The biochemical changes of ultrasonic waves on protein metabolism.

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ABSTRACT

The biochemical changes in liver marker enzymes and protein metabolism after repeated exposure to diagnostic ultrasonic waves in rabbits. Thirty-five male rabbits were used for the study; animals were classified to five equal groups. Group I (control group) not exposed to ultrasound (control group) while other groups (II, III, IV and V) were exposed to diagnostic ultrasound (5.5 MHz) 2 times per week for different time (15, 30, 45 and 60min) for 5 weeks, respectively. Our results revealed that a highly significant increase of serum liver marker enzymes (ALT, AST, GGT, Alp activities, total protein), protein concentration in liver tissue, and changes in protein electrophoresis were observed after ten times of ultrasound exposure. Also a significant increase of serum urea and uric acid concentrations were observed in groups III, IV and V. with changes in electrophoretic pattern of liver protein electrophoresis after five and ten times of exposure to ultrasound. Our results showed that, ultrasound exposure had positive anabolic effect on protein metabolism when used for short period as 15 min. However, it had a negative effect on protein metabolism when used for long durations more than 15 min of exposure.

Keywords: Protein metabolism, Ultrasound, rabbits, protein electrophoresis, liver marker enzymes

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1. INTRODUCTION

The proteins are responsible for the maintenance of structural, functional and organization of the cells. They play a vital role in respiration, enzyme catalysis, and transport of materials, regulation of metabolism, movement and in body defense. The total protein content of the cell includes both structural and soluble portions, of which the former plays an important role in cellular metabolism (Jyothi and Suneetha, 2010). Ultrasound energy is attenuated when the waves propagates through a medium. Attenuation is the result of absorption and scattering effects (Ensminger and Stulen, 2008). Absorption of ultrasound in soft tissues can be the result of two phenomena: classical absorption (due to viscosity) and relaxation (due to existence of a time-lag between the application of an ultrasonic wave to a tissue particle and oscillation of that particle in response to the wave) (Shung, 2008). Therefore, this study aims to investigate the biochemical changes in protein metabolism in rabbits after exposure to ultrasonic waves.

2. MATERIAL AND METHODS:

2.1. Experimental animals:

Thirty-five males' moshtohor rabbits of 3months old and of average body weight 1.75 kg to 2kg used in the experimental investigation of this study. Rabbits were housed in separate metal cages, fresh and clean water was supplied.

2.2. Experimental groups:

Rabbits were divided into five groups, each group consists of seven rabbits as follows:

Group I not exposed to ultrasound, served as control group, while other groups (II, III, IV and V) were prepared for ultrasound exposure, where the area of the last two ribs and the abdomen was clipped, shaved and prepared for liver examination. The transducer was placed over the hepatic area at 5.5 MHz of ultrasound for different time as (15, 30, 45 and 60min), 2 times per week for 5 weeks, respectively. Blood samples for serum separation were obtained by vein puncture of the marginal ear vein after the end of exposure to ultrasound by 15 to 30 min .The collected blood was allowed to coagulate at room temperature then centrifuged at 2500 r. p.m. for 15 mins , the clear sera were aspirated carefully by Pasteur pipette and transferred into dry and sterile labeled glass vials then kept in a deep freez at – 20c^{o} for subsequent biochemical analysis: Alanine aminotransferase (ALT),Aspartate aminotransferase (AST), γ-Glutamyl Transferase (GGT), Alkaline phosphatase, Uric acid, Urea, Total protein, Protein concentration in liver tissue, and protein electrophoresis. The biochemical analysis were determined according to the method described by Reitman and frankel, 1957(for ALT and AST), Szasz and Persijn 1974, Belfield and Goldberg 1971, Trivedi and Rebar 1908, Fawcett and Scott 1960, Gornall, Bardawill and David 1949, Kjeldahl 1883, and Werner 1993, respectively.

3. RESULTS

By using ANOVA for statistical analysis of data. Ultrasonographically in control group, all over the experiment, the liver tissue appeared with homogenous echogenicity evenly distributed. The hepatic circulation appeared as an echoic tubular and round structure with a hyperechoic wall. In all remaining four groups, there were no any significant changes with echogenicity of the liver tissue structure and the hepatic circulation all over the periods of the experiment. It is evident from the results recorded in tables (1, 2, and 3) that, serum ALT, AST and ALP showed significant increase all over the periods of experiment in group II exposed to ultrasound for (15 min) as compared to control group. However, there was significant decrease in serum ALT and AST activities were observed after 2, 3, 4 and 5 weeks in group V as compared to control group. The obtained result in table (4) clarifies that, mean values of serum GGT activity showed significant increase all over the period of experiments in group II (15 min) as compared to control group. Additionally, there was significant increase in group IV after 1st week when compared to control group. Table (5) demonstrated significant increase of mean values of serum urea after 3rd week, 4th week and 5th week in group IV and group V as compared to control group. Also, there was significant increase was obtained after 3, 4 and 5 weeks in group III as compared to control group. For data presented in table (6) showed significant decrease in serum uric acid level after 1,2,3, 4 and 5 weeks in group II as compared to control group. Also, a significant decrease in serum uric acid concentration was observed after all over the durations periods in group III as compared to control group. However, a significant increase in serum uric acid was observed after 4th week and 5th week in group IV and group V as compared to group I and group II. Table (7) showed significant increase in serum total protein after 2nd week, 3rd week, 4th week and 5th week in group II. Also there was significant decrease after 5th week in group IV. In addition, there were significant decrease in group V after 5 weeks when compared to control group. Our results in table (8) show that there were a significant increase in total protein levels in liver in group II and group III after 5 weeks. In addition, there were significant decrease in total protein levels in liver in group IV and group V after 5th week. Our results in table (9) clarifies the mean values of protein electrophoresis after 5 times of exposure. It showed significant increase of alpha 1 globulin in group II,

group III and group IV and group V . Also there were significant increases of Alpha $_2$ globulin in group IV and group V. Moreover, there was significant decrease of Alpha$_2$ globulin in group III. Our results in table (10) clarify the mean values of protein electrophoresis after 10 times of exposure to ultrasound. There was significant increase of alpha$_1$ globulin in group II, group III, group IV and group V. Also, there were significant decrease of Alpha$_2$ globulin in group IV and group V. In addition there was significant increase of Alpha$_2$ globulin in group III. This table also shows highly significant decrease of Beta globulin in groups (II, III, IV and V).

Table (1) Effect of ultrasound on serum ALT activity (U/ml) in all experimental groups of rabbits.

Experimental animals	Duration of ultrasound exposure / week				
	1st week	2nd week	3rd week	4th week	5th week
G I	59.57±2.38^{C}	59.57±2.38^{C}	59.57±2.38^{C}	59.57±2.38^{C}	59.57±2.38^{C}
G II	106.57±1.98^{A}	110±2.03^{A}	108.28±0.86^{A}	108.57±1.46^{A}	109.28±1.44^{A}
G III	68.43±1.19^{B}	67.71±0.87^{B}	65.85±1.06^{B}	65.42±0.87^{B}	64.85±0.91^{B}
G IV	54.85±2.19^{D}	47.43±0.75^{E}	43.57±0.78^{EF}	39.71±0.56^{FG}	33.29±2.89^{HI}
G V	43.28±0.87^{F}	36.28±0.68^{HG}	29.86±0.51^{JI}	29±0.44^{J}	27.29±0.28^{J}

Data are represented as (X± S.E). X= mean. S.E= standard error. Mean values with the same superscript letters are not significantly different. Mean values with different superscript letters are significantly different.

Table (2) Effect of ultrasound on serum AST activity (U/ml)in all experimental groups of rabbits.

Experimental groups	Duration of ultrasound exposure / week				
	1st week	2nd week	3rd week	4th week	5th week
G I	60.42±2.38^{FGH}	60.42±2.38^{FGH}	60.42±2.38^{FGH}	60.42±2.38^{FGH}	60.42±2.38^{FGH}
G II	129.28±4.53^{A}	120±1.58^{B}	120.14±2.45^{B}	119±2.30^{B}	118.85±2.37^{B}
G III	70.28±2.29^{C}	67.28±1.82^{CD}	65.71±1.71^{CDE}	64.71±1.68^{DEF}	63±1.54^{DEF}
G IV	67.71±0.42^{CD}	61.42±0.48^{EFG}	56.57±0.84^{GHI}	51.57±1.23^{IJ}	43.42±0.94^{LK}
G V	55.57±2.49^{HI}	48.42± 0.48 ^{JK}	42.85±0.88^{L}	39.14±0.50^{L}	33.57±0.48 ^{M}

Table (3) Effect of ultrasound on serum ALP activity (IU/L) in all experimental groups of rabbits.

Experimental groups	Duration of ultrasound exposure / week				
	1st week	2nd week	3rd week	4th week	5th week
G I	69±2.22^{J}	69±2.22^{J}	69±2.22^{J}	69±2.22^{J}	69±2.22^{J}
G II	118±2.39 ^{AB}	113.14 ±2.83^{ABCD}	109.42±1.82^{BC} DEF	108.28±2.37^{CD} EF	107.57±1.11^{CD} EF
G III	109.28±2.71 BCDEF	112.29±1.54^{BC} DE	110.29±1.82^{BC} DEF	111.71±1.47^{BC} DE	110.71±1.78^{BC} DE
G IV	122.28±4.69^{A}	116.85±1.29^{AB} C	110.43±0.64^{BC} DEF	103.43±8.42^{EF} G	96.14±5.59^{GHI}
G V	110.43±0.64^{BC} DEF	106±0.82^{DEF}	103.71±0.68^{EFG}	92.43±0.57^{HI}	89.29±7.96^{I}

Table (4) Effect of ultrasound on serum GGT activity (U/ml) in all experimental groups of rabbits.

Experimental groups	Duration of ultrasound exposure / week				
	1st week	2nd week	3rd week	4th week	5th week
G I	6.15±1.56 H	6.15±1.56 H	6.15±1.56 H	6.15±1.56 H	6.15±1.56 H
G II	9.07±0.15 A	8.74±0.07 A	8.54±0.11 ABC	8.41±0.11 ABC	8.08±0.05 ABCD
G III	8.67±0.1 AB	8.24±0.09 ABCD	7.97±0.07 ABCDE	7.51±0.12 BCDEF	7.2±0.07 DEFGH
G IV	8±0.53 ABCDE	7.11±0.09 DEFGH	6.88±0.08 FGH	6.51±0.11 FGH	6.2±0.07 GH
G V	7.37±0.12 FGH	7.42±0.37 CDEF	6.85±0.51 EFGH	6.57±1.58 FGH	6.85±0.51 EFGH

Table (5) Effect of ultrasound on serum urea concentration in all experimental groups of rabbits (mg/dl).

Experimental groups	Duration of ultrasound exposure / week				
	1st week	2nd week	3rd week	4th week	5th week
G I	22.57±0.75 HI	22.57±0.75 HI	22.57±0.75 HI	22.57±0.75 HI	22.57±0.75 HI
G II	24.43±1.13 FGHI	22.29±0.74 I	23±1.27 HI	23.29±1.15 GHI	22.43±0.89 I
G III	23.86±0.51 FGHI	24.71±0.71 FGHI	25.71±0.52 EFG	27.71±0.42 CDE	30.86±0.51 B
G IV	22.29±0.42 I	25±0.89 FGH	28±0.53 CDE	29.57±0.84 BC	31.85±0.59 B
G V	23.85±1.05 FGHI	25±2.2 FGH	26±0.75 DEF	28.29±0.56 CD	35.28±0.81 A

Table (6) Effect of ultrasound on serum uric acid concentration in all experimental groups of rabbits (mg/dl).

Experimental groups	Duration of ultrasound exposure / week				
	1stweek	2nd week	3rd week	4th week	5th week
G I	3.35±0.75 AB	3.35±0.75 AB	3.35±0.75 AB	3.35±0.75 AB	3.35±0.75 AB
G II	1.1±0.09 H	1.51±0.08 GH	1.71±0.06 FGH	1.74±0.09 FG	1.9±0.05 FG
G III	1.67±0.11 FGH	1.91±0.07 FG	2±0.017 FG	2.17±0.09 DEF	2.27±0.07 DEF
G IV	1.9±0.24 FG	2.27±0.2 DEF	2.64±0.22 CDE	3.07±0.28 BC	3.41±0.27 AB
G V	2.05±0.12 DEFG	2.7±0.18 DC	2.05±0.05 EFG	3.2±0.18 ABC	3.75±0.16 A

Table (7) Effect of ultrasound on serum total protein concentration in all experimental groups of rabbits (g/dl).

Experimental groups	Duration of ultrasound exposure / week				
	1st week	2nd week	3rd week	4th week	5th week
G I	6.31 ± 0.22^{EFGH}	6.31 ± 0.22^{EFGH}	6.31 ± 0.22^{EFGH}	6.31 ± 0.22^{EFGH}	6.31 ± 0.22^{EFGH}
G II	6.61 ± 0.28^{CDEF}	6.97 ± 0.22^{BC}	7.07 ± 0.29^{BC}	7.47 ± 0.27^{A}	7.75 ± 0.27^{BCD}
G III	6.1 ± 0.24^{FGHIJ}	6.39 ± 0.51^{EDFGH}	6.25 ± 0.29^{EFGHI}	6.21 ± 0.21^{EFGHI}	5.63 ± 0.14^{IJK}
G IV	6.54 ± 0.17^{CDEFG}	6.21 ± 0.17^{EFGHI}	6.17 ± 0.18^{FGHIJ}	5.86 ± 0.22^{HIJK}	5.42 ± 0.07^{K}
G V	6.54 ± 0.18^{CDEFG}	6.81 ± 0.08^{CDE}	5.57 ± 0.22^{CDEFG}	5.56 ± 0.12^{jk}	5.95 ± 0.03^{GHIJK}

Table (8) Effect of ultrasound on liver tissue of total protein concentration in all experimental groups of rabbits (%).

Experimental groups	Protein percentage (%)
G I	56.20 ± 0.51^{C}
G II	75.78 ± 1.43^{A}
G III	62.89 ± 0.57^{B}
G IV	52.15 ± 0.29^{D}
G V	48.20 ± 0.38^{E}

Table (9) Effect of exposure to ultrasound 5 times on electrophoretic patterns of serum protein in all experimental groups of rabbits.

	GI	GII	GIII	GIV	GV
Albumin%	31 ± 0.53^{CD}	25.05 ± 0.51^{C}	33.58 ± 3.59^{D}	30.46 ± 0.37^{CD}	33.22 ± 0.77^{C}
Alpha 1globulin%	2 ± 0.16^{D}	11.85 ± 0.59^{B}	8.97 ± 1.01^{CB}	7.04 ± 0.29^{C}	17.29 ± 1.27^{A}
Alpha 2 globulin%	14 ± 0.49^{B}	11.91 ± 1.04^{BC}	10.08 ± 1.07^{C}	21.55 ± 0.37^{A}	19.96 ± 1.42^{A}
Beta globulin%	32 ± 0.53^{B}	40.36 ± 1.25^{A}	30.11 ± 1.86^{B}	7.68 ± 0.82^{EF}	7.13 ± 0.65^{F}
Gamma globulin%	21 ± 0.49^{CD}	10.89 ± 0.29^{F}	17.25 ± 2.45^{DE}	33.12 ± 0.77^{A}	22.39 ± 0.99^{C}

Table (10) Effect of exposure to ultrasound 10 times on electrophoretic patterns of serum protein in all experimental groups of rabbits.

	GI	GII	GIII	GIV	GV
Albumin%	31 ± 0.53^{CD}	28.48 ± 0.66^{CD}	30.01 ± 0.85^{CD}	47.19 ± 5.62^{B}	56.38 ± 2.22^{A}
Alpha 1globulin%	2 ± 0.16^{D}	11.74 ± 2.17^{B}	11.18 ± 1.65^{B}	11.94 ± 1.61^{B}	9.62 ± 0.96^{CB}
Alpha 2 globulin%	14 ± 0.49^{B}	13.47 ± 0.83^{BC}	22.59 ± 2.20^{A}	9.99 ± 1.78^{C}	5.76 ± 1.11^{D}
Beta globulin%	32 ± 0.53^{B}	10.78 ± 1.36^{DE}	8.41 ± 0.56^{DEF}	16.19 ± 1.90^{C}	11.67 ± 0.97^{D}
Gamma globulin%	21 ± 0.49^{CD}	36.25 ± 1.97^{A}	27.81 ± 2.17^{B}	14.62 ± 0.99^{FE}	16.87 ± 1.92^{DE}

4. DISCUSSION

The obtained data in tables (1-8) revealed that, ultrasound effect on enzymatic activities showed significant increase. Similarly, (Jyothi and suneetha, 2010) who indicated that ultrasound induced enhancement in protein metabolism and enzymatic activity. Ultrasound waves generate vibrations in the tissue components, including intracellular and extracellular fluids and cell membranes, when penetrating these tissues. They cause movements or displacements of tissue particles when transmitted in it. These motions of ultrasound treatment produce micro-massage effect in tissue, which produces mechanical stimulation (kossof 2000, and Ziskin 1993). The acoustic vibration has thermal and non-thermal effects in biological tissue, the energy attenuated by tissues leads to increase thermal effects (Claes and Willie, 2007). Liver enzymes, such as AST and ALT are the most sensitive indicator of hepatocyte injury. Both AST and ALT are normally present in low concentrations. With cellular injury or changes in cell membrane permeability, these enzymes leak into circulation. ALT is the more sensitive and specific test for hepatocyte injury, also AST can be elevated in the state of cardiac arrest or muscle injury. Serum AST, ALT, and ALP are elevated between (50% to 200%) from baseline when compared with normal levels (Jeschke et al., 2007&2008). Thermal effects including increased in metabolic activity, blood flow and an analgesic effect on nerves. In additional, it increased collagen extensibility (Williams 1983; and Lehmann and Lateur 1990). Non-thermal effects divided by (Ter Haar, 1988) into cavitation and other mechanical effects. She suggested that the beneficial effects of ultrasound were due to "non-thermal interaction mechanisms" rather than heating. Our results recorded in tables 7 and 8 indicated that, exposure to ultrasound for short period as in GI influences the serum protein concentration and protein percent in liver tissue. Brian D et al 2003, observed that, ultrasound induce a positive anabolic effect when it treated with pulsed ultrasound. Cheung et al., 2010 showed that, ultrasound enhances the intra-scleral penetration of protein, increasing the diffusibility 1.6 folds while causing no damage of retinal tissue, and suggest that cavitation is possible mechanism for increasing the permeability of sclera for diffusive transport. Moreover, Harvey et al, (1975) suggest that, ultrasound enhances protein synthesis in fibroblast. In addition, tables (1-8) clarified that, long exposure to ultrasound with repeated intervals decreased enzymatic activities, serum total protein and protein percent in the liver tissue. Moreover, it caused changes in protein electrophoretic pattern as indicated in tables (9 and 10). Over exposure (time or intensity) to ultrasound could cause the structure of the cell membrane to be destroyed, with many enzymes in cells deactivated and the cell metabolism disrupted that could lead to extremely low cell survival and proliferation (Liu., et al 2006). High ultrasound intensity could destroy the ultrastructure of the cell such as cell membrane, cytoskeleton, chloroplast, and mitochondria (Sawidis and Reiss, 1995). The heating of globular protein disrupts some of the forces responsible for the stability of tertiary or secondary protein structures. These forces included hydrogen interactions between the polar groups and interactions of non-polar groups (hydrophobic interactions) through the surrounding water molecules which form cages around hydrophobic groups. Also, electrostatic bonds and Vander Waals interactions were involved in this heat denaturation process, although to a lesser extent (Relkin 1994, Nygren-Babol and Karonen 2009). In addition, on prolonged heat treatment, the process is reversed and heat enhanced protein aggregation. Also, Sonication might result in disruption of intra-molecular forces of proteins due to shear forces and lead to the formation of

aggregates on prolonged sonication as is the case for heating.

5. CONCLUSION

It was concluded that exposure to ultrasound for short period (15 min) had positive effect on protein metabolism and if the exposure for long period more than (15 min) had a negative effect on protein metabolism. So, it was adviced that during examination of the liver, the maximum time to ultrasound should not be more than 15 min to prevent adverse effects of ultrasound on liver protein metabolism.

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