

Side Effects of Chemotherapy on the Molecular Structure of rat's retina and the Possible Protective Role of Antioxidants

*Rehab A. Fouad**, *Gehan M. Kamal**, *Sahar M. Awad**

* Physics department, Faculty of Science (girls), Al-Azhar University, Cairo.

*Corresponding author, Email: rerefouad.1212@gmail.com, Tel: 01146904148

Abstract

Ifosfamide is an alkylating oxazaphosphorine antitumor prodrug. Although it is an effective chemotherapeutic agent, it has been shown to induce many side effects. The objective of the present study, therefore, was to investigate the possible protective roles of lecithin and quercetin either singly or combined against ifosfamide-induced molecular structure changes in retina of female rats using Fourier transform infrared spectroscopy (FTIR). Seventy female albino rats were randomly divided into seven groups. Ifosfamide (Ifo; 80mg/kg b.wt.) was administered for five consecutive days intraperitoneally (i.p.), while quercetin (50mg/kg b.wt.) and lecithin (100mg/kg b.wt) were given orally either singly or in-combination with IFO for six consecutive days. Our results indicate that Ifosfamide was affected on the lipid components of the retina, NH-OH region changes revealed unusual interface/binding mechanism that related to different surrounding environment due to ifosfamide intraperitoneal injection. The co-administration of Lec+Que with the intraperitoneal injection of Ifosfamide preventing the side effect of Ifosfamide. We suggest that synergistic effect of quercetin and lecithin in the combined therapy results in marked neuroprotective effect in part through its antioxidant properties and down regulation of molecular structure in retina.

Introduction

Systemic drug-induced ocular side effects are increasing because of the vast numbers of new drugs being introduced. Reports of drug-induced ocular toxicity must be well documented, and the other causes of these side effects ruled out to help establish causality. Systemic anticancer therapies can produce acute and chronic organ damage, but the eye is usually considered a protected site (**Moorthy RS, 1999**). Nonetheless, the oculovisual system has a potentially high degree of sensitivity to toxic substances. Ocular toxicity induced by cancer

*Corresponding author, Email: rerefouad.1212@gmail.com, Tel: 01123220010

chemotherapy includes a broad spectrum of disorders, reflecting the unique anatomical, physiological and biochemical features of the eye. Understanding the ocular side effects will assist the ophthalmologist and oncologist to recognize them early and intervene before blindness occurs.

Ifosfamide is from alkylating agents that directly damage DNA to prevent the cancer cell from reproducing. It is mainly metabolized through CYP 3A4 and CYP 2B6 enzymes. Ifosfamide is used in the treatment of a variety of solid tumors including those of the cervix, endometrium, lung, ovary, testes and thymus as well as in sarcoma and in the treatment of Burkitt's lymphoma (Yang, H. et al., 2015).

Lecithins are usually phospholipids, composed of phosphoric acid with choline, glycerol acid or other fatty acids usually glycolipids or triglyceride. Glycerophospholipids in lecithin include phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and phosphatidic acid (Najafi A, 2014).

Quercetin is a flavonol, it is plant derived flavonoid used as a nutritional supplement found in fruits and vegetables. Quercetin is thought to have potent antioxidant, Antidiabetic and anti tumour, and antiviral, anti inflammatory benefits (Spencer et al., 2008).

The present study aimed to investigate the possible protective roles of lecithin and quercetin either singly or combined against ifosfamide-induced molecular structure changes in retina of female rats.

Materials and methods

Chemicals

All chemicals utilized were obtained from Sigma Company (St. Louis, MO, USA) with the highest purity commercially available.

Animals grouping

Healthy seventy female *Rattus norvegicus* rats weighing 180g \pm 10 were divided into seven groups, each containing ten rats. The first group served as control, which received the vehicle only (0.9% NaCl, 0.25 ml intraperitoneally for 5 days), Groups 2 and 3 were administered orally lecithin (Lec) at a dose of 100 mg/kg bwt (Lee H.S, et al., 2013) or quercetin (Que) at a dose of 50 mg/kg bwt (Francescato H, et al., 2004) suspended in distilled water, respectively for six days whereas group 4 was used as positive control were administered

ifosfamide (Ifo) at a dose of 80mg/kg bwt (**Chen N., et al.,2008**) intraperitoneally for five days. Groups 5 and 6 were administered either Que or Lec along with Ifo by the same treatments regimens, respectively. Group 7 received a combination of both protective agents along with Ifo and treated similarly. Retina from each eye of all groups was used for FTIR.

FTIR spectroscopy measurements

The KBr (100 mg) powder was crushed in an agate mortar with pestle to yield fine particles of nearly 100 nm diameter; then pressed hydraulically under vacuum to form a transparent KBr disks that will be used for the measurement. Weighted retinae , corneas and lens were freeze-dried separately and mixed with KBr powder (5mg retina: 95 mg KBr) then pressed to prepare the transparent KBr disks that will be used for FTIR investigations. Measurements were done using infrared spectrophotometer (Thermo Fisher Scientific Inc, USA). The resolution was set at 2 cm^{-1} with the wavenumber range 4000-400 cm^{-1} and using a dry N_2 gas to remove interference. Savitsky Golay filter were used to remove the noise before Fourier transformation. The spectra were baseline corrected, then smoothed with Three spectra from each sample were obtained and averaged using Origin 2016 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 FTIR figures (**Eman S.M and Eman M.A.,2011**).

Statistical analysis

The data obtained are represented as Mean \pm Standard deviation. One-way analysis of variance (ANOVA) followed by Duncan at $P < 0.05$ and were carried out using SPSS-11 for windows, SPSS Inc., Chicago, IL, USA.

Results

The spectra results from FTIR to retinal tissue extracted from control rats and all studied groups analyzed in three frequency regions; 4000-3000 cm^{-1} , 3000-2800 cm^{-1} and 1600-900 cm^{-1} corresponding to NH-OH, C-H and fingerprint region, respectively.

NH-OH region

Figure (1) shows the NH-OH region of the retinal FTIR spectra in the range 4000-3000 cm^{-1} for all studied groups. The spectra of the NH–OH region of different groups were subjected to Fourier deconvolution and non-linear curve fitting to resolve the contour to its underlying

structural bands. Table (1) indicated the assignments of different peaks appeared in the NH-OH spectra and the corresponding bandwidth for each peak.

For control pattern the mean peak was centered at 3454cm^{-1} and it can be resolved into six peaks. These underlying peaks were observed at 3852 ± 3 , 3742 ± 6 and 3454 ± 3 that correspond to (1) stretching O-H (strOH), 3454 ± 3 corresponding to (2) asymmetric OH (asymOH), 3268 ± 4 related to (4) stretching O-H symmetric (symOH) and the last band at 3077 ± 2 correspond to (5) CH_{ring} as previously described by (**Dovbeshko et al. (2000)**).

The lecithine- positive control –group shows the same six underlying bands as control group. On the other hand the quercitine- another positive control -group was characterized by the presence of five underlying peaks with different vibrational asymNH_3 mode at $3314\pm 3\text{ cm}^{-1}$ and disappear of CH_{ring} vibration mode.

The intraperitoneal injection of Ifo lead to resolve the mean NH-OH band into seven underlying peaks with two newly strOH vibrational modes at $3695\pm 3\text{ cm}^{-1}$ and $3640\pm 4\text{ cm}^{-1}$. Oral supplementation of Lec for five days in conjunction with intraperitoneally injection of Ifo resulted in different FTIR pattern. Regarding the positive control pattern, the main band was resolved into five underlying peaks but with different characteristics as shown in table (1). Comparing this pattern with the Ifo pattern, it is clear that the strOH band is greatly affected, where three broad band was noticed compared to four bands for Ifo group, this is concomitant with increased vibrational frequency for the asymOH band and decreased vibrational frequency of the symOH band and their width were significant increased relative to the Ifo group. Adjunct supplementation of Que with Ifo for five days was characterized by marked changes when compared with negative and the corresponding positive control. New vibrational strOH mode at $3614\pm 7\text{ cm}^{-1}$ and restricted CH_{ring} mode were observed. On the other hand the vibrational frequency of OH_{asym} bond was decreased while that of OH_{sym} was increased relative to the negative and positive controls. Comparing this Que-Ifo pattern with the Ifo one it can be noticed that the vibrational frequency of asymOH was decreased and that of symOH was increased, this is was concomitant to significant increase in their bandwidth. The strOH band shows four underlying peaks but with different characteristics.

The co-administration of Lec+Que with the intraperitoneally injection of Ifo was also characterized by changes in the vibrational frequency of asymOH and symOH when compared to

the Ifo group. The vibrational frequency peak of the $_{\text{asym}}\text{OH}$ was decreased and width was significantly increased ($p < 0.05$) relative to the Ifo group as shown in table (1).

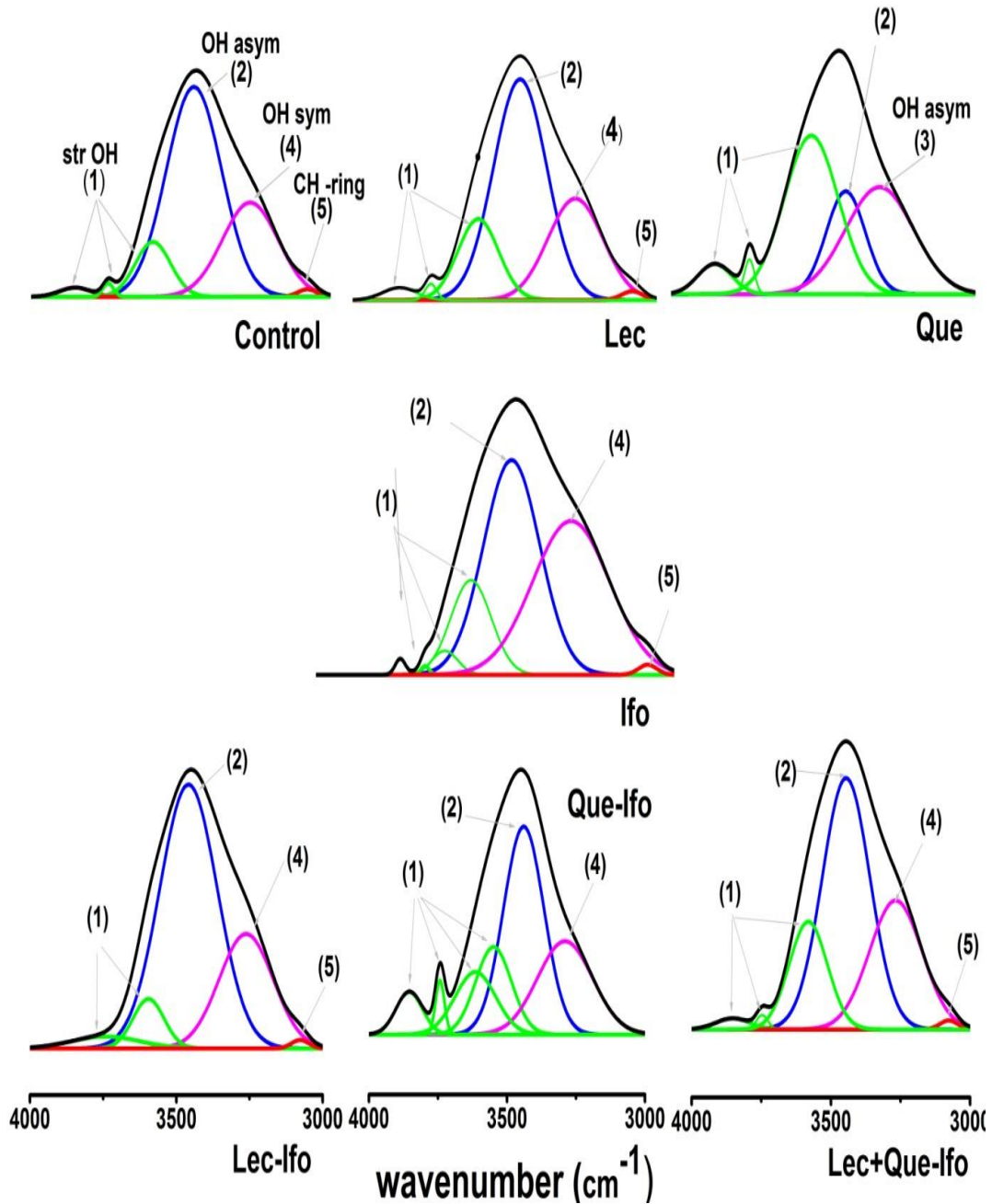


Fig. (1) FTIR spectra for all groups in the range $4000\text{-}3000\text{cm}^{-1}$ where (1) str OH (2) OH asym (3) OH asym (4) OH sym and (5) CH-ring.

Table (1) Vibrational frequencies, bandwidth and the estimated structural components of the NH-OH region of all groups.

	(1) Str-O-H-					(2) asym OH	(3) asym NH ₃	(4) sym O-H	(5) CH _{ring}	
	Gr.I Control	3852±3 124±6	3742±6 42±6				3593±3 139±8	3454±3 217±9		3268±4 219±4
Gr.II Lec	3846±4 131±9	3742±3 45±3				3586±2† 157±5	3449±3 205±5		3267±3 213±5	3080±3 84±5
Gr.III Que	3856±3 133±10	3742±3 39±8				3539±3† 209±3†	3426±3† 151±3†	3314±3 263±3		
Gr.IV Ifo		3764±2† 33±6	3695±3 28±3		3640±4 85±5	3567±4† 132±6	3453±1 185±7†		3287±3† 253±2†	3074±1 62±1†
Gr.V Lec-Ifo		3741±2 277±10†				3580±1† 125±3†	3458±1 231±6		3261±4 217±4	3077±2 72±2
Gr.VI Que-Ifo	3854±1 116±9	3742±4 38±5		3614±7 174±6		3548±7† 153±4	3439±2† 172±6†		3289±4† 235±2†	
Gr.VII Lec+Que- Ifo	3852±2 144±12	3746±4 49±5				3581±4† 161±6†	3445±1† 201±7		3267±5 222±4	3076±3 76±1

*The first line in each cell indicates the frequency of the corresponding band in cm^{-1} , while the second line indicates the band width in cm^{-1} .

† Statistically significant

C-H region

The spectra of the CH region of different groups were shown in figure (2) in the IR range 3000-2800 cm^{-1} . For control pattern, The curve enhancement procedure that used to resolve any overlapping peaks confirms the presence of four bands that were observed at 2962±3 cm^{-1} , 2925±3, 2876±1 cm^{-1} and 2852±3 cm^{-1} . These bands can be assigned as (1) asym CH_3 , (2) asym CH_2 , (3) sym CH_3 and (4) sym CH_2 respectively as shown in table (2). The assignment of the bands has been previously mentioned by (Severcan et al., (2000)).

The Lecithine –positive control-group and the Quercitine- positive control group shows the same four underlying bands as control group.

The intraperitoneal injection of Ifo lead to increase the number of peaks to seven with presence of newly $_{\text{asym}}\text{CH}_3$ vibrational mode at $2993\pm 1\text{cm}^{-1}$ and newly $_{\text{sym}}\text{CH}_2$ vibrational modes at $2821\pm 6\text{cm}^{-1}$ and 2805cm^{-1} .

Adjunct supplementation of Lec with Ifo for five days was characterized by no changes when compared with negative and the corresponding positive control expect there was significant decrease ($p<0.05$) in CH_2 symmetric and asymmetric bands width comparing with positive control.

Oral supplementation of Que for five days in conjunction with intraperitoneally injection of Ifo resulted no changes when compared with negative and positive controls expect there was significant change in $_{\text{asym}}\text{CH}_2$ bandwidth.

The co-administration of Lec+Que with the intraperitoneally injection of IFO was also characterized by changes in the vibrational frequency of $_{\text{asym}}\text{CH}_3$ and $_{\text{sym}}\text{CH}_2$ when compared with IFO group.also their was significant changes in bandwidth of both CH_2 symmetric and asymmetric bands as shown in table (2)

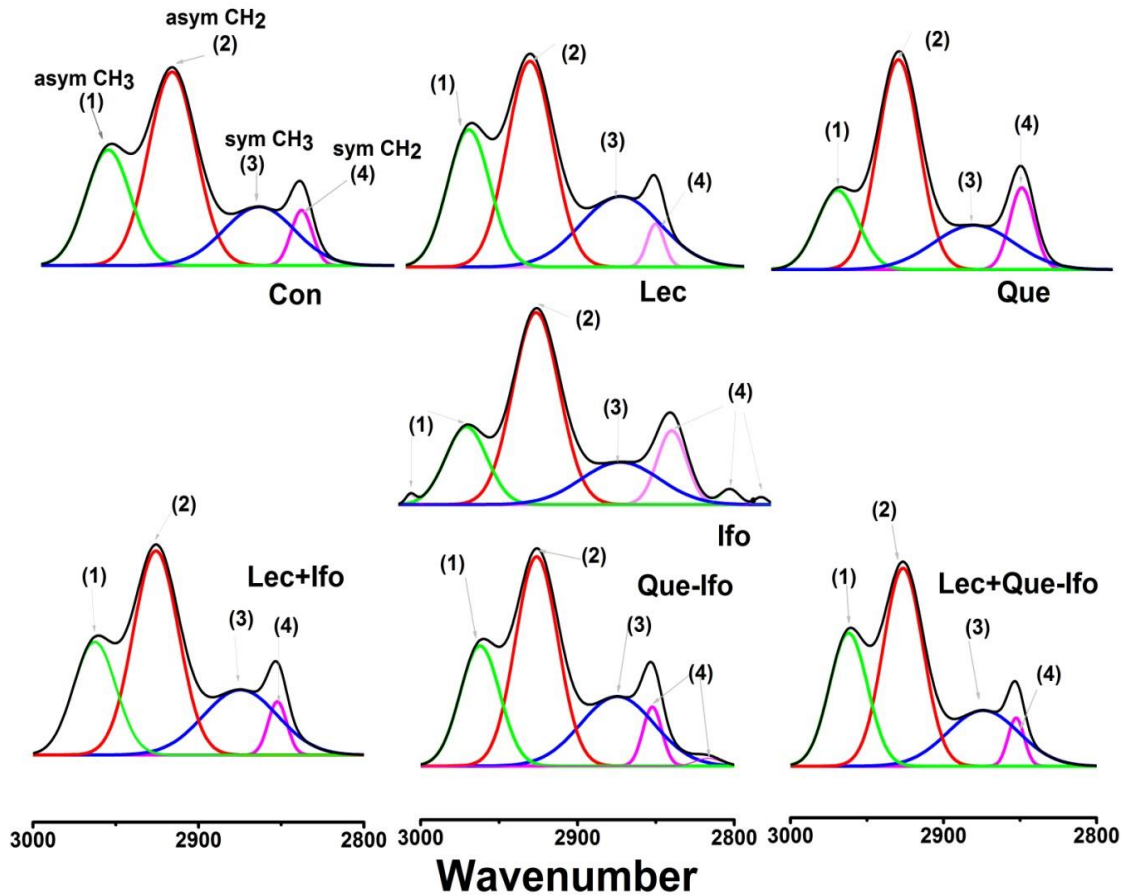


Fig.2: Stretching CH region of retinal tissue in the range $3000\text{-}2800\text{cm}^{-1}$ where (1) asym CH_3 (2) asym CH_2 (3) sym CH_3 (4) and sym CH_2 .

Table 2: C-H region ($3000\text{-}2800\text{ cm}^{-1}$) of retinal tissues for all studied groups.

*The first line in each cell indicates the frequency of the corresponding band in cm^{-1} , while the

Groups	(1) asym CH_3		(2) asym CH_2	(3) sym CH_3	(4) sym CH_2		
	group I Control	2962±3 29±1	2925±3 31±1	2876±1 47±7	2852±3 14±2		
Gr.II Lec	2962±4 29±1	2926±1 31±1	2873±1 55±5	2852±1 11±2			
Gr.III Que	2961±3 28±1	2925±1 28±1	2881±2† 55±4	2853±1 16±3			
Gr.IV Ifo	2993±1 5±1.39	2963±2 26±5	2926±3 29±2	2880±2† 49±6	2853±5 18±20	2821±6 11±2	2805±5 7±2
Gr.V Lec-Ifo	2962±7 30±1	2925±3 32±1	2874±2 52±1	2852±4 13±5			
Gr.VI Que-Ifo	2962±3 29±1	2925±1 31±1	2874±6 55±3	2852±5 14±4	2818±4 10±1		
Gr.VII Lec+Que -Ifo	2962±3 29±1	2926±4 30±2	2874±2 54±2	2852±6 12±3			

second line indicates the band width in cm^{-1} .

Fingerprint region

Figure (3) illustrated the fingerprint region ($1600\text{-}900\text{ cm}^{-1}$) that corresponding to the lipid and protein parts of the retinal tissues for all studied groups. It can be noticed for control group, five bands were observed: (1) Amide II at 1536 ± 1 , (2) CH_2 bend at 1453 ± 3 , (3) Str COO^- sym at 1398 ± 3 , (4) str PO_2^- asym at 1237 ± 4 and Str PO_2^- sym 1079 ± 3 according previously to (Jung (2000)).

As a results in table (3) some observations was found in our studied groups:

Amide II: No changes in band position for all groups expect there is significant decrease ($p < 0.05$) in band position for Ifo group.

CH_2 bend: this band was not observed for all groups while it observed in Lec+Que-Ifo group.

Str COO^- sym and Str PO_2^- asym: There is no change in band position for all groups expect for Ifo group this band were not observed.

Str PO_2^- sym: There is no change in band position for all groups.

str COC: This band was not observed for negative and positive control groups also not found in Ifo group .While this band were observed in Lec-Ifo group, Que -Ifo group and Lec+Que-Ifo group and there were assigned at 940 ± 2 , 948 ± 1 and 961 ± 3 , respectively.

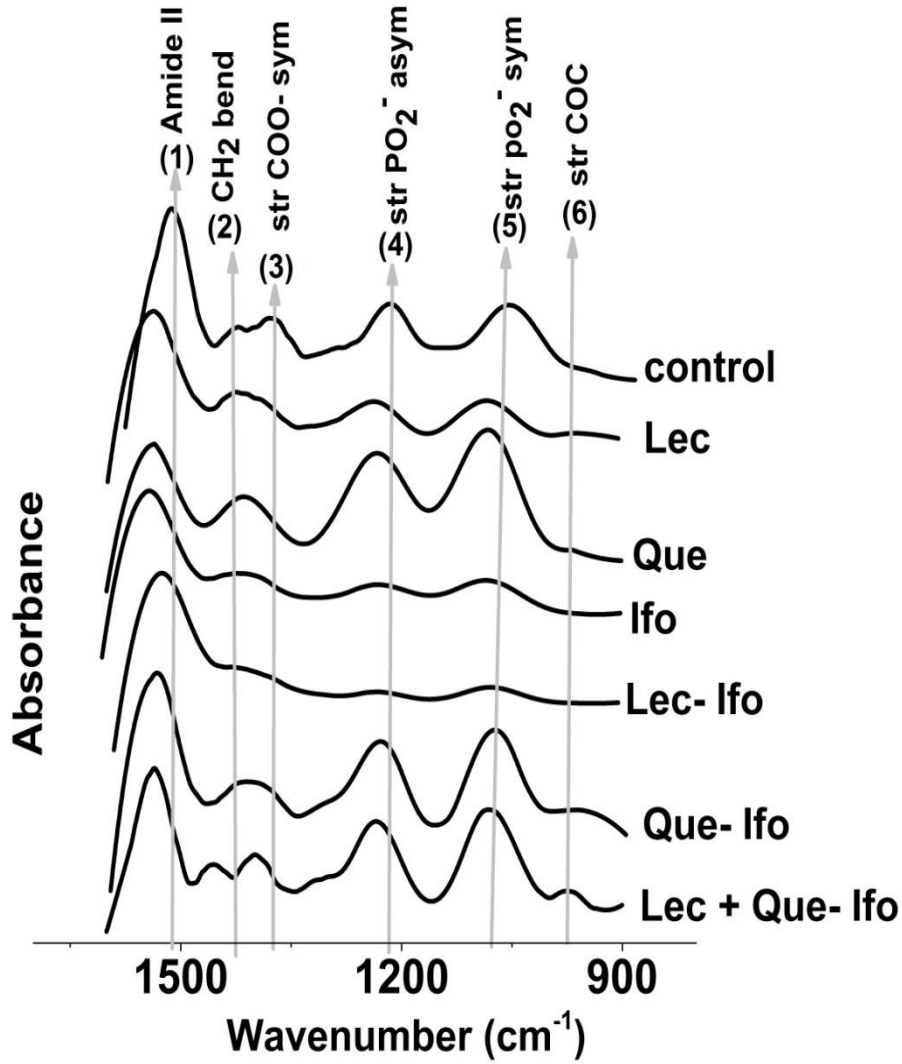


Fig.3: The fingerprint region (1600-900 cm-1) of the retinal tissues for all groups.(1) amide II , (2) CH₂ bend, (3) strCOO⁻sym, (4) Str PO₂⁻asym, (5) strPO₂⁻sym,and (6) str COC.

Table 3: Fingerprint region (1600-900) cm⁻¹ for all studied groups.

Groups	(1) Amide II	(2) CH ₂ bend	(3) Str COO- sym	(4) Str PO ₂ ⁻ asym	(5) Str PO ₂ ⁻ sym	(6) str COC
group I (Con)	1536±1	1453±3	1398±3	1237±4	1079±3	-
group II (Lec)	1537±2	-	1393±2	1233±4	1076±1	-
group III (Que)	1537±2	-	1394±2	1234±3	1076±1	-
group IV (IFO)	1529±2†	-	-	-	1082±2	-
group V (Lec- IFO)	1533±2	-	1393±3	1234±2	1082±2	940±2
group VI (Que-IFO)	1536±3	-	1392±4	1235±3	1082±3	948±1
group VII (Lec+ Que-IFO)	1533±2	1453±2	1393±4	1233±1	1082±2	961±3

† Statistically significant

Discussion

Ocular toxicities are a common side-effect of systemic chemotherapeutic drugs and have also emerged as an important clinical concern for newer molecularly-targeted agents entering standard oncology practice.

Fourier Transform Infrared (FTIR) micro-spectroscopy is an emerging technique for the biochemical analysis of tissues and cellular materials. It provides objective information on the holistic biochemistry of a cell or tissue sample and has been applied in many areas of medical research (Zohdi, V. ,et al.,2015). In the last twenty years, the infrared spectra of single cells and intact tissues have not only provided important information regarding the macromolecular contents and their distribution in a cell or tissue sample but have also demonstrated the ability of FTIR spectroscopy to differentiate between diseased and non-diseased states (Rubin, S., et al.,(2008), determine cell cycle stage (Whelan, D. R., et al.,2013) and monitor cell death (Gu, L., , et al.,2004). By measuring the absorption of infrared light by a sample, the characteristic energies and intensities of absorbance bands of cellular macromolecules can be detected and assigned, including carbohydrates lipids proteoglycans collagens, nucleic acids and proteins.

The region between 850 and 1680 cm^{-1} (fingerprint region) contains information about the retinal chromophore. Besides bands attributed to C=C stretching vibrations and located between 1500 and 1580 cm^{-1} , this region also contains chromophore bands (above 1600 cm^{-1}) attributed to vibrations of the aldimine (C=NH) group of the Schiff base. C–C and C–C–H stretching vibrations are localized in the fingerprint region (between 1100 and 1400 cm^{-1}). Frequencies of all types of deformational vibrations are located in the region below 1000 cm^{-1} .

New band (str COC) was observed in Lec-Ifo group, Que –Ifo group and Lec+Que-Ifo group which confirming the presence of collagen. The PO₂ stretching modes (Table 3) are characterized by no changes in their environment. Moreover PO₂ asymmetric stretching mode cannot be observed while intraperitoneal injection of Ifo which indicated that phospholipids, genetic material or phosphate sugar that represents the structural constituents of the retina tissue were affected.

In our study, NH-OH region changes (table1) revealed unusual interface/binding mechanism that related to different surrounding environment due to ifosfamide intraperitoneal injection. Ifosfamide was found to be affected on the lipid components of the retina, the strOH bands in both Lec-Ifo, Que-Ifo groups are found in different structural environments than the normal one, which may give the impetus that there is binding/interaction mechanism(s). This OH band can be found in many membrane constituents as the cholesterol (**Sherif MS ,et al.,2017**).

The co-administration of Lec+Que with the intraperitoneal injection of Ifo caused increased of the vibrational frequency of _{asym}OH and decreased in other vibrational peak associated with significant increase in bandwidth compared with Ifo group. This means preventing the side effect of Ifosfamide and the pattern return to control one in spite of the difference of peaks characteristics.

The CH vibrational region (table2) is used generally to characterize the lipid molecules. The highest values of the vibrational frequency that noticed in Table 2 for the symCH₃ mode of Que and Ifo groups may be due to change in their environment, indicating a decrease in the order of the lipid hydrocarbon chains. The splitting of symCH₂ band in Ifo and Lec+ Que –Ifo groups reflects different interaction binding mechanism and variation of the surrounding environment.

Conclusion

Supplementation with lecithin and quercetin in the current study significantly modulated the biophysical molecular structure markers of ifosfamide in retina of rats. Therefore, we suggest that synergistic effect of quercetin and lecithin in the combined therapy results in marked neuroprotective effect in part through its antioxidant properties and down regulation of molecular structure in retina.

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الأثار الجانبية للعلاج الكيميائي على البنية الجزيئية لشبكية الفئران والدور الوقائي المحتمل لمضادات الأكسدة

رحاب أحمد فؤاد*، جيهان محمد كمال*، سحر محمود عوض*

*قسم الفيزياء ، كلية العلوم (بنات) ، جامعة الأزهر، القاهرة

الملخص العربي :

يعد الايفوسفميد دواء مساعد كمضاد للأورام . وعلى الرغم من انه عامل فعال للعلاج الكيميائي الا انه ثبت انه يحدث العديد من الاثار الجانبية. والهدف من هذه الدراسة هو دراسة الادوار الوقائية الممكنة لليثيسين والكيرسيتين، سواء منفردة او مجتمعة مع الايفوسفميد والتغيرات التي تحدث على البنية الجزيئية بفعل الايفوسفميد فى شبكية انثى الفئران باستخدام التحليل الطيفى بالأشعة تحت الحمراء (فورييه) .
تم تقسيم سبعون أنثى من الفئران البيضاء بشكل عشوائي إلى سبع مجموعات. الايفوسفميد (Ifo، ٨٠ ملليجرام لكل كيلوجرام من الوزن) كان يعطى لمدة خمسة ايام متتالية بالحقن داخل الصفاق بينما تم اعطاء الكيرستين (50) ملليجرام لكل كيلوجرام من الوزن، اما الليثيسين (100) ملليجرام لكل كيلوجرام من الوزن) شفويا باحداهم او مقترنا مع الايفوسفميد لمدة ستة ايام متتالية. نتائجنا تشير إلى أن الايفوسفميد اثر على مكونات الدهون في الشبكية ، وأظهرت منطقة NH-OH تغييرات غير عادية / آلية ملزمة التي تتعلق بالبيئة المحيطة بسبب حقن الايفوسفميد داخل الصفاق.

نقترح أن التأثير التآزري للكيرستين والليثيسين في العلاج المشترك مع الايفوسفميد يؤدي إلى تأثير اعصاب

ملحوظ في جزء منه من خلال خصائصه المضادة للأكسدة والتنظيم الهيكلية للجزيئي في شبكية العين.