

Effect of ultrasound on rabbit cornea and the protective role of antioxidant

Ayman O. Ahmed^a, Mona M. Gamal^b, Mervat A. Ali^b, Abdel Sattar M. Sallam^a, Elsayed M. Elsayed^a

^aBiophysics Department, College of Science, Ain Shams University, Cairo, ^bBiophysics and Laser Science, Research Institute of Ophthalmology, Giza, Egypt

Correspondence to Prof. Mona Mostafa Gamal, PhD in Biophysics, Research Institute of Ophthalmology, Giza, 12511, Egypt.
Tel: + 20 111 425 6787;
fax : + (202) 35733033;
e-mail: monamg1@yahoo.com

Received: 20 July 2020

Revised: 9 August 2020

Accepted: 26 August 2020

Published: xx xx 2020

Journal of The Arab Society for Medical Research 2020, 15:25–35

Background/aim

Applications of ultrasound in medicine for therapeutic purposes have been an accepted and beneficial use for many years. The present study aimed to investigate the harmful effect of exposure to therapeutic ultrasound on the cornea and the possible protective role of ascorbic acid and β -carotene.

Materials and methods

A total of 50 healthy mature New Zealand albino rabbits were enrolled in this study and classified into four groups. Group I contained five rabbits and were used as a control group. The remaining rabbits were classified into three groups of fifteen rabbits each. The eyes of group II were insonicated with continuous ultrasound waves (1.5 W/cm^2 at 2.8 MHz) for 20, 40, and 60 min. Group III and group IV were topically treated with vitamin C and supplemented with β -carotene, respectively, before insonicated with ultrasound for 20, 40, and 60 min. All the rabbits were decapitated 24 h after ultrasound exposure. Total soluble protein content and Fourier-transform infrared (FTIR) analyses were carried out on cornea.

Results

Soluble protein content of cornea recorded highly significant decreased ($P < 0.001$) by increasing exposure time to ultrasound. The most prominent change in FTIR was detected in NH-OH and finger print regions of cornea after insonification with therapeutic ultrasound for 60 min. The uses of antioxidants reduce the adverse effect of ultrasound on FTIR analysis and total protein content of corneal tissue.

Conclusion

Ultrasound waves have the possibility to cause significant change in the molecular structure of corneal tissue. The use of antioxidants such as vitamin C and β -carotene preserves the corneal tissue, especially at short insonification time.

Keywords:

cornea, ultrasound, vitamin C and β -carotene

J Arab Soc Med Res 15:25–35

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1687-4293

Introduction

The biological effects that may be induced by ultrasound in the eye have been studied experimentally for decades to examine the safety of ophthalmic diagnostic systems and to clarify the therapeutic applications of intense ultrasound. The therapeutic use of ultrasound owing to its hyperthermia has accepted much interest in ophthalmology. The therapeutic applications of ultrasonic energy are also considered for the treatment of glaucoma, ocular tumor, retinal detachments, coagulation of lens protein, disruption of vitreous membranes, and hemorrhages [1].

The biological effects of ultrasound energy are related primarily to the production of heat. Heat is produced whenever ultrasound energy is absorbed, and the amount of produced heat is related to the intensity of the ultrasound, exposure time, and the specific absorption structures of the tissue [2].

Approximately 70% of the total temperature increase related to ultrasound occurs within the first minute of tissue exposure [2], but temperature continues to increase as exposure time is prolonged. Minimizing the time of exposure is probably the particular most important factor for confirming patient safety from thermal injury [3]. Ultrasound in aqueous solution makes cavitation directly producing water molecule disintegration and leads to the formation of free radical [4]. The high temperature induced by cavitation bubble collapse enables the pyrolysis of water and leads to the formation of $\cdot\text{OH}$ radicals and H atoms [5].

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Formation of reactive oxygen species was detected in incubated medium after exposure to 1-MHz continuous ultrasound at the intensities of 0.61–2.44 W/cm². Free radicals in addition to hydrogen peroxide produced by exposure of cells to ultrasound can lead to DNA damage [6]. The resulting damage to DNA, which is also called oxidative damage to DNA, is implicated in mutagenesis, carcinogenesis, and aging. Mechanisms of damage involve abstractions and addition reactions by free radicals leading to carbon-centered sugar radicals and OH-adduct or H-adduct radicals of heterocyclic bases. Further reactions of these radicals yield numerous products [7].

Free radical causes damage to biological tissue and is a critical event in the etiopathogenesis of different diseases [8]. The harmful effect of free radicals causing potential biological damage called oxidative stress arises in biological systems when there is an overproduction of reactive oxygen species on one side and an insufficiency of antioxidants on the other [9]. Ocular tissues and fluids have both low-molecular-weight antioxidants (such as ascorbic acid, glutathione and alpha-tocopherol) and high-molecular-weight antioxidants (such as catalase, superoxide dismutase, glutathione peroxidase, and reductase) to protect ocular structures from oxidative damage [10,11]. In fact, under physiological conditions, these factors protect the cornea tissue from oxidative stress [12].

Systemic and topical use of antioxidant substances for the medical treatment of numerous diseases as well as additional protection of the eye against the negative action of free radicals and other reactive species has become widespread during the past years. Many researches are dedicated on reducing free radical during ultrasound exposure by using ascorbic acid [13–15].

Carotenoid pigments are naturally occurring and found in most fruits and vegetables, plants, algae, and photosynthetic bacteria. Humans cannot synthesize carotenoids and must ingest them in food or via supplementation. Li *et al.* [16] reported that carotenoid supplementation can improve human visual performance. The incidence of age-related eye diseases is estimated to rise with the aging of people. Oxidation and inflammation are implicated in the etiology of these diseases. There is evidence that dietary antioxidants and anti-inflammatories may provide benefit in decreasing the risk of age-related eye disease. Nutrients of interest are vitamins C and β -carotene [17,18].

The present study was designed to assess the molecular changes of rabbit cornea after insonification with therapeutic ultrasound and the possible protective role of ascorbic acid (vitamin C) and β -carotene.

Materials and methods

Experimental animals

A total of 50 healthy mature New Zealand rabbits of both sexes, weighing 2–2.5 kg, were used in this study. All animals were housed two to three rabbits per cage at a central temperature of 22–25°C and fed on a laboratory balanced diet. All procedures were conducted according to the principles enunciated in the guide for care and use of laboratory animals. The rabbits were classified into four groups according to the following:

- (1) Group I: it included five rabbits that served as a control group.
- (2) Group II (ultrasound exposure group): it included 15 rabbits were subdivided into three subgroups (five rabbits and 10 eyes each), according to the time of ultrasound exposure for 20, 40, and 60 min.
- (3) Group III (ultrasound exposure and ascorbic acid-treated rabbit group): it contained 15 rabbits subdivided into three subgroups (five rabbits and 10 eyes each), as the previous group. The three subgroups were topically treated with one drop (~50 μ l) of 10% ascorbic acid as eye drops every 15 min, starting 1 h before insonification, until the end of ultrasound exposure.
- (4) Group IV (ultrasound exposure and β -Carotene-treated rabbit group): it contained 15 rabbits subdivided into three subgroups (five rabbits and 10 eyes each), as the previous group. The three subgroups were supplemented with 15 mg/kg β -carotene for 2 weeks. The eyes of the three subgroups were insonified for 20, 40, and 60 min with ultrasound and decapitated after 24 h of ultrasound exposure.

Ethical consideration

Use of animals in this study was in compliance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research, and this study was also approved by the Research Institute of Ophthalmology Ethical Committee.

Chemicals

All the chemicals used in this study were obtained from Sigma Company (St. Louis, Missouri, USA) with the highest purity commercially available.

Ultrasound insonification

The rabbits were anesthetized by injection with 0.1 ml/kg separeine as muscle relaxant, and after 15 min, 50 mg/kg ketamine hydrochloride was injected intramuscularly. The rabbits were placed in a lateral position on an operating table, and their body temperature was maintained at 37°C. The eyes were dilated with one drop of 1% mydriacyl. After adequate anesthesia was obtained, the eye lid was open with a stainless-steel speculum. Insonification of rabbit eyes was carried out with continuous ultrasound waves (1.5 W/cm^2 at 2.8 MHz) by using a focus piezoelectric transducer in a direct contact with the cornea. Good coupling was maintained with 10% viscous phenylephrine. The transducer was positioned so that the ultrasonic beam was aligned to achieve perpendicular transmission through the cornea. Ultrasound transducer consists of a piezoelectric crystal (barium zirconate, titanium). Type SVHSP101 and a conical base ended with a fine tip transducer with very low quality factor (Q) to give a very wide nearly flat, frequency/output power. The transducer converts the pulsating voltage output of the generator into a train of mechanical pulses in the ultrasonic range with a power proportional to the applied voltage [19].

The corneas of all insonified groups as well as control ones were subjected to total protein content and Fourier-transform infrared (FTIR) analysis.

Quantitative analysis of soluble cornea protein

The total protein content was measured according to the method of Lowry *et al.* [20].

FTIR spectroscopy analysis

FTIR spectra of pool samples of rabbit cornea were recorded by a Thermo Scientific Nicolet iS5 FTIR spectrometer (USA), in the range $4000\text{--}1000 \text{ cm}^{-1}$. The spectrometer is operated under a continuous dry nitrogen gas purge to remove interference from atmospheric carbon dioxide and water. The data are baseline corrected and smoothed by Savitzky-Golay to eliminate the noise before Fourier transformation. A total of 100 scans are taken for each interferogram at 2 cm^{-1} resolution. Corneas are weighed, lyophilized, and then mixed with KBr powder (98 mg KBr: 2 mg retina) to prepare the KBr disks for FTIR analysis. The average of spectra for each group is obtained using Origin Pro 9.3 software (USA). The spectral analysis was performed in three distinct frequency ranges, namely, NH–OH region ($3800\text{--}3000 \text{ cm}^{-1}$), CH stretching region ($3000\text{--}2800 \text{ cm}^{-1}$), and finger print region ($1800\text{--}900 \text{ cm}^{-1}$) [21,22].

Statistical evaluation

Statistical comparison is performed between control and treated eyes using the Student *t*-test. All data are represented as the mean $\pm$ SD, and studies were repeated at least four times. Significance level was set at *P* less than 0.05.

Results

Total protein content

Table 1 illustrates the total protein content of corneas from control rabbits, rabbits insonified for 20, 40, and 60 min with ultrasound radiation only, and rabbits insonified with ultrasound in the presence of antioxidant. The total protein of cornea from control rabbits was $29.21 \pm 2.4 \text{ mg/g}$ wet wt. Total protein content significantly decreased ($P < 0.001$) when the corneas were insonified with ultrasound only. When rabbits were treated with antioxidant (vitamin C or β -carotene) before and after ultrasound insonification, the decrease in protein content of cornea is less than that detected in group insonified with ultrasound only.

FTIR Spectroscopy

NH–OH region

Figure 1 illustrates the NH–OH region ($3800\text{--}3000 \text{ cm}^{-1}$) for cornea from control rabbits, rabbits insonified with continuous ultrasound waves (1.5 W/cm^2 at 2.8 MHz) for 20 min, and rabbits insonified with ultrasound in the presence of vitamin C and β -carotene. The main band of control rabbit was resolved into three structural components centered at $3610 \pm 4 \text{ cm}^{-1}$ (strOH), $3477 \pm 5 \text{ cm}^{-1}$ ($\text{strOH}_{\text{asym}}$), and $3261 \pm 3 \text{ cm}^{-1}$ ($\text{strOH}_{\text{sym}}$). When rabbit's eye was insonified for 20 min with ultrasound without antioxidant supplementation, the $\text{strOH}_{\text{sym}}$ band disappeared and a new band of $\text{strNH}_{\text{asym}}$ was detected. Moreover, there was a change in band width and band position of strOH and $\text{strOH}_{\text{asym}}$ bands (Table 2).

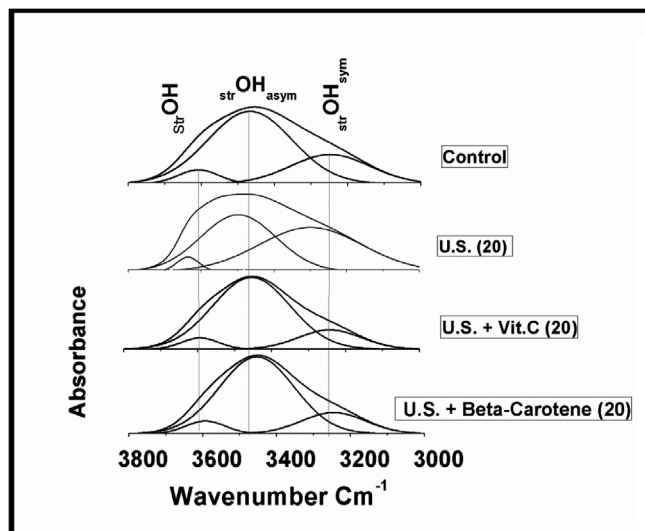
When rabbits were treated with antioxidant before and after insonification for 20 min, the FTIR pattern matches the control.

Table 1 Total soluble protein content of rabbit's cornea after 20, 40, and 60 min of ultrasound insonification

Groups	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Group I: control	29.21 $\pm$ 2.4	29.21 $\pm$ 2.4	29.21 $\pm$ 2.4
Group II: US only	19.72 $\pm 1.98^\dagger$	18.17 $\pm 1.82^\dagger$	15.72 $\pm 1.91^\dagger$
Group III: US+vitamin C	23.55 $\pm 1.57^\dagger$	20.25 $\pm 1.74^\dagger$	18.29 $\pm 1.77^\dagger$
Group IV: US + β -carotene	26.14 $\pm$ 1.69	24.43 $\pm 2.11^\dagger$	19.16 $\pm 1.82^\dagger$

US, ultrasound. † Statically significant than control group at $P < 0.05$.

Figure 1

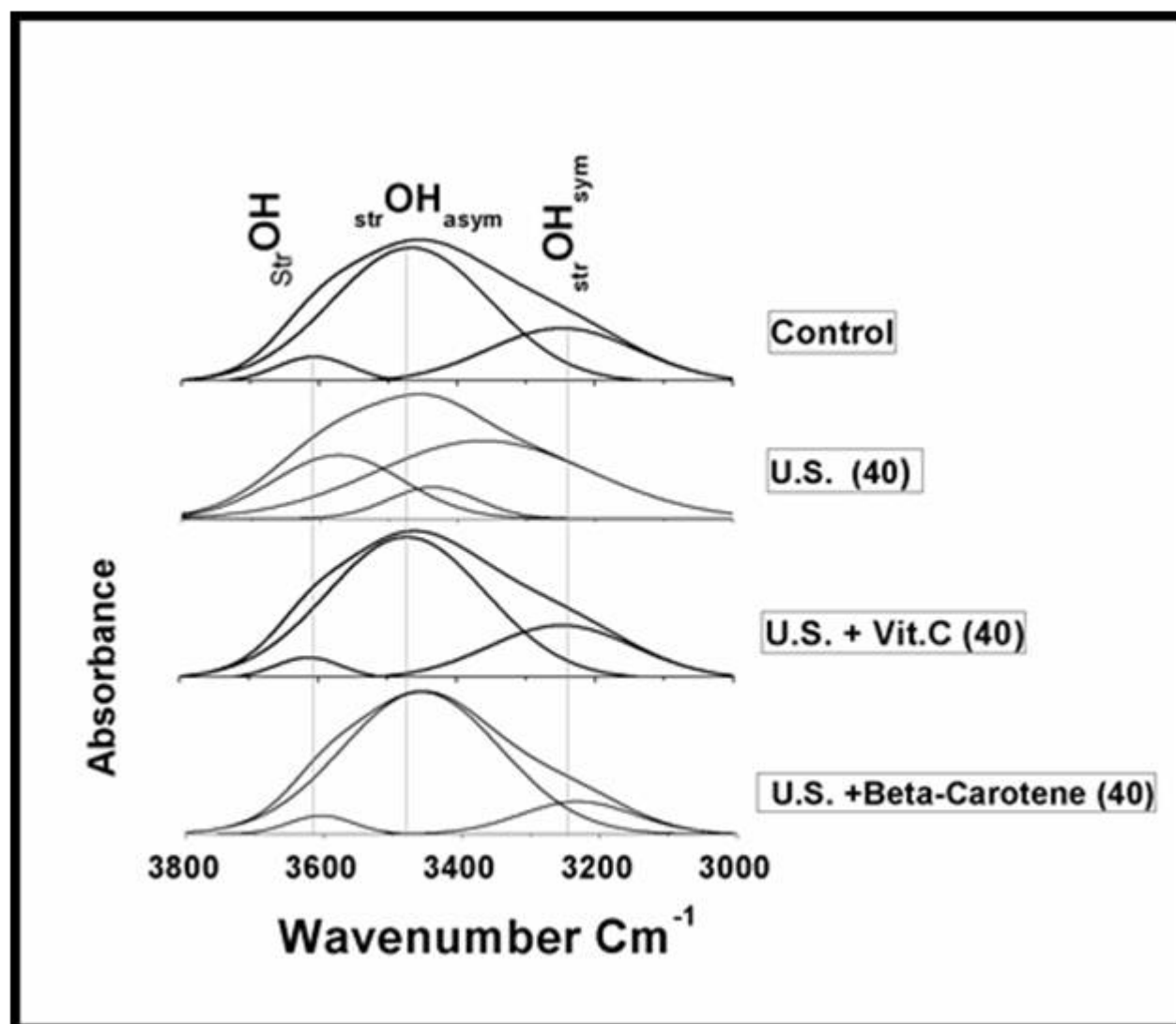


NHOH region of rabbit cornea insonicated with ultrasound for 20 min ($3800\text{--}3000\text{cm}^{-1}$).

When the eyes of rabbits were insonicated for 40 min with ultrasound without antioxidant supplementation (Fig. 2), the strOH band was split into two bands: $\text{strOH}_{\text{asym}}$ and $\text{strOH}_{\text{sym}}$ disappeared, and a new band of $\text{strNH}_{\text{asym}}$ was detected (Fig. 2). There is no significant change in FTIR pattern after antioxidant treatment (Table 3).

Figure 3 illustrates the NH–OH region ($3800\text{--}3000\text{cm}^{-1}$) for cornea from control rabbits, rabbits insonicated with continuous ultrasound waves (1.5 W/cm^2 at 2.8 MHz) for 60 min, and rabbits insonicated with ultrasound in the presence of antioxidants. When rabbit eyes were insonicated for 60 min with ultrasound without antioxidant supplementation, the main band was resolved into four bands where the strOH band split into two bands: a new band of $\text{strNH}_{\text{asym}}$ was appeared and $\text{strOH}_{\text{sym}}$ disappeared (Table 4). When rabbit's eye was subjected to vitamin C with ultrasound,

Figure 2



NHOH region of rabbit cornea insonicated with ultrasound for 40 min ($3800\text{--}3000\text{cm}^{-1}$).

Table 2 NHOH region of rabbit's cornea for different studied groups insonificated for 20 min with ultrasound

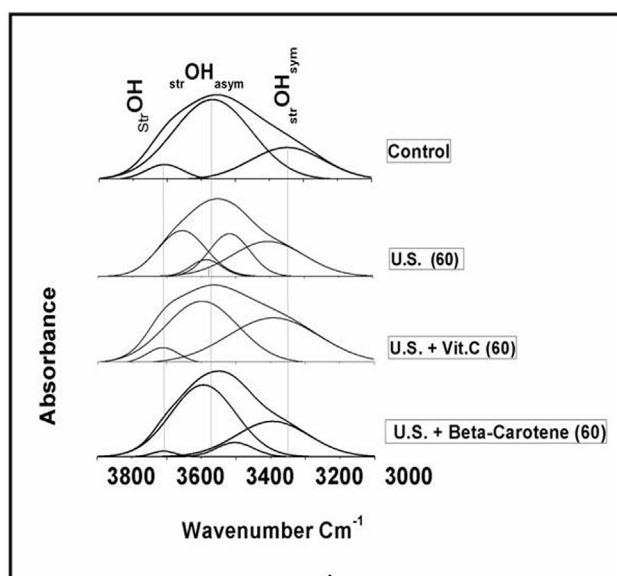
Groups	Peaks assignment			
	strOH	strOH _{asym}	strNH _{asym}	strOH _{sym}
Group I: control	3610±4	3477±5		3261±3
	93±0.9	210±1.3		194±1.5
Group II: US only	3583±5 [†]	3448±6 [†]	3362±6	
	135±0.4 [†]	185±0.7 [†]	245±1.1	
Group III: US+vitamin C	3603±5	3471±2		3261±4
	94±1.6	240±2.1		184±1.6
Group IV: US+β-carotene	3600±5	3470±4		3260±2
	92±0.3	311±0.8 [†]		213±1.2

First line, in each raw, indicates the frequency of the band in cm^{-1} , whereas the second line indicates the band width in cm^{-1} . US, ultrasound. [†] Statically significant than control group at $P < 0.05$.

Table 3 NHOH region of rabbit's cornea for different studied groups insonificated for 40 min with ultrasound

Groups	Peaks assignment			
	strOH	strOH _{asym}	strNH _{asym}	strOH _{sym}
Group I: control	3610±4	3477±5		3261±3
	93±0.9	210±1.3		194±1.5
Group II: US only	3664±43509±6		3342±6	
	78±0.4249±2.4		258±2.9	
Group III: US+vitamin C	3610±3	3477±3		3260±4
	87±1.6	253±2.1 [†]		172±2.9
Group IV: US+β-carotene	3606±4	3477±4		3265±4
	93±0.3	241±2.8 [†]		149±1.9 [†]

First line, in each raw, indicates the frequency of the band in cm^{-1} , whereas the second line indicates the band width in cm^{-1} . US, ultrasound. [†] Statically significant than control group at $P < 0.05$.

Figure 3

NHOH region of rabbit cornea insonificated with ultrasound for 60 min (3800–3000 cm^{-1}).

strNH_{asym} band was still detected, and there is change in wave number of all bands. After supplementation with β-carotene, there was significant change in wave number of strOH_{asym} and strNH_{sym} bands (Table 4).

CH Stretching region

Tables 5–7 illustrate the CH stretching region (3000–2800 cm^{-1}) for cornea from control rabbits and rabbits insonificated with continuous ultrasound waves (1.5 W/cm² at 2.8 MHz) for 20, 40, and 60 min, respectively, with and without antioxidants. The CH stretching region (3000–2800 cm^{-1}) indicates the presence of 4 bands in control rabbit centered at 2962±5, 2925±5, 2878±3, and 2852±4 cm^{-1} , which correspond to CH₃ asymmetric, CH₂ asymmetric, CH₃ symmetric, and CH₂ symmetric, respectively (Figs. 4–6), as previously described by Severcan *et al.* [23].

The main change in CH stretching region was obvious in rabbit cornea insonificated for 40 and 60 min with ultrasound only, as CH₂_{asym} was splitting into two bands. The IR pattern of rabbit cornea treated with antioxidant for all the studied periods mimics the control.

Finger print region

The finger print region, ranges of 1800–1000 cm^{-1} , of normal rabbit cornea was characterized by eight absorption bands centered at 1721±6 cm^{-1} (Ester), 1648±7 cm^{-1} (Amide I), 1544±5 cm^{-1} (Amide II), 1458±4 cm^{-1} (CH₂ bend), 1402±4 cm^{-1} (strCoo_{sym}), 1317±3 cm^{-1} (Amide III), 1234±4 cm^{-1}

Table 4 NHOH region of rabbit's cornea for different studied groups insonicated for 60 min with ultrasound

Groups	Peaks assignment			
	StrOH	strOH _{asym}	strNH _{asym}	strOH _{sym}
Group I: control	3610±4	3477±5		3261±3
	93±0.9	210±1.3		194±1.5
Group II: US only	3580±5 [†] 3503±6	3457±6	3312±4	
	172±2.5 [†] 95±1.4	148±1.7	217±1.2	
Group III: US+vitamin C	3639±5 3518±6		3325±7	3290±5 [†]
	62±1.6 [†] 234±2.2		98±3.2	208±1.9
Group IV: US+β-carotene	3612±5	3497±4		3291±4 [†]
	81±0.8	210±2.1		269±2.7

First line, in each raw, indicates the frequency of the band in cm^{-1} , whereas the second line indicates the band width in cm^{-1} .US, ultrasound.[†] Statically significant than control group at $P<0.05$.

Table 5 CH stretching region of rabbit's cornea for different studied groups insonicated for 20 min with ultrasound

Groups	Peaks assignment			
	StrCH _{3asym}	strCH _{2 asym}	StrCH _{3sym}	StrCH _{2sym}
Group I: control	2962±5	2925±5	2878±3	2852±4
	28±0.8	28±0.4	39±0.9	12±0.5
Group II: US only	2962±5	2924±5	2878±6	2852±4
	27±0.7	26±0.3	97±0.9 [†]	11±0.6
Group III: US+vitamin C	2962±4	2925±5	2878±6	2852±4
	27±0.13	26±0.7	35±0.8	10±0.8
Group IV: US+β-carotene	2962±3	2925±5	2878±6	2852±4
	26±0.8	26±0.5	40±1.2	11±0.8

First line, in each raw, indicates the frequency of the band in cm^{-1} , whereas the second line indicates the band width in cm^{-1} .US, ultrasound.[†] Statically significant than control group at $P<0.05$.

Table 6 CH stretching region of rabbit's cornea for different studied groups exposed for 40 min to ultrasound

Groups	Peaks assignment			
	StrCH _{3asym}	strCH _{2 asym}	StrCH _{3sym}	StrCH _{2sym}
Group I: control	2962±5	2925±5	2878±3	2852±4
	28±0.8	28±0.4	39±0.9	12±0.5
Group II: US only	2972±6	2959±5 [†] 2926±5	2879±4	2852±5
	27±0.7	26±0.5 37±0.9	33±0.5	11±0.6
Group III: US+vitamin C	2962±4	2925±6	2876±5	2852±4
	28±0.8	26±0.7	39±0.8	12±0.4
Group IV: US+β-carotene	2962±3	2925±5	2876±5	2852±3
	27±0.7	26±0.8	35±0.9	10±0.3

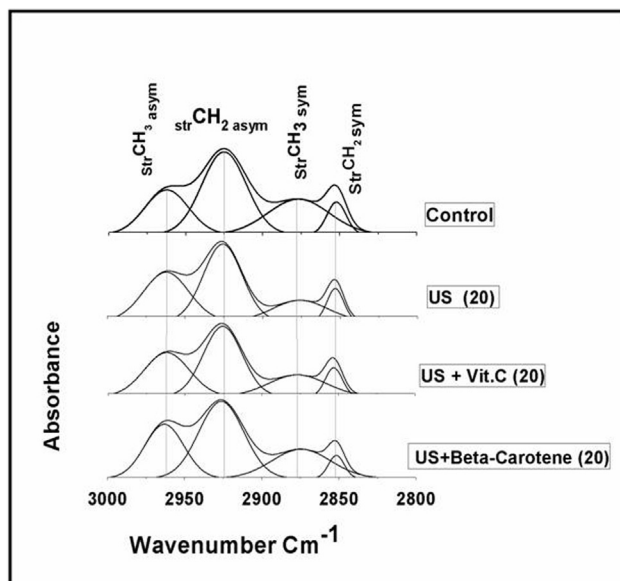
First line, in each raw, indicates the frequency of the band in cm^{-1} , whereas the second line indicates the band width in cm^{-1} .US, ultrasound.[†] Statically significant than control group at $P<0.05$.

Table 7 CH stretching region of rabbit's cornea for different studied groups exposed for 60 min to ultrasound

Groups	Peaks assignment			
	StrCH _{3asym}	strCH _{2 asym}	StrCH _{3sym}	StrCH _{2sym}
Group I: control	2962±5	2925±5	2878±3	2852±4
	28±0.8	28±0.4	39±0.9	12±0.5
Group II: US only	2972±3	2959±5 [†] 2926±5	2875±5	2852±5
	24±0.7	26±0.5 36±0.9	33±0.5	10±0.6
Group III: US+vitamin C	2962±3	2926±6	2878±5	2852±4
	28±0.8	26±0.7	39±0.8	12±0.4
Group IV: US+β-carotene	2962±4	2925±5	2875±5	2852±3
	22±0.7	26±0.8	35±0.9	10±0.3

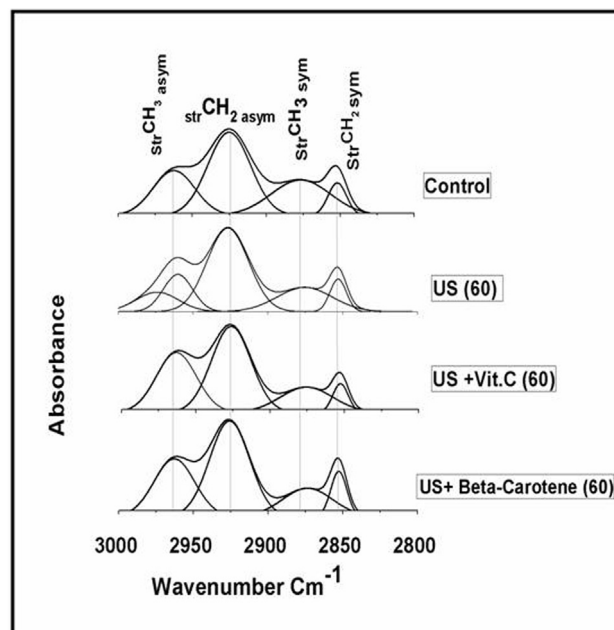
First line, in each raw, indicates the frequency of the band in cm^{-1} , whereas the second line indicates the band width in cm^{-1} .US, ultrasound.[†] Statically significant than control group at $P<0.05$.

Figure 4



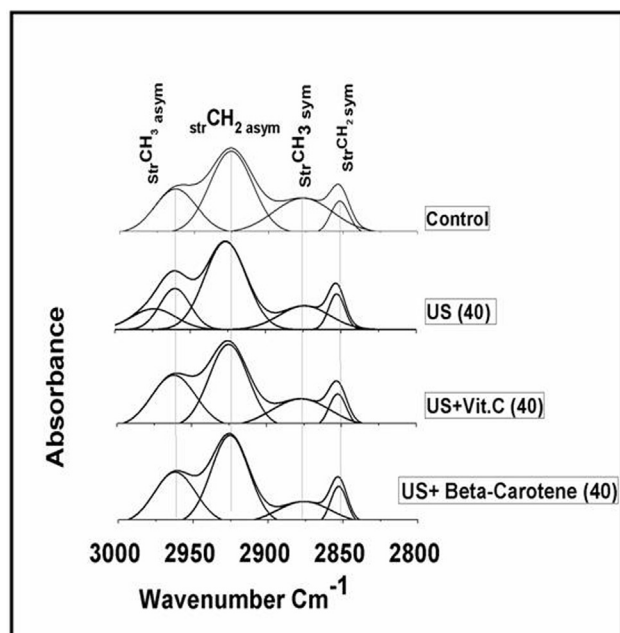
CH stretching region of rabbit cornea insonicated with ultrasound for 20 min ($3000\text{--}2800\text{ cm}^{-1}$).

Figure 6



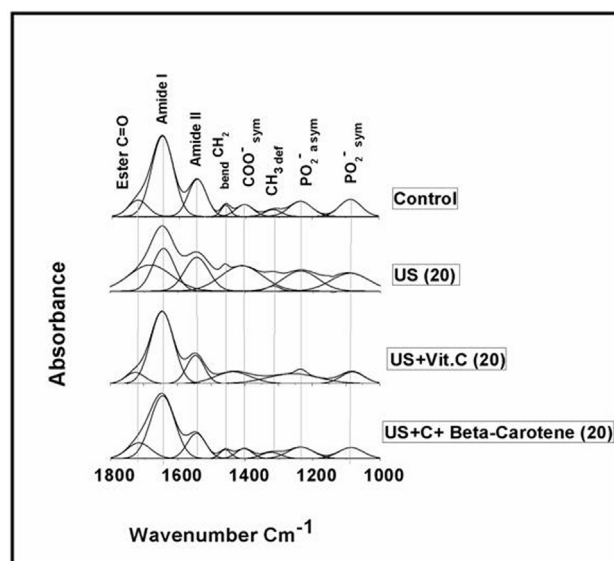
CH stretching region of rabbit cornea insonicated with ultrasound for 60 min ($3000\text{--}2800\text{ cm}^{-1}$).

Figure 5



CH stretching region of rabbit cornea insonicated with ultrasound for 40 min ($3000\text{--}2800\text{ cm}^{-1}$).

Figure 7



Finger print region of rabbit cornea insonicated with ultrasound for 20 min ($1800\text{--}1000\text{ cm}^{-1}$).

($\text{strPO}_2^- \text{ asym}$), and $1084 \pm 3\text{ cm}^{-1}$ ($\text{strPO}_2^- \text{ asym}$) (Figs. 7–9). When rabbit eyes were insonicated with continuous ultrasound waves (1.5 W/cm^2 at 2.8 MHz) for 20, 40, and 60 min strCoo-sym and amide III bands disappeared in all studied groups. The band positions of ester, amide I, and amide II were changed in all groups insonicated with ultrasound. After antioxidant treatment,

strCoo-sym appeared in vitamin C groups only (Tables 8–10).

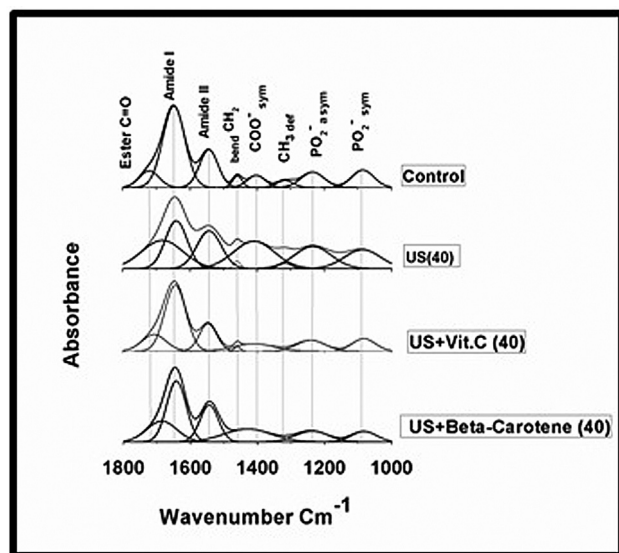
Discussion

Applications of ultrasound in medicine for therapeutic purposes have been an accepted and beneficial use for many years. Ultrasound energy exerts important cellular, genetic, thermal, and mechanical effects. Ultrasound

waves are capable of producing destructive changes in living tissues. Some of these changes are mechanical beating with rupture of the tissues and heat generation [24]. Free radical formation has been postulated to be another cause of the damage [25,26].

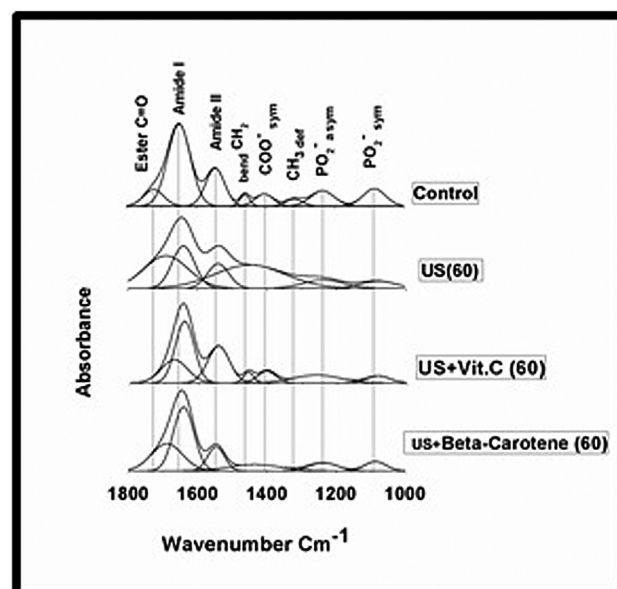
In the present study, the detected decrease in total protein content after corneal insonification with ultrasound may be owing to free radical generation. This is supported by the work of Nemet *et al.* [14], who confirmed that in phacoemulsification, ultrasound

Figure 8



Finger print region of rabbit cornea insonified with ultrasound for 40 min (1800–1000 cm^{-1}).

Figure 9



Finger print region of rabbit cornea insonified with ultrasound for 60 min (1800–1000 cm^{-1}).

Table 8 Finger print region of rabbit's cornea for different studied groups exposed for 20 min to ultrasound

	Peaks assignment							
	Ester	Amide I	Amide II	CH ₂ bending	COO _{sym}	Amide III	PO _{2asym}	PO _{2sym}
Group I: control	1721±6 55±1.2	1648±7 88±1.4	1544±5 54±0.8	1458±4 25±0.5	1402±4 49±1.2	1317±3 32±0.7	1234±4 55±1.3	1084±3 58±1.2
Group II: US only	1687±4 [†] 76±1.5	1641±3 58±0.8 [†]	1543±5 62±0.9	1435±6 63±1.1 [†]			1234±7 67±0.9	1084±5 68±0.7
Group III: US+vitamin C	1715±5 54±1.1	1648±5 87±1.6	1544±3 54±1.5	1458±5 24±0.6	1402±3 47±0.7	1323±4 33±0.5	1234±4 53±0.7	1084±4 56±1.1
Group IV: US+β-carotene	1710±3 52±1.0	1644±5 83±1.7	1544±4 53±0.9	1458±4 47±1.3			1234±3 58±1.4	1084±4 51±1.3

First line, in each row, indicates the frequency of the band in cm^{-1} , whereas the second line indicates the band width in cm^{-1} . US, ultrasound. [†] Statically significant than control group at $P < 0.05$.

Table 9 Finger print region of rabbit's cornea for different studied groups exposed for 40 min to ultrasound

Groups	Peaks assignment							
	Ester	Amide I	Amide II	CH ₂ bending	COO _{sym}	Amide III	PO _{2asym}	PO _{2sym}
Group I: control	1721±6 55±1.2	1648±7 88±1.4	1544±5 54±0.8	1458±4 25±0.5	1402±4 49±1.2	1317±3 32±0.7	1234±4 55±1.3	1084±3 58±1.2
Group II: US only	1671±4 [†] 77±2.0 [†]	1635±6 58±0.8 [†]	1532±7 66±1.4	1435±5 [†] 10±0.4 [†]			1234±6 68±1.7	1084±7 69±1.4
Group III: US+vitamin C	1684±5 [†] 64±1.4	1642±5 79±1.1	1544±3 54±0.8	1458±7 34±0.8	1405±6 62±1.4		1234±5 59±1.4	1084±4 57±1.2
Group IV: US+β-carotene	1684±6 [†] 61±1.2	1644±5 81±1.7	1544±4 54±1.1	1458±6 14±0.9 [†]			1234±3 57±1.7	1084±4 57±0.9

First line, in each row, indicates the frequency of the band in cm^{-1} , whereas the second line indicates the band width in cm^{-1} . US, ultrasound. [†] Statically significant than control group at $P < 0.05$.

Table 10 Finger print region of rabbit's cornea for different studied groups exposed for 60 min to ultrasound

Groups	Peaks assignment							
	Ester	Amide I	Amide II	CH ₂ bending	COO _{sym}	Amide III	PO _{2asym}	PO _{2sym}
Group I: control	1721±6	1648±7	1544±5	1458±4	1402±4	1317±3	1234±4	1084±3
	55±1.2	88±1.4	54±0.8	25±0.5	49±1.2	32±0.7	55±1.3	58±1.2
Group II: US only	1677±7 [†]	1631±6	1530±6	1433±5			1234±6	1084±6
	86±2.1 [†]	55±1.4	44	89±2.1 [†]			62±1.2	57±1.7
Group III: US+vitamin C	1684±5 [†]	1642±5	1544±3	1458±5	1402±3		1234±4	1084±4
	63±± 1.4	80±2.8	54±1.6	8±0.4 [†]	64±1.8		78±0.8 [†]	59±1.3
Group IV: US+β-carotene	1684±3 [†]	1634±5	1544±4	1453±4			1234±3	1084±4
	72±1.8	80±2.5	46±1.9	32±1.1			58±1.8	55±0.7

First line, in each raw, indicates the frequency of the band in cm⁻¹, whereas thesecond line indicates the band width in cm⁻¹.US, ultrasound.[†] Statically significant than control group at $P<0.05$.

induces hydroxyl radical (OH) formation, damaging corneal endothelium.

When rabbits were treated with oral and topical antioxidant, the decrease in protein content of cornea is less than that detected in group insonified with ultrasound only. Nemet *et al.* [14] reported that adding the antioxidants ascorbic acid and oxidize glutathione to the irrigation solution significantly reduced the endothelial corneal cell damage.

FTIR spectroscopy is an established method for the structural characterization of proteins, DNA, and RNA of biological tissues [27]. The obtained data revealed that the NH–OH region of cornea is affected by insonification with ultrasound. The $\nu_{\text{str}}\text{OH}$ band was split into two bands. The splitting of the bands may be owing to the changes caused in corneal tissues upon insonification with ultrasound. Barth [28] and Yang *et al.* [29] reported that the splitting of the band is indicative for the presence of variation in molecular structure of biological tissue.

The split in CH₂ stretching bands after exposure of rabbit cornea to ultrasound may enhance lipid peroxidation process. The CH stretching region of the infrared spectroscopy may be used to estimate lipid level in biological tissues. CH₂ stretching vibration gives valuable information about the state order of hydrocarbon tails in lipids because they are good monitors of the changes in acyl chain [30]. The results of this study clearly demonstrated that unsaturated bonds of cholesterol and fatty acids in the membranes can readily react with free radicals and undergo peroxidation. The loss of the fatty acids of cellular membranes, the formation of lipid peroxides, plus the uptake of oxygen by lipid-containing structures all suggest that peroxidation occurs. Thus, lipid peroxides have a sufficient lifetime, which means that they can migrate and damage other cellular

components, including DNA, apart from the membranes [31].

In finger print region, the peaks observed at 1648 and at 1544 cm⁻¹ correspond to amide I and amide II vibration of structural proteins, respectively [32], and the band observed at 1317 cm⁻¹ correspond to amide III components of protein [33,34]. The change in band position of amide I and II bands and disappearance of amide III band after ultrasound insonification reflect alterations in the composition of protein secondary structure. The decrease of total protein content in rabbit cornea after ultrasound exposure confirms this finding. COO_{sym} band at 1402 cm⁻¹ is vibrational mode owing to amino acid side chain and fatty acid. The disappearance of this band after ultrasound insonification may indicate change in amino acid side chains of corneal tissue and change in fatty acid tail of membrane. The physiologic temperature of the rabbit cornea is 34±1°C, and several minutes of hyperthermia at 43°C may have produced functional effects in the epithelial cells (such as production of heat shock proteins) [35]. The heating of the cornea is of great concern in ultrasound applications because these structures are largely composed of collagen, which is an efficient absorber of ultrasonic energy. In addition, the cornea is avascular structures in which no heat dissipation owing to perfusion occurs [36]. The thresholds for 50% probability of corneal damage were found to be at 65 and 59°C for 10 and 60 s of hyperthermia, respectively [36].

The detected change in corneal tissue was reduced when rabbits were treated with antioxidant. Rubowitz *et al.* [15] found that addition of ascorbic acid to the irrigating solution significantly reduced the amount of corneal endothelial cell loss during phacoemulsification by ~70%. The protective topical ascorbic acid enhances the activity of the antioxidant defense system and inhibits lipid peroxidation [37].

β -Carotene exert its mode of action as antioxidants owing to one of the following hypotheses: first, radical addition; second, electron transfer; or third, allylic hydrogen abstraction. It has been proposed that a lipid peroxyl radical (ROO*) might add at any place across polyene chain of carotenoids, resulting in the formation of a resonance-stabilized carbon-centered radicals (ROO-CAR). As this radical should be quite stable, it would interfere with the propagating step in lipid peroxidation and would explain the antioxidant effect of carotenoids [38,39].

Conclusion

Ocular ultrasound has become an essential diagnostic tool for ocular examination and therapy. The cornea is the first defense tissue in the eye, and it is sensitive to ultrasound exposure and coagulation owing to ultrasound insonification. The corneal tissue damage increases by increasing the time of ultrasound exposure. The present findings indicate that receiving antioxidants, at the lowest exposure time (20 min), counterbalanced the damage completely, whereas in the group exposed for long time (60 min), the deleterious effects were significantly minimized. Moreover, to some extent, β -carotene and vitamin C have the same efficacy in protection of cornea from ultrasound insonification.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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