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Investigating the effect of therapeutic ultrasound irradiation on the liver and kidney function of male albino mice

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Abstract

In the current explanatory study an experimental methods were conducted to anticipate the effect of therapeutic Ultrasound irradiation on liver and kidney of male albino mice. The histopathological studies revealed a convenient apparent aberration. These finding were supported by hematological investigation of complete blood count (CBC), blood enzymes like aspartate amino transferase (AST or SGOT) and alanine amino transferase (ALT or SGPT), also Creatinine and Urea levels were detected**.** The results showed no significant alteration in the physiological function of both liver and kidney. The level of malondialdehyde (MDA) and total antioxidant contents were carried out. the level of Glutathione (GST), Superoxide dismutase (SOD) were still in normal ranges.

Keywords: Non-ionizing radiation, ultrasound, free radicals, antioxidants.

Introduction

Nowadays, Cutting–edge technology increases the exposure rate of electromagnetic waves (EM) irradiation. The daily lifestyle sources may be industrial noising, cell phones, and computers, home-aid tools like microwave, oven, and electric heater. Consequently, a great attention has paid to the effects of electromagnetic radiation upon living organism[1].

EM includes ionizing (such accelerated atomic particles, infrared laser) and non-ionizing radiations (high-intensity ultrasound).Non-Ionizing radiations have sufficient energy only for excitation, instead of producing charged ions when passing through matter like ionizing[2,3].

Ultrasound waves are widely used in ordinary lifestyle Industry exposure (ultrasonic washers, welding and erosion machines), consumer devices (burglaralarms), dog whistles, bird and rodent repellents, humidifiers and inhalers. These types of US waves are of low frequency of average 20 kHz. While higher frequency (over 0.8 MHz) is used in medical therapeutic and diagnostic purposes[1-3].

The biologic effects of ultrasound can be thermal and non-thermal. Although most of the biologic reactions are due to the thermal effect, the non-thermal effects of ultrasound includes, e.g., cell membrane gaseous cavitations[4].

Radiation exposure, especially ionizing one, may result in liberation of oxygen and nitrogen reactive species known as free radicals within the

cells [5] .these radicals have unpaired electrons making them highly reactive species[6].In mammals, a sophisticated system of antioxidants is produced within liver to counteract the action of these reactive species. Catalase, Superoxide dismutase, and glutathione peroxidase are enzymatic A lot of risk factors, including alcohol, drugs, environmental pollutants and irradiation, may induce oxidative stress in liver, which in turn results in severe liver diseases**Error! Bookmark not defined.**, [6].The current explanatory study an experimental methods are conducted to anticipate the effect therapeutic ultrasound irradiation on liver and kidney of male albino mice. The study protocol will be conducted at cellular level.

Material and methods

Ultrasonic unit

An ultrasonic therapy instrument was used (Model CSl Shanghai, No. 822 Factory. China). It operates at a frequency 0.8 MHz and power output which converted to ultrasonic mechanical energy by means of ultrasonic transducer (calcium zirconate –titanate). The mechanical ultrasonic energy has a beam power density which can be adjusted from 0.5 to 3W/cm^2. Sonocation time can be adjusted up to 30 minutes, while the set-time is over, the power supply cut off automatically and intermittent alarming sound may be given. This instrument operates at both continuous wave mode with output power from 0.5 - 3W/cm^2 adjustable in 11 steps and pulsed mode (pulse frequency 1000 Hz, duty ratio 1/3 and average power density from 0.15-1 W/cm^2).

Methods

Sample groups: The mice sample size was 15 albino mice were divided into groups as shown below:

- **Group I: 5mice non radiated act as control group.**
- **Group III: 10 mice, ultrasound group as follow:**
  - **a) 5 mice:** Were exposed to pulsed ultrasound at power density of 3W/cm^2 for 3 minutes.
  - **b) 5 mice:**Were exposed to continuous ultrasound at power density of 3W/cm^2 for 3 minutes.

Histopathological Examination

Small pieces of freshly excised organs of (liver, and kidney) of all the experimental groups were processed and examined by Hematoxylin and Eosin (H&E) method as follows:The small pieces of organs were fixed at 10% formaldehyde. Dehydration in ascending grades using alcohol was performed. Paraffin block were performed embedded in paraffin.Then Cleanwith xylene followed by rehydration in descending grades of alcohol.Stain the samples with Haematoxlin and Eosin stain, then Cleaned again by ethylene. Finally the slides were prepared to be examined by light microscopy

Haematological and serological study

The protocol of blood collection for hematological studies was quoted from *M Salahudd et al*[9]*.* The collected blood was divided to three portions. About 2 ml portion was taken in evacuated EDTA-containing tube for hematological studies in the same day. The remaining blood was used for the collection in a plain tube for serological studied. Serum was separated and centrifuged to remove unwanted blood cells where necessary.

The samples were stored at -20°C. Liver enzymes such" Alanine amino transferase, GPT, and serum albumin were assayed. Kidney function was assayed by conventional "urea, creatinine and total protein contents". using a Hitachi 911 automated analyzer using *spectrum* Kits according to the manufacturer's specifications.

Statistical analysis: The data were analyzed statistically between normal and treated values by one way ANOVA with post-hoc Duncan's multiple range test.

Biochemical and serological study

Estimation of Serum AspartateTransaminase (AST/GOT) and AlanineTransaminase (ALT/POT)[10]

ALT enzyme is highly concentrated in liver and lower extent in kidney and heart muscles, pancreas and lungs. It can be elevated in case like Hepatitis, cirrhosis, obstructive jaundice, liver carcinoma,.MeanwhileAST enzyme concentrates mainly in heart, heart, liver, muscles and kidney. Although both enzymes are elevated whenever liver cell affected, ALT is the liver specific one. Assay principle of both enzymes is the same. ALT activity is monitored by the concentration of pyruvatehydrazone, formed with 2,4-dinitrophenylhydrazine.While in AST by concentration oxaloacetatehydrazone.

Alkaline phosphatase

Alkaline phosphatase was estimated by Kinetic method according to the recommended reference method of DGKC. Liquid stable double reagent. (ALP) hydrolyzes the colorless p-nitrophenyl phosphate to p-nitrophenol and phosphate in the presence of magnesium ions. The product of enzyme hydrolysis p-nitrophenol, has a yellow color at the pH of the reaction. The working procedure was followed according to determination kits.

Creatinine and Urea levels[11]

Creatine is a metabolite synthesized in kidney, liver and pancreas. It is transported other organs such as muscle and brain where it isophosphorylated to phosphocreatine. Some free creatines converted to creatinine in muscles cells daily. Urea is the main end product of protein nitrogen metabolism. It is synthesized throughoutthe urea cycle from ammonia in liver cells.Both serum creatinine and urea levels are elevated inrenal malfunction, especially decreasedglomerularfiltration.unlikecreatinineserum urea levels may be affected by dehydration, diet and proteinmetabolism. Thus serum creatinine isa significantly more reliable renal functionscreening test than serumurea.

Creatinine is monitored by a colored complex formed by the reaction of creatinine reacts with picric acid in alkaline solution. While urea is hydrolyzed to ammonia a carbon dioxide by ureasein alkaline media, acolored complex is formed in the presence of an indicator which is proportional to urea concentration.

Estimation of serum Malondialdehyde (MDA)

The procedure was adopted from Deepa D'souza et al8 as follow; 2 mL of blood was collected. Serum was separated by centrifuging the blood sample at 3000 rpm for 5 min. Following which the serum MDA was measured using the method of Buege (1978). Serum-100 µL serum is diluted to 500 µL with distilled water. The samples are kept in boiling water bath for 15 min. To the diluted sample 1 mL of Trichloroacetic acid TCA-2-thiobarbituric acid (TBA)–HCl reagent is added. The reaction mixture is cooled and centrifuged. The supernatant is taken and the optical density of the pink color formed is read at 535 nm. The concentration of MDA in the sample is got by plotting the obtained absorbance against the standard graph. The optical density of the pink color formed is directly proportional to the concentration of serum MDA in the given sample. Sample concentration is calculated from the following : sample = A sample ÷A standard ×10 nmol/ml

Estimation of plasma total antioxidant capacity

The determination of the antioxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H_2O_2). The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H_2O_2 is determined colorimetrically by an enzymatic reaction which involves the conversion of 3, 5, dichloro –2–hydroxy benzensulphonate to a colored product. Total antioxidant Sample concentration is calculated from the following = absorbance blank-sample absorbance×3.33 (m M/L)

Superoxide dismutase (SOD)

Colorimetric that produces a water-soluble formazan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to the xanthineoxidase activity, and is inhibited by SOD. Therefore, the inhibition activity of SOD can be determined by a colorimetric method.

Glutathione reductase (GR)

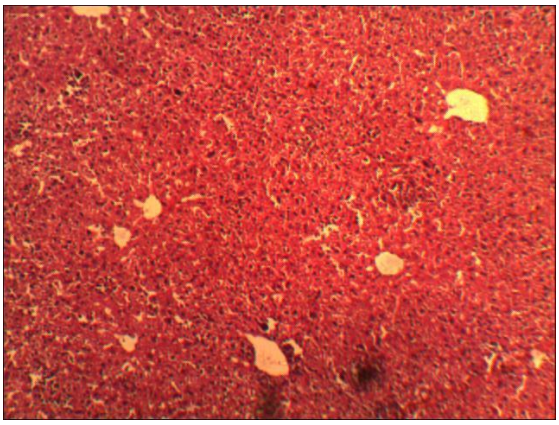
GR reduces GSSG to GSH, which reacts with 5, 5'-Dithiobis (2-nitrobenzoic acid) (DTNB) to generate TNB2- (yellow color, ?max = 405 nm).

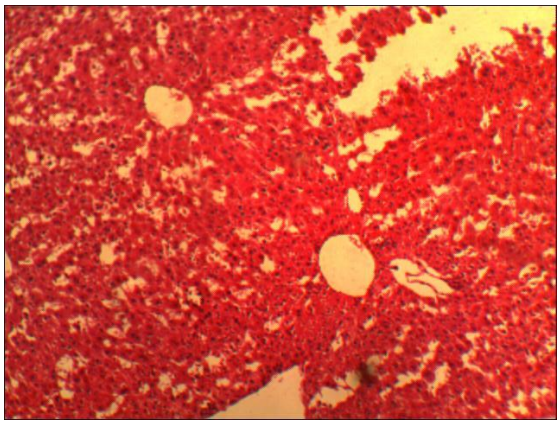
Catalase

It catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. Catalase is a tetramer of four polypeptide chains, and contains four porphyrinheme (iron) groups that allow the enzyme to react with hydrogen peroxide. The optimum pH for human catalase is approximately pH 7, with a fairly broad maximum as the rate of reaction does not change appreciably between pH= 6.8-7.5.

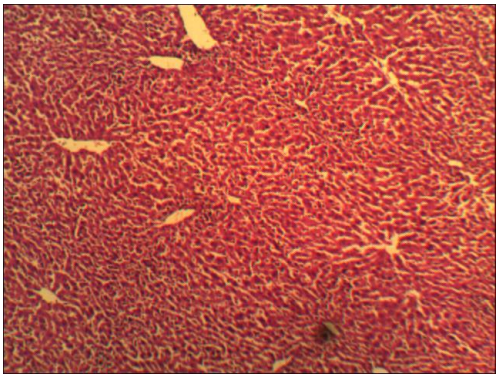
Results and discussion

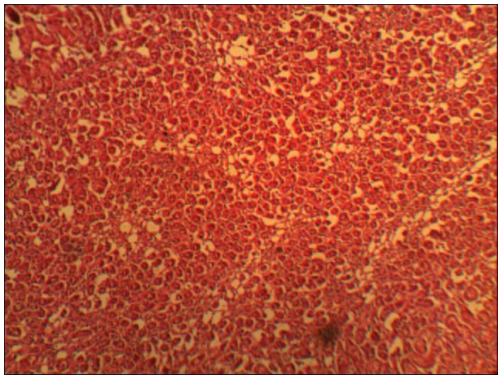
The macroscopic photographs were captured to investigated histopathological integrity for both liver and kidney of irradiated samples relative to control group .The microscopic photos in Figure 1 show no aberrant alteration in the cellular integrity for both liver and kidney samples compared with control.





A: LIVER KINDEY





B: US irradiated Liver US irradiated Kidney

Figure 1 Microscopic photos showing a morphological details of study groups for liver and kidney tissue samples, A: Control samples, and B: US irradiated samples.

Hematological studies

CBC parameters for sample groups were tabulated in Table 1(Mean ± SE). The results are still within normal ranges. There was no significant difference (P<0.01) in values of hemoglobin (HGB), red blood cells (RBCs), and other red blood indices such as haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular heamoglobin content (MCHC) between research groups Table 1.

Table 1:Complete blood picture parameters for study groups.

	RBC (M/µL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (mm/µL)	WBC (mm/µL)
Control	11.4±1.8	14±1.2	44.6±2.4	59.4±2.5	18±1	38±3.2	**671**	**1.4**
US pulsed	12±0.6	15±1.2	46±2.4	61±2.5	20±2	40 ±2.2	559	1.5
US continuous	9.8±1.6	14±1.2	51.6±2.4	66.4±2.5	18±1	38±1	633	1.1

Total Antioxidant activity

The lipid peroxidation activity and malonaldehyde levels are shown in Table2. According to our study, US irradiated groups exhibited significantly high levels of MDA, as compared with the control group. Regarding the antioxidant capacity, all study groups showed decreased activities of antioxidants (SOD, CAT, GR, GST and TAC) in comparison with normal animals(p>0.05). Table2.

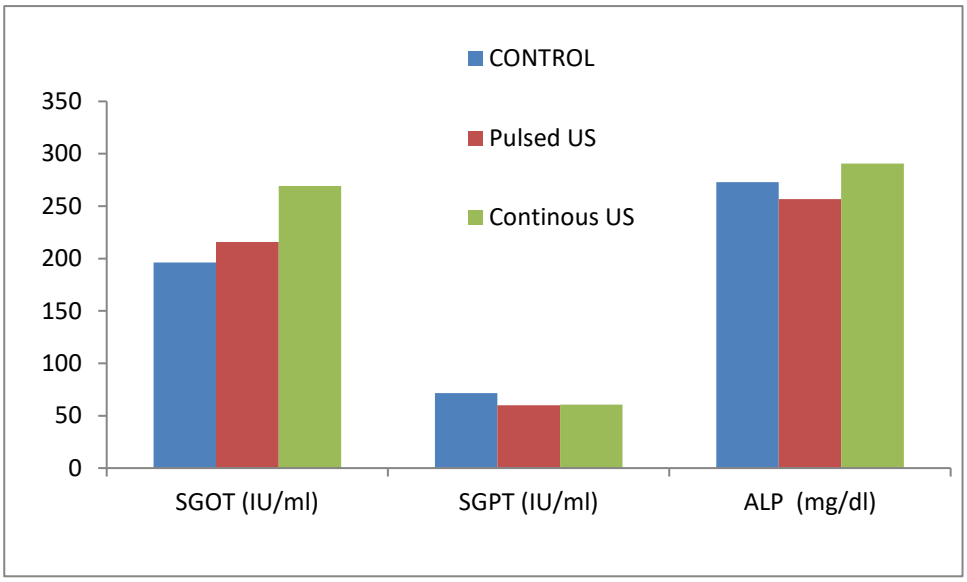
F: F value for ANOVA test, a: Significant with Normal group*: Statistically significant at p ≤ 0.05Data was expressed by using mean ±SD.

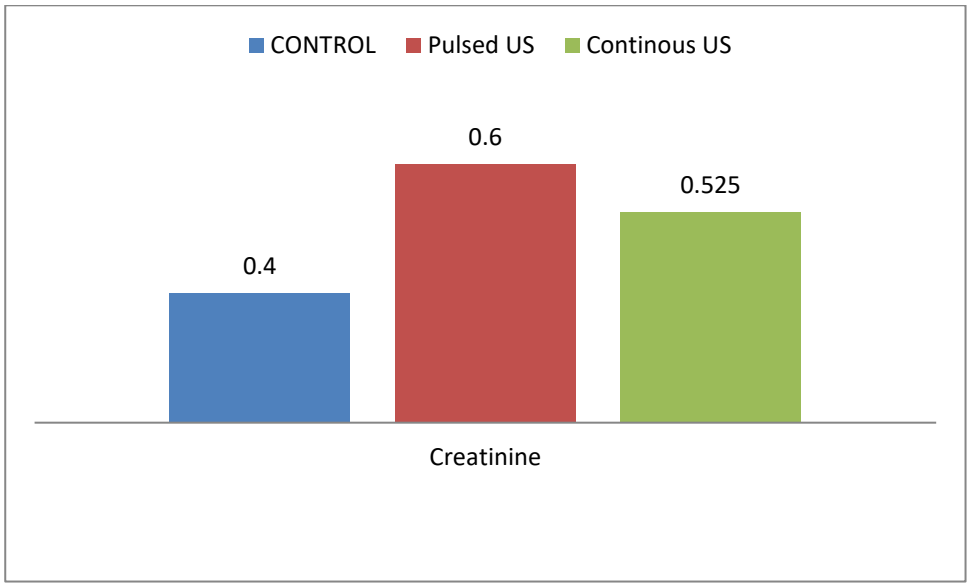
Effects on biochemical parameters

Liver enzymes (ALT, AST) and kidney function parameters (urea and creatinine) are represented in .there is no significant alteration in study groups compared with control group.

Table2 Antioxidants and malonaldehyde levels of study groups.

Group Name	GST (U/ml)	GR (mU/ml)	CAT (mU/ml)	TAC (mM/L)	SOD (U/ml)	MDA (nmol/ml)
Normal	3.95 ± 0.92	0.082 ± 0.0	814.26 ± 3.66	0.73 ± 0.0	1771.5 ± 5.32	24 ± 0.05
Puls. US	1.11 [a] ± 0.11	0.033 [a] ± 0.0	276.35 [a] ± 2.81	0.32 [a] ± 0.0	375 [a] ± 1.16	195.12 [a] ± 1.04
Cont. US	1.32 [a]± 0.0	0.037 [a] ± 0.0	361.52 [a] ± 5.54	0.36 [a] ± 0.0	375.68 [a] ± 0.33	168 [a] ± 1.58
F	**74820.546***	**133.934***	**14462.300***	**206084.277***	**445465.141***	**27015.816***
P	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***





Discussion

Upgrading technology makes our present world as a pool of electromagnetic waves irradiation1.our daily lifestyle aids in increase the rate of exposure including industrial noising, working environment, cell phones, Home-aid tools, microwave, oven, and electric heater and computers.

In the current study, the effect of therapeutic ultrasound waves irradiation on the liver and kidney function of male albino mice was investigated. The outcome results showed a significant no alteration at the hematological and biochemical levels (liver enzymes and kidney function tests) in comparing with non irradiated control group. Histopathological studies revealed no obvious change in cellular ultrastrncture of mice liver and kidney cells in comparing with control groups[12].

Monitoring the oxidative stress parameters, lipid peroxidation indication was evaluated by MDA level, irradiated groups with I.R.L. or U.S. exhibited significantly high levels of MDA, as compared with the control group. This finding was

coincident with work of Luis DT ET al and Errki J V. [4,13,12]. The antioxidants capacity was estimated as preventive line for liver and kidney injury. Enzymatic (like SOD), and nonenzymatioc antioxidants (such as GST) showed a decreased activities in comparison with control group.

In summary, from our finding on irradiated male albino mice, we can occlude that using therapeutic US irradiation with output power from 0.5 - 3W/cm2 and pulsed mode (pulse frequency 1000 Hz, duty ratio 1/3 and average power density from 0.15-1 W/cm^2) can be safely used for therapeutic purpose with no significant alteration in cell integrity of both liver and kidney of male albino mice**.**

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