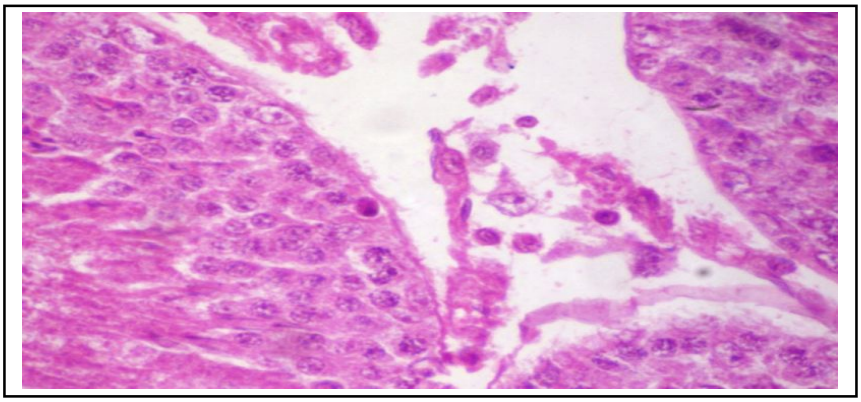


Figure 1: Degenerated pachytene in testes of diabetic rat of Group II (H & E, 400x).

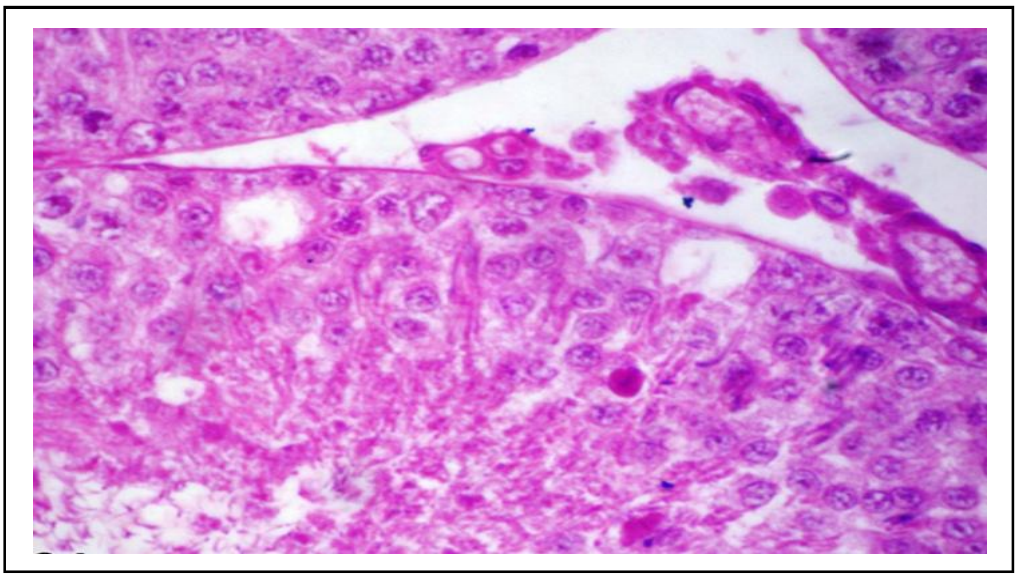


Figure 2: Vacuolization in seminiferous tubule of testis in diabetic rat of Group II (H & E, 400x).

In diabetic rats, particularly in Group II, the epithelium of the seminal vesicle revealed atrophy of cuboidal epithelial cells, accompanied by minor disorganization of the muscular (longitudinal) layer. Additionally, small vacuoles appeared in this region along with hyperchromatic nuclei when compared to the Group I (control) rats (Figure 3).

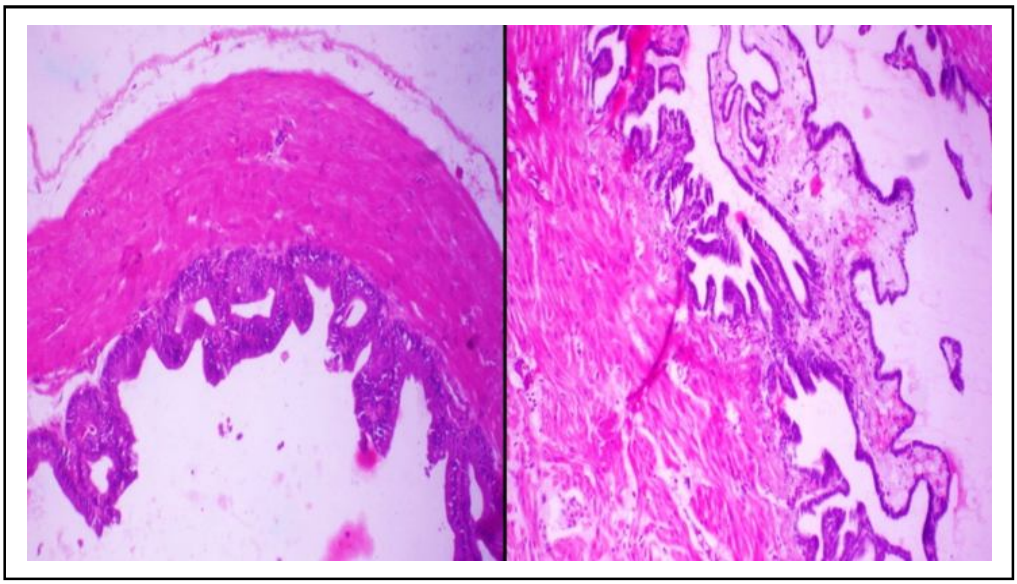


Figure 3: Seminal vesicle: Atrophy of cuboidal epithelial cells along with hyperchrmoatic nuclei in positive control diabetic rats (H and E, 100x Left; 400x Right).

The most prominent morphological aberration in the parotid gland of STZ-NT-induced diabetic rats manifested as vacuolization in serous acinar cells, accompanied by varying degrees of atrophic columnar in serous acini (Figure 4). Around 10% of acinar cells exhibited nuclei displacement, towards the cell corners. Furthermore, there was a mild infiltration of inflammatory cells surrounding the acini, and some ducts exhibited dilation.

The histopathological examination of the sub-mandibular gland in STZ- NT-induced diabetic rats revealed enlargement of inter-acinar spaces, infiltration of inflammatory cells, and atrophy of acinar ducts (Figure 5).

Furthermore, serous acini exhibited atrophic columnar cells, depletion of eosinophilic granules, clear vacuolization, and spherical and/or ellipsoid nuclei located in the basal region in STZ-NT induced diabetic rats, as shown in Figure 6.

In diabetic rats (Group III, IV, and V) treated with various spices, all histopathological observations showed improvement compared to untreated diabetic rats (Group II). In the current investigation, histopathological examination of the trachea in STZ-NT-induced diabetic rats revealed squamous metaplasia of the epithelium, concomitant with degenerated cells within the tracheal lumen (Figure 7, Left).

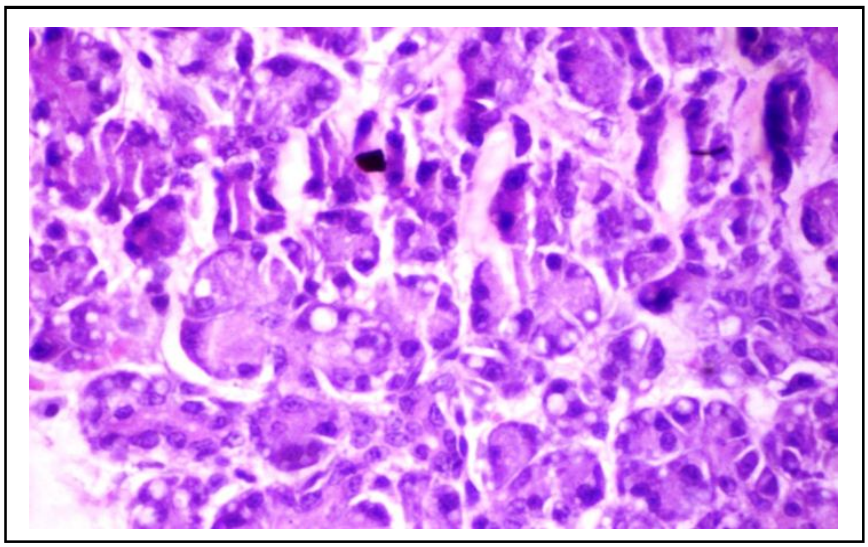


Figure 4: Depletion of eosinophilic granules, clear vacuolization, and spherical and/or ellipsoid nuclei in parotid gland of diabetic rats (H and E, 400x).

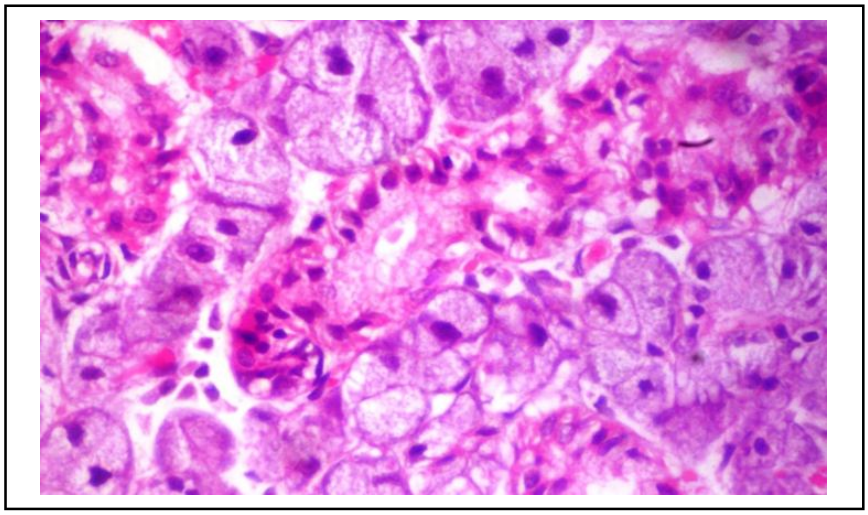


Figure 5: Atrophy of duct, hyperchromatic nuclei in sub-mandibular acini epithelium in diabetic rat of Group II (H & E, 400x).

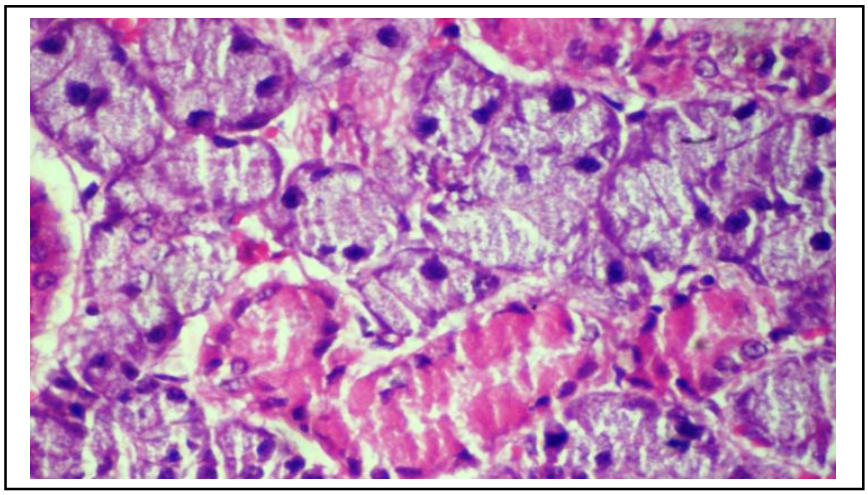


Figure 6: Atrophic columnar cells of serous acini of sub-mandibular gland in diabetic rat of Group II (H & E, 400x).

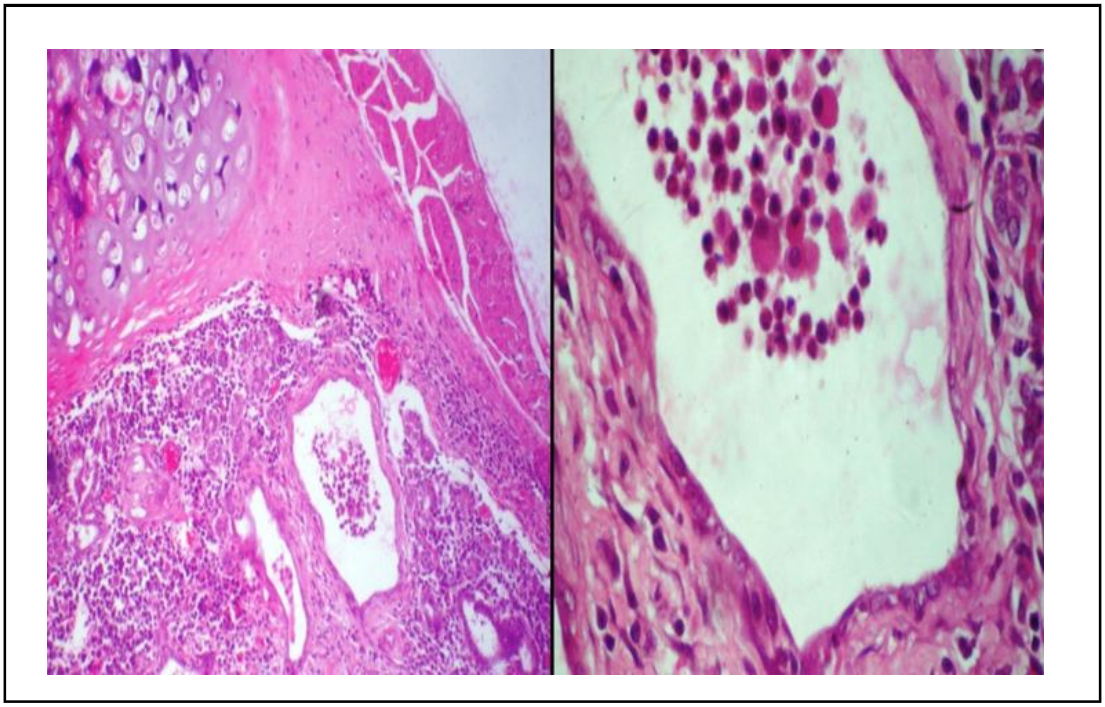


Figure 7: Squamous metaplasia of the epithelium, concomitant with degenerated cells within the tracheal lumen (H and E, 400 × Left). Trachea showed loss of cilia and submucosal gland infiltrated by momonuclear cells left (H and E100 × Right).

Moreover, tracheal cilia were lost and sub-mucosal glands were infiltrated with mononuclear cells, accompanied by dilatation (Figure 7, Right). It is noteworthy, that there is a scarcity of literature documenting the histopathology of the trachea in diabetic rats.

4. Discussion

To prevail over diabetes mellitus and its associated complications, researchers are exploring novel therapies and treatments. Various Indian spices, *e.g.,* black cumin, fenugreek and garlic are rich natural sources of pharmaceutically active ingredients. Therefore, we have evaluated the ameliorative effect of these spices on oxidative stress-induced damage in the glandular tissue of diabetic rats. The findings suggest that the spices utilized in the current study (Black Cumin, Fenugreek, and Garlic), may possess the potential to mitigate hyperglycemia. The hypoglycaemic activity of these spices may be attributed through the inhibition of endogenous glucose production or by improving insulin sensitivity (Balyan and Ali, 2022; Kuroda *et al*., 2003). Furthermore, the active ingredients in the spice, *viz.,* thymoquinone, alkaloids (trigonelline), 4-hydroxyisoleucine, allicin, polyphenols and other antioxidants (Balyan *et al.,* 2022; Rani *et al.,* 2022; Goyal *et al*., 2016; Forouzanfar *et al*., 2014, Shang *et al*., 2019), known for their bioactive antidiabetic properties, may modulate various metabolic actions, contributing to glucose level reduction.

STZ-NT-induced diabetes resulted in an elevation in lipid peroxidation (LPO) levels, predominantly driven by the β-oxidation of fatty acids. This oxidation process is initiated by the destruction of beta cells of Islets of Langerhans within the pancreas. Lipid peroxidation (LPO) within cellular organelles is regarded as a pivotal biomarker indicative of free radical-mediated lipid degeneration associated with diabetes (Shodehinde and Oboh, 2013, Sakul *et al*., 2013; Bandeira *et al*., 2012). The heightened LPO levels adversely impact membrane function by reducing fluidity and altering the activity of membrane-bound enzymes and receptors, ultimately affecting their vital functions. The elevation of thiobarbituric acid (TBA) levels within red blood cells, accompanied by a simultaneous reduction in the activity of erythrocyte antioxidant enzymes, has also been observed in diabetic conditions (Salgueiro *et al*., 2013; Singh and Shin, 2009). Our findings in the context to Group II rats were consistent with the studies conducted by Bikkad *et al*. (2014), Al-Koofee (2013), Kumawat *et al*. (2012) and Padalkar *et al*. (2012). In diabetes, the free radical generation is increased due to chronic exposure to high blood glucose, which favors to increase oxidative stress levels (Bikkad *et al*., 2014). For, antioxidant activity, present findings suggest that spices might have the potential to alleviate the degradation of SOD induced by STZ-NT in rats by lowering oxidative stress levels (Patel *et al*., 2023). The outcome of SOD aligns with prior studies conducted by Lucchesi *et al.* (2013), Shukla *et al.* (2012), and Taheri *et al.* (2012). Superoxide dismutase (SOD) holds a pivotal role in shielding cells from damage caused by reactive oxygen species (ROS). Fundamentally, SOD functions as the primary defense mechanism against harm inflicted by free radicals, facilitating the conversion of oxide radicals into oxygen and water (Davari *et al*., 2013). Our study suggests that active ingredients in spices can be able to diminish or inhibit the lipid peroxidation generation specifically garlic and fenugreek through the lowering of elevated glucose levels.

For hematological values, the present study results are consistent with earlier research, indicating that individuals with diabetes mellitus exhibit altered hematological parameters (Akpan and Ekaidem 2015; Keskin *et al*., 2016). Numerous studies have provided substantiating evidence that the etiology of the observed hematological changes is attributed to the oxidative stress inherent in diabetes mellitus (Shurtz-

Swirski *et al*., 2001). Furthermore, the manifestation of anemia in diabetic conditions is ascribed to the glycosylation of membrane proteins in red blood cells (Oyedemi *et al*., 2011), the concurrent presence of chronic renal disease (Gupta and Lal, 2022; Thomas, 2003), and various other tissue lesions (Weiss, 2005; Arun and Ramesh, 2002).

In the histopathological study, testis microscopic lesions appeared to be mitigated in treated diabetic rats, which was comparable to earlier records (Ozturk *et al*., 2002; Sanguinetti *et al*., 1995). This implies that spices might possess the capability to alleviate the detrimental impacts of diabetes on testicular structure through the reduction of hyperglycemia and lipid peroxidation levels. The pathological lesion in seminal vesicles is ascribed to an abundance of oxidative stress and a vigorous cellular reaction to diabetes-induced damage. Moreover, the application of aqueous extracts led to a more favorable histological profile compared to the diabetic group. The extract of spices could alleviate hypercontractility by restoring the histology of the seminal vesicle, resulting in significantly improving the concentration of oxidative stress enzymes. In the parotid gland, the displacement of acinar cell nuclei could potentially be attributed to lipid deposition. Although, the exact mechanism of lipid accumulation remains unclear, but might be as a source of energy in the absence of glucose transport as it does in cardiac and skeletal muscle (Stearns *et al*., 1979) or it may result from nonspecific endocytosis of excess circulating lipid (Nehemiah and Novikoff, 1974). Top of FormThe aforementioned pathological lesions in submandibular glands were not solely attributed to hypo-insulinemia; oxidative stress also played a significant role. The accumulation of lipid droplets in the acinar cells of the salivary gland in diabetic animals is associated with reduces lipid utilization, leading to the storage of lipid droplets in the cytoplasm. This is attributed to the suppression of protein synthesis and secretory granule secretion in response to perturbed insulin secretion (Anderson *et al.,* 1994). These findings indicate that spices have the potential to mitigate the adverse effects on glandular tissue by reducing elevated blood glucose levels.

5. Conclusion

The administration of black cumin, fenugreek, garlic, and their mixture resulted in reducing blood glucose levels and lipid peroxidation (LPO) levels, and in enhancing the activity of superoxide dismutase (SOD), a critical antioxidant enzyme. This suggests that spices have potent hypoglycemic and antioxidant properties that mitigate oxidative stress, a key contributor to the pathological effect of diabetes and its complications. By reducing LPO and glucose level, they limit the oxidative damage to tissue histology. This could be attributed to various bioactive compounds present in these spices. The findings suggest that incorporating these spices into the diet or as part of a treatment regimen could be a viable to manage diabetes and its complications.

Acknowledgements

Financial Support Acknowledgment: The authors express gratitude to the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Dantiwada, for their generous financial assistance.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Akpan, H. D. and Ekaidem, I. (2015). Modulation of immunological and hematological disturbances of diabetes mellitus by diets containing combined leaves of *Vernonia amygdalina* and *Gongronema latifolium*. British Journal of Applied Science and Technology, **6**:534-544.

AL-Koofee, D. A. (2013). Study of malondialdehyde, reduced glutathione, and peroxy nitrite levels in type 2 diabetics patients. Journal of Applied Chemistry, **2**:1581-1588.

AL-Rawi, N. H. (2011). Oxidative stress, antioxidant status and lipid profile in the saliva of type 2 diabetics. Diabetes and Vascular Disease Research, **8**:22-28.

Anderson, L.C.; Suleiman, A. H. and Garrett, J. R. (1994). Morphological effects of diabetes on the granular ducts and acini of the rat submandibular gland. Microscopy Research and Technique, **27**:61-70.

Arun, G. S. and Ramesh, K. G. (2002). Improvement of insulin sensitivity by perindopril in spontaneously hypertensive and streptozotocin diabetic rats. Indian Journal of Pharmacology, **34**:156-164.

Atkinson, M. A. and Maclaren, N. K. (1994). The pathogenesis of insulindependent diabetes mellitus. The New England Journal of Medicine, **331**:1428-1436.

Ballester, J.; Muñoz, M. C.; Domínguez, J.; Rigau, T.; Guinovart, J. J. and Rodríguez-Gil J. E. (2004). Insulin-dependent diabetes affect testicular function by FSH- and LH-linked mechanisms. Journal of Andrology, **25**:706-719

Balyan, P.; Akhter, J.; Kumar, P and. Ali, A. (2022). Traditional and modern usage of *Nigella sativa* L. (Black cumin). Ann. Phytomed., **11**(2):255-265.

Balyan, P. and Ali, A. (2022). Comparative analysis of the biological activities of different extracts of *Nigella sativa* L. seeds. Ann. Phytomed., **11**(1):577-587.

Bandeira, S.deM.; Guedes, G. daS.; Fonseca, L. J. da.; Pires, A. S.; Gelain, Moreira, D. P.; Rabelo, J. C. L. A.; Vasconcelos, S. M. and Goulart, M. O. (2012). Characterization of blood oxidative stress in type 2 diabetes mellitus patients: increase in lipid peroxidation and SOD activity. Oxid Med Cell Longev Oxidative Medicine and Cellular Longevity, 819310

Bell G. W.; Large, D. M. and Barclay, S. C. (2000). Oral health care in diabetes mellitus. The South African Dental Journal, **55**:158-65.

Bikkad, M. D.; Somwanshi, S. D.; Ghuge, S. H. and Nagane, N. S. (2014). Oxidative stress in type II diabetes mellitus. Biomedical Research, **25**:84-87.

Cakatay, U. (2005). Protein oxidation parameters in type 2 diabetic patients with good and poor glycaemic control. Diabetes and Metabolism, **31**:551-557

Davari, S.; Talaei, S. A.; Alaei, H. and Salami, M. (2013). Probiotics treatment improves diabetes-induced Impairment of synaptic activity and cognitive function: Behavioral and electrophysiological proofs for Microbiome-gut-brain axis. Neuroscience, **240**:287-296

Farman, U. K.; Durrani, F. R.; Asad, S.; Rifat, U. K. and Shabana, N. (2009). Effect of fenugreek (*Trigonella foenum-graecum*) seed extract on visceral organs of broiler chicks. ARPN Journal of Agricultural and Biological Science, **4**(1):58-60.

Forouzanfar, F.; Bazzaz, B. S. and Hosseinzadeh, H. (2014). Black cumin (*Nigella sativa*) and its constituent (thymoquinone). a review on antimicrobial effects. The Iranian Journal of Basic Medical Sciences, **17**(12):929-938.

Goyal, S.; Gupta, N. and Chatterjee, S. (2016). Tinvestigating therapeutic potential of *Trigonella foenum-graecum* L. as our defense mechanism against several human diseases. Journal of Toxicology, 1250387.

Gupta, V. and Lal, K. M. (2022). Assessment of hepato and nephroprotective potential of polyherbal combinations against STZ-induced diabetic liver and kidney complications in wistar rats. Ann. Phytomed., **11**(1):311-319.

Ivanovski, K.; Naumovski, V.; Kostadinova, M.; Pesevska, S.; Drijanska, K. and Filipce, V. (2012). Xerostomia and salivary levels of glucose and urea in patients with diabetes. Prilozi, **33**:219-29.

Kasim, S. H.; Al-Mayah.; Nada, M.; Al-Bashir.; Bader, M. and Al-Azzaw. (2012). In vivo efficacy of *Nigella sativa* aqueous seed extract against metacestode of *Echinococcus granulosus*. Medical Journal of Babylon, **9**(1):140-151.

Keskin, E.; Donmez, N.; Kýlýcarslan, G. and Kandir, S. (2016). Beneficial effect of quercetin on some haematological parameters in streptozotocin-induced diabetic rats. Bulletin of Environment, Pharmacology and Life Sciences, **5**(6):65-68.

Knas, M.; Karaszewska, K.; Szajda, S. D.; Zarzycki, W.; Dudzik, D. and Zwierz, K. (2006). Saliva of patients with Type 1 diabetes: effect of smoking on activity of lysosomal exoglycosidases. Oral Diseases, **12**(3):278-282.

Kuhn-Velten N.; Waldenburger, D. and Staib, W. (1982). Evaluation of steroid biosynthetic lesions in isolated leydig cells from the testes of streptozotocin-diabetic rats. Diabetologia, **23**:529-533.

Kumawat, M.; Singh, I.; Singh, N.; Singh, V. and Kharb, S. (2012). Lipid peroxidation and lipid profile in type 2 diabetes mellitus. Webmed Central Biochemostry, **3**:2-9.

Kurek, K.; Wiesio³ek-Kurek, P.; Piotrowska, D. M.; £ukaszuk, B.; Chabowski, A. and ¯endzianendzian-Piotrowska, M. (2014). Inhibition of ceramide de novo synthesis with myriocin affects lipid metabolism in the liver of rats with streptozotocin-induced type 1 diabetes. Bio. Med. Research International, **2014**:980815.

Kuroda, M.; Mimaki, Y.; Sashida, Y.; Mae, T.; Kishida, H.; Nishiyama, T.; Tsukagawa, M.; Konishi, E.; Takahashi, K.; Kawada, T.; Nakagawa, K. and Kitahara, M. (2003). Phenolics with PPAR-ligand-binding activity obtained from liquorice (*Glycyrhizza uralens* roots) and ameliorative effects of glycerine on genetically diabetic KK-Ay mice. Bioorganic and Medicinal Chemistry Letters, **13**:4267-4272.

Lucchesi, A. N.; Freitas, N. T.; Cassettari, L. L.; Marques, S. F. and Spadella, C. T. (2013). Diabetes mellitus triggers oxidative stress in the liver of alloxan-treated rats: A mechanism for diabetic chronic liver disease. Acta Cirúrgica Brasileira, **28**(7):502-508.

Lushchak, V. I. (2014). Free radicals, reactive oxygen species, oxidative stress and its classification. Chemico-Biological Interactions, **224**:164-175.

Madesh, M. and Balasubramanian, K. A. (1998). Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian Journal of Biochemistry and Biophysics, **35**(3):184-188.

Melendez-Ramirez, L. Y.; Richards, R. J. and Cefalu, W. T. (2010). Complications of type 1 diabetes. Endocrinology and Metabolism Clinics of North America, **39**:625-640.

Morimoto, S.; Mendoza-Rodriguez, C. A.; Hiriat, M.; Larrieta, M. E.; Vital, P. and Cerbón, M. A. (2005). Protective effect of testosterone on early apoptotic damage induced by streptozotocin in rat pancreas. Journal of Endocrinology, **187**:217-224.

Nehemiah, J. L. and Novikoff, A. B. (1974). Unusual lysosomes in hamster hepatocytes. Experimental and Molecular Pathology, **21**:398-423.

Orth, J. M.; Murray, F. T. and Bardin, C. W. (1979). Ultrastructural changes in Leydig cells of streptozotocin-induced diabetic rats. The Anatomical Record, **195**:415-430.

Oyedemi, S. O.; Yakubu, M. T. and Afolayan, A. J. (2011). Antidiabetic activities of aqueous leaves extract of Leonotis leonurus in streptozotocin induced diabetic rats. Journal of Medicinal Plants Research, **5**:119-125.

Ozturk, F.; Gul, M.; Agkadir, M. and Yagmurca, M. (2002). Histological alterations of rat testes in experimental diabetes. Turkiye Klinikleri Journal of Medical Sciences, **22**:173-178.

Padalkar, R. K.; Shinde, A. V. and Patil, S. M. (2012). Lipid profile, serum malondialdehyde, superoxide dismutase in chronic kidney diseases and type 2 diabetes mellitus. Biomedical Research, **23**:207-10.

Patel, N.; Prata.p S. S. and Thakur. V. (2023). Study on functional properties and health importance of Fenugreek (*Trigonella frenum-graecum L.*): A review. Ann. Phytomed., **12**(1):665-673.

Pedraza-Chaverrí, J.; Gil-Ortiz, M.; Albarrán, G.; Barbachano-Esparza, L.; Menjívar, M. and Medina-Campos, O. N. (2004). Garlic's ability to prevent in vitro Cu2+-induced lipoprotein oxidation in human serum is preserved in heated garlic: effect unrelated to Cu2+-chelation. The Journal of Nutrition, **3**:10.

Pellegrino, M.; Christophe, B.; Rene, G.; Michele, R.; Michele, M.; Dominique, H. B.; Michela, N. and Gerard, R. (1998). Development of a new model of type II diabetes in adult rats administered with streptozotocin and nicotinamide. Diabetes, **47**:224.

Premkumar, L. S. and Pabbidi, R. M. (2013). Diabetic peripheral neuropathy: role of reactive oxygen and nitrogen species. Cell Biochemistry and Biophysics, **67**:373-83.

Rani, V.; Nandal, U.; Reena. and Sindhu, C.S. (2022). Nutrient milieu of products developed for prediabetic population using fenugreek seeds debittered by traditional techniques. Ann. Phytomed., **11**(2):344-350

Rees, T. D. (2000). Periodontal management of the patient with diabetes me-llitus. Journal of Periodontology, **23**:63-72.

Rehman, S. (1984). Lead-induced regional lipid peroxidation in brain. Toxicology Letters, **21**:333-337.

Sakul, A.; A. Cumaoglu, E. Aydýn, N. Ar, N. Dilsiz, C. Karasu (2013). Age-and diabetes-induced regulation of oxidative protein modification in rat brain and peripheral tissues: consequences of treatment with antioxidant pyridoindole. Experimental Gerontology, **48**(5):476-484.

Salgueiro, A. C.; Leal, C. Q.; Bianchini, M. C.; Prado, I. O.; Mendez, A. S.; Puntel, R. L.; Folmer, V.; Soares, F. A.; Avila, D. S. and Puntel, G. O. (2013). The influence of Bauhinia forficata Link sub sp. pruinosa tea on lipid peroxidation and non-protein SH groups in human erythrocytes exposed to high glucose Concentrations. Journal of Ethnopharmacology, **148**(1):81-87.

Sanguinetti, R. E.; Ogawa, K.; Kurohmaru, M. and Hayashi, Y. (1995). Ultrastructural changes in mouse Leydig cells after streptozotocin administration. Experimental Animals, **44**:71-73.

Shang, A.; Cao, S. Y.; Xu, X. Y.; Gan, R. Y.; Tang, G. Y.; Corke, H.; Mavumengwana, V. and Li, H. B. (2019). Bioactive compounds and biological functions of garlic (*Allium sativum* L.). Foods, **8**(7):246.

Shodehinde , S. A., G. Oboh (2013). Antioxidant properties of aqueous extracts of unripe Musa paradisiaca on sodium nitroprusside induced lipid peroxidation in rat pancreas in vitro. Asian Pacific Journal of Tropical Biomedicine, **3**(6):449-457.

Shukla, K.; Dikshit, P. P.; Tyagi, M. K.; Shukla, R. and Gambhir, J. K. (2012). Ameliorative effect of *Withania coagulans* on dyslipidemia and oxidative stress in nicotinamide streptozotocin induced diabetes mellitus. Food and Chemical Toxicology, **50**(10):3595-3599.

Shurtz-Swirski, R.; Sela, S.; Herskovits, A. T.; Shasha, S. M.; Shapiro, G.; Nasser, L. and Kristal, B. (2001). Involvement of peripheral polymorphonuclear leukocytes in oxidative stress and inflammation in type 2 diabetic patients. Diabetes Care, **24**(1):104-110.

Singh, M. and Shin, S. (2009). Changes in erythrocyte aggregation and deformability in diabetes mellitus: A brief review. Indian Journal of Experimental Biology, **47**(1):7-15.

Soysa, N.S.; Samaranayake, L. P. and Ellepola, A. N. (2006). Diabetes mellitus as a contributory factor in oral candidosis. Diabetic Medicine, **23**:455-459.

Stearns, S. B.; Teperman, H. M. and Tepperman, J. (1979). Studies on the utilization and mobilization of lipid in skeletal muscles from streptozotocin-diabetic and control rats. Journal of Lipid Research, **20**:654-662.

Taheri, E.; Djalali, M.; Saedisomeolia, A.; Moghadam, M. A.; Djazayeri, A. and Qorbani, M. (2012). The relationship between the activities of antioxidant enzymes in red blood cells and body mass index in Iranian type 2 diabetes and healthy subjects. Journal of Diabetes and Metabolic Disorders, **11**:2-5.

Thomas, M. C.; MacIsaac, R. J.; Tsalamandris, C.; Power, D. and Jerums, G. (2003). Unrecognized anemia in patients with diabetes: A cross sectional survey. Diabetes Care, **26**(4):1164-1169.

Weiss, G. and Goodnough, L. T. (2005). Anemia of chronic disease. The New England Journal of Medicine, **352**:1011-1023.

Citation **T. J. Sheikh, D.V. Joshi, B.J. Patel, S.H. Raval, Ranjan Rajeev and C.M. Modi (2024). Ameliorative effect of spices on oxidative stress induced damage in glandular tissues in diabetic rats: A chronic study. Ann. Phytomed., 13(1):558-566. http://dx.doi.org/10.54085/ap.2024.13.1.58.**