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The Study of Anti-Cancer Efficacy of Therapeutic Ultrasound in Rats

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Abstract: Globally, cancer is the second most common cause of death, with 70% of deaths due to cancer occurring in low and middleincome people in the countries. Many treatments have been reported for their anticancer potential but most of the treatment options for cancer are not completely effective. Ultrasound is emerging as a novel treatment agent, though the trials are still in their infancy. The advantage of using ultrasound is that it is not an electromagnetic radiation; hence it does not produce the undesired harmful effects encountered through the repeated use of electromagnetic radiation. The present study was aimed to evaluate the chemotherapeutic potential of ultrasound in 7, 12-dimethyl benz(a)anthracene(DMBA) induced sarcoma in rats. Application of the rapeutical (low intensity 1MHz) ultrasound to sarcoma tumor bearing rats for 10 min (continuous mode)was foundtobe effective against DMBA induced sarcoma in female Wistar rats. There were significant increases in the body weight and tumor weight of treated rats. Theincreased activities of lipid peroxidation(MDA), serum and liver path physiological enzymes AST, ALT, ALP, ACP, and LDH in serum and liver of ultrasound treated rats were significantly higher than normal levels indicating loss of redox homeostasis. This has implications for cell death and apoptosis. The levels ofenzymatic antioxidants such as CAT, GPx, SOD, and non-enzymatic antioxidantssuch as GSH, Vitamin C, and Vitamin E weredecreased significantly by administration of DMBA. The levels of antioxidants were further decreased by the application of ultrasound therapy indicating that the tumor cells were unable to counteract the oxidative stress produced due to ultrasound therapy. The histopathological analysis of sarcoma tissues indicated evidence of the anti-tumorigenic nature of ultrasound by showing extensive hemorrhage and necrosis. The results of the present study indicate thatultrasoundsignificantly suppresses DMBA induced sarcoma in rats.

Key words: Ultrasound, Sarcoma, Therapeutics, Cancer, Anti-tumorigenic

I. INTRODUCTION

Cancer is the second leading cause of death globallyand was responsible for 8.8 million deaths in 2015. Globally 1 in 6 deaths is due to cancer. Approximately 70% of deaths from cancer occur in low- and middle-income people of countries[1].Cancer is the name given to a collection of related diseases. In all types of cancer, some of the bodycells begin to divide without stopping and spread into surrounding tissues. There are more than 100 types of cancer. Which are usually named for the organs or tissues where the cancers form.Soft tissue sarcomas can develop from soft tissues like fat, muscle, nerves, fibrous tissues, blood vessels, or deep skin tissues[2].The American Cancer Society estimates that in 2017 in the United States 12,390 new soft tissue sarcomas will be diagnosed and 4,990 Americans are expected to die of soft tissue sarcomas[3,4]. The analysis of large SEER database shows that the age adjusted incidence of sarcomas arising in soft tissues is 3.1/100,000 irrespective of gender. There are no large scale studies in India however an ICMR study finds that among all cancer patients in India, the incidence in children varies between 3.6% at Delhi to 14.8% at Barshi (Maharashtra) among males and 3.7% at Bangalore to 9.5% at Bhopal among females[5].Most of the treatment options available for cancer are not completely effective i.e. the chances of a relapse of tumor or the inability to treat the tumor exist. Most of the options are very costly and a vast majority of the population especially in the Indian context cannot afford them. Consequently there is a need for developing alternative strategies in the fight against cancer. Ultrasound is one of the modalities which is emerging as a novel treatment agent, though the trials are still in their infancy. The advantages of using Ultrasound are that this modality is not an electromagnetic radiation; hence it does not produce the undesired harmful effects encountered through the repeated use of electromagnetic radiation. The mechanisms of ultrasound action on biological material can thus be divided into

thermal and non-thermal. Thermal effects occur when acoustic energy is absorbed and transformed to heat. Production of heat depends on the absorption and dissipation of ultrasound energy. Cavitation leading to the formation of reactive oxygen species and its consequences are of primary interest[6].Recent clinical studies have demonstrated that cancer cells can be targeted and destroyed by a single blast of ultrasound[7].However the extent to which ultrasound affects cancerous tissue is an area of ongoing research and needs to be explored further[8,9,10,11,12].To a large extent, the biophysical effects of therapeutic ultrasound have been examined through in vitro studies. There is relatively little evidence that these changes occur in vivo, and extrapolation of these results to humans is therefore conjectural[13].The potential of ultrasound to provide local tumor control and to enhance other therapy modes has motivated the current efforts by several groups to further study and understand it actions on malignancies.

Our study aimed to study the effect of ultrasound therapy on morphological, biochemical and histopathological changes in sarcoma cancer.

II. MATERIALS AND METHODS

A. Chemicals

7,12-Dimethyl benz (a) anthracene is a known carcinogen which produces mammary and sarcomas in rats[14]. 7, 12-DMBA was purchased from Sigma chemical company (St. Louis MO, USA). All other chemicals used were of analytical grade procured from local commercial sources. The carcinogen mixture was prepared in bio-safety level II lab conditions.

B. Experimental animals

Virgin female Wistarrats,7 weeks of age were purchased from Central Animal House AIIMS New Delhi and were used in the experiment. They were housed spaciously in individual cages and maintained under standard experimental conditions: temperature 25±1^{o}C, relative humidity 60±5% and 12±1 h (light/dark cycle) and fed with a balanced diet of commercially available pellet diet laboratory grade and water ad libitum. The animals were acclimatized for 2 days prior to the start of experiments. The experimental design was performed in accordance with the current ethical norms approved by the CPCEAGovernment of India and Institutional Animal Ethics Committee Guidelines and approved by Institutional Ethics Committee vide approval No. IAEC No: ITS/01/IAEC/2013.

C. Group Allocations

The rats were divided into four groups of ten rats each as follows:

Group 1: Normal control rats.

Group 2: Control rats administered with ultrasound therapy (2.6W/cm^2) Frequency also.

Group 3: DMBA induced sarcoma cancer rats with sham treatment.

Group 4: DMBA induced sarcoma cancer rats administered with ultrasound therapy (2.6W/cm^2)Frequency.

D. Carcinogen treatment

Abdominal sarcoma tumors were induced by DMBA using the “airpouch technique” as described by Arun*et al*. (1984)[15]with slight modifications. Briefly, the air pouch was produced using a 10ml capacity glass syringe in the abdominal region. About 2-3ml of air was drawn into the syringe, and a rubber cork was fixed to the needle tip in such a way that the plugging was airtight. The whole set was wrapped with aluminum foil and autoclaved at 15 psi for 20 min. The sterile air (1-2 ml) was carefully injected subcutaneously just beneath the abdominal fat pad so as to produce a pouch containing sterile air. The air inside was allowed to remain for a day to form a pouch. A single dose of 7, 12-DMBA (25 mg/kg BW/rat) in 0.5 ml of corn oil was vortexed to obtain a uniform emulsion and carefully injected into the air pouch.

E. Ultrasound Therapy

1) Preparation: Before ultrasonic therapy application in rats a tail vein is catheterizedand general anaesthesia is induced and maintained with 2% is of lurane and air. A depilatory cream was used to remove the hair coat from the tumor site and US coupling gel is applied to the skin.

2) Tumor insonation: After the tumor has grown (over 3 to 4 weeks) to a minimum size of 1 cm in at least one dimension, the tumor was insonated with a physiotherapy ultrasound machine (1-MHz , continuous output, power level = 2; Chattanooga Corp, USA). In the experimental group the tumor was insonated at 2.6 W cm−2, 1 MHz, continuous duty cycle) for a total of 10 minutes with cooling allowed after each 5 minutes. There will be a gap of 5 min between each 5-min period of insonation, during which the face of the probe will be cooled in tap water at room temperature. In a further group of animals (sham group); the ultrasonic probe will

be applied to the tumor for a total of 10 min, with 5-min intervals between each 5 min of application, as described above, but the machine will not be switched on.

3) Optimum Dose Fixation: Graded doses (2.2W/cm^2,2.6W/cm^2,3.0 W/cm^2)Frequency of ultrasound therapy was administeredtotumor bearing rats to fix the optimum dosage of ultrasound therapy. It was observed that ultrasound treatment at doses of 2.6W/cm^2 and 3.0 W/cm ^{2} significantly altered the levels of tumor markers as well as activities of liver marker enzymes. However, there was no significant difference in the levels of tumor markers and liver marker enzymes activity in rats treated with 2.2 W/cm ^{2} of ultrasound therapy. Hence, a minimum dose of 2.6 W/cm^2 was fixed as the optimum dose for the study.

4) Blood Sample Collection: All rats were palpated every week to monitor the onset of tumor genesis z. Tumor yield and size were stabilized 90 days after the initiation with DMBA. After the experimental period, all animals were fasted overnight and sacrificed by sodium pentothal anesthesia followed by cardiac puncture. Blood was collected with and without anticoagulant (EDTA) and the serum was centrifuged at 5000 rpm for 15 min to obtain a clear supernatant and stored at -80^0 C until its use for further biochemical analysis. Liver tissues from control and experimental groups of rats were immediately excised, washed in ice-cold PBS to remove the blood stains, blotted, weighed and homogenized in Tris-HCl buffer (0.1 M, pH 7.4) using a Teflon homogenizer to prepare 10% (w/v) tissue homogenate. This homogenate was centrifuged at 12,000 g for 30 min at 4^o C to obtain a clear supernatant. This supernatant was pooled and used for further analysis.

5) Body and tumor weight; The total body weight gain of the control and experimental animals was recorded periodically throughout the experimental period. The tumor volume was estimated according to the method of Gera*net al.* (1972)[16]. Briefly, the resultant solid tumor was considered to be prelate ellipsoid with one long axis and two short axes. The two short axes were measured with verniercalipers. The tumor volume was calculated using the formula:

Tumor volume = [length (cms) x width^{2}cms] / 2

6) Biochemical analysis

a) Estimation of liver marker enzymes: The products of lipid peroxidation, lipid hydroperoxides, and aldehydes, such as MDA, were measured via a thiobarbituric acid reactive substances (TBARS) assay. In this assay, performed on in cell lysate, MDA combines with thiobarbituric acid (TBA) to form a fluorescent adduct that can be detected at an excitation wavelength of 530 nm and an emission wavelength of 550 nm. The results are then expressed as MDA equivalents, normalized to total cellular protein (determined by Bradford assay)[17].The activity of cytosolic marker enzymes such as AST and ALT were assayed by the method of Bergmeyer*et al.* (1978)[18].Alkaline phosphatase and Acid phosphatase activities were estimated by the method of King (1965a)[19] as described by Balasubramanian*et al.* (1983)[20]using disodium phenyl phosphate as substrate. Lactate dehydrogenase (LDH) was assayed by the method of King (1965b)[21]using lithium lactate as substrate.

b) Assay for liver and serum enzymatic and non-enzymatic antioxidants: The enzymatic antioxidant, superoxide dismutase (SOD) activity was measured spectrophotometrically as the degree of inhibition of autoxidation of pyrogallol in an alkaline pH at an absorbance of 420 nm[22]. In the catalase (CAT) activity assay, dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2) with the formation of perchloric acid as unstable intermediate and chronic acetate thus formed was measured spectrophotometrically at 570 nm. The results were expressedin terms of µmol H_2O_2 liberated/min/mg protein[23]. For the glutathione peroxidase (GPx) activity assay, the reaction mixture containing 0.2 ml of EDTA (0.8 mM pH 7.0), 0.4 ml of phosphate buffer (10 mM) and 0.2 ml of enzyme source were incubated with 0.1 M of H_2O_2 and 0.2 ml of glutathione for 10 min. Oxidation of glutathione by the enzyme was measured spectrophotometrically at 420 nm. The activity of GPx, was expressed asmmol glutathione oxidized/mg protein[24]. The non-enzymatic antioxidants Vitamin C, Vitamin E and reduced glutathione (GSH) were also estimated. In the reduced glutathione (GSH) assay, 1 ml of the sample was precipitated with 1 ml of TCA and centrifuged at 1200 g for 20 min. To 0.5 ml of supernatant, 2 ml of 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was added and the color developed was read immediately at 412 nm using a spectrophotometer[25]. Vitamin E (a-tocopherol) assay estimated the levels of ferric ions which were reduced to ferrous ions in the presence of tocopherol and bathophenanthroline to form a pink colored substance, which was read at 520 nm using a spectrophotometer[26]. In the Vitamin C (ascorbic acid) assay, 1 ml of ethanol was added to 1 ml of sample and then mixed thoroughly after which 3 ml of petroleum ether was added and the reaction mixture was centrifuged. The supernatant was evaporated to dryness and 0.2 ml each of bathophenanthroline, ferric chloride and O-phosphoric acid were added to reach a total volume of 3 ml with ethanol. The color developed was measured at 530 nm[27].

7) Histopathology: The tumor tissue was immediately fixed in 10% neutral buffered formalin, embedded in paraffin, 5 µm section was cut using a microtome and then rehydrated with xylene and graded series of ethanol. The specimens were then stained with

Hematoxylin and Eosin. The H & E stained breast specimens were examined by apathologist to histopathologically classify the tumors as described by Russo et al. (1990)[28].

8) Statistical analysis: Statistical analysis was performed using SPSS (SPSS Inc.,Chicago) statistical package. The results were expressed as Mean, Standard Error Mean (SEM). One-way analysis of variance (ANOVA) followed by post hoc test least significant difference (LSD) was used to correlate the difference between the variables. Values were considered statistically significant if $p < 0.05$.

III. RESULTS

A. *Analysis of Body Weight and Tumor weight of Rats*

Table 1 Effect of Ultrasound therapy treatment on total body weight and tumor weight of control and DMBA treated rats.

Groups	Body Weight (g)	Tumor Weight (g)
Control	219.46 ± 4.18	-
Control + US	211.82± 6.35[a]	-
DMBA	154.33±4.87[b,c]	15.45±1.11
DMBA + US	176.21±3.12 [d,e]	11.28±1.32[e]

Results are expressed as Mean± S.E.M (n=10). [a]P>0.05 compared with control group of rats. [b]P<0.05 compared with control group of rats, [c]P<0.05 compared with Control+US group of rats, [d]P<0.05 compared with Control+US group of rats, [e]P<0.05 compared with DMBA induced group of rats.

Table 1 shows the body weight of control and experimental rats. The body weight of control Group I rats (219.46 g) was significantly higher as compared to Group III rats (154.33 g) following DMBA treatment (P<0.05). The body weight of DMBA induced Group IV rats following ultrasound therapy treatment was significantly higher (176.21 g) as compared to Group III rats(P<0.05). But, no statistically significant changes could be observed in the body weight of Group II rats treated with Ultrasound therapy (211.82 g) as compared to Control Group I rats (219.46 g) (P>0.05).

Table 2 Effect of ultrasound therapy on plasma malondialdehyde level

Group/Enzyme (U/L)	Control	Control + Ultrasound	DMBA	DMBA +Ultrasound
MDA	0.8±0.02	2.87±0.06 [a]	1.82±0.03[b, c]	4.13±0.02[d,e]

Results are expressed as Mean± S.E.M (n=10). [a]P<0.05 compared with control group of rats. [b]P<0.05 compared with control group of rats, [c]P<0.05 compared with Control+US group of rats, [d]P<0.05 compared with Control+US group of rats, [e]P<0.05 compared with DMBA induced group of rats.

Table 2 shows the plasma malondialdehyde (MDA) level of control and experimental rats. The serum MDA level of control Group I rats (0.8 nmol/mg of protein) significantly increased to 2.87 nmol/mg of protein in group II rats following ultrasound therapy treatment (P<0.05). Ultrasound therapy treatment to DMBA induced Group IV rats following ultrasound therapy treatment significantly increased the MDA level to 4.13 nmol/mg of protein as compared to Group III rats where the MDA level was 1.82 nmol/mg of protein (P<0.05).

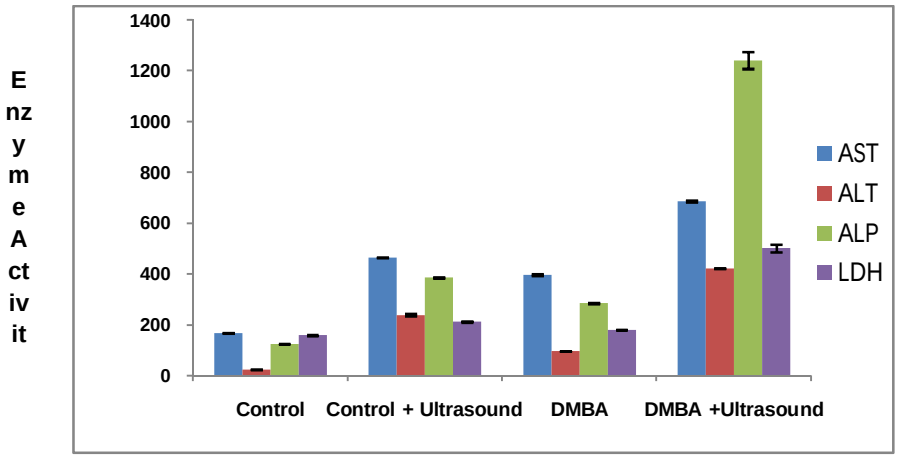


Figure1: Comparison of Serum Enzyme Activity Levels in Control and Experimental rats,Results are expressed as Mean± S.E.M (n=10). [a]P<0.05 compared with control group of rats. [b]P<0.05 compared with control group of rats, [c]P<0.05 compared with Control+US group of rats, [d]P<0.05 compared with Control+US group of rats, [e]P<0.05 compared with DMBA induced group of rats.

Figure 1 shows the serum level of pathophysiological enzymes AST, ALP, ALT, LDH in control and experimental rats. The serum level of pathophysiological enzymes in control Group II rats following ultrasound therapy treatment was significantly increased as compared to control Group I rats (P<0.05). Ultrasound therapy treatment to DMBA induced Group IV rats following ultrasound therapy treatment significantly increased the level of pathophysiological enzymes as compared to Group III rats(P<0.05). The treatment of DMBA induced rats with ultrasound therapy significantly increased the level of serum pathophysiological enzymes viz.,AST from 395.41 to 686.87 U/L, ALT from 95.65 to 422.22 U/L, ALP from 285.64 to 1242 U/L, LDH from 179.90 to 501.48 U/L (P<0.05).

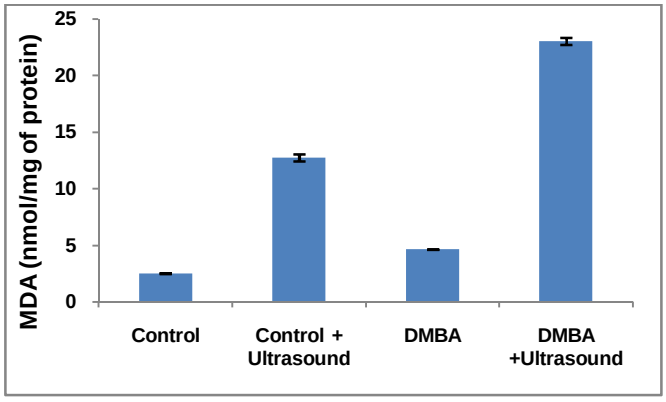


Fig. 2:Comparison of Liver Malondialdehyde Activity Levels in Control and Experimental rats, Resultsare expressed as Mean± S.E.M (n=10). [a]P<0.05 compared with control group of rats. [b]P<0.05 compared with control group of rats, [c]P<0.05 compared with Control+US group of rats, [d]P<0.05 compared with Control+US group of rats, [e]P<0.05 compared with DMBA induced group of rats.

Figure 2 shows the liver Malondialdehyde (MDA) level of control and experimental rats. The liver MDA level of control Group I rats (2.5 nmol/mg of protein) was significantly increased to 12.73 nmol/mg of protein in group II rats following ultrasound therapy treatment(P<0.05). Ultrasound therapy treatment to DMBA induced Group IV rats following ultrasound therapy treatmentsignificantly increased the MDA level to 23.03 nmol/mg of protein as compared to Group III rats where the MDA level was 4.61 nmol/mg of protein(P<0.05).

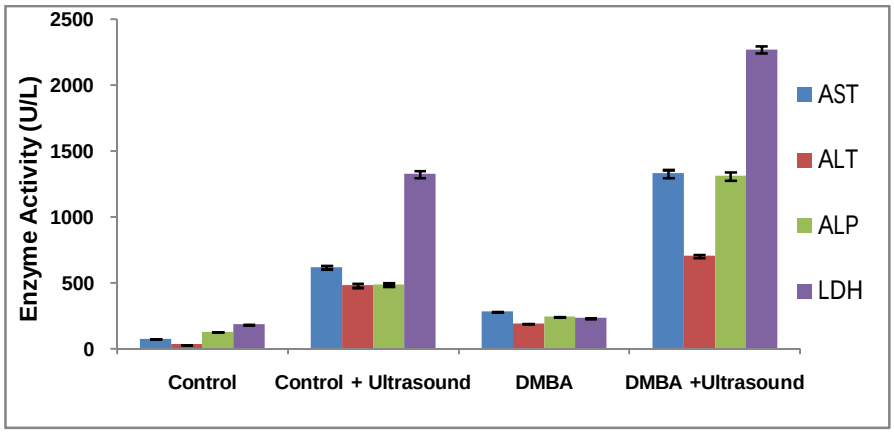


Fig. 3:Comparison of Liver Enzyme Activity Levels in Control and Experimental rats, Resultsare expressed as Mean± S.E.M (n=10). [a]P<0.05 compared with control group of rats. [b]P<0.05 compared with control group of rats, [c]P<0.05 compared with Control+US group of rats, [d]P<0.05 compared with Control+US group of rats, [e]P<0.05 compared with DMBA induced group of rats.

Figure 3 shows the level of pathophysiological enzymesAST,ALP,ALT,LDH in control and experimental rats in the liver. The level of pathophysiological enzymes in liver of control Group II rats following ultrasound therapy treatment was significantly higher as compared to control Group I rats (P<0.05). Ultrasound therapy treatment to DMBA induced Group IV rats significantly increased the level of pathophysiological enzymes as compared to Group III rats. The treatment of DMBA induced rats with ultrasound therapy significantly increased the level of liver pathophysiological enzymes viz.,AST from 280.48 U/L to 1328 U/L, ALT from 190.46 U/L to 703.30 U/L, ALP from 242.28 to 1309 U/L, LDH from 231.35 U/L to 2269 U/L (P<0.05).

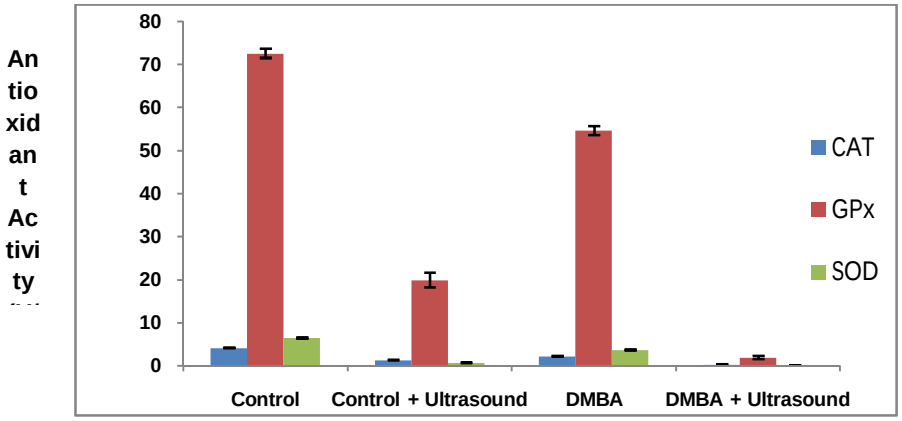


Fig. 4:Comparison of Serum Antioxidant Enzyme Activity in Control and Experimental rats, Results are expressed as Mean± S.E.M (n=10). [a]P<0.05 compared with control group of rats. [b]P<0.05 compared with control group of rats, [c]P<0.05 compared with Control+US group of rats, [d]P<0.05 compared with Control+US group of rats, [e]P<0.05 compared with DMBA induced group of rats.

In the present study, changes in the activities of Catalase (CAT), Glutathione peroxidase (GPx), Superoxide dismutase (SOD) were investigated. Fig. 4 shows the level of anti-oxidant enzymes CAT,GPx,SOD in control and experimental rats in serum. The serum level of anti-oxidant enzymes in control Group II rats following ultrasound therapy treatmentwas significantly lower as compared to control group I rats (P<0.05). Ultrasound therapy treatment to DMBA induced Group IV rats lowered the level of anti-oxidant enzymes as compared to Group III rats (P<0.05). The treatment of DMBA induced rats with ultrasound therapy lowered the level of serum anti-oxidant enzymes CAT from 2.31 U/L to 0.36 U/L, GPx from 54.70 U/L to 1.99 U/L, SOD from 3.70 U/L to 0.10 U/L (P<0.05).

Figure 5 shows the level of anti-oxidant enzymes CAT,GPx,SOD in control and experimental rats in liver. The liver level of anti-oxidant enzymes in control Group II rats following ultrasound therapy treatmentwas significantly lower as compared to control group I rats (P<0.05).

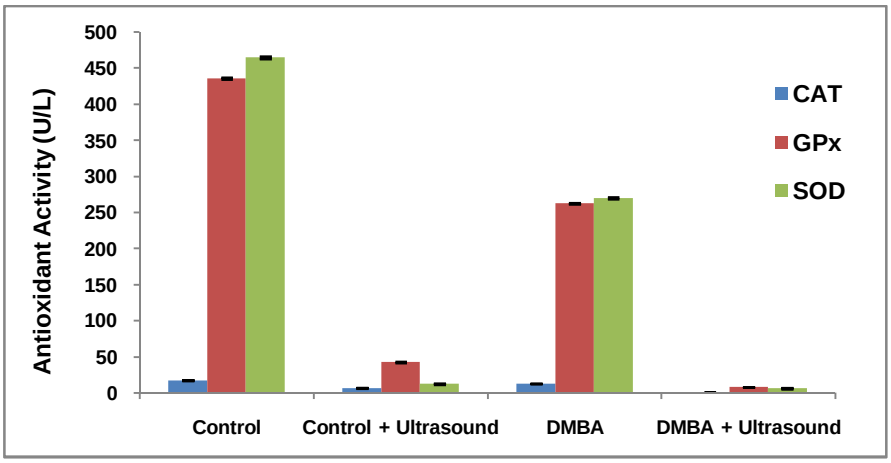


Fig. 5: Comparison of Liver Antioxidant Enzyme Activity levels in Control and Experimental rats

Results are expressed as Mean± S.E.M (n=10). [a]P<0.05 compared with control group of rats. [b]P<0.05 compared with control group of rats, [c]P<0.05 compared with Control+US group of rats, [d]P<0.05 compared with Control+US group of rats, [e]P<0.05 compared with DMBA induced group of rats.

Ultrasound therapy treatment to DMBA induced Group IV rats lowered the level of anti-oxidant enzymes as compared to Group III rats (P<0.05).The treatment of DMBA induced Group 4 rats with ultrasound therapy lowered the level of serum anti-oxidant enzymes CAT from 13.39 U/L to 1.47 U/L, GPx from 262.93 U/L to 8.44 U/L, SOD from 270.72 U/L to 7.03 U/L (P<0.05).

B. Effect of ultrasound therapy on serum non-enzymatic antioxidants

The levels of serum non-enzymatic antioxidants namely total glutathione (GSH), vitamin C and vitamin E are presented in figure 8.

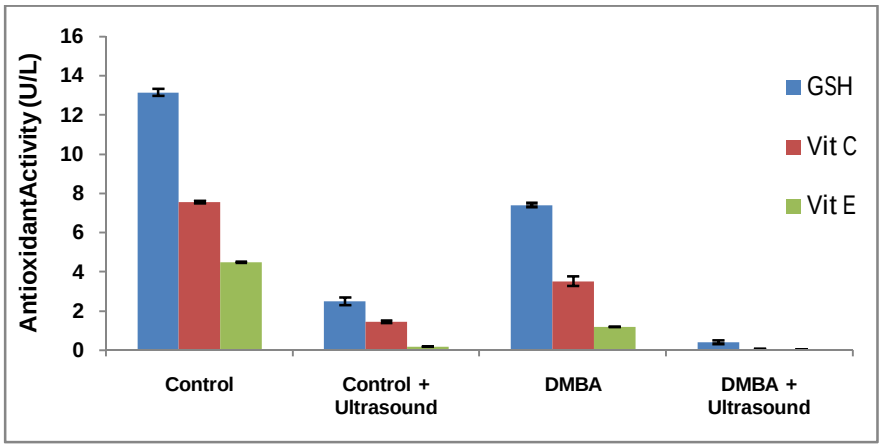


Fig. 6: Comparison of Serum Non-Enzymatic Antioxidant Activity Levels in Control and Experimental rats, Results are expressed as Mean± S.E.M (n=10). [a]P<0.05 compared with control group of rats. [b]P<0.05 compared with control group of rats, [c]P<0.05 compared with Control+US group of rats, [d]P<0.05 compared with Control+US group of rats, [e]P<0.05 compared with DMBA induced group of rats.

Figure 6 shows the level of non-enzymatic anti-oxidants GSH,Vit C, Vit E in control and experimental rats in serum. The serum level of non-enzymatic anti-oxidants in control Group II rats following ultrasound therapy treatment was significantly lower as compared to control group I rats (p<0.05). Ultrasound therapy treatment to DMBA induced Group IV rats significantly lowered the level of non-enzymatic anti-oxidants as compared to Group III rats (p<0.05).The treatment of DMBA induced rats with ultrasound therapy significantly lowered the level of serum non-enzymatic anti-oxidants namely GSH from 7.42 U/L to 0.43 U/L, Vit C from 3.54 U/L to 0.08 U/L, Vit E from 1.2 U/L to 0.05 U/L (p<0.05).

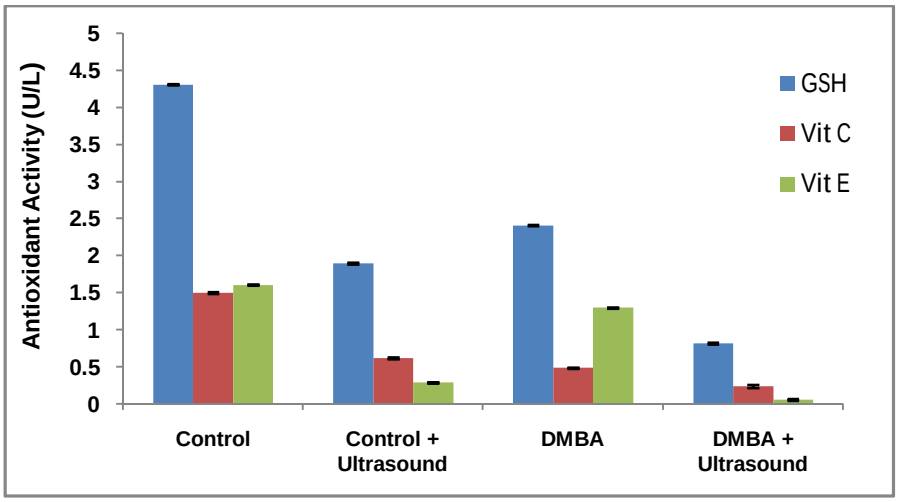


Fig. 7: Comparison of Liver Non-Enzymatic Activity levels in Control and Experimental rats

Results are expressed as Mean± S.E.M (n=10). [a]P<0.05 compared with control group of rats. [b]P<0.05 compared with control group of rats, [c]P<0.05 compared with Control+US group of rats, [d]P<0.05 compared with Control+US group of rats, [e]P<0.05 compared with DMBA induced group of rats.

Figure 7 shows the level of non-enzymatic anti-oxidants GSH,Vit C, Vit E in control and experimental rats in liver. The serum level of non-enzymatic anti-oxidants in control Group II rats following ultrasound therapy treatment was significantly lower as compared to control group I rats ($p<0.05$).. Ultrasound therapy treatment to DMBA induced Group IV rats significantly lowered the level of non-enzymatic anti-oxidants as compared to Group III rats. The treatment of DMBA induced rats with ultrasound therapy significantly lowered the level of liver non-enzymatic anti-oxidants namely GSH from 2.41 U/L to 0.82 U/L, Vit C from 0.50 U/L to 0.24 U/L, Vit E from 1.3 U/L to 0.06 U/L.

C. Histopathology analysis of Sarcoma

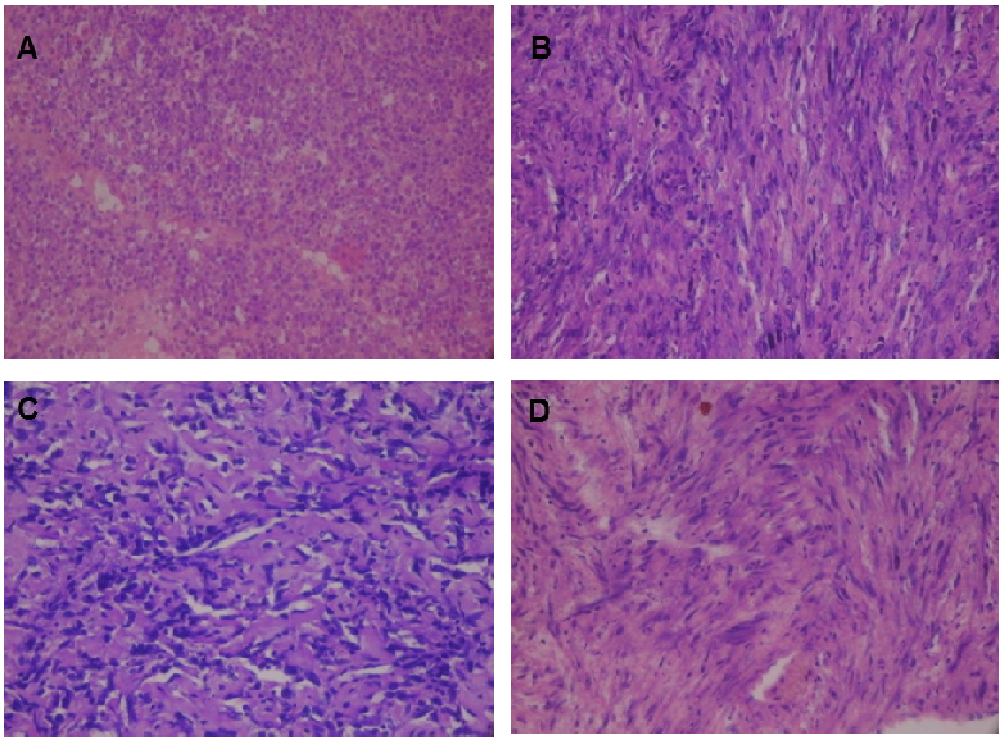


Fig. 8: Histological analysis of sarcoma tissue in control and experimental group of rats,

(A)Control rats showed normal architecture i.ehyalinizedstroma composed of round cell population. (B) Control + Ultrasound Therapy treated animals exhibited marked areas of necrosis surrounded by mixed inflammatory infiltrate of acute and chronic cells. (C) DMBA induced tumor rats showed sarcoma with loss of normal architecture. Round cell population showing marked anaplastic features of pleomorphism, hyperchromatism, anisonucleosis and anisocytoses with 4-5 mitotic figures. Prominent vasculature can be appreciated with proliferating endothelial cells. (D) DMBA and Ultrasound therapy treated rats in addition to all the features of (C) above showed marked areas of necrosis and haemorrage.

IV. DISCUSSION

Various mechanisms contribute to weight loss of the host in cancerous condition. No significant changes could be observed in the final body weight of control Group II rats treated with ultrasound therapy in comparison to control Group I rats. This shows that ultrasound therapy in control group did not alter the anabolic metabolism of the rats. Group 3 (DMBA) rats and in Group 4 (DMBA + ultrasound) rats showed significant reduction in body weight. This is because cancer causes cachexia i.egeneralized weight loss[29]. On comparison Group 4 rats showed positive effect of ultrasound therapy by significant reduction in tumor volume. We believe that it may be due the arrest of tumor progression in Group 4 rats[30,31].

In our study there was a marked increase in plasma/serum and liver concentrations of pathophysiological enzymes in Group 2, Group 3 and Group 4 in comparison to Group 1. These changes further exacerbate the whole body inflammatory response into vicious cycle of accelerating organ dysfunction[32-39]. We postulate that the increase in serum and liver pro-oxidant enzymes are attributable to the thermal effects and chemical effects caused by ultrasound on normal cells[40-50].

Ultrasound therapy can cause rapid increase in reactive oxygen species (ROS) levels even in normal Group 2 cells**.**.This may be due to an increase in lipid peroxidation and resultant increase in plasma levels of lipid peroxide after a thermal injury[51-57].

The elevated levels of pro-oxidant enzymes in Group 3 (DMBA group) indicate that there was an oxidative stress environment within the cancerous cells. This is in agreement with studies which show that a moderate increase in ROS can promote cell proliferation and differentiation[58-64].

DMBA is a chemical carcinogen and causes gradual changes in the redox homeostasis of the cells, whereas ultrasound therapy causes sudden oxidative stress. The oxidative stress response in Group 2 caused damage to cells in the form of necrosis. The rapid increase in oxidative stress within a short span of time may lead to cellular damage as postulated by many studies[65-74]. Ultrasound therapy to cancerous cells caused an exorbitant rise in the level of oxidative enzymes and leads to cell death[75-77]. We believe that this is because redox homeostasis within the cancerous cells has been lost and hence the cancerous cells may not be able to produce anti-oxidants at a rate required to neutralize the oxidative stress during exposure to ultrasound therapy[78]. In Group 2 (Control + Ultrasound group) the level of oxidative stress enzymes was lower because the normal cells are able to produce some amounts of anti-oxidant enzymes which can neutralize the oxidative stress enzymes[79-80].There was cellular damage, haemorrhage and necrosis after ultrasound therapy. These effects range from haemorrhage to complete cellular disruption. This is attributable to collapse of bubbles of inertial cavitations[81-83]. Sonication can trigger apoptosis in both normal and malignant cells[84-88]. Our findings revealed that low intensity ultrasound markedly kills cells by damaging the ultrastructure and morphology[89-92]. In our study the sarcoma tumor was particularly susceptible to ultrasonic therapy. This correlated well with other in vitro studies[93-94]. Emerging evidence has confirmed that low-intensity ultrasound markedly inhibits the proliferation and clone formation of tumor cells through heat, mechanical effects and acoustic cavitation[70,71]. Cellular necrosis may be due to autophagy in the tumors[95-96]. The induction of autophagy by ultrasound therapy may lead to a substantial improvement in antitumor therapy.

V. CONCLUSION

In conclusion, the results of the present studyclearly establish the anticancer efficacy of ultrasound therapy against DMBA induced sarcomain rats. Also, the alteration inthe levels of tumorbiomarker marker enzymes indicates the antitumor activity of ultrasound therapy. Our results underlie the potency of ultrasound therapy as an effective therapeutic agent in the treatment of cancer.However, further studies are warranted to elucidate the exactmolecular mechanism underlying the action of ultrasound in reducing the toxic effects of DMBA in sarcoma cancer.

REFERENCES

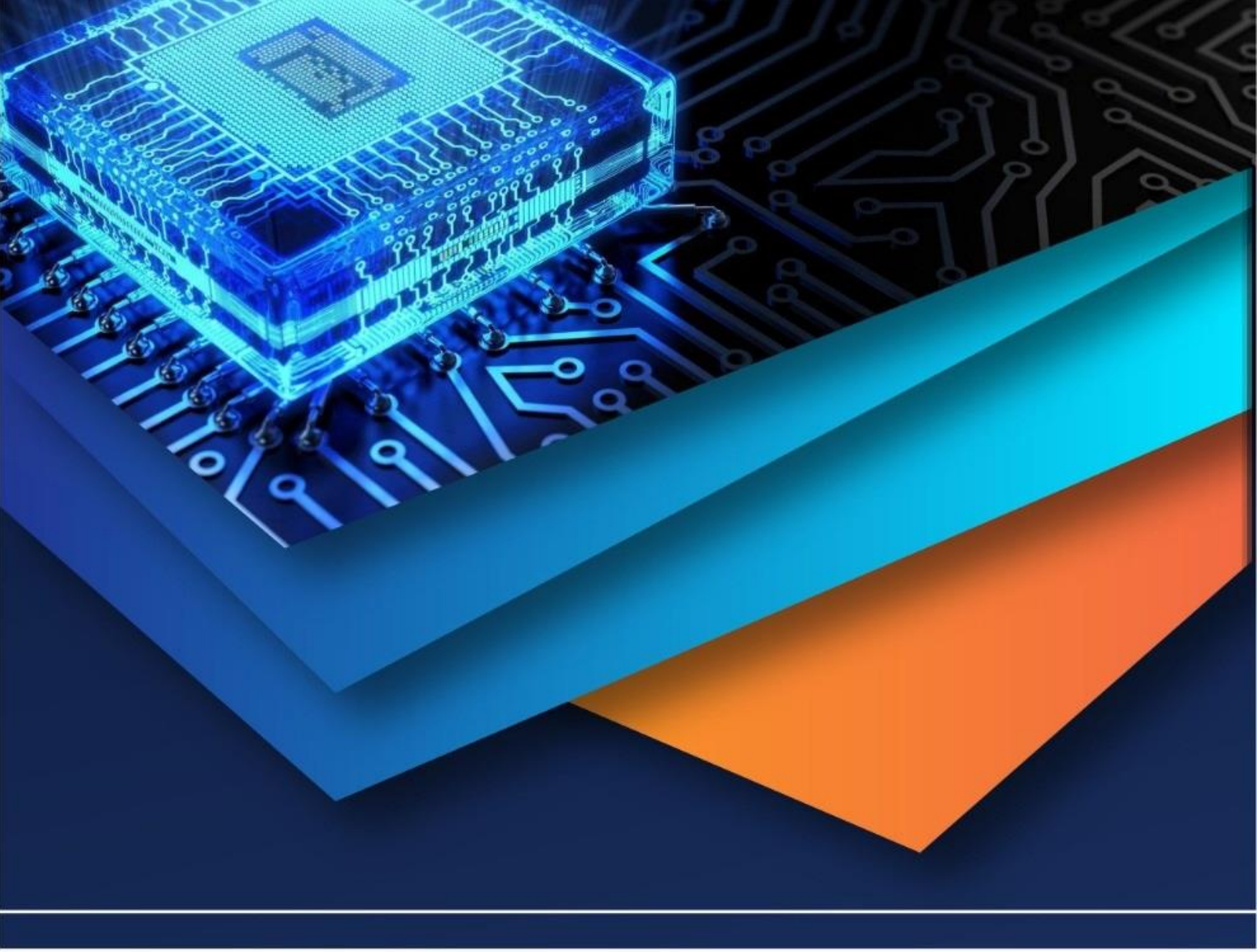
[1] Cancer [Homepage on the Internet].World Health Organization: Media Centre. 2017[updated Feb 2017, cited Dec 2017]. Available from http://www.who.int/mediacentre/ factsheets /fs297/en/

[2] What is cancer.[Homepage on the Internet] NIH. National Cancer Institute. 2017 [updated 9 Feb 2015, cited 18 Dec 2017]. Available from https://www.cancer.gov/about-cancer/ understanding/what-is-cancer

[3] All Cancers (excluding non-melanoma skin cancer) Estimated Incidence, Mortality and Prevalence Worldwide in 2012: Globocan Fact Sheets by Cancer.[Homepage on Internet]. International Agency for Research on Cancer. World Health Organization. 2012 [updated 18 Dec 2017, cited 18 Dec 2017] Available from http://globocan.iarc.fr/Pages/ fact_sheets_ cancer.aspx.
[4] Understanding Advanced Cancer, Metastatic Cancer, and Bone Metastasis.[Homepage from Internet]. American Cancer Society. 2017 [updated 15 Dec 2016, cited 18 Dec 2017]. Available from https://www.cancer.org/treatment/understanding-your-diagnosis/advanced-cancer/what-is.html
[5] Consensus Document for Management of Soft Tissue Sarcoma and Osteosarcoma.[Homepage on Internet]. Indian Council of Medical Research: 2016 [cited 18 Dec 2017]. Availablefromhttp://www.icmr.nic.in/guide/cancer/SARCOMA%20AND%20OSTEO SARCOMA%207.7.2017%20(1).pdf
[6] Baker, Kerry G; Robertson, Valma J; Duck, Francis A,A Review of Therapeutic Ultrasound: Biophysical Effects:PhysTher. 2001 Jul;81(7):1351-8.
[7] Paul A. Prentice, Donald McLean, Alfred Cuschieri, KishanDholakia, Mark R. Prausnitz& Paul Campbell Membrane disruption by optically controlled cavitation Nature-Physics 1 107-110 (2005)
[8] Husseini GA, Pitt WG.The use of ultrasound and micelles in cancer treatment.JNanosciNanotechnol. 2008 May;8(5):2205-15.
[9] Milowska K, Ultrasound--mechanisms of action and application in sonodynamic therapy. PostepyHig Med Dosw (Online). 2007 Jun 1;61:338-49.
[10] Huber PE, Debus J., Tumor cytotoxicity in vivo and radical formation in vitro depend on the shock wave-induced cavitation dose. Radiat Res. 2001 Sep;156(3):301-9.
[11] Husseini GA, Pitt WG, Ultrasonic-Activated Micellar Drug Delivery for Cancer Treatment. , J Pharm Sci. 2009 Mar; 98(3): 795–811.
[12] Oerlemans C, BultW,Polymeric Micelles in Anticancer Therapy: Targeting, Imaging and Triggered Release. Pharm Res. 2010 Dec; 27(12): 2569–2589.
[13] WD O'Brien, Therapeutic Ultrasound Mechanism to Applications, Chap2: Thermal and Non-Cavitational Mechanisms, 7-38.
[14] James W Flesher et. al., Carcinogenicity of the derivatives of 7,12Dimethylbenz(a)anthracene., Cancer Research Dec 1971, Vol 31, 1951-1954.
[15] B. Arun, M. Udayachander, A. Meenakshi, 7, 12-Dimethylbenzanthrace induced mammary tumors in Wistar rats by “air pouch” technique - a new approach, Cancer Lett. 25 (2) (1984) 187-194.
[16] R. Geran, N. Greenberg, M. MacDonald, A. Schumacher, B. Abbott, Protocols for screening chemical agents and natural products against animal tumors and other biological systems, CancerChemother. Rep. 3 (1972) 61-63.
[17] Potter TM, Neun BW, Stern ST. Assay to detect lipid peroxidation upon exposure to nanoparticles. Methods Mol Biol. 2011;697:181-9.
[18] H.U. Bergmeyer, P. Schiebe, A.W. Wahlefeld, Optimization of methods for aspartate aminotransferase and alanine aminotransferase, Clin. Chem. 24 (1978) 58-73.
[19] J. King, The transferases-alanine and aspartate transaminases, in: Practical and Clinical Enzymology, Van Nostrand Co. Ltd, London, 1965a, pp. 121- 138.
[20] M.P. Balasubramanian, S. Dhandayuthapani, Comparative studies on phosphomonoesterase in helminthes, Helminthologia 20 (1983) 111-120.
[21] J. King, The dehydrogenases or oxidoreductase-lactate dehydrogenase, in:Practical Clinical Enzymology, Van D. Nostrand Co. Ltd, London, 1965, pp. 83-93.
[22] S. Marklund, G. Marklund, Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, Eur. J. Biochem. 47 (1974) 469-474.
[23] A.K. Sinha, Colorimetric assay of catalase, Anal. Biochem. 47 (1972) 389-394.
[24] J.T. Rotruck, A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman, W.G. Hoekstra, Selenium: biochemical role as a component of glutathione peroxidase, Science 179 (1973) 588-590.
[25] M.S. Moron, J.W. DePierre, B. Mannervik, Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver, Biochim.Biophys. Acta 582 (1979) 67-78.
[26] I.D. Desai, Vitamin E analysis methods for animal tissues, Meth. Enzymol. 105 (1984) 138- 147.
[27] S.T. Omaye, J.D. Turnbull, H.E. Sauberlich, Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids, Meth. Enzymol. 62 (1979) 3-11.
[28] J. Russo, B.A. Gusterson, A.E. Rogers, I.H. Russo, S.R. Wellings, M.J. van Zwieten, Comparative study of human and rat mammary tumorigenesis, Lab Invest. 62 (1990) 244-273.
[29] T.P. Stein, Cachexia, gluconeogenesis and progressive weight loss in cancer patients, J. Theor. Biol. 73 (1978) 51-59.
[30] KeshavarziA(1), Vaezy S, Noble ML, Chi EY, Walker C, Martin RW, Fujimoto VY. Treatment of uterine leiomyosarcoma in a xenograft nude mouse model using high-intensity focused ultrasound: a potential treatment modality for recurrent pelvic disease. GynecolOncol. 2002 Sep;86(3):344-50.
[31] Huber PE, Debus J. Tumor cytotoxicity in vivo and radical formation in vitro depend on the shock wave-induced cavitation dose.Radiat Res. 2001 Sep;156(3):301-9.
[32] Gibran NS, Heimbach DM. Current status of burn wound pathophysiology. ClinPlast Surg. 2000 Jan;27(1):11-22.
[33] Miyoshi K, Tsukada S, Yasuda Y, Kawakami S, Sakurai T, Matsuda Y, Ito T, Matsuno H. Hepatic disorder in burn patients. Burns InclTherm Inj. 1985 Oct;12(1):49-53.
[34] Nagane NS, Bhagwat VR, Subramanium M. Increased free radical activity in burns. Indian J Med Sci. 2003 Jan;57(1):7-11.
[35] Nagane NS, Ganu JV, Bhagwat VR, Subramanium M. Efficacy of topical honey therapy against silver sulphadiazine treatment in burns: A biochemical study. Indian J ClinBiochem. 2004 Jul;19(2):173-6.
[36] Chiarelli A, Casadei A, Pornaro E, Siliprandi L, Mazzoleni F. Alanine and aspartate aminotransferase serum levels in burned patients: a long-term study. J Trauma. 1987 Jul; 27(7):790-4.
[37] Kumar R(1), Seth RK, Sekhon MS, Bhargava JS. Serum lipid peroxide and other enzyme levels of patients suffering from thermal injury. Burns. 1995 Mar;21(2):96-7.
[38] LathaB(1), Ramakrishnan M, Jayaraman V, BabuM.Serum enzymatic changes modulated using trypsin: chymotrypsin preparation during burn wounds in humans.Burns. 1997 Nov-Dec;23(7-8):560-4.
[39] HalkesS(1), van den Berg A, Hoekstra M, du Pont J, Kreis R. Transaminase and alkaline phosphatase activity in the serum of burn patients treated with highly purified tannic acid. Burns. 2002 Aug;28(5):449-53.
[40] Flanagan SW, Moseley PL, Buettner GR. Increased flux of free radicals in cells subjected to hyperthermia: detection by electron paramagnetic resonance spin trapping. FEBS Lett. 1998 Jul 17; 431(2):285-6.
[41] Riesz P, Kondo T., Free radical formation induced by ultrasound and its biological implications. Free RadicBiol Med. 1992 Sep;13(3):247-70.

[42] Riesz P, Kondo T, Krishna CM. Free radical formation by ultrasound in aqueous solutions. A spin trapping study. Free Radic Res Commun. 1990;10(1-2):27-35.
[43] Finkelstein E, Rosen GM, Rauckman EJ. Spin trapping of superoxide and hydroxyl radical: practical aspects. Arch BiochemBiophys. 1980 Mar;200(1):1–16.
[44] terHaar, G. R. and Daniels, S., 1981, Evidence for Ultrasonically Induced Cavitation in vitro, Phys. Med. Biol. ,26:1145–1149.
[45] Edmonds PD, Sancier KM. Evidence for free radical production by ultrasonic cavitation in biological media. Ultrasound Med Biol. 1983 Nov-Dec;9(6):635–639.
[46] terHaar, G., Daniels, S., Eastaugh, K. C. and Hill, C. R., 1982, Ultrasonically Induced Cavitation in vivo, Br. J. Cancer 45 (Suppl V) :151–155.
[47] K.I.Morton, G.R.TerHaar, I.J.StratfordC.R.Hill, Subharmonic emission as an indicator of ultrasonically-induced biological damage.,Ultrasound Med Biol. 1983 Nov-Dec: 9 (6): 629-633.
[48] Hynynen K, Watmough DJ, Mallard JR, Fuller M. Ultrasound Med Biol. 1983 Nov-Dec; 9(6):621-7. Local hyperthermia induced by focused and overlapping ultrasonic fields--an in vivo demonstration.
[49] Makino K, Mossoba MM, Riesz P. Formation of .OH and .H in aqueous solutions by ultrasound using clinical equipment. Radiat Res. 1983 Nov;96(2):416–421.
[50] Duco W, Grosso V, Zaccari D, Soltermann AT. Generation of ROS mediated by mechanical waves (ultrasound) and its possible applications.Methods. 2016 Oct 15;109:141-148.
[51] Sasaki, J, Cottam, GL, Baxter, CR. Lipid peroxidation following thermal injury. J Burn Care Rehabil. 1983;4: 251–254.
[52] O. Cetinkale, A. Belce, D. Konukoglu, C. Senyuva, M.K. Gumustas, T. Tas, Evaluation of lipid peroxidation and total antioxidant status in plasma of rats following thermal injury. Burns. 1997 Mar; 23(2):114-6.
[53] Hiramatsu, M, Izawa, Y, Hagahara, M. Serum lipid peroxide levels of patients suffering from thermal injury. Burns. 1984;11:111
[54] Sparkes, BG, Monge, G, Marshall, S, Peters, W, Allgower, M, Schoenenberger, G. Plasma levels of cutaneous burn toxin and lipid peroxides in thermal injury. Burns. 1990;16:118.
[55] Nguyen, TT, Cox, CS, Traber, DL et al, Free radical activity and loss of plasma antioxidants, vitamin E, and sulfhydryl groups in patients with burns. J Burn Care Rehabil. 1993;14:602–609.
[56] Sun X, Xu H, Shen J, Guo S, Shi S, Dan J, Tian F, Tian Y, Tian Y. Real-time detection of intracellular reactive oxygen species and mitochondrial membrane potential in THP-1 macrophages during ultrasonic irradiation for optimal sonodynamic therapy. UltrasonSonochem. 2015 Jan;22:7-14.
[57] Yumita N, Iwase Y, Watanabe T, Nishi K, Kuwahara H, Shigeyama M, Sadamoto K, Ikeda T, Umemura S. Involvement of reactive oxygen species in the enhancement of membrane lipid peroxidation by sonodynamic therapy with functionalized fullerenes. Anticancer Res. 2014 Nov; 34(11):6481-7.
[58] Giuseppina Barrera, "Review Article: Oxidative Stress and Lipid Peroxidation Products in Cancer Progression and Therapy", International Scholarly Research Network ISRN Oncology Volume 2012, Article ID 137289, 21 pages.
[59] T. P. Szatrowski and C. F. Nathan, "Production of large amounts of hydrogen peroxide by human tumor cells," Cancer Research, vol. 51, no. 3, pp. 794–798, 1991.
[60] S. Kawanishi, Y. Hiraku, S. Pinlaor, and N. Ma, "Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis," Biological Chemistry, vol. 387, no. 4, pp. 365– 372, 2006.
[61] Y. Zhou, E. O. Hileman, W. Plunkett, M. J. Keating, and P.Huang, "Free radical stress in chronic lymphocytic leukemia cells and its role in cellular sensitivity to ROS-generating anticancer agents," Blood, vol. 101, no. 10, pp. 4098–4104, 2003.
[62] A. S. Kamiguti, L. Serrander, K. Lin et al., "Expression and activity of NOX5 in the circulating malignant B cells of hairy cell leukemia," Journal of Immunology, vol. 175, no. 12, pp.8424–8430, 2005.
[63] F. Q. Schafer and G. R. Buettner, "Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple," Free Radical Biology and Medicine, vol. 30, no. 11, pp. 1191–1212, 2001.
[64] S. Toyokuni, "Persistent oxidative stress in cancer," FEBS Letters, vol. 358, no. 1, pp. 1–3, 1995.
[65] G Filomeni, D De Zio and F Cecconi, Oxidative stress and autophagy: the clash between damage and metabolic needs Cell Death and Differentiation (2015) 22, 377–388;
[66] Droge W. Free radicals in the physiological control of cell function. Review. Physiol. Rev. 2002;82:47–95.
[67] Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Review. Crit. Rev. Food. Sci. Nutr. 2004;44:275–295.
[68] Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol. Rev. 2007;87:315–424.
[69] Genestra M. Oxyl radicals, redox-sensitive signalling cascades and antioxidants. Review. Cell Signal. 2007;19:1807–1819.
[70] Halliwell B. Biochemistry of oxidative stress. Biochem. Soc. Trans. 2007;35:1147–1150.
[71] Young I, Woodside J. Antioxidants in health and disease. J. Clin. Pathol. 2001;54:176–186.
[72] Davies KJ. Protein damage and degradation by oxygen radicals. I. general aspects. J Biol Chem. 1987;262:9895–9901.
[73] Halliwell B. Biochemical mechanisms accounting for the toxic action of oxygen on living organisms: the key role of superoxide dismutase. Cell BiolInt Rep. 1978;2:113–128.
[74] Imlay JA, Linn S. DNA damage and oxygen radical toxicity. Science. 1988;240:1302–1309.
[75] Wang X, Liu Q, Wang Z, Wang P, Hao Q, Li C. Bioeffects of low-energy continuous ultrasound on isolated sarcoma 180 cells. Chemotherapy. 2009;55(4):253-61.
[76] Wang P, Wang X, Liu Q. Cell damage of hepatoma-22 cells exposed to continuous wave ultrasound. Tumori. 2012 Jul-Aug;98(4):523-31.
[77] Li T, Hao Q, Wang X, Liu Q. The effect of focused ultrasound on the physicochemical properties of Sarcoma 180 cell membrane. Sheng Wu Yi Xue Gong Cheng XueZaZhi. 2009 Oct;26(5):941-6.
[78] Nathan FM, Singh VA, Dhanoa A, Palanisamy UD. Oxidative stress and antioxidant status in primary bone and soft tissue sarcoma. BMC Cancer. 2011 Aug 27;11:382.
[79] Prieur F, Pialoux V, Mestas JL, Mury P, Skinner S, Lafon C. Evaluation of inertial cavitation activity in tissue through measurement of oxidative stress.UltrasonSonochem. 2015 Sep;26:193-9.

[80] Feng Y, Tian Z, Wan M. Bioeffects of low-intensity ultrasound in vitro:apoptosis, protein profile alteration, and potential molecular mechanism. J Ultrasound Med. 2010 Jun;29(6):963-74.
[81] Hu X, Cai H, Zhou M, He H, Tian W, Hu Y, Chen L, Deng Y. New clinical application of high-intensity focused ultrasound: local control of synovial sarcoma. World J SurgOncol. 2013 Oct 8;11:265.
[82] Wu F, Chen WZ, Bai J, Zou JZ, Wang ZL, Zhu H, Wang ZB. Pathological changes in human malignant carcinoma treated with high-intensity focused ultrasound.Ultrasound Med Biol. 2001 Aug;27(8):1099-106.
[83] Despa F, Orgill DP, Neuwalder J, Lee RC.The relative thermal stability of tissue macromolecules and cellular structure in burn injury.Burns. 2005 Aug;31(5):568-77.
[84] Ashush H, Rozenszajn LA, Blass M, Barda-Saad M, Azimov D, Radnay J, ZiporiD,RosenscheinU.Apoptosis induction of human myeloid leukemic cells by ultrasound exposure. Cancer Res. 2000 Feb 15;60(4):1014-20.
[85] Vykhodtseva N, McDannold N, Martin H, Bronson RT, Hynynen K. Apoptosis in ultrasound-produced threshold lesions in the rabbit brain. Ultrasound Med Biol. 2001;27:111–7.
[86] Lagneaux L, de Meulenaer EC, Delforge A, Dejeneffe M, Massy M, Moerman C, Hannecart B, Canivet Y, Lepeltier MF, Bron D. Ultrasonic low-energy treatment: a novel approach to induce apoptosis in human leukemic cells. ExpHematol. 2002;30:1293–301.
[87] H. Honda, Q.L. Zhao, T. Kondo, Effects of dissolved gases and an echo contrast agent on apoptosis induced by ultrasound and its mechanism via the mitochondria-caspase pathway, Ultrasound Med. Biol. 28 (2002) 673–682.
[88] Böhm I, SchildH.,Apoptosis: the complex scenario for a silent cell death. Mol Imaging Biol. 2003 Jan-Feb;5(1):2-14.
[89] Wang P(1), Leung AW, Xu C. Low-intensity ultrasound-induced cellular destruction and autophagy of nasopharyngeal carcinoma cells. ExpTher Med. 2011 Sep;2(5):849-852.
[90] Yu T(1), Bai J, Hu K, Wang Z. Biological effects of ultrasound exposure on adriamycin-resistant and cisplatin-resistant human ovarian carcinoma cell lines in vitro.UltrasonSonochem. 2004 Apr;11(2):89-94.
[91] Takeuchi S, Udagawa Y, Oku Y, Fujii T, Nishimura H and Kawashima N: Basic study on apoptosis induction into cancer cells U-937 and EL-4 by ultrasound exposure. Ultrasonics 44: e345-e348, 2006.
[92] Ivone M, Pappalettere C, Watanabe A, Tachibana K. Study of cellular response induced by low intensity ultrasound frequency sweep pattern on myelomonocytic lymphoma U937 cells.J Ultrasound. 2016 Apr 4;19(3):167-74.
[93] Wang X, Liu Q, Wang P, Wang Z, Tong W, Zhu B, Wang Y, Li C. Comparisons among sensitivities of different tumor cells to focused ultrasound in vitro. Ultrasonics. 2009 Jun;49(6-7):558-64.
[94] Fitzgerald PJ, Takagi A, Moore MP, et al: Intravascular sonotherapy decreases neointimal hyperplasia after stent implantation in swine. Circulation 103: 1828-1831, 2001.
[95] Feril LB Jr, Kondo T, Cui ZG, et al: Apoptosis induced by the sonomechanical effects of low intensity pulsed ultrasound in a human leukemia cell line. Cancer Lett 221: 145-152, 2005.
[96] Sasnauskene A, Kadziauskas J, Vezelyte N, Jonusiene VM and Kirveliene V: Apoptosis, autophagy and cell cycle arrest following photodamage to mitochondrial interior. Apoptosis 14: 276-286, 2009.



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