Journal of Global Biosciences
ISSN 2320-1355
Volume 4, Number 8, 2015, pp. 3229-3237
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

CHARACTERIZATION OF CONDUCTIVITY AND VISCOSITY FOR RABBITS' EYE LENS FOLLOWING ULTRASOUND EXPOSURE

Fathia M. Elrefaei[1], Mona S. Hassan [2], Ibrahim H. Ibrahim [2] and Rehab N . Afify [2]

1-Vision Sciences Department, Research Institute of Ophthalmology Giza, Egypt.
2-. Physics Department, Faculty of Science, Ain Shams University, Egypt..

Abstract

This work aimed to study the effect of continuous ultrasound (US) waves on the conductivity and viscosity of eye lens protein for rabbits. Thirty New Zealand rabbits (60 eyes), divided into ten groups ((G1-G10) of three rabbits each were used in this study. Groups G_1 and G_2 were served as controls. Groups (G3-G10) were exposed to US of power intensity0.5 W/cm^2 or 3W/cm^2 at the frequency of 2.8 and 10.8 MHz for 10 and 40 minutes respectively. Total soluble lens protein evaluation, conductivity and viscosity measurements for the soluble lens protein were carried out .The obtained results showed that there was a noticeable decrease in the soluble lens protein content which was inversely proportional to US power intensity, frequency and exposure time. In contrast there was an increase in the conductivity of the soluble lens protein which was directly proportional to the power intensity, frequency and exposure time. After sonification with 3 W/cm^2 viscosity was significantly affected at the high frequency (10.8 MHz) and at exposure time of 40 minutes .On the other hand, the relation between the shear rate and shear stress was fluctuated within small range in comparison with the control group. It is concluded that short exposure time of US (10 minutes) ,with low power intensity(0.5 W/cm^2) at low frequency are recommended for the safety treatment with US.

Key words: Ultrasound, rabbits, eye lens protein, conductivity, viscosity.

INTRODUCTION

The therapeutic applications of ultrasound are being studied for the treatment of glaucoma, ocular tumor, retinal detachments, coagulations of lens protein, disruption of vitreous membranes and vitreous hemorrhages [1, 2]. This is depended on the amount of ultrasound energy absorbed by the eye tissues and the shape of the ultrasonic beam used which differed from exposure to another [(3, 4, 5].

Ultrasound therapy was applied either continuously, thereby completely covering the tear, or in a discrete exposure pattern around the tear. Both methods prevent the formation of generalized cataract [6, 7]. No harmful side effect of US associated with the clinical applications had been reported in humans. Bioeffects had been reported in non human mammalian species with continuous and pulsated wave forms. Earlier studies on the effects of plane and focused US waves on the eye found lens opacities that occurred following application of focused ultrasound on the bovine lens in vitro. The mean temperature rise in human lens and ciliary body using the maximum exposure settings of US for the US scanner was 2.27^{o}C and 1.93^{o}C, respectively [8, 9, 10].

Diagnostic ultrasonography is now widely accepted technique, which employs low intensity ultrasound to image the eye and orbit. These low intensities pose no known treat to patient safety [6, 8, 9]. The basic methods are A- scan, B- scan, Doppler techniques and three-dimensional approaches; unique for ophthalmology (ultrasound biomicroscopy); utilizing ultrasound frequencies of 50 MHz and higher [9, 10, 11]. High frequency ultrasound (50 MHz) was used in diagnostic imaging system in ocular tissue and simulations showed that, rapid dissipation of heat, and low total acoustic power produced by the transducer. High intensity focused ultrasound was employed to seal lens capsular tears in rabbit model [12,13].
Present study is aiming to determine the injuries induced by ultrasound therapeutic doses to the conductivity and viscosity of the eye lens protein.

MATERIALS AND METHODS

Animals:

Thirty New Zealand rabbits (60 eyes)of both sexes (males and females) weighing 2.2±0.25 Kg were housed in special cages in which temperature and humidity were controlled and fed on balanced diet throughout the duration of the experiment. The research protocol was approved by the local ethical committee that applies the ARVO (the association for research in vision and ophthalmology) statements for using animals in ophthalmic and vision research. The rabbits were divided into ten groups of three rabbits each .Group 1 (G1) and G2 were served as control. G3 and G4 were exposed to US of power intensity 0.5W/cm^{2}at the frequency 2.8 MHz for 10 and 40 minutes respectively. G5 and G6 were exposed to US of power intensity0.5W/cm^{2}at the frequency 10.8 MHz for 10 and 40 minutes respectively. G7 and G8 were exposed to US of power intensity 3W/cm^{2}at the frequency 2.8 MHz for 10 and 40 minutes respectively. G9 and G10 were exposed to US of power intensity 3W/cm^{2}at the frequency 10.8 MHz for 10 and 40 minutes respectively [14, 15, 16].

Insonification:

The rabbits were anesthetized by injection of 1mlL/Kg Xylazine (Rompun manufactured by Bayer AG Leverkusen, Germany) intravenously as muscle relaxant at the beginning and after 15 minutes. One ml/Kg were administrated by Ketalar (Ketamine supplied by Ayerst Labs Ontario, Canada) intramuscularly. The eyes were covered after anesthetization of the rabbit, and eye lid was opened with a stainless steel speculum. For preventing impedance coupling, a gel was used between the eye of rabbit and ultrasound prop [6,8]. Sonification of rabbits' eyes was carried out with continuous ultrasound waves after anesthetization as shown in table (1). Insonification was done using focus piezoelectric transducer (Barium zirconate, titanate crystal), type SVHSP101, manufactured by an Egyptian electronic company in a direct contact with the cornea. The transducer was positioned so that the ultrasonic beam was aligned to achieve perpendicular transmission through the lens. The transducer was calibrated in the Faculty of Science, South Valley University, Egypt.

Sample preparation:

After decapitation of the animals, eyes were enucleated immediately. The lenses without their capsules were weighed and homogenized in bi-distilled water so that the concentration of the lens in the solution was kept constant for all samples (each 0.69 wet weight lens homogenized in 3 ml bi-distilled water). The lenses homogenate were then centrifuged in a cooling high speed centrifuge at 16.000 r.p.m for 20 minutes. The following measurements were carried out on the supernatant solution.

- **Estimation of total soluble protein content:**

Total protein in the soluble part of the crystalline lens was determined using Bio-Rad protein microassay according to Bradford [17]. It depends on preliminary treatment of proteins with an alkaline copper reagent followed by foline-ciocalteu phenol reagent. The developing color for different proteins depends on tyrosin, tryptophan content and the sequence of various amino acids with functional side groups especially, histidine, arginine and glutamic acid.

- **Conductivity measurement:**

Conductivity of the soluble lens protein samples was measured using conductance bridge model Griffin and George LTD, serial NO.AV838/1761. The cell have two stainless steel electrodes 1 mm diameter and separated by distance 3mm, which was filled by the soluble lens protein samples from normal and treated rabbits [6,8,9]. The conductivity was calculated from the relation: $\sigma = GL/A$ where σ is the conductivity in $\Omega^{-1}m^{-1}$, G is the conductance in Ω^{-1}, L is the distance between the two electrodes in meter and A is the area of the electrode in m^2.

- **Viscosity measurement:**

Viscosity is the ratio of shear stress to shear rate. Shear stress is related to the summation of torque (T) over conical surface. Shear rate is related to the cone rotational speed (ώ) and gap width (c) at any radial distance (r) from the center of the rotating cone [11]. The ratio of (ώr) and (c) is constant for any value of (r)the shear rate is related to (ώ)and sine (θ). For cone plate viscometer the mechanical relationships are:

$$\text{Shear stress (dyne/cm}^2) = \frac{T}{2/3\pi r^3} \quad \text{---------- (1)}$$

$$\text{Shear rate (1/ sec)} = \frac{ώ}{\text{Sine}\theta} \quad \text{---------- (2)}$$

$$\text{Viscosity (mPa.S)} = \frac{\text{Shear stress x 100}}{\text{Shear rate}} \quad \text{---------- (3)}$$

T = % full scale torque (dyne.cm), r = cone radius (cm), ώ = cone speed (rad/sec) and θ =cone angle (degrees)

To study viscosity of soluble lens proteins samples 0.5 ml from normal cases, sonificated cases were placed in CP 40 cone of Brookfield programmable DV- Π + viscometer that used to describe the interrelation between force, deformation, time, temperature of the used sample [6, 9.10.11].

Statistical analysis

The significance of the measured data was considered as follows:
Non significant (N.S.) when $P > 0.5$, Significant (S.) when $P < 0.05$, High significant (H.S.) when $P < 0.01$ and Very High significant (V.H.S.) when $P < 0.001$ Where, P is the probability (reflect of null hypothesis).

RESULTS

In the present work the effect of ultrasound on total soluble protein, conducity and viscosity of the rabbit's eye lens protein were studied. The obtained results represent the effect of 0.5 and 3 W/cm^2 ultrasound intensities on rabbits' eye at frequencies 2.8, and 10.8 MHz for exposure time of 10and 40 minutes. Since the lens crystallin structure is changed by aging; all the studied groups have their own controls.

Effect of ultrasound on total soluble lens protein

Table (2) and figures (1 and 2) illustrate the effect of ultrasound on total soluble proteins for the control and treated studied groups. The soluble lens protein content of control group was about 325.14 ±1.32 mg/gm wet weight lens. The percentage change in the total soluble lens proteins for the rabbits' groups exposed to the power intensity 0.5 W/cm^2 for 10 minutes showed non significant decrease in the total soluble protein. In contrast with the obtained results after exposure to 40 minutes; this showed a significant decrease in soluble lens protein. Generally, there were decrease in the soluble lens protein compared with the control which became more remarkable by increasing the power intensity, frequency and exposure time for all the studied groups.

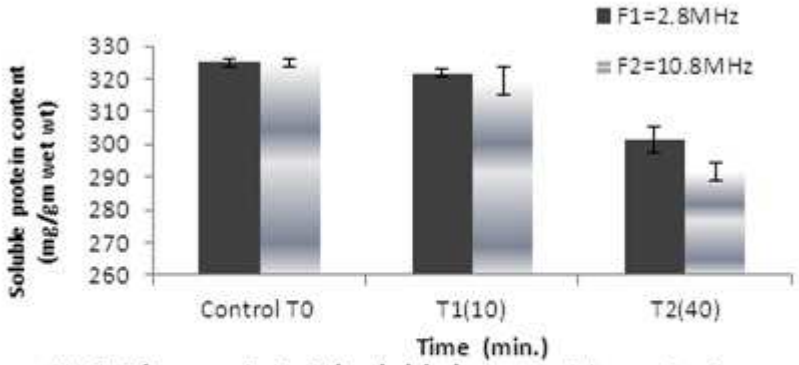

Fig 1. Changes in total soluble lens protein content after sonification with the power intensity 0.5 W/cm^2 as a function of time and frequency.

Effect of ultrasound on the conductivity

The conductivity of control group for soluble lens protein of rabbit's eye for the studied groups was 6.07± 0.1x10^{-3} ohm^{-1} m^{-1}. The obtained data showed an increase in the conductivity of the total soluble lens proteins by increasing the power intensity, frequency and the time of exposure of ultrasound. These results are confirmed by the results obtained by Tosk et al (1990) [13] which showed a remarkable increase in the permiability of the eye tissues under the action of ultrasound which is frequency dependent.

Table (3) and figures (3 and 4) reported the conductivity of the control and insonified groups at power intensities 0.5 W/cm^{2}and 3W/cm^2 as a function of frequency 2.8 and 10.8 MHz and exposure time (10 and 40 minutes). After exposure to low ultrasound intensity (0.5 W/cm^2) at the frequency 2.8 MHz, there were no significant changes in the conductivity. While at the frequency of 10.8 MHz and the exposure time 40 minutes there were significant increase in conductivity. The obtained results after exposure to the power intensity 3 W/cm^2 showed significant increase for all the studied groups especially those exposed for longer time (40 minutes) at frequency 10.8 MHz.

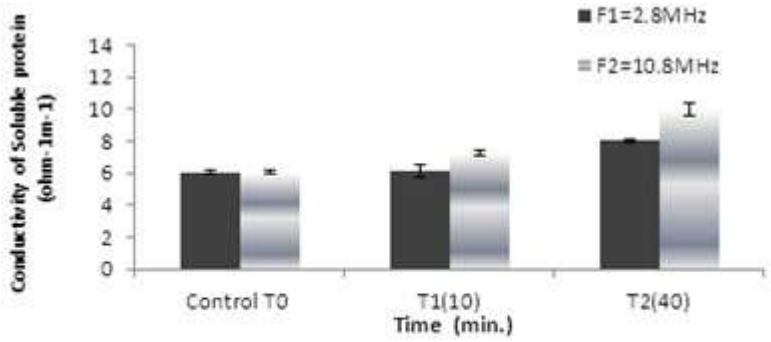

Fig3. Changes in conductivity of soluble lens protein after sonification with the power intensity 0.5 W/cm^2 as a function of time and frequency.

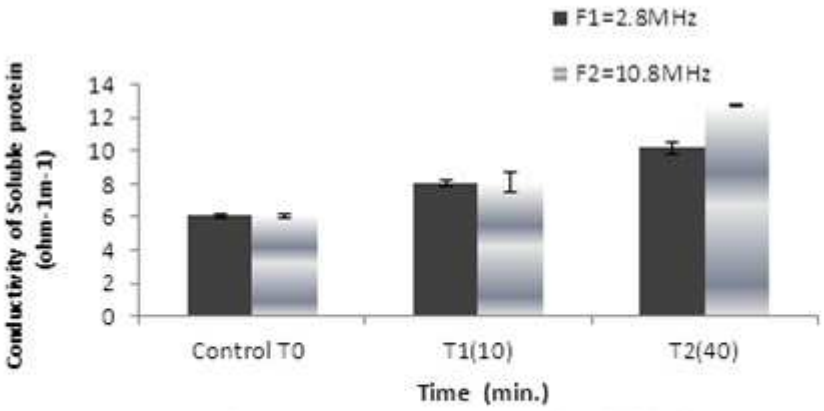

Fig 4. Changes in conductivity of soluble lens protein content after sonification with the power intensity 3 W/cm^2 as a function of time and frequency.

Effect of ultrasound on the viscosity

Tables (4 and 5) showed the variations in viscosity and shear stress for different shear rates of soluble lens protein for control and exposed groups at power intensity 0.5 W/cm^2, frequencies 2.8 and10.8MHz as a function of time (10 and 40 minutes).There was no significant changes for the different periods of sonification except a small variation in the case of 40 minutes exposure at frequency 10.8 MHz.

Tables (6 and 7) showed the variations in viscosity and shear stress with different share rates of soluble lens protein for control and exposed groups to power intensity 3 W/cm^2 at frequencies 2.8 and 10.8MHz and sonified periods (10and40 minutes), there were no significant decrease for the different periods of sonification.

DISCUSSION

Ultrasound energy is a mechanical altering energy which produces cycles that create little bubbles or cavities. Under the right conditions of US exposure and critical point, these bubbles implode with tremendous energy and heat [18,19,20]. The high intensity and frequency dependent, focused US might facilitate this mechanism (cavitation & heating effect) which causes the lens protein denaturation , affecting the ion permeability and so, its conductivity (molecular charges) and viscosity as shown in the obtained results [21-25].

The obtained changes in the lens protein may lead to cataract formation. It is known that during cataract formation, the decrease of biosynthesis of lens crystallins is followed by their aggregation that leads to lens opacification. Also the changes in the protein pattern which related to the formation of aggregated covalently linked high molecular weight protein would play an important role in opacification of the lens.

From the obtained data of the conductivity, the risk of thermal injury increased by increasing exposure duration, and it was a function of intensity and frequency [26,27,28]. Ultrasound effects on the lens protein viscosity indicated a remakable decrease which depended on the sonification parameters. The viscosity decrease was more pronounced at power intensity 3 W/cm.^{2}, frequency 10.8MHz and exposure time 40minutes.

The obtained results are in agreement with previous study [14] which reported that sonification may alter the confirmation of the protein subunits at or near its surface. Moreover, this may be also due to temperature rise accompanying sonification which affected the concentration of alpha-crystalline which resulted in the increase of alpha helix with compensatory decrease in beta sheets [22-25].

The obtained data showed an increase in the conductivity of the total soluble lens proteins by increasing the power intensity, frequency and the time of exposure of ultrasound. These results are confirmed by the results previousely obtained [14] which showed a remarkable increase in the permiability of the eye tissues under the action of US [12-16].

From the obtained results of total, protein content, it was found that there is a protein denaturation resulted from exposure to ultrasound, this denaturation may increase the conductivity. That may decrease the viscosity and as the denaturation increased, the viscosity of lens protein decreased which is confirmed in the obtained results of lens protein viscosity [22, 28, 29].

The increase in the conductivity may be produced by free radicals which generated in the cell due to US exposure. These free radicals may lead to damage of the lens protein which in turn lead to changes in the structure and solubility of lens protein.

Moreover, it is clear that the lens protein is typically a non-newtonian fluid defined as for which the shear rate is varied, the shear stress doesn't vary in the same proportion. The viscosities of such fluids will therefore changes as the share rate is varied. This happens when the fluid has a mixture of molecules with different shapes and sizes. As they pass by each others during flow, their size , shape and cohesiveness will determined how much forces is required to move them. So at each specific shear rate,the alignment may be different and more or less force may be required[17-20].

It is concluded from the obtained results that this study reflect the safety levels for therapeutic doses of ultrasound based on injuries induced to the eye tissues. (1) Exposure of the eye to US of low intensity (0.5 W/cm^2) and short time (10minutes) is safe to be used in medical therapy. (2) Exposure of the eye to high intensity of ultrasound (3 W/cm$^{2)}$ is harmful to lens protein, which may lead to cataract formation. (3) There is a need for the reconsideration of the therapeutic doses of ultrasound based on injuries induced to the eye tissues. (4) Recommendations for research studies on antioxidants supplementation for the US users.

Table (1): Insonification of rabbits' eyes

Power intensity (W/cm^2)	**F1 = 2.8MHz**		**F2 =10.8MHz**	
	T1 (10)	**T3 (40)**	**T1 (10)**	**T3 (40)**
P1 = 0.5	G3	G4	G5	G6
P2 = 3	G7	G8	G9	G10

Table (2) Total soluble protein content (mg/gm wet weight lens) of rabbit lens for all studied groups at different sonification periods (10, 40 minutes) with different power intensities (0.5 and 3 W/cm^2) and frequencies (2.8 and 10.8 MHz).

Frequency	**Time**	**Conductivity**			
		P_1=0.5	**%Difference**	**P_2=3**	**%Difference**
F_1=2.8 MHz (Mean±S.D)	T_0	6.07±0.1		6.07±0.1	
	T_1	6.09±0.4***	+0.33	8.05±0.15***	+32.62
	T_2	8.01±0.1***	+31.96	10.17±0.4***	+67.55
F_2=10.8 MHz (Mean±S.D)	T_0	6.07±0.1		6.07±0.1	
	T_1	7.27±0.2***	+19.76	8.06±0.6***	+32.78
	T_2	10.01±0.4***	+64.91	12.76±0.1***	+110.21

*T_0: Control, ***: p<0.001: very high significant, ** P < 0.05 High significant, and * P < 0.05: significant.*

Table (3) Conductivity of soluble protein ($ohm^{-1}m^{-1}$) of rabbit lens for the studied groups as a function of time (10, 40 minutes) and frequency (2.8 and 10.8 MHz) after sonification with power intensities (0.5 and 3W/cm^2)

Frequency	Time	Total soluble protein content			
		P_1=0.5	%Difference	P_2=3	%Difference
F_1=2.8 MHz (Mean±S.D)	T_0	325.1±1.32		325.1±1.32	
	T_1	321.8±1.32**	-1.02	317±1.32*	-2.43
	T_2	301.5±3.91**	-7.26	292.5±3.91**	-10.03
F_2=10.8 MHz (Mean±S.D)	T_0	325.1±1.32		325.1±1.32	
	T_1	319.3±4.36*	-1.78	311.6±4.36*	-4.15
	T_2	291.8±2.74**	-10.24	289.1±2.74*	-11.07

*T_0: Control, ***: p<0.001: very high significant, ** P < 0.05 High significant, and * P < 0.05: significant.*

Table (4) The variation in viscosity and shear stress with shear rate for the studied groups as a function of time (T1=10 and T_2=40 min) at frequency 2.8 MHz and power intensity 0.5 W/cm^2

Shear rate (Sec^{-1})	Viscosity(cP)			Shear stress (mPa)		
	T_0 Normal	T_1 10 min.	T_2 40 min.	T_0 Normal	T_1 10 min.	T_2 40 min.
7.9	305	304	289	23.5	23.4	22.5
19.8	119	118	115	22.9	22.8	22.3
37.8	62	61.8	60.4	22.5	22.3	21.5
75.5	27	26	24.8	22.2	22	20.8
150	15.5	15.4	14.8	22.1	21.5	20.4
375	5.62	5.61	5.5	22	21.9	20.2

Table (5) The variation in viscosity and shear stress with shear rate for the studied groups as a function of time (T_1=10 and T_2=40 min) at frequency 10.8 MHz and power intensity 0.5 W/cm^2

Shear rate (Sec^{-1})	Viscosity(cP)			Shear stress (mPa)		
	T_0 Normal	T_1 10 min.	T_2 40 min.	T_0 Normal	T_1 10 min.	T_2 40 min.
7.9	305	304	281	23.5	23.1	22
19.8	119	118	103	22.9	22.5	21.1
37.8	62	61.8	47.6	22.5	21.9	20.4
75.5	27	25.5	18.2	22.2	21.4	19.9
150	15.5	15.2	8.1	22.1	21.1	19.8
375	5.62	5.59	3.65	22	21.3	19.5

Table (6): The variation in viscosity and shear stress with shear rate for the studied groups as a function of time (T_1=10 and T_2=40 min) at frequency 2.8 MHz and power intensity 3 W/cm²

Shear rate (Sec^{-1})	Viscosity(cP) for normal and sonified lens			Shear stress (mPa) for normal and sonified lens		
	T_0 Normal	T_1 10 min.	T_2 40 min.	T_0 Normal	T_1 10 min.	T_2 40 min.
7.9	305	302	292	23.5	23.2	22.3
19.8	119	118	105	22.9	22.7	21.9
37.8	62	61.8	51.8	22.5	22.1	21.4
75.5	27	26	17.3	22.2	21.9	20.4
150	15.5	15.4	9.3	22.1	21.8	19.7
375	5.62	5.6	4.46	22	21.5	19.5

Table (7) The variation in viscosity and shear stress with shear rate for the studied groups as a function of time (T_1=10 and T_2=40 min) at frequency 10.8 MHz and power intensity 3 W/cm²

Shear rate (Sec^{-1})	Viscosity(cP)			Shear stress (mPa)		
	T_0 Normal	T_1 10 min.	T_2 40 min.	T_0 Normal	T_1 10 min.	T_2 40 min.
7.9	305	305	267	23.5	22.8	21.8
19.8	119	120	98	22.9	22.4	21.5
37.8	62	62	46.1	22.5	21.8	20.5
75.5	27	25	11.1	22.2	21.3	19.7
150	15.5	15.1	3.8	22.1	21.1	18.5
375	5.62	5.55	0.31	22	20.6	18.4

REFERENCES

1. Moyer L.C., Timbie K.F., Sheeran P.S., Price R. J., Miller G.W. and Dayton P. A., 2015, High-intensity focused ultrasound ablation enhancement in vivo via phase-shift nanodroplets compared to microbubbles. Journal of Therapeutic Ultrasound, 3 (7):27,7 P.
2. Spratt HG Jr , Levine D, Tillman L., 2014, Physical therapy clinic therapeutic ultrasound equipment as a source for bacterial contamination. Physiother Theory Pract. Oct;30(7):507-11.
3. Tabandeh H., Wilkins M., Thompson G., Nassiri D. and Karim A. J., 2000, Hardness and ultrasonic characteristics of the human crystalline lens. J Cataract Refract Surg. 26(6):838-41.
4. Thijssen J. M., 1993, The history of ultrasound techniques in ophthalmology. Ultrasound Med .Biol. 19(8):599-618.
5. Flededelius H. C., 1997, Ultrasound techniques in ophthalmology. Ultrasound Med. Biol. 23(3):365-75.
6. Afify R. N., 2008. Characterization of some biophysical changes in protein of excieted eye lens. Msc.thesis, Ain Shams University, Egypt.
7. Anonymous. Section 3; 2000, Selected biological properties tissues Potential determinants of susceptibility to ultrasound induced bioeffects. American J. Ultrasound Med. Biol; 2 : 85 –96.
8. Elabrak E.S.and Aly E.M., 2011, Effect of ultrasound radiation on the aqueous humor of rabbits eye, Journal of American Science, 7 (2) : 613-19.
9. Foster F .S, Pavlin C. J, Haraaswiewicz K. A, Christooher D. A. and Turnbull. D. H., 2000, Advances in ultrasound biomicroscopy, Ultrasound Med. Biol., 26(1) : 1-27.

10. Avetisov K.S., 2010, The methods for studying the lens, Vesten Oftalmo., 126(2): 37-42.
11. Andries C., Backhovens H.Clauwaert J.De; Block J.,De Voeght F.,and Dhopnt C., 1982, Physical-chemical studies on bovine eye lens proteins. .Light- scattering and viscosity studies of low-molecular weight alpha –crystallin isolated from adult and embryonic bovine lenses. Exp. Eye Res.; 34(2):239-55.
12. Pacifico R L., May 1994. Ultrasnic energy in Phacoemulsification: mechanical cutting and cavitation" J Cataract Refract Surg., 20 (3): 338 – 41.
13. Tsok R. M., Gereliuk I., Tsok O.B. and kamins k. I., 1990, The effect of ultrasonic oscillations of different frequencies on radionuclide accumulation in the tissues, Oftalmol., (1): 46-9.
14. Nemet A.Y., Assia E.I., Meyerstein D., Gedanken A., Topaz M., 2007, Protective effect of free-radicals scavengers on corneal endothelial damage in phacoemulsification. J Cataract Refract. Surg., 33(2): 310-5.
15. Eibaid A., , Bashari M., Miao M., and Jiang B., 2014 , Effect of Ultrasound Radiation on the Activity of Cyclodextrin Glucanotransferase , Journal of Academia and Industrial Research (JAIR) Volume 2, Issue 10
16. Plvinskis V.P., 1982, Effect of high frequency ultrasound on adaptive change of cell resistance, Arkh Anat Gistol Embriol., 82(3); 66-9.
17. Bradford M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72 : 248 – 254.
18. Rutzen A. Roberts C. W., Driller J., Gomez D., Lizzi F. L. and Coleme0n D. J., 1990, Production of corneal lesion using high intensity focused ultrasound. Cornea, 9 (4): 324 – 30.
19. Silverman R. H., Lizzi F.L and Ursea B.G., 2001, Safety levels for exposure of cornea and lens to very high frequency ultrasound, J Ultrasound Med., 20(9): 979-986.
20. Lenhart P.D., Hutchinson A. K., Lynn M.J. and Lambert S.R., 2010, Partial coherence interferometry versus immersion ultrasonography for axial length measurement in children, J Cataract Refract Surg., 36(12): 2100-4.
21. Lavine O., Langenstrass K., Boweyer C., 1955, Effect of ultrasonic waves on refractive media of the eye. Arch. Ophthalmo., 47: 204- 219.
22. El-Refaei F.M., Hassan M.S., Ibrahim I.H. and Afify R.N., 2009, Ultrasound effects on lens protein and its membrane of rabbit's eye. J. Arab Society Med. Res., 4(1): 101-110.
23. Erichev V. P., Khoroshilova I. P., Kalinina O.M., Bessmertnyi A. M., Solontsova L.V. and Dmitriev V. N., 1994, Focused ultrasound experimental morphological research, Vestn Oftalmol., 110 (2): 30-2.
24. Wesley L. N., 2000, Biological effects of ultrasound: development of safety guidelines. Ultrasound in Medicine and Biology, 26: 911-64.
25. Putilina T., Zhang Z.W., Augusteyn R. C., 1991, The effects of sonication on alpha- crystalline. Current eye research, 10(2): 113-20.
26. Kodama M., Matsuura T., Hara Y., 2013, Structure of vitreous body and its relationship with liquefaction, J. Biomedical Science and Engineering, 6, 739-745.
27. Silverman, R. H., Urs, R., Lloyd, H. O., 2013, Effect of Ultrasound Radiation Force on the Choroid. Investigative Ophthalmology & Visual Science, 54(1), 103–109.
28. Lucas B. C., Diller J., Iwamoto T., Silverman R. H., Lizzi F. L. and Colemen D. J., 1998, Ultrasconically induced disruption and hemolysis of vitreous hemorrhages, Ultrasound. Med. Biol. 15 (1): 29-37.
29. Cuvinic V., Brown A.S and Foster F. S., 2005, Thermal assessment of 40-MHz pulsed Duppler ultrasound in human eye. Ultrasound. Med. Biopl., 31(4): 565-573.