Effect of Ultrasound Radiation on the Aqueous Humor of Rabbits' Eye

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Abstract: The present work aimed to evaluate the protein rabbit aqueous humor changes after exposure to ultrasound. Sixteen New Zealand rabbits (male and female) weighing 2.0-2.5 Kg, divided into four groups, group I served as control and the other three groups exposed to ultrasound of power intensity 3W/cm^2 at frequency 10.8MHz for 10, 20 and 40 minutes exposure time. Estimation of protein content, gel filtration chromatography and Sodium Dodecyl Sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE) were carried out to aqueous humor for all the studied groups. The results showed a significant decrease of protein content of rabbits aqueous humor of all groups reached to maximum decrease (-41.3%) at 40 minutes of exposure. A change in the molecular structure of aqueous humor protein was observed in the shift of the protein fractions to high molecular weight and decrease in the mobility of all peaks in the electrophoretic pattern. It is concluded that aqueous humor protein is sensitive to the ultrasound exposure as a function of time of exposure and may lead to denaturation of proteins.
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Key words: Ultrasound, Aqueous humor, Column chromatography, Electrophoresis, rabbits.

1. Introduction:

Diagnostic ultrasonography is now widely accepted and used technique which employs low intensity ultrasound to image the eye and the orbit[1,2]. The basic methods are A-scan, B-scan, Doppler techniques, and three dimensional approaches[3].Unique for ophthalmology is newly invented, highly resolving equipment utilizing ultrasound frequencies of 50 MHz and higher so-called ultrasound biomicroscopy[4]. The therapeutic use of ultrasound by hyperthermia has gained much interest in ophthalmology. The therapeutic applications of ultrasonic energy are also being studied for the treatment of glaucoma, ocular tumor ,retinal detachments, coagulations of lens proteins disruption of vitreous membranes and vitreous hemorrhages[5] . Exposure to high power density ultrasound may produce adverse biological effects. High power ultrasound characterized by high intensity out puts (20-100 KHz) which has a wide range of applications throughout industry [6]. Doses of ultrasound (3W/cm^2) reduce the electroretinograph-b wave amplitude and induce irreversible destructive change in cells of all retinal layers while therapeutic doses (0.6 W/cm^2) of ultrasound increased the b-deflection amplitude and a decrease in the osmiophlicty of rods and cons[7]. The degree of corneal endothelial damage caused by phocoemulsification depended on ultrasonic duration ultrasonic power, the distance of phacotip and the angle of phagotip [8]. The morphology of rabbit eye encleated in various periods after ultrasound exposure illustrated two mechanisms of focused ultrasound effects, inhibited secretion of intraocular fluid and creation of transscleral route of chamber fluid discharge [9] .The heating capability of ultrasound beams of lens and cilliary body was studied and measuring of the mean temperature rise in human lens and cilliary body using the maximum exposure settings of ultrasound scanner and they were 2.27^{o}C and 1.93^{o}C , respectively[10].

In the present work the effect of ultrasound at power intensity of 3W/cm^2 at 10.8Hz for 10, 20 and 40 minutes on the molecular structure of aqueous humor protein of rabbits were studied.

2. Material and Methods

Animals

Sixteen male and female NewZealand rabbits of either sex weighing 2.0-2.5 Kg were used for this study. The animals were divided into four groups, group I (8 eyes) served as control ,groups II,III and IV exposed to ultrasound (US) of power intensity 3 W/cm^2 at frequency 10.8 MHz for 10,20 and 40 minutes, respectively .

Insonification

The rabbits were anesthetized by injection of 1ml/Kg Xylazine (Rompun manufactured by Bayer AG Leverkusen, Germany) intravenously as muscle relaxant at the beginning and after 15 minutes 1ml/Kg were administered 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. Sonification of rabbit's eyes was carried out with continuous ultrasound waves from ultrasound generator and transducer which is consists of a piezoelectric crystal (type SVHSP 101). The transducer is calibrated in Faculty of Science, South Valley University, Egypt.

Sample preparation

After decapitation, the eyes of rabbits were enucleated then the aqueous humor was collected by direct aspiration through transcorneal puncture of the anterior chamber using a 27-gauge needle with 1ml sterile syringe.

Quantitative analysis of total protein

The total protein content of all samples of aqueous humor was measured with the Bio-Rad protein microassay (Bio-Rad Chemicals, CA) according to Bradford (1976) [(11)] and Tripathi *et al.* [(12)].Twenty µL of each sample was diluted to 0.8 ml with bidistilled water and combined with 0.2 ml dye reagent (Coomassie blue G-250). The spectrophotometric reading was taken at 595 nm with spectrophotometer (type UV-visible Recording 240 Graphical, Shimadzu, Japan). Standard bovine albumin was used for generating of the standard curve.

Gel chromatography

A volume of 0.2 ml of sample was injected on column (1.6 cm diameter and 30 cm height) loaded with sephacryl S-300 (Pharmacia Fine Chemicals AB Uppsala, Sweden). The elution was carried out by phosphate buffer with pH 7.4. Fractions of 1 ml/min were collected using fraction collector (type Haake Buchler Instruments, Inc. Saddle Brooke, USA). Gel filtration calibration kits (Pharmacia Fine Chemicals) were used in setting up the calibration curve by which the approximate molecular weights of the protein peaks in the aqueous humor chromatogram can be evaluated.

SDS polyacrylamide electrophoresis

Aqueous proteins were separated according to their molecular weights by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli [(13)] using 5% stacking gel and 12% separating gel. The data represented graphically with an automatic scanner (model R-112, manufactured by Beckman).

Statistical analysis

All values were statistically analyzed by a computerized statistical package SPSS and expressed as mean ±S.D. Comparison of the values was performed using one way ANOVA test. Statistical significance was set at 95% confidence level.

3. Results:

Table(1) represents the concentrations of aqueous humor proteins for normal rabbits and those exposed to ultrasound of power 3W/cm at 10.8 MHz for 10,20 and 40 minutes. The total protein content of normal aqueous humor was 55.4 ±2.0 mg/100 ml. The results showed a significant decrease ($p<0.001$) in total protein content for all groups exposed to ultrasound for the different times of exposure, reached the maximum decrease for group exposed to US for 40 minutes (32.5±6).The percentage decrease of total protein content were 15.5%, 35.6% and 41.3%for groups exposed to US for 10, 20 and 40 minutes, respectively. Figure (1) summarizes the obtained proteins values for all the studied groups in comparison with control to indicate the direct proportional relationship between the time of exposure to US and the total protein content, as the time of exposure increase the total protein content of aqueous humor decrease.

Figure (2) illustrates the chromatographic elution pattern of aqueous humor proteins of normal and exposed to US (3W/cm at 10.8 Hz) for 10 min. The normal pattern of aqueous humor was eluted in 3 peaks; the first peak had a molecular weight of about 170KDa, the second peak was the highest peak in the pattern and had a molecular weight of about 141KDa and the third peak had a molecular weight of about 105KDa. There was a shift of all peaks to high molecular weight (199,145,117KDa, respectively).

Figure (3) represents the chromatographic elution pattern of aqueous humor exposed to 3W/cm for 20 min as compared to the normal. There was a shift of the first and the last peaks to high molecular weights (209 and 129KDa, respectively). In the same time, the second peak was fractionated into two peaks 170KDa and 151KDa.

By increasing the time of exposure to 40 min, all peaks showed high molecular weights (Fig. 4). This shift was appeared clearly in the first peak which contain high molecular weight proteins and partially excluded from the gel. Also, the fractionation of the second peak was increased and had molecular weights of 178 and 162KDa, respectively. The last peak had a molecular weight of 135KDa which was higher than its corresponding peak in normal rabbits.

Figure (5) shows the electrophoretic patterns of aqueous humor for control rabbits and exposed group to ultrasound radiation (3W/cm at 10.8 Hz) for 10 minutes. The control pattern was characterized by the presence of 9 peaks, which reflect the different protein fractions with specific intensities and

broadening that covered the molecular weight range 40-220 KDa. The patterns of aqueous humor of the animals exposed to ultrasound for 10 minutes revealed a shift of all peaks to high molecular weight indicating decrease in the protein mobility and covered the molecular weight range 50-230KDa. Also intensity of most peaks was slightly decreased in comparison with the control.

Figure (6) shows the electrophoretic patterns of aqueous humor of animals exposed to ultrasound for 20 minutes compared to the patterns of the normal aqueous humor. Decrease in the mobility of all proteins fractions was observed and the intensity of all peaks was decreased after exposure to ultrasound. The molecular weight range for this group was 55-235KDa.

Figure (7) shows the electrophoretic patterns of aqueous humor for rabbits exposed to ultrasound for 40 minutes compared to the normal. The most important observation in this group was continuing of the decrease the mobility and the intensity of all peaks fractions till the last peak began to diminish. The molecular weight range is 65-245KDa.

Table 1: Effect of ultrasound of power 3W/cm^{2}at frequency 10.8MHz on aqueous humor proteins of rabbit eyes after different times of exposure

	Total protein (mg/100ml)	Decrease %
control) (Group I	55.4±2	
Group II (10 min)	46.8±3†	15.5
Group III (20 min)	35.7±2†	35.6
Group IV (40 min)	†32. 5±6	41.3

† Statistically significant , P < 0.001

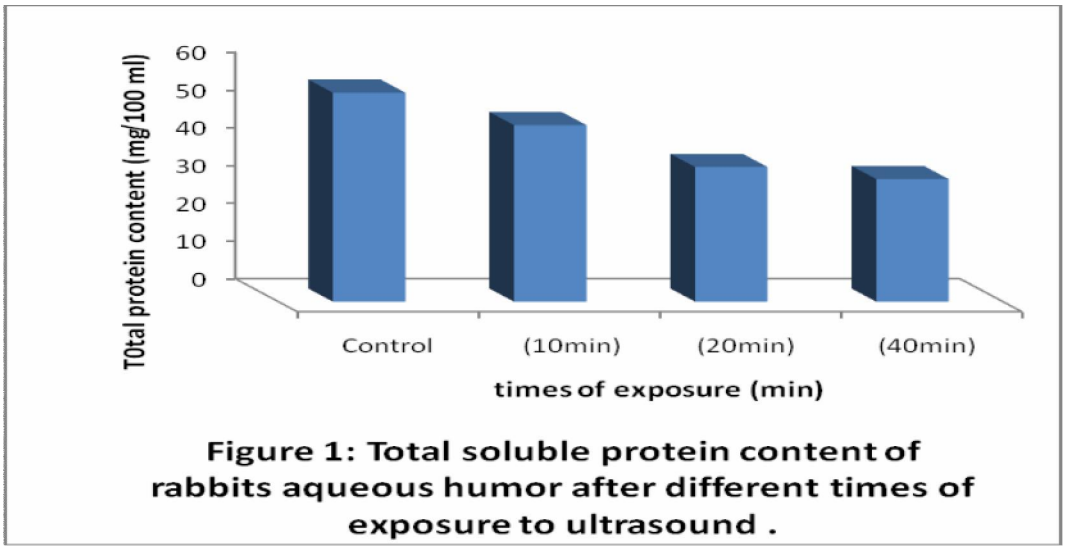

Figure 1: Total soluble protein content of rabbits aqueous humor after different times of exposure to ultrasound .

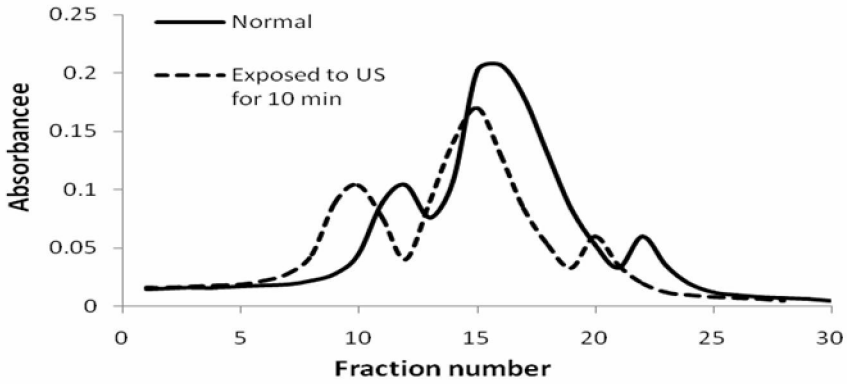

Fig 2: The chromatographic pattern of aqueous humor proteins for normal and exposed to ultrasound for 10 minutes.

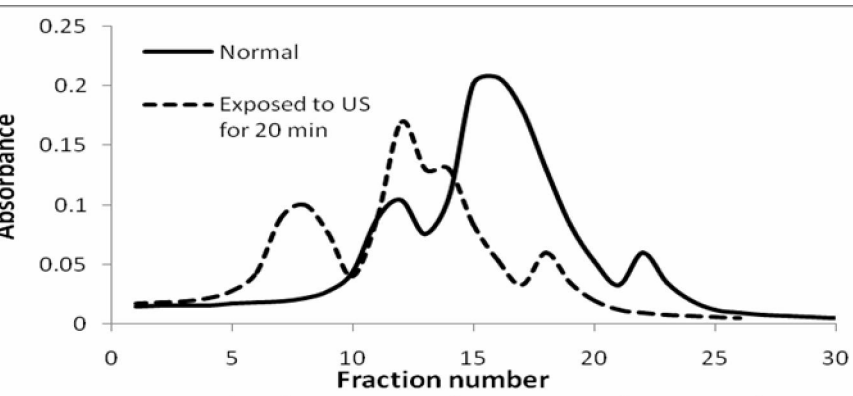

Fig 3: The chromatographic pattern of aqueous humor proteins for normal and exposed to ultrasound for 20 minutes.

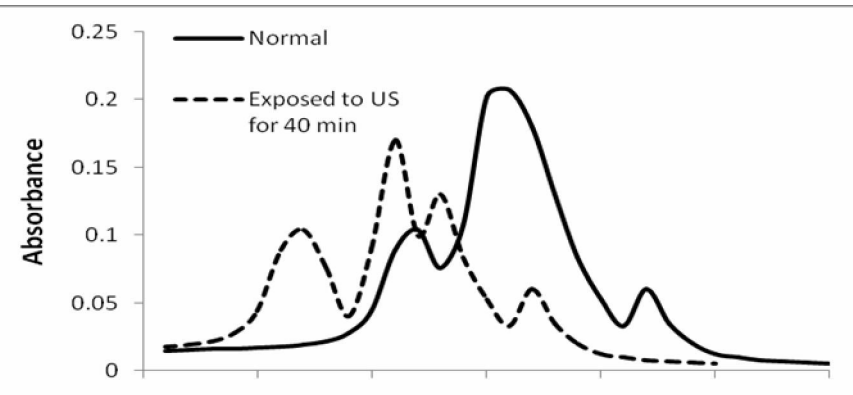

Fig 4 : The chromatographic pattern of aqueous humor proteins for normal and exposed to ultrasound for 40 minutes.

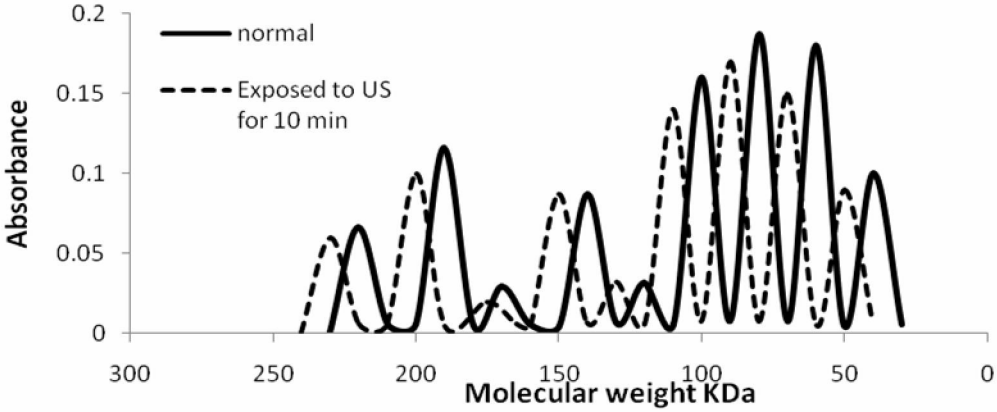

Fig 5 Electrophoretic pattern of normal rabbits aqueous humor and exposed eyes to ultrasound for 10 minuts

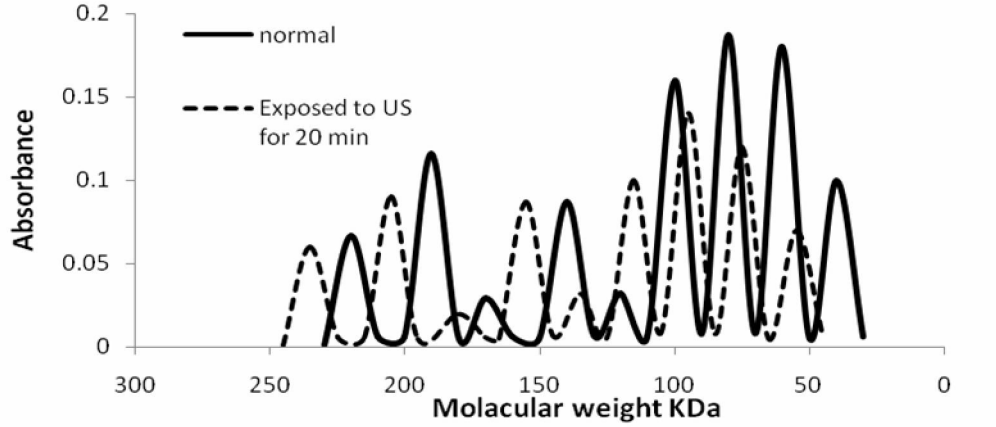

Fig 6 Electrophoretic pattern of normal rabbits aqueous humor and exposed one to ultrasound for 20 minutes

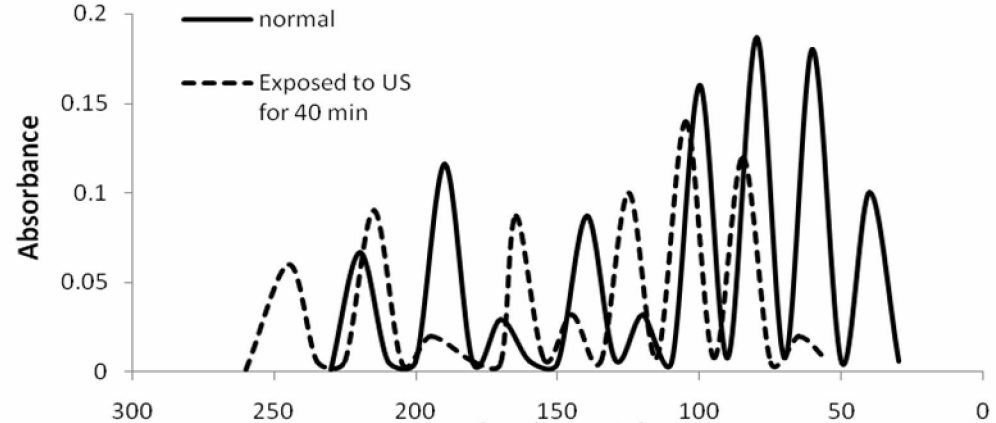

Fig 7 Electrophoretic pattern of normal rabbits aqueous humor and exposed one to ultrasound for 40 minutes

4. Discussion:

After the end of World War II, advances in ultrasound technology brought improved possibilities for medical applications. The first major efforts in this direction were in the use of US to treat diseases. Medical studies were accompanied by experiments with laboratory animals and other model systems to investigate basic biological questions and to obtain better understanding of mechanisms. Also, improvements were made in methods for measuring and controlling acoustical quantities such as power, intensity and pressure. When diagnostic US became widely used, the scope of biological and physical studies was expanded to include conditions for addressing relevant safety matters[14,15].Two primary forms of ultrasound include diagnostic and therapeutic. Diagnostic ultrasound is used for medical imaging while its therapeutic counterpart is used in the treatment of various physical ailments; the combined. Diagnostic involves the emission of pulsed waveforms of less than 1 W/cm^{2}intensity while therapeutic US typically employs incident waves of either 1 or 3 MHz, transmitted as either pulsed or continuous waveforms depending on the desired physiological effect[16].

Aqueous humor provides optical transparency, structural, integrity and nutrition of the eye [17,18,19]. There have been numerous studies about the protein structure of aqueous humor in normal and diseased eyes [20,21].

In the present study, the effect of continuous wave ultrasound with intensity 3W/cm^2 at frequency 10.8 MHz for different duration (10, 20 and 40 minutes) on aqueous humor protein of rabbits was investigated. The protein content in the normal aqueous humor is in agreement with data of Waters *et al.* [22]. The results of electrophoretic separation are in agreement with data of Tripathi *et al.*[12]. Litin *et al.* [23] also reported that human aqueous humor contains many high and low molecular weight bands which are common to serum. The decrease that appeared in aqueous humor protein of the exposed animals is time-dependant and propagated with time increase to 40 minutes. This decrease may be due to temperature rise and this is in agreement with Cuvenic *et al.* [10] who reported that the main effect of ultrasound exposure for long time lead to rise in the temperature and known as thermal effect. These changes in aqueous humor content is supported by the molecular weight distribution where shift of all peaks for all groups to high molecular weight and the degradation and the aggregation of protein molecules. Moreover, it was noticed from the electrophoretic studies decrease of the mobility of all protein fractions due to either loss of surface charge or increase in the molecular weight. This finding is in agreement with Putilina *et al.*[24] who reported that sonification may alter the confirmation of the protein subunit at or near its surface. Also other effects of ultrasound are due to non thermal mechanisms mediated by a process called cavitation. Cavitation essentially describes the biophysical interaction of gaseous inclusions (bubbles) within tissues when exposed to an incident waveform as ultrasound. Typically, bubbles will expand and contract as the peak positive and negative pressures propagate through the tissue which causes protein denaturation, affecting the ion permeability that induces biophysical effects[25].

In conclusion ultrasound can produce a variety of bioeffects through thermal and non thermal effects. Aqueous humor protein is sensitive to ultrasound of power intensity 3W/cm^{2}at 10.8Hz as a function of time of exposure and may lead to denaturation of protein.

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