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Original Article

FTIR ANALYSIS FOR RETINA ASSOCIATED WITH DIABETIC CHANGES AND TREATMENT WITH OAT

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ABSTRACT

Objective: Diabetes is known to induce oxidative stress along with deranging various metabolisms. One of the most serious complications of diabetes, a disease that has seen a worldwide increase in the prevalence, is diabetic retinopathy, which is a leading cause of acquired blindness. The aim of this study is to evaluate the effect of oat on the diabetic-induced oxidative stress and if this can attenuate the development of diabetic retinopathy.

Methods: Changes on retina structure were performed by using the application of Fourier transform infrared spectroscopy.

Results: The results demonstrated that diabetic retinopathy was associated with changes on the retina structure which appear after received a single dose of streptozotocin (STZ) 60 mg/kg. These changes clearly appeared in the NH-OH, CH and fingerprint regions. The use of oat in case of diabetic was associated with different beneficial effects on the retina constituents, as showed by the changes toward control of the same Fourier transform infrared spectroscopy bands.

Conclusion: Oat can be considered as a novel treatment modality for diabetic retinopathy and further studies is required to optimize dosing and formulations that are maximally effective.

Keywords: Rats, Diabetic, Streptozotocin, Retina, FTIR-Oat.

INTRODUCTION

Diabetes increases oxidative stress in the retina and in its capillary cells, which considered as one of the major metabolic abnormalities associated with the development of diabetic retinopathy (DR) [1-4]. Diabetic retinopathy is a classic chronic micro vascular complication of the retina caused by the deleterious metabolic effects of hyperglycemia, which results in extensive and early neuro degeneration. Neuroretinal degeneration initiates several metabolic and signaling pathways that participate in the microvasculopathy process as well as in disturbances of the blood-retinal barrier (BRB), a key phenomenon in the pathogenesis of DR [5]. In diabetes, retinal mitochondria become dysfunctional and mitochondrial DNA (mtDNA) is affected [6-8]. All the blood vessels of the retina have tight junctions that help to protect them against leaking, but prolonged high concentrations of glucose damage these tight junctions and the vessels become leaky allowing the fluid and/or blood to seep into the retina, which results in the swelling of the retina [9]. Due to progressive dysfunction, the capillaries die prematurely resulting in ischemia that can be followed by neovascularization and finally retinal detachment and blindness [10].

In the progress of DR, the basement membrane thickens and the blood pressure is altered. In addition pericytes and endothelial cells undergo accelerated apoptosis resulting in pericyte ghosts and acellular capillaries [11]. The leukocytes become less deformable, and retinal leukostasis is increased affecting the endothelial function [12, 13]. In addition to increase in reactive oxygen species (ROS) in the retina, the antioxidant defense system is also compromised in diabetes [14-16].

Cumulative studies demonstrated that dietary fiber can significantly reduce the risk of cardiovascular disease and diabetes [17]. This is due in part to the ability of fiber to reduce postprandial glycemia and improve long-term glycemic control [18]. It was assumed that the rheological properties of soluble dietary fibers are highly related to their effects on control of the glucose concentration [19]. For instance, the ability of oat-derived β-glucan to reduce postprandial glycaemia has been strongly correlated with its viscosity [20], demonstrating an inverse linear relationship between the logarithm of viscosity measures and peak postprandial plasma glucose and insulin responses after consuming various doses of purified oat. Despite these findings, the levels of viscosity required to achieve specific glucose-lowering effects are not well understood. Still, the majority of trials investigating dietary fiber have not represented the principles of polysaccharide solubility and viscosity as the main determinants of its physiological outcome. While a small number of studies have shown the effect of oat on diabetes [21, 22], none examine its effect on the development and progression of DR.

The aim of this study is to evaluate the effect of oat-meal supplementation (10 and 20% w/w) on the diabetic-induced oxidative stress and its potential effect to attenuate the development of DR. The results of this study may provide an alternative for enhancing nutrition and diabetic control during DR.

MATERIALS AND METHODS

Streptozotocin induced diabetic retinopathy and study design

Streptozotocin (STZ) induced DR according to the model previously suggested by Sayed (2012) [23]. Nine-week-old 200±20g male Albino rats were randomly selected from the animal house facility at the Research Institute of Ophthalmology, Giza, Egypt. The animals were kept separately under good ventilation and adequate standard diet. They were housed in specially designed cages and maintained under constant air flow and illumination during the experimental periods. The animals were handled according to the ARVO (The Association for Research in Vision and Ophthalmology) statements for the use of animals in research, and the research protocol was approved by the local ethical committee. The animals were kept under observation for one week prior to the start of the experiment. 10 rats were randomly selected as a control group (group 1), which received a single tail vein injection of 0.1mol /L citrate buffer only. The other rats received a single dose of STZ (Sigma S-0130) in citrate buffer pH 4.5 through the indwelling catheters over 2 min, at a fixed dose of 60mg/kg . Only rats, with blood glucose higher than 250 mg/dL after two days were considered as being a diabetic in the fasting state. Blood glucose was measured by using Accu-Chek Active GS392 (Germany). Rats with blood glucose levels, lower than 200 mg/dL were excluded from the study. All studies were carried out two days after STZ injection. Diabetic rats were classified to three groups each contains ten rats: group 2, untreated diabetic untreated rats and groups 3, 4 (10 rats each), oat treated diabetic

rats. Rats of these groups were supplemented with oat 10 and 20%, respectively, on the diet (W/W). Treatment was continued for 12 w starting from day two after STZ administration. At the end of the experiment, all groups were killed. Eyes were enucleated, and then opened by corneal section through the ora serrata where the anterior segment constituents can be removed so that the retina is exposed and can easily be obtained. Each retina was immediately processed for IR-characterization; if not, it was kept for 10 min (maximum) in a sterilized dark glass vial, flushed with dry nitrogen gas and stored at-20^{o}C.

Fourier transforms infrared spectroscopy (FTIR)

Retinae were freeze-dried separately for 1 h, and then mixed with KBr powder (2 mg retina: 98 mg KBr) to prepare the KBr disks that will be used for the FTIR investigation. FTIR measurements were carried out using Nicolet-iS5 infrared spectrometer (Thermo Fisher Scientific Inc, Madison, USA) with effective resolution of 2 cm^{-1}. Each spectrum was taken from 100 sample interferograms. The spectrometer was subject to a continuous dry N_2 gas purge to remove interference from atmospheric CO_2 and H_2O vapor. The spectra were baseline corrected, then smoothed with Savitsky–Golay filter to remove the noise before Fourier transformation. Three spectra from each sample were obtained and averaged using OriginPro8 software (Origin Lab Corporation, Northampton, MA, USA) to obtain the final average group spectrum which was normalized according to certain peaks and used in the figures.

Statistical evaluation

Data was represented as the mean±SD For comparison between multiple groups the analysis of variance (ANOVA) procedure was used, where a commercially available software package (SPSS-11, for windows) was used and the significance level was set at P <0.05.

RESULTS AND DISCUSSION

FTIR spectroscopy is a non-destructive technique, which provides quantitative biochemical information about biological samples. It is a valuable technique due to its high sensitivity in detecting changes in the molecular constituents of tissues.

The FTIR spectra of retina for control, diabetic and diabetic rats supplemented oat 10 and 20%, on the diet (W/W) groups covering the range 4000-1000 cm^{-1} was shown in fig. 1. The FTIR spectra of retinal tissues are quite complex and contain several bands arising from the contribution of different functional groups belonging to lipids, proteins, and others. Therefore, the detailed spectral analyses were performed in three distinct frequency ranges; 4000–3000 cm^{-1}(NH-OH region), 3000–2800 cm^{-1} (CH stretching region), and 1800–1000 cm^{-1} (fingerprint region).

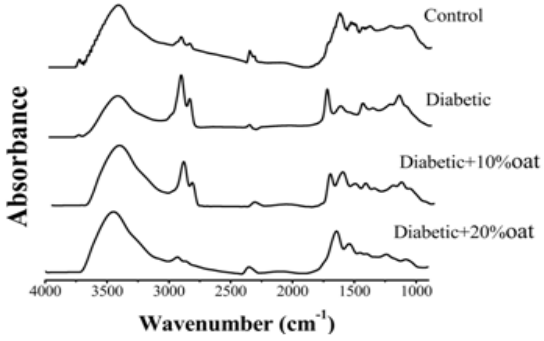

Fig. 1: Overlaid FTIR spectra for all studied groups

NH-OH region

Fig. 2 demonstrates that the main band of the control pattern was found at 3445±3 cm^{-1}. After deconvolution procedure this main band was resolved into three bands at 3553±3, 3412±5 and 3235±3 cm^{-1} that corresponds to stretching OH ($_{str}$OH) labeled as 1, stretching OH asymmetric ($_{str}OH_{asym}$) labeled as 2 and stretching OH symmetric ($_{str}OH_{sym}$) labeled as 3 respectively, as previously mentioned by Dovbeshko *et al.* [24]. After induction of diabetes, there are detectable changes in the retinal structure appeared in NH-OH region. A significant decrease of band position and significant increase in bandwidth of $_{str}$OH is observed in relation to control which indicates the formation of hydrogen bonds with different structural states. There is a shifting of $_{str}OH_{asym}$ and $_{str}OH_{sym}$ mode band toward higher wave number, with changes in bandwidth, which indicates that the hydrogen bond has been destructed and/or weakened [25].

After treatment with oat, the $_{str}$OH and $_{str}OH_{asym}$ mode bands were mimicking the control with changes of bandwidth only as showed in table (1). But $_{Str}OH_{sym}$ is very sensitive to diabetic even after oat administration.

The above findings indicate that there are changes in the protein structure of the retina associated with diabetes and oat administration has protective effects on the retina. These findings were supported by the findings of Tapola *et al.* [21] and Kowluru *et al.* [26].

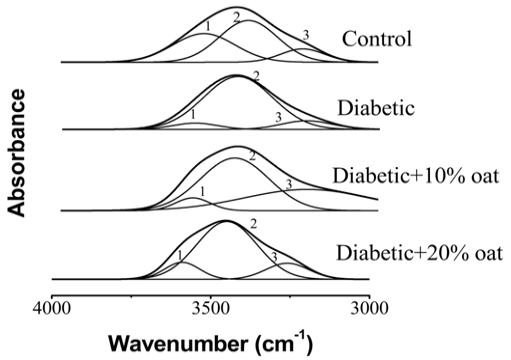

Fig. 2: FTIR spectra in the frequency range 4000–3000 cm^{-1} that corresponds to the NH-OH region of the normal retinae and those of different animal groups, showing the underlying bands as deduced by curve fitting analysis. The numbers to facilitate the identification of the bands

CH region

Fig. 3 indicates the vibrational frequency range 3000-2800 cm^{-1} that corresponds to the CH stretching region of the lipid part of the sample. The control pattern indicates the presence of three bands in the pattern and the curve enhancement procedure confirm the presence of these bands that was centered at 2967±2, 2924±2 and 2859±2 cm^{-1} and can be assigned to $CH_{3\ asym}$ (labeled as 1), $CH_{2\ asym}$(labeled as 2), and $CH_{2\ sym}$ (labeled as 3),stretching vibrations respectively as previously mentioned by Severcan *et al.* [27].

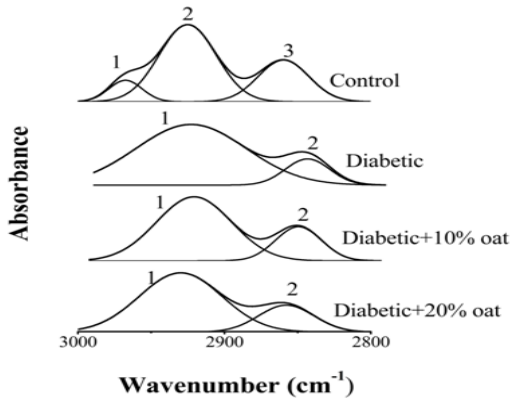

Fig. 3: Stretching CH region (3000–2800 cm^{-1}) of retinae that were isolated from all animal groups involved in the study

Table 1: Estimated structural components and their vibrational frequencies of the NH-OH region

Functional group	Control	Diabetic	Diabetic+10%oat	Diabetic+20%oat
(1) $_{str}OH$	3553±3	3490±4*	3540±8	3545±7
	59±2	230±3*	183±4*	170±3*
(2) $_{str}OH_{asym}$	3412±5	3469±5*	3407±4	3420±4
	212±3	190±2*	147±3*	125±4*
(3) $_{str}OH_{sym}$	3235±3	3252±2*	3249±5*	3299±3*
	418±3	121±2*	125±2*	198±4*

First line in each cell indicates the vibrational frequency, while second line reflects the bandwidth, *Statistically significant ($p<0.05$)

In the data of the CH stretching region shown in table (2), the CH_{3asym} vibrational mode was appeared in control only. The CH_{2asym} and $CH_{2\ sym}$ mode of vibrations shows a significant change ($P<0.05$) in wave number (increase and decrease respectively) for diabetic group, that indicating an environmental change indicating change in the order of the lipid hydrocarbon chains. On the other hand they go back to their ranges in control for the groups treated with oat.

Table 2: Estimated structural components with its vibrational frequencies of CH region

Functional group	Control	Diabetic	Diabetic+10%oat	Diabetic+20%oat
(1) $CH_{3\ asym}$	2967±2			
	23±1			
(2) CH_{2asym}	2924±2	2953±1*	2928±3	2929±3
	39±2	75±1*	51±1*	56±1*
(3) $CH_{2\ sym}$	2859±2	2840±2*	2856±2	2858±2
	35±2	32±1	31±1	35±2

First line in each cell indicates the vibrational frequency, while second line reflects the bandwidth *Statistically significant ($p<0.05$)

Table 3: Assignment of the fingerprint region (1800–1000 cm^{-1}) of retinae from normal and different animal groups (the vibrational frequency in cm^{-1})

Functional group	Control	Diabetic	Diabetic+oat 10%	Diabetic+oat 20%
(1)	1695±1	1746±1*	1743±1*	1689±4
Ester C=O	128±4	41±1*	64±4*	120±3
(2)	1643±1	1639±2	1640±3	1635±4
Amide I	87±1	100±4*	64±3*	61±4*
(3)	1539±1	1559±2*	1540±1	1539±1
Amide II	115±2	98±11*	64±3*	89±2*
(4)	1460±2	1458±1	1458±1	1456±1
CH2 Bending	30±2	51±3*	64±7*	37±8
(5)	1408±1			1404±3
COOsym	133±5			215±12*
(6)		1381±2	1386±2	
CH3 deform		145±13	64±8	
(7)	1245±1	1246±2	1244±2	1243±1
asymPO2	316±10	148±11*	64±8*	127±5*
(8)		1165±1	1166±2	
COOCasym		58±4	64±7	
(9)		1103±5		
P–O–Csym		184±18		
(10)	1086±4		1090±3	1085±2
symPO2	178±7		64±8*	323±7*

First line in each cell indicates the vibrational frequency, while the second line reflects the bandwidth, *Statistically significant ($p<0.05$)

Fingerprint region

Fig. 4 indicates the vibrational frequency range 1800-1000 cm^{-1} corresponds to the fingerprint region that presents the lipid and protein parts of all samples [28]. The control pattern was characterized by seven bands and the curve enhancement procedure also confirms the presence of these bands that were centered at 1710±1, 1643±1, 1539±1, 1460±2, 1408±1, 1245±1 and 1086±4 cm^{-1} that correspond to ester c=o (labeled as 1), amide I (labeled as 2), amide II (labeled as 3), CH_2 bending (labeled as 4), $_{sym}COO$ (labeled as 5), $_{asym}PO_{2,}$ (labeled as 7) and $_{sym}PO_2$ (labeled as 10) respectively [24]. Table 3 indicated the changes in band frequency and width associated with diabetic and treatment with oat.

The absorption band 1710 cm^{-1} of the spectra which ascribed to C=O stretching vibrations of carboxylic acid of the amino acid was shifted to higher wave number and there were a significant increase in the diabetic group and diabetic treated with 10% oat group. But in diabetic treated with 20% oat group, there was no significant change in the wave number of C=O. Also the significant changes in bandwidth in diabetic group and those treated with 10% oat were disappeared in the group treated with 20% oat.

Amide I band and CH_2 bending had no significant change in vibrational frequency for all groups but the bandwidth was significant increase after diabetic and decrease for oat groups. Amide II derives mainly from in-plane NH bending and from the CN stretching vibration, shows protein conformational sensitivity [29]. Amide II shift to the higher frequency in the diabetic group which indicates a different vibration mode and returns to its mode in oat groups but bandwidth still significant changes. Disappearance of the vibrational mode of COO_{sym}

and $_{sym}PO_2$ in the diabetic group was observed. Also appear of three bands: $CH_{3deform}$ (labeled as 6) and $COOC_{asym}$ (labeled as 8) in the diabetic group and 10% oat and $P–O–C_{sym}$ (labeled as 9) in the diabetic group only. These changes confirm the change in the environment rather than a retina function disorder.

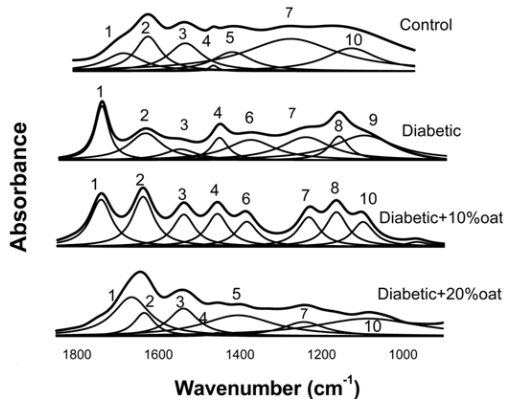

Fig. 4: Representative IR spectra in the fingerprint region (1800–1000 cm^{-1}). The numbers above the peaks are to facilitate their assignment and to facilitate their identification. The assignment of these peaks was given in table 3 as well as in the text

In summary, our data demonstrate that the oat-meal nutritional supplementation, which is currently in preclinical trials, has potential effects to maintain the structure and function of the retina of human subjects with long-term diabetes. This is achieved, possibly, via changes in the protein structure of the retina. Supplementation with oat appears as an inexpensive adjunct therapy to inhibit retinal dysfunction.

CONCLUSION

STZ-induced DR (60 mg/kg, single dose) was associated with changes in the retina structure. These changes clearly appeared in the NH-OH, CH and fingerprint regions. The use of oat in case of diabetes was associated with different beneficial effects on the retinal constituents, as showed by the changes toward control of the same FTIR bands. In summary, oat can be considered as a novel treatment modality for DR and further studies are required to optimize dosing and formulations that are maximally effective.

REFERENCES

1. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005;54:1615–25.
2. Madsen-Bouterse SA, Kowluru RA. Oxidative stress and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. Rev Endocr Metab Disord 2008;9:315–27.
3. Zheng L, Kern TS. Role of nitric oxide, superoxide, peroxynitrite and PARP in diabetic retinopathy. Front Biosci 2009;14:3974–87.
4. Giacco F. Brownlee M: Oxidative stress and diabetic complications. Circ Res 2010;107:1058–70.
5. Silva KC, Rosales MA, Hamassaki DE. Green tea is neuroprotective in diabetic retinopathy. Invest Ophthalmol Visual Sci 2013;54:1325-36.
6. Kowluru RA. Mitochondria damage in the pathogenesis of diabetic retinopathy and in the metabolic memory associated with its continued progression. Curr Med Chem 2013;20:3226-33.
7. Madsen-Bouterse S, Zhong Q, Mohammad G, Ho YS, Kowluru RA. Oxidative damage of mitochondrial DNA in diabetes, and its protection by manganese superoxide dismutase. Free Rad Res 2010;44:313-21.
8. Santos JM, Tewari S, Goldberg AFX, Kowluru RA. Mitochondria biogenesis and the development of diabetic retinopathy. Free Rad Biol Med 2011;51:1849-60.
9. Kern TS, Kowluru R, Engerman RL. Abnormalities of retinal metabolism in diabetes or galactosemia: ATPases and glutathione. Invest Ophthalmol Visual Sci 1994;35:2962–7.
10. Bhaskar JJ, Shobha MS, Sambaiah K, Salimath PV. Beneficial effects of banana (Musa sp. var. elakki bale) flower and pseudostem on hyperglycemia and advanced glycation end-products (AGEs) in streptozotocin-induced diabetic rats. J Physiol Biochem 2011;67:415–25.
11. Kern TS, Tang J, Mizutani M. Response of capillary cell death to aminoguanidine predicts the development of retinopathy: comparison of diabetes and galactosemia. Invest Ophthalmol Visual Sci 2000;41:3972–8.
12. Joussen AM, Poulaki V, Le ML. A central role for inflammation in the pathogenesis of diabetic retinopathy. FASEB J 2003;18:1450–2.
13. Nakagawa T, Yokozawa T, Terasawa K, Nakanishi K. Therapeutic usefulness of Keishi-bukuryo-gan for diabetic nephropathy. J Pharm Pharmacol 2003;55:219–27.
14. Kowluru RA, Kern TS, Engerman RL. Abnormalities of retinal metabolism in diabetes or experimental galactosemia. IV. Antioxidant defense system. Free Rad Biol Med 1997;22:587–92.
15. Kowluru RA, Tang J, Kern TS. Abnormalities of retinal metabolism in diabetes and galactosemia. VII. Effect of long-term administration of antioxidants on retinal oxidative stress and the development of retinopathy. Diabetes 2001;50:1938–42.
16. Kowluru R, Kern TS, Engerman RL. Abnormalities of retinal metabolism in diabetes or galactosemia II. Comparison of gamma-glutamyl transpeptidase in retina and cerebral cortex, and effects of antioxidant therapy. Curr Eye Res 1994;13:891–6.
17. Würsch P, Pi-Sunyer FX. The role of viscous soluble fiber in the metabolic control of diabetes: a review with special emphasis on cereals rich in β-glucan. Diabetes Care 1997;20:1774–80.
18. Vuksan V, Sievenpiper JL, Xu Z. Konjac-mannan and American ginseng: emerging alternative therapies for type 2 diabetes mellitus. J Am Coll Nutr 2001;20:370S–80S.
19. Hallfrisch J, Behall KM. Mechanisms of the effects of grains on insulin and glucose responses. J Am Coll Nutr 2000;19:S320–S25.
20. Wood PJ, Braaten JT, Scott FW, Riedel KD, Wolynetz MS, Collins MW. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Br J Nutr 1994;72:731–43.
21. Tapola N, Karvonen H, Niskanen L, Mikola M, Sarkkinen E. Glycemic responses of oat bran products in type 2 diabetic patients. Nutr Metab Cardiovasc Dis 2005;15:255–61.
22. Panahi SA, Ezatagha F, Temelli T, Vasanthan Vuksan V. β-glucan from two sources of oat concentrates affect postprandial glycemia in relation to the level of viscosity. J Am Coll Nutr 2007;26:639–44.
23. Sayed AR. Thymoquinone and proanthocyanidin attenuation of diabetic nephropathy in rats. Eur Rev Med Pharmacol Sci 2012;16:808–15.
24. Dovbeshko GI, Gridina NY, Kruglova EB, Pashchuk OP. FTIR spectroscopy studies of nucleic acid damage. Talanta 2000;53:233–46.
25. Yang L, Xu Y, Su Y. Study on the variations of molecular structures of some biomolecules induced by free electron laser using FTIR spectroscopy. Nucl Instrum Methods Phys Res Sect B 2003;258:362–8.
26. Kowluru RA, Zhong Q, Santos JM. Thandampallayam beneficial effects of the nutritional supplements on the development of diabetic retinopathy. Nutr Metab 2014;11:8.
27. Severcan F, Toyran N, Kaptan N, Turan B. Fourier transform infrared study of the effect of diabetes on rat liver and heart tissues in the C-H region. Talanta 2000;53:55–9.
28. Sekiya N, Kishigami A, Naoki H. Fourier transform infrared spectroscopic study on retinochrome and its primary photoproduct lumiretinochrome. FEBS Lett 1991;280:107-11.
29. Kong J, YU S. Fourier transform infrared spectroscopic analysis of protein secondary structures. Acta Biochim Biophys Sin 2007;39:549–59.