

## Molecular Structure Changes of Rat's Retina After Sodium Iodate Injection and the Effect of Taurine

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**T**HE aim of present work is to investigate the mechanism of action of sodium iodate ( $\text{NaIO}_3$ ) on the molecular structure of retina and whether the nutritional intake of taurine may delay the development of age macular degeneration (AMD) disease induced by  $\text{NaIO}_3$  using Fourier Transform Infrared spectroscopy (FTIR). One hundred and five albino rats of both sex (200-230 g) were classified into three groups. Group I (15 rats) used as control, group II (45 rats) received a single intravenous injection of 25, 50, or 75 mg/kg  $\text{NaIO}_3$ , and group III (45 rats) supplemented with 4 g taurine /100 g diet for two weeks before injection of  $\text{NaIO}_3$ . The injection continued until decapitation. All injected doses of  $\text{NaIO}_3$  are associated with vibrational changes that include decrement of the modes of vibration related to stretching OH and amide II, significant increases ( $p < 0.05$ ) in wavenumber and width of asymmetrical OH and disappear of asymmetrical  $\text{NH}_3$ . Also, there are significant diminish ( $p < 0.05$ ) in  $\alpha$ -helix content in the same context, in addition to significant increments ( $p < 0.05$ ) in  $\beta$ -Sheet indicating the conformational changes of the secondary structure of protein due to  $\text{NaIO}_3$  injection. Enhancement of most changes that previously detected in FTIR spectra is noticed after taurine treatment especially before and after low dose of  $\text{NaIO}_3$ .  $\text{NaIO}_3$  is considered to be specific for animal model as age macular degeneration. Taurine action is efficient as antioxidant to protect retina from oxidative stress limits in range of 25 mg of  $\text{NaIO}_3$  and decreases the efficiency by increasing the dose of  $\text{NaIO}_3$  up to 75 mg.

**Keywords:** Age macular degeneration (AMD), Retina, FTIR, Antioxidant.

### Introduction

Retinal degeneration, a feature of neurodegenerative diseases such as AMD that characterize by degeneration of the macular section of the retina can lead to strict visual injury and finally blindness. Millions of people worldwide suffer varying degrees of permanent vision loss because of these not curable, as degenerative eye disorders<sup>(1,2)</sup>. There are several risk factors, including age, race, smoking, and diet<sup>(3)</sup>. But the etiology and pathogenesis of the disease remain largely unclear. Vital advances in the understanding of AMD pathogenesis have been paying attention to the role of oxidative stress (OS) or damage into the retina. It is clearly established that reactive oxygen species (ROS) and oxidized lipoproteins are crucial sources of

cell and tissue stress creating enough background for parainflammation in the aging retina. This persistent situation lead to the development and/or progression of AMD<sup>(4)</sup>. Oxidative stress results from the imbalance between the prooxidants and the antioxidant resistance leading to cellular damage.

Sodium iodate ( $\text{NaIO}_3$ ) injection has been extensively used as a pre-clinical model of AMD<sup>(5,6)</sup>.  $\text{NaIO}_3$  is directly affecting the retinal pigment epithelial (RPE) cells with resultant effects on photoreceptors and the choriocapillaris and has been shown to induce the production of reactive oxygen species causative to damages in retinal cells<sup>(7,8)</sup>.

Taurine or 2-aminoethanesulfonic acid is a derivative of cysteine, an amino acid which

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contains a sulfhydryl group. Taurine antioxidant activity has been shown to play role as a cytoprotectant and in the attenuation of apoptosis. There is a growing consensus that oxidative stress is linked to mitochondrial dysfunction<sup>(9,10)</sup>, and that the beneficial effects of taurine are a result of its antioxidant properties<sup>(11,12)</sup>, as well as its ability to improve mitochondrial function by stabilizing the electron transport chain and inhibiting the generation of reactive oxygen species<sup>(13)</sup>.

To understand the molecular mechanisms structure of disease induction and progression for retina, and to develop therapeutic strategies for vision preservation, the present study evaluates the action of NaIO<sub>3</sub> that a model can already serve as tool to investigate beneficial effects of experimental treatments on the molecular structure of the retina and whether the dietary intake of taurine can help as an antioxidant to retina or may delay the progression of AMD.

## Materials and Methods

### Experimental Animals

One hundred and five albino rats of both sexes, weighing 200–230 g were randomly selected from the animal house facility at the research institute of ophthalmology, Giza, Egypt. The rats were maintained in standard 12 hr light-dark cycle with free access to water and balanced diet at a temperature of 22± 2 °C, 50 % humidity, and kept separately under good ventilation. Animals were handled according to the Association for Research in Vision and Ophthalmology (ARVO) statements and regulations for the use of animals in research. The rats were classified into 3 groups I, II and III according to the following: Group I: contains 15 rats were used as control and receive intravenous injection of saline. Group II: contains 45 rats and were subdivided into 3 subgroups (15 rats each). All subgroups receive a single intravenous injection of 25, 50, or 75 mg/kg NaIO<sub>3</sub><sup>(14)</sup>. Group III: contains 45 rats and were subdivided into 3 subgroups (15 rats each). All rats are supplemented with 4 g taurine /100 g diet 2 weeks before intravenous injection of NaIO<sub>3</sub><sup>(15)</sup>. Taurine supplementation is continued until decapitation. All groups were decapitated after 1 day of NaIO<sub>3</sub> injection.

### Preparation of samples

The albino rats were sacrificed by decapitation, where the eyes were enucleated, and then opened by corneal section through the

ora serrata. After removing the corneas, the iris was pulled out by a forceps where the eye lens and the vitreous humour (jelly structure) were jointly removed. The remaining eye cub, which contains the retina, removed to a previously sterilized and weighted dark brown glass vials. The glass vials were flushed by dry N<sub>2</sub> gas and kept at -20°C for FTIR measurements.

### FTIR Spectrophotometer measurements

Weighted retinæ were freeze-dried separately and mixed with KBr powder (2 mg retina: 98 mg KBr) then hard-pressed to prepare the transparent KBr disks that will be used for the FTIR investigations. Using Nicolet-iS5 infrared spectrometer (ThermoFisher Scientific Inc, Madison, USA) with resolution of 2 cm<sup>-1</sup>, spectrum is derived from 100 sample interferogram. The spectrometer operation was done under a continuous dry N<sub>2</sub> gas to remove interference from atmospheric CO<sub>2</sub> and H<sub>2</sub>O vapor. Spectra baseline are corrected, and then smoothed with Savitsky–Golay filter. The average of three spectra using OriginPro9 software (Origin Lab Corporation, Northampton, MA, USA) is normalized according to obtain the definite peaks<sup>(16)</sup>.

### Statistical analysis

Mean standard deviations are expressed through statistical analysis. Comparisons among multiple groups are made by using analysis of variance (ANOVA). Available statistical software package (SPSS-11, Windows) was used and the significance level is established at P<0.05.

## Results

The FTIR spectra of retina were obtained for normal and injected animals with different doses of NaIO<sub>3</sub> with or without treated by taurine after one day. The spectra covering the range of 4000–900 cm<sup>-1</sup> were recorded. Figure (1) summarized all overlaid FTIR spectra of all groups compared to normal spectra for animals decapitated after 1 day. Panel (a) of Fig. (1) shows all vibrational frequency range corresponding to retinal tissue of control group and the groups injected with 25, 50 and 75 mg of NaIO<sub>3</sub>. Panel (b) shows overlaid FTIR spectra of normal rats retina and those of 4 g taurine supplement /100 g, diet two weeks before intravenous injection of NaIO<sub>3</sub> which continued until decapitation after 1 day compared to control.

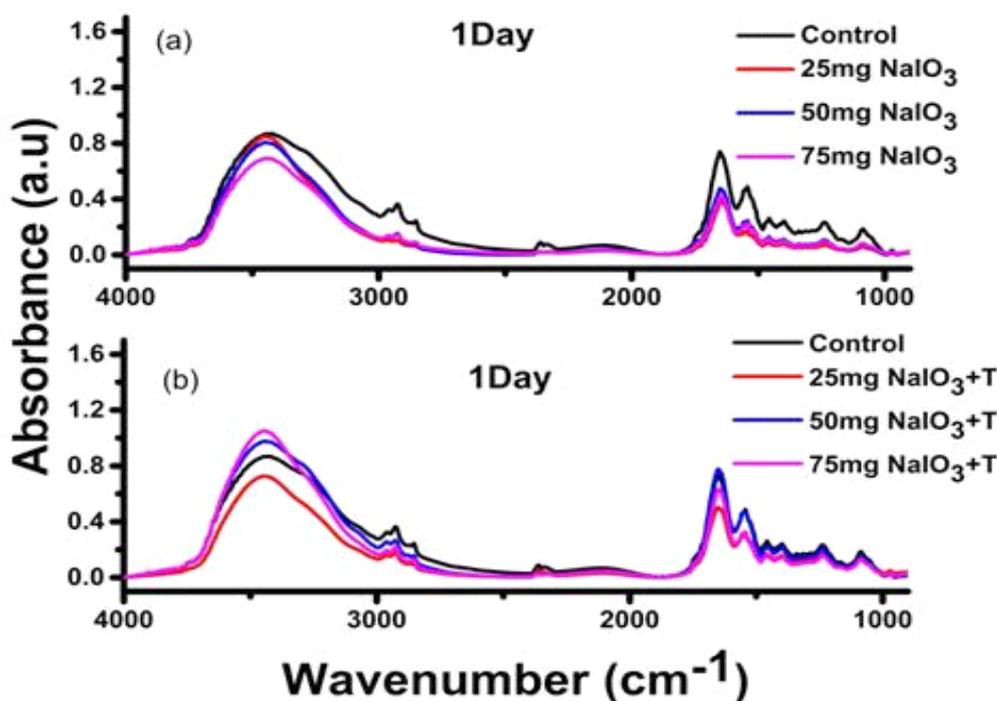


Fig. 1. FTIR spectra for rats injected with different doses of NaIO<sub>3</sub>(a) and rats supplemented with taurine before and after same doses of NaIO<sub>3</sub> (b) after one day.

The detailed spectral analyses are performed in 3 distinct wavenumber ranges; 4000–3000 cm<sup>-1</sup> (NH-OH region), 3000–2800 cm<sup>-1</sup> (C-H stretching region) and 1500–900 cm<sup>-1</sup> (Fingerprint region). The amide I band that appears at 1650 cm<sup>-1</sup> was measured separately since it consists of a number of underlying bands which corresponding to different protein secondary structural components.

#### NH-OH Region

Figure (2) shows the NH-OH region of the retinal FTIR spectra that has the range 4000–3000 cm<sup>-1</sup>, for control rats, rats injected with different doses of NaIO<sub>3</sub> (25, 50 and 75 mg) and rats taurine supplement before and after injection of NaIO<sub>3</sub> then decapitated after 1 day. The main band of the normal pattern is found at 3454±2 cm<sup>-1</sup>. The curve enhancement procedure resolved this band into 4 structural components (Table 1) correspond to (1) stretching O-H (<sub>str</sub>OH), (2) asymmetric O-H (<sub>asym</sub>OH), (3) symmetric O-H (<sub>sym</sub>OH) and (4) asymmetric NH<sub>3</sub> (<sub>asym</sub>NH<sub>3</sub>) respectively as assignments previously mentioned by Dovbeshko et al.,<sup>(17)</sup>. Stretching O-H component vibrates in 3 modes of vibration.

Table (1) indicates the changes in vibrational frequency and the width in all bands in the NH-OH region after 1 day. For all groups injected with NaIO<sub>3</sub>, the 3 modes of vibrations related to <sub>str</sub>OH appeared at wave length 3740±2, 3615±3, 3532±2 cm<sup>-1</sup> which observed in control pattern are decreased to one vibration mode and changed to a range between 3585±3 to 3580±3 cm<sup>-1</sup>. In the same context, appear of 2 modes of vibrations for <sub>str</sub>OH to all groups taurine supplemented two weeks before intravenous injection of NaIO<sub>3</sub> then continued until capitation after 1 day. There are statistically significant increments (p<0.05) in modes of vibration of <sub>asym</sub>OH and width in all doses injected of NaIO<sub>3</sub> in contrast to <sub>asym</sub>OH vibration modes in taurine supplemented groups that had no significant differences in frequency and width compared to control. Mode of vibration related to <sub>sym</sub>OH suffered from statistically significant increases (p<0.05) in width for NaIO<sub>3</sub> groups. Also disappear of <sub>asym</sub>NH<sub>3</sub> in NaIO<sub>3</sub> groups and appear in taurine treated groups injected with 25 and 50 gm of NaIO<sub>3</sub>.

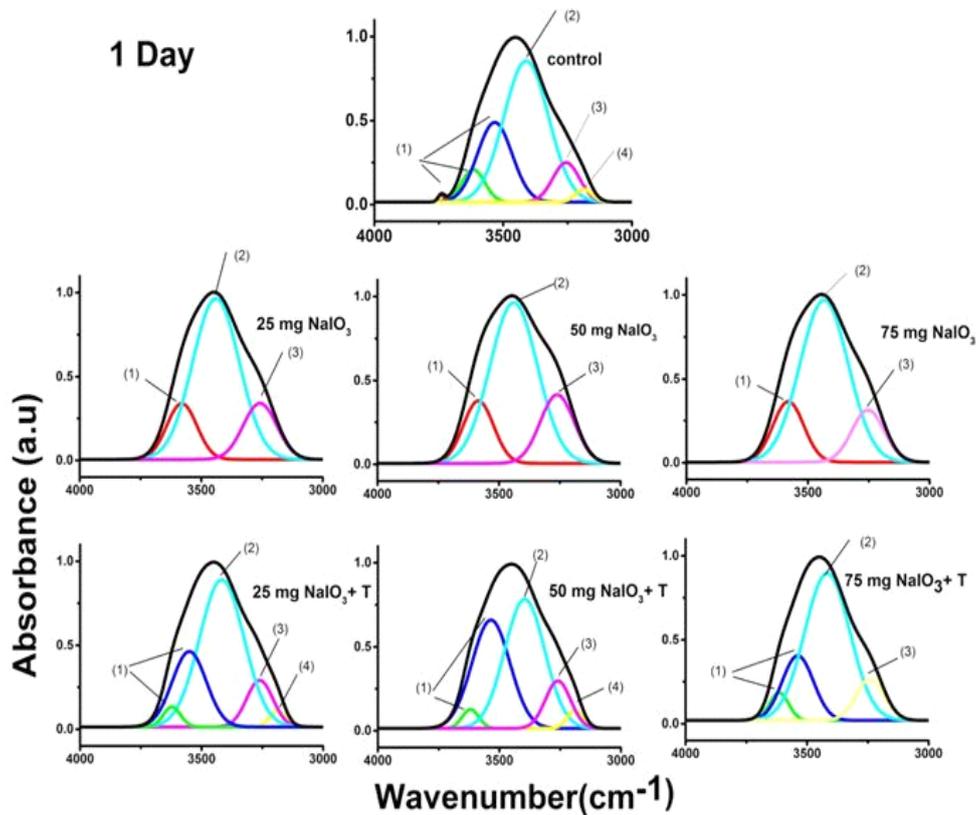


Fig.2.NH-OH region of the retinal FTIR spectra in the range 4000-3000  $\text{cm}^{-1}$  for control rats, rats injected with different doses of  $\text{NaIO}_3$  (25, 50 and 75 mg) and rats supplemented taurine before and after injection of  $\text{NaIO}_3$  after 1 day. (1) strOH, (2)asymOH,, (3) symOH and (4) sym  $\text{NH}_3$ .

TABLE 1. Fourier deconvolution and nonlinear curve fitting of the NH-OH region of retina from normal and different groups of decapitated animals after 1 day.

	(1) Str O-H-	(2) asym OH	(3) sym O-H	(4) sym $\text{NH}_3$
Control	3740±2 30±1	3615±3 105±5	3532±2 158±4	3408±4 204±6
25 mg $\text{NaIO}_3$			3580±3 145±5	3439±1† 226±3†
50 mg $\text{NaIO}_3$			3585±3 147±5	3439±2† 230±4†
75 mg $\text{NaIO}_3$			3581±2 149±5	3434±2† 234±4†
25 mg $\text{NaIO}_3$ +T		3620±2 85±2†	3551±3† 164±8	3413±4 215±7
50 mg $\text{NaIO}_3$ +T		3618±1 81±4†	3535±2 173±4†	3403±3 194±5
75 mg $\text{NaIO}_3$ +T		3615±2 92±2†	3536±3 151±5	3413±2 218±7
				3256±3 121±8
				3258±3 162±3†
				3260±3 164±3†
				3253±2 156±3†
				3260±2 127±8
				3188±2 78±5
				3188±2 78±6
				3187±2 79±7

First line in each cell indicates the vibrational frequency in  $\text{cm}^{-1}$ ± standard deviation, while second line reflects the bandwidth, †Statistically significant

### C-H Region

The infrared absorption pattern of control retina that is shown in Fig (3) compared to different groups studied after 1 day. The control pattern is characterized by 3 absorption bands in the IR range 3000-2800  $\text{cm}^{-1}$ . The curve enhancement procedure that used to resolve any overlapping peaks confirms the presence of four bands that are centered at  $2960 \pm 2 \text{ cm}^{-1}$  with bandwidth of  $21 \pm 1 \text{ cm}^{-1}$ ,  $2925 \pm 2 \text{ cm}^{-1}$  with bandwidth of

$31 \pm 1 \text{ cm}^{-1}$ ,  $2874 \pm 3$  with band width  $46 \pm 2$  and  $2852 \pm 2 \text{ cm}^{-1}$  with corresponding bandwidth of  $14 \pm 1 \text{ cm}^{-1}$ . These bands can be assigned as  $\text{CH}_3_{\text{asym}}$ ,  $\text{CH}_2_{\text{asym}}$ ,  $\text{CH}_3_{\text{sym}}$  and  $\text{CH}_2_{\text{sym}}$  respectively as shown in Table (2). The assignment of the bands has been previously mentioned by Severcan et al.,<sup>(18)</sup>. As observed from their assigned, there are no significant changes in band absorbance or band width to all groups compared to control.

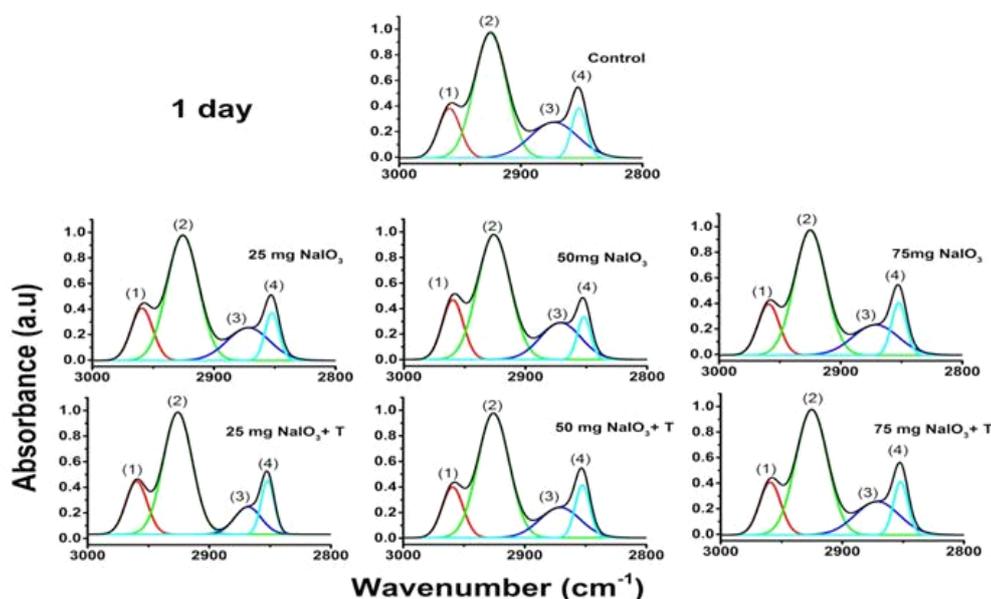


Fig. 3. CH region of retina from normal group, animals injected with  $\text{NaIO}_3$  and treated by taurine after 1 day showing the deconvoluted FTIR spectrum. (1) asym  $\text{CH}_3$ , (2) asym  $\text{CH}_2$ , (3) sym  $\text{CH}_3$  and (4) sym  $\text{CH}_2$ .

TABLE 2. Estimated structural components with its vibrational frequencies, bandwidth and area of the CH region of retina for normal, different doses of  $\text{NaIO}_3$  injection and treated with taurine before and after injection after 1 day.

	(1) $\text{CH}_3_{\text{asym}}$	(2) $\text{CH}_2_{\text{asym}}$	(3) $\text{CH}_3_{\text{sym}}$	(4) $\text{CH}_2_{\text{sym}}$
Control	$2960 \pm 2$ $21 \pm 1$	$2925 \pm 2$ $31 \pm 1$	$2874 \pm 3$ $46 \pm 2$	$2852 \pm 2$ $14 \pm 1$
25 mg $\text{NaIO}_3$	$2960 \pm 1$ $21 \pm 1$	$2926 \pm 2$ $31 \pm 1$	$2872 \pm 2$ $39 \pm 2 \dagger$	$2852 \pm 1$ $14 \pm 1$
50 mg $\text{NaIO}_3$	$2960 \pm 3$ $20 \pm 1$	$2926 \pm 4$ $32 \pm 1$	$2871 \pm 4$ $39 \pm 1 \dagger$	$2852 \pm 3$ $13 \pm 2$
75 mg $\text{NaIO}_3$	$2959 \pm 2$ $21 \pm 1$	$2925 \pm 2$ $30 \pm 1$	$2871 \pm 2$ $43 \pm 3$	$2852 \pm 4$ $14 \pm 2$
25 mg $\text{NaIO}_3$ +T	$2960 \pm 3$ $21 \pm 2$	$2926 \pm 3$ $28 \pm 2$	$2868 \pm 4$ $41 \pm 3$	$2852 \pm 2$ $13 \pm 1$
50 mg $\text{NaIO}_3$ +T	$2959 \pm 1$ $20 \pm 1$	$2926 \pm 2$ $30 \pm 2$	$2870 \pm 3$ $41 \pm 2$	$2853 \pm 2$ $14 \pm 2$
75 mg $\text{NaIO}_3$ +T	$2959 \pm 4$ $21 \pm 1$	$2925 \pm 2$ $31 \pm 2$	$2871 \pm 2$ $42 \pm 3$	$2852 \pm 3$ $14 \pm 1$

First line in each cell indicates the vibrational frequency in  $\text{cm}^{-1} \pm$  standard deviation, while second line reflect the bandwidth.

### Fingerprint Region

Figure (4) shows the FTIR absorption spectra in the range 1600-900  $\text{cm}^{-1}$  for control group, animals injected with  $\text{NaIO}_3$  (25, 50 and 75 mg) after one day and those which are taurine supplemented before and after injection of  $\text{NaIO}_3$ . This figure shows the presence of eleven bands in the normal pattern, that are centered at  $1542 \pm 2 \text{cm}^{-1}$  and  $1521 \pm 1$  (amide II  $\text{N}=\text{H}_{\text{bend}}$ ),  $1457 \pm 4 \text{cm}^{-1}$  ( $\text{bend}_{\text{CH}_2}$ ),  $1399 \pm 5 \text{cm}^{-1}$  ( $\text{strCOO}^-_{\text{sym}}$ ),  $1337 \pm 3 \text{cm}^{-1}$  and  $1315 \pm 3 \text{cm}^{-1}$  ( $\text{CH}_{3\text{def}}$  deformation),  $1284 \pm 3 \text{cm}^{-1}$  and  $1233 \pm 2 \text{cm}^{-1}$  ( $\text{strPO}_2^-_{\text{asym}}$ ),  $1167 \pm 2 \text{cm}^{-1}$  ( $\text{strCOOC}_{\text{asym}}$ ),  $1085 \pm 4$ , ( $\text{strPO}_2^-_{\text{sym}}$ ) and  $1052 \pm 3 \text{cm}^{-1}$  ( $\text{strCOC}$ ) as shown in table (3). The bands assignments have been indicated by Jung. <sup>(19)</sup> The most obvious observation due to  $\text{NaIO}_3$  injection is disappearance in mode of vibration related to Amide II that appeared in control and the re-appearance in all groups treated with taurine. Some fluctuation observed in number of modes of vibrations related to  $\text{strPO}_2^-_{\text{asym}}$ ,  $\text{COOC}_{\text{asym}}$  and  $\text{COC}$  in a way that cannot be attributed to  $\text{NaIO}_3$  injection or taurine treated as indicated in Table (3).

### Amide I band

Analysis of the amide I band Fig.(5) shows the curve enhancement procedure to control group, animal groups injected with 25, 50 and 75 mg of

$\text{NaIO}_3$  and groups treated with taurine after 1 day. The analysis resolved the contour of the normal band into 3 compositional bands that are centered at  $16732 \text{cm}^{-1}$  ( $\beta$ -Turns),  $16481 \text{cm}^{-1}$  ( $\alpha$ -helix), and  $16271 \text{cm}^{-1}$  ( $\beta$ -Sheet) (Lin et al., 1998). Table (4) indicates that the distribution of normal protein secondary structure components - that are calculated as the area percentage - are the following; 192% for  $\beta$ -Turns, 604 % for  $\alpha$ -helix and 202 % for  $\beta$ -Sheet. The assignment of the bands are indicated by Fuller et al.,<sup>(20)</sup>. After 25, 50 and 75 mg of  $\text{NaIO}_3$  injection, the number of estimated bands were increased to 4, 5 and 6 bands respectively. For all doses of  $\text{NaIO}_3$  injection, there are statistically significant decrements ( $p < 0.05$ ) in  $\alpha$ -helix associated with statistically significant increases ( $p < 0.05$ ) in  $\beta$ -Sheet structures. By taurine treatment before and after injection of 25 mg of  $\text{NaIO}_3$ , the number of estimated peaks returned back to the control pattern of 3 peaks and no significant differences appeared in  $\alpha$ -helix content or  $\beta$ -Sheet structures. However, after high doses of  $\text{NaIO}_3$  (50 and 75 mg) the number of peaks related to the protein structure components became 5 and 6 respectively and statistically significant diminish ( $p < 0.05$ ) in  $\alpha$ -helix associated with statistically significant increment ( $p < 0.05$ ) in  $\beta$ -Sheet structures were observed.

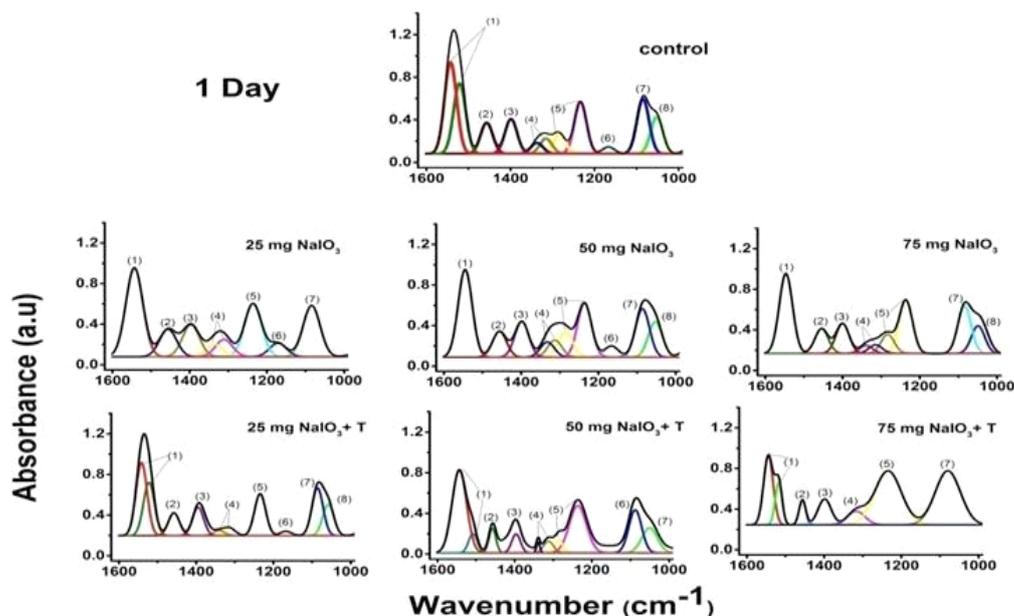


Fig.4. FTIR spectra of the fingerprint region of all groups compared to control. The numbers above the peaks are to facilitate their assignment. (1) Amide II, (2)  $\text{CH}_2$  bend, (3)  $\text{strCOO}^-_{\text{sym}}$ , (4)  $\text{CH}_3\text{def}$ , (5)  $\text{strPO}_2^-_{\text{asym}}$ , (6)  $\text{strCOOC}_{\text{asym}}$ , (7)  $\text{strPO}_2^-_{\text{sym}}$  and (8)  $\text{strCOC}$ .

**TABLE 3. Vibrational frequencies of the estimated bands and width in fingerprint region of control and different groups injected with  $\text{NaIO}_3$  and treated with taurine after 1 day.**

	(1) Amide II N=H	(2) CH <sub>2</sub>	(3) COO <sup>-</sup>	(4) CH <sub>3</sub>	(5) PO <sub>2</sub> <sup>-</sup>	(6) COOC	(7) PO <sub>2</sub> <sup>-</sup>	(8) COC
Control	1542±2 31±5 1521±1 24±2	1457±4 11±4	1399±5 12±3	1337±3 6±3	1284±3 7±2	1167±2 4±2	1085±4 19±3	1052±3 13±1
25 mg $\text{NaIO}_3$	1543±2 48±5†	1456±2 14±2	1397±3 17±4	1336±4 7±2	1236±2 26±4	1171±2 7±3	1084±3 28±3	
50 mg $\text{NaIO}_3$	1545±4 40±3†	1456±2 12±3	1398±3 16±3	1333±3 7±3	1285±2 11±3	1166±4 4±1	1085±3 21±2	1053±12 16±3
75 mg $\text{NaIO}_3$	1547±3 34±6	1454±3 10±2	1400±3 13±3	1339±2 6±1	1283±3 8±1	1236±1 22±4	1084±3 20±3	1049±3 12±1 1058±1
25 mg $\text{NaIO}_3$ +T	1542±4 26±3 1522±1 19±3†	1458±2 8±3	1394±2 10±3	1341±3 2±1	1312±2 4±1	1168±2 2±1	1085±2 17±2	12±2
50 mg $\text{NaIO}_3$ +T	1543±1 38±4 1518±2 5±1	1458±14 5±1	1396±2 5±2	1339±3 9±2	1312±2 4±1	1236±2 26±6	1087±2 18±2	1050±3 12±2
75 mg $\text{NaIO}_3$ +T	1544±2 26±4 1517±2 9±1	1456±2 5±1	1398±1 11±1	1317±2 7±1	1234±2 46±2		1079±3 43±3	

First line in each cell indicates the vibrational frequency, while second line reflects the bandwidth, †Statistically significant.

**TABLE 4. Summary of curve fitting analysis of amid I bands expresses summation of area under peak percentage (%) of main protein secondary structural components of rat retinas for control group, animals injected with  $\text{NaIO}_3$  and treated by taurine after 1 day.**

	β- turn	α- helix	β- sheet
Control	19.29±2	60.34±4	20.37±2
25mg $\text{NaIO}_3$	24.44±3	49.65±2†	25.91±1†
50mg $\text{NaIO}_3$	21.07±3	49.77±3†	29.16±2†
75mg $\text{NaIO}_3$	22.79±3	48.63±2†	28.58±1†
25mg $\text{NaIO}_3$ +T	26.51±4	56.34±2	17.15±1
50mg $\text{NaIO}_3$ +T	20.41±2	53.00±1†	25.59±1†
75mg $\text{NaIO}_3$ +T	20.95±5	53.27±1†	25.78±1†

†Statistically significant

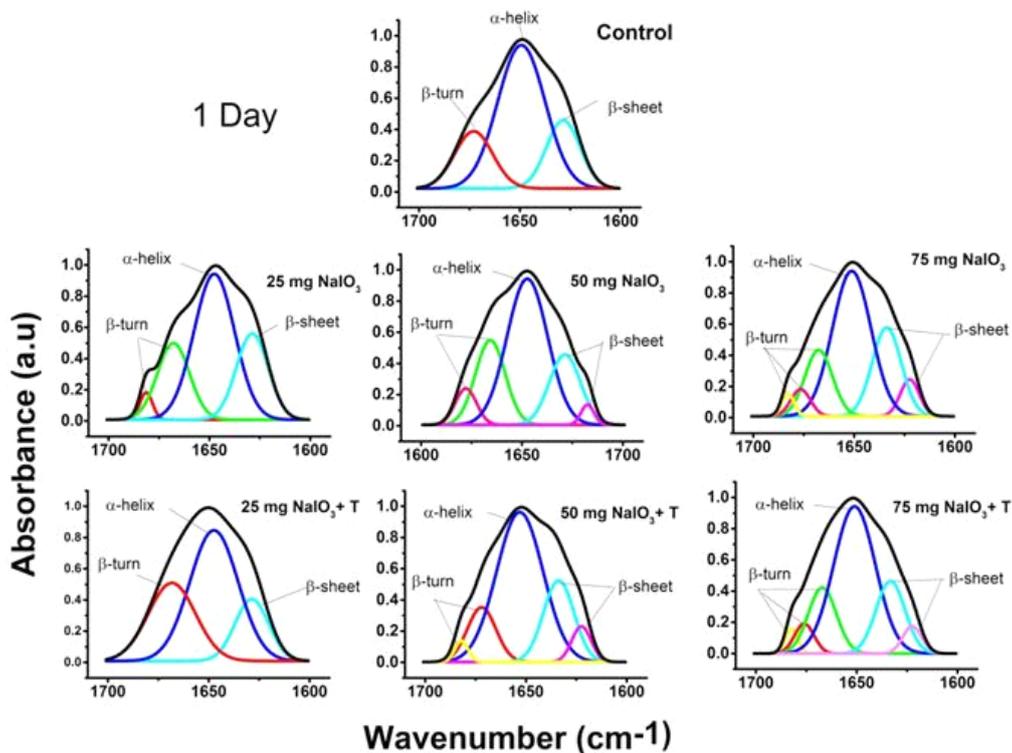


Fig. 5. Amide I region (1700:1600  $\text{cm}^{-1}$ ) of deconvoluted FTIR spectrum to retina for control group, animals injected with  $\text{NaIO}_3$  and treated by taurine after 1 day.

## Discussion

This study reveals that NH-OH region is affected by injection of  $\text{NaIO}_3$  where there is a different interaction/binding mechanism and production of various states in the retinal system, as well as different configurations and conformations co-exist in the system. There are changes in modes and the number of vibration for  $\text{OH}_{\text{str}}$  are related to  $\text{NaIO}_3$  injection, The observed recovery is connected to taurine treatment and it appears to be dose dependent. In addition, the disappearance of  $\text{NH}_3^{\text{sym}}$  in all  $\text{NaIO}_3$  injected groups reflect changes in membrane structure of retinal cells since NH bond exists in several membrane constituents that contain protein and lipid. These changes are in line with the work by Aboualizadeh et al., 2015<sup>(21)</sup> who observed changes in FTIR spectra of retinal cells due to oxidative stress. The appearance of  $\text{NH}_3^{\text{sym}}$  in most taurine treated groups indicates the recovery following the taurine treatment. Also,  $\text{NaIO}_3$  injection enhances the asymmetric vibrational motion of the OH band which may be indicative

for different types of membrane disorder. The observed positive effect exerted by taurine could be ascribed to its ability to induce more vibrational motion in the taurine treated groups (25,50,75 mg of  $\text{NaIO}_3$ ) compared to control. Analysis of fingerprint region is concerning with the changes in the amide II band vibration mode, due to coupling of CN stretching and in-plane bending of the N-H group. The decrease of amide II vibration modes in all groups of  $\text{NaIO}_3$  injection groups revealed its action. The appearance or disappearance of modes of vibration infrared bands may be due carbonization, degeneration of biological tissues and losing its function that is in agreement with Zezell et al., 2015<sup>(22)</sup> and/or transcriptional regulation effect as observed by Lima et al., 2015<sup>(23)</sup>. However, the amide II band is not often used for structural studies because it is less sensitive and subject to interference from absorption bands of amino acid side-chain vibrations as described by Al-Muslet and Ali 2012<sup>(24)</sup>. The noticeable fluctuation changes in the fingerprint region recognized to the oxidative

stress after injection of all doses of NaIO<sub>3</sub> leading to increase the stress with the dose which is consistent with the work of Zhang et al., 2016<sup>(25)</sup> that suggested accumulation of reactive oxygen species in retina due to injection of NaIO<sub>3</sub> and resemble condition of AMD.

The protein secondary structure is affected by all doses of NaIO<sub>3</sub> injection where a change in the  $\alpha$ -helix,  $\beta$ -sheet and  $\beta$ -turn contents is observed. It has been suggested by Mitra et al., 2017<sup>(26)</sup> that protein insolubility depends on the content of the  $\beta$ -sheet structure: more  $\beta$ -sheet structure means more insoluble protein that agree with our results in decrease  $\alpha$ -helix, and the disordered chains aggregate to form  $\beta$ -sheet structure. Changes in the profile and the pattern of the amide I band are clearly observed for all NaIO<sub>3</sub> groups compared with the control pattern. These changes in the amide I band profile are due to differences in hydrogen-bonding strengths, differences in transition dipole coupling and consequently in molecular geometry of proteins, which may induce damages in protein folding and result in definitive loss of protein biological function as these are the factors that give the amide I band its sensitivity to protein secondary conformational changes. These findings are in line with the results of study by Zhou et al., 2014<sup>(1)</sup> who reported that the molecular mechanism of RPE damage that occurs after NaIO<sub>3</sub> administration is thought to be mediated through induction of oxidative stress through accumulation of ROS that co-localizes with mitochondria.

All changes observed in the presented study may cause the retina to lose function. Such function loss could be due to two factors. First, NaIO<sub>3</sub> treatment could cause synaptic damage, as a defect in synaptic transmission between the photoreceptors and depolarizing bipolar cells can lead to a weaken ERG b-wave as observed by Pang et al., 2006<sup>(27)</sup>. This idea is supported by Hariri 2013<sup>(28)</sup> who used SD-OCT to describe early changes associated with injection of high doses of NaIO<sub>3</sub> in rats and demonstrating a rapid swelling of photoreceptor outer segments due to hydropic change. Secondly, NaIO<sub>3</sub> could directly alter photoreceptor function and cell survival with a special stress on oxidative stress leading to phototransduction processes changes as reported by Wang et al., 2014<sup>(29)</sup>.

A complete recovery is observed in our study following the treatment with taurine before and

after the 25mg of NaIO<sub>3</sub> injection and a partial role with the 50 and 75 mg of injection. The effect of taurine is correlated to its antioxidative activity as confirmed by Imaki et al.<sup>(30)</sup>, and Ishikawa et al.,<sup>(31)</sup>. Dietary taurine is absorbed via the digestive system and then is transported by the Na<sup>+</sup>-dependent taurine transporter into the retina through the blood-retinal barrier. The role taurine may play in the retina is the promotion of retinal cell differentiation during rod photoreceptor development<sup>(32)</sup>.

Various physiological roles have been suggested for taurine, among which are modulation of Ca<sup>2+</sup> transport, antioxidation, neurotransmission, osmoregulation, membrane stabilization, and regulation of protein phosphorylation. Many of the actions of taurine may be considered as membrane based, in which taurine is modify membrane phospholipids, thus modifying membrane function<sup>(33)</sup>.

### Conclusion

- FTIR spectroscopy has been an integral part of understanding the mechanisms behind protein misfolding diseases including MDA
- NaIO<sub>3</sub> is considered to be highly specific for animal model to AMD and oxidative stress to retina even in low dose.
- Taurine is required for maintaining retinal health since it fights oxidative stress. Evidence is strong that taurine is vital in maintaining optimal retinal function. Taurine action is appeared in range of 25 mg of NaIO<sub>3</sub> and decrease the efficiency by increasing the dose of NaIO<sub>3</sub> to 75 mg.

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## التغيرات الجزيئية في بنية شبكية العين للجرذان بعد الحقن بأبودات الصوديوم وتأثير التورين

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الهدف من هذا العمل هو التحقق من تأثير أبودات الصوديوم على شبكية عين الجرذان وما إذا كان تناول مادة التورين في النظام الغذائي يؤخر من تطور مرض الضمور البقعي الناتج عن مركب أبودات الصوديوم وذلك باستخدام التحليل الطيفي بالأشعة تحت الحمراء حويل فورية . تم اختيار مائة وخمسة (105) من الفئران البيضاء من كلا الجنسين والتي يتراوح وزنها من 200 - 230 جم تم تقسيمها إلى ثلاث مجموعات , المجموعة الأولى الضابطة ( 15 ) فأر المجموعة الثانية ( 45 ) تتلقى حقنة وريدية من أبودات الصوديوم بتركيزات مختلفة 25 , 50 , 75 مجم \ كجم , المجموعة الثالثة ( 45 ) فأر تتناول التورين في الغذاء قبل أسبوعين من الحقن بأبودات الصوديوم . بعد إجراء القياس لوحظ أن التورين لهو دور فعال كمضاد للأكسدة خاصة في التركيزات المنخفضة من أبودات الصوديوم