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A STUDY ON THERAPEUTIC EFFECTS OF ULTRASOUND FOR THE TREATMENT OF SARCOMA CANCER.

C. S. Ram[1], R. K. Saxena[2], Jayanand[3], Sonali Gangwar[4], Maya Datt Joshi[5] and D.V. Rai*[6]

[*1]Professor- I.T.S Paramedical College, Murad Nagar, Ghaziabad.

[2]Professor, Center for Biomedical Engineering, Indian Institute of Technology, Hauz Khas, New Delhi.

[3]Professor, Noida International University, Noida UP.

[4]Assistant Professor, Center for Biological Engineering, Shobhit University, Gangoh, Saharanpur, U.P, India.

[5]Assoc. Prof -Centre for Biomedical Engineering, Shobhit University, Modipuram, Meerut.

[6]Professor, Center for Biological Engineering, Shobhit University, Gangoh, Saharanpur.

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***Corresponding Author**
D. V. Rai
Professor, Centre for Biological Engineering, Shobhit University, Gangoh.

ABSTRACT

Context: Ultrasound is emerging as a novel treatment agent for cancer. 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. **Aims:** The present study was aimed to evaluate the therapeutic potential of ultrasound in 7, 12-dimethyl benz(a)anthracene (DMBA) induced sarcoma in rats. **Settings and Design:** Forty female wistar rats were used in the experimental study. They were allocated in four groups.7, 12-dimethyl benz(a)anthracene (DMBA) was used to induce sarcoma in 20 rats. Therapeutic ultrasound was applied at 2.6W/cm^2 for 10 min (continuous mode) to 10 sarcoma tumor bearing rats and normal 10 rats. **Materials and Methods:** 7, 12-dimethyl benz(a)anthracene (DMBA) was used to induced sarcoma in rats. Body weight, Tumor weight, Serum enzymes were determined following treatment with therapeutic ultrasound (Chattanooga Corp Inc. USA). Statistical analysis used: 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. **Results:** There were significant increases on the body weight and tumor weight of treated rats. The increased activities of serum pathophysiological enzymes AST, ALT, ALP, ACP, and LDH of ultrasound treated rats were significantly ($p < 0.05$) higher than control levels indicating loss of redox homeostasis. The histopathological analysis of sarcoma tissues showed extensive haemorrhage and necrosis indicating the anti-tumor nature of ultrasound. **Conclusions:** The results of the present study indicate that ultrasound significantly suppresses DMBA induced sarcoma in rats.

KEYWORDS: Therapeutic, Ultrasound, Sarcoma, Cancer.

INTRODUCTION

Ultrasound cavitation leads to the formation of reactive oxygen species and its consequences are of primary interest[1]. Recent clinical studies have demonstrated that cancer cells can be targeted and destroyed by a single blast of ultrasound[2]. However the extent to which ultrasound affects cancerous tissue is an area of ongoing research and needs to be explored further[3]. The biophysical effects of therapeutic ultrasound have been examined through in vitro studies. Extrapolation of these results to humans is therefore conjectural.[4] Our study aimed to study the effect of ultrasound therapy on morphological, biochemical and histopathological changes in sarcoma cancer in rats.

SUBJECTS AND METHODS

Virgin female Wistar rats, 7 weeks of age were purchased from Central Animal House AIIMS New Delhi and were used in the experiment. The experimental design was performed in accordance with the current ethical norms approved by the CPCEA Government of India and Institutional Animal Ethics Committee Guidelines and approved by Institutional Ethics Committee vide approval No. IAEC No: ITS/01/IAEC/2013. 7, 12-Dimethyl benz (a) anthracene is a known carcinogen which produces mammary and sarcomas in rats.[5] 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. 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 ($2.6W/cm^2$), Group 3: DMBA induced sarcoma cancer rats with sham treatment, Group 4: DMBA induced sarcoma cancer rats administered with ultrasound therapy ($2.6W/cm^2$). A single dose of 7, 12-DMBA (25 mg/kgbw/rat) in 0.5 ml of corn oil was injected into the rat abdomen. After the tumor had 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 (Chattanooga Corp, USA) at 1-MHz , continuous output, power level = 2.6W/cm^2.

Blood was collected and the serum was centrifuged at 5000 rpm for 15 min to obtain a clear supernatant for use in further biochemical analysis. The total body weight gain of the control and experimental animals was recorded periodically throughout the experimental period. Enzymes levels of oxidants and anti-oxidants in serum were determined by assay. 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 specimens were examined by a pathologist to histopathologically classify the tumors as described by Royal College of Pathologists UK (1990).[6]

RESULTS

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*	-
DMBA	154.33±4.87$^{\dagger, \ddagger}$	15.45±1.11
DMBA + US	176.21±3.12 $^{\S, \|}$	11.28±1.32$^{\|}$
Results are expressed as Mean± S.E.M (n=10). *P>0.05 compared with control group of rats. †P<0.05 compared with control group of rats, ‡P<0.05 compared with Control+US group of rats, §P<0.05 compared with Control+US group of rats, $^{\|}$P<0.05 compared with DMBA induced group of rats.		

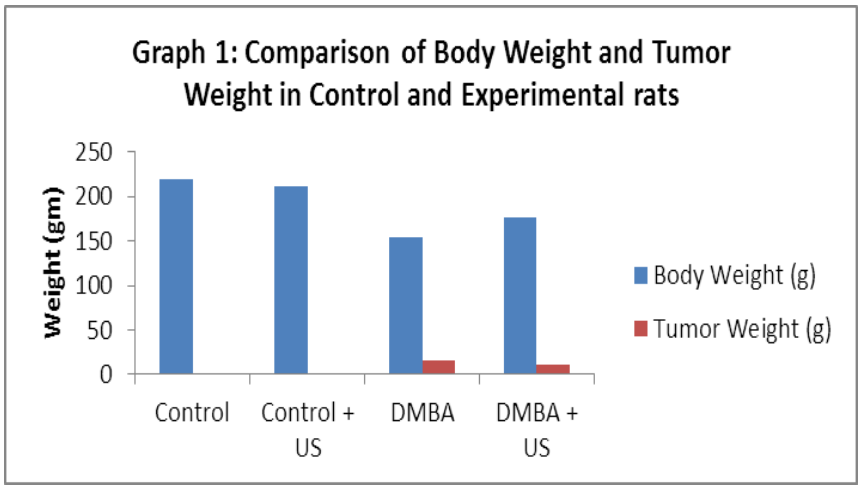

Table 1 & Graph 1 shows the body weight of control and experimental rats. The body weight of control Group I rats (219.46 g) was significantly higher as compared to Group III rats (154.33 g) following DMBA treatment (P<0.05). The body weight of DMBA induced Group IV rats following ultrasound therapy 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).

Effect of ultrasound therapy on serum pathophysiological enzymes

Table 2: Effect of ultrasound therapy on serum pathophysiological enzymes.

Group/ Enzyme (U/L)	Control	Control + Ultrasound	DMBA	DMBA + Ultrasound
AST	167.43 ±1.76	464.66± 1.79 *	395.81 ± 3.49 †,‡	686.87± 2.71 $^{\S,\|}$
ALT	23.41 ± 0.76	238.22 ± 4.79 *	95.65 ± 1.01 †,‡	422.22±0.73 $^{\S,\|}$
ALP	124.52 ± 1.35	385.69 ± 3.36 *	285.64 ± 2.28 †,‡	1242±33.05 $^{\S,\|}$
LDH	158.17 ± 1.58	212.14 ± 1.95 *	179.90 ± 1.95 †,‡	501.58±15.67 $^{\S,\|}$

AST=Amino-s-transferase, ALT=Alanine transferase, ALP=Alkaline phosphatise, LDH=Lactate dehydrogenase. Results are expressed as Mean± S.E.M (n=10). *P<0.05 compared with control group of rats. †P<0.05 compared with control group of rats, ‡P<0.05 compared with Control+US group of rats, §P<0.05 compared with Control+US group of rats, $^{\|}$P<0.05 compared with DMBA induced group of rats.

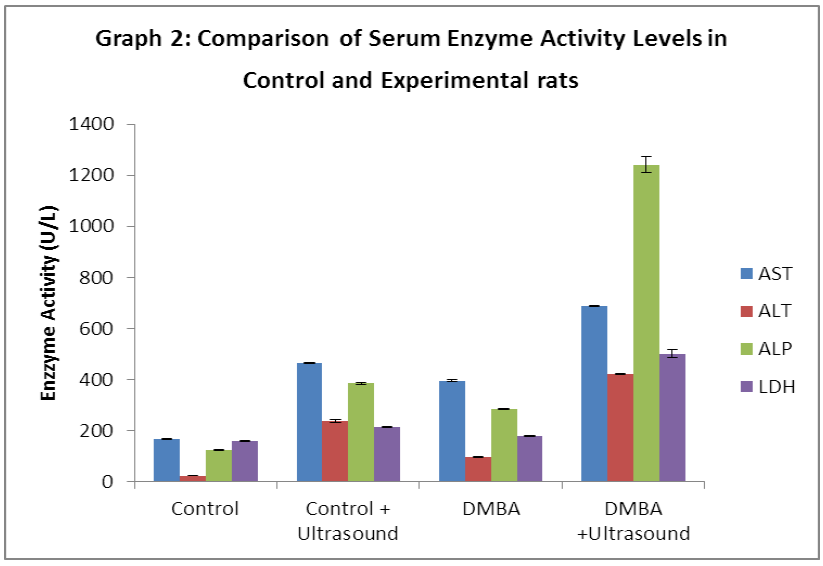

Table 2 & Graph 2 shows the serum level of pathophysiological enzymes AST, ALP, ALT, LDH in control and experimental rats. The serum level of pathophysiological enzymes in control Group II rats following ultrasound therapy treatment was significantly increased as compared to control Group I rats (P<0.05). 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 ($P<0.05$).

Effect of ultrasound therapy on serum antioxidants

The levels of serum antioxidants namely total glutathione (GSH), vitamin C and vitamin E are presented.

Table 3 Effect of ultrasound therapy on serum antioxidants.

Group/Enzyme U/L	Control	Control + Ultrasound	DMBA	DMBA + Ultrasound
GSH	13.16±0.18	2.5±0.20*	7.42±0.10$^{\dagger, \ddagger}$	0.43±0.10$^{\S, \|}$
Vit C	7.57±0.07	1.46±0.07*	3.54±0.24$^{\dagger, \ddagger}$	0.08±0.02$^{\S, \|}$
Vit E	4.49±0.03	0.19±0.01*	1.2±0.01$^{\dagger, \ddagger}$	0.05±0.01$^{\S, \|}$

GSH=Glutathione, Vit C= Ascorbic acid, Vit E= α-tocopherol. Results are expressed as Mean± S.E.M (n=10). $^{*}P>0.05$ compared with control group of rats. $^{\dagger}P<0.05$ compared with control group of rats, $^{\ddagger}P<0.05$ compared with Control+US group of rats, $^{\S}P<0.05$ compared with Control+US group of rats, $^{\|}P<0.05$ compared with DMBA induced group of rats.

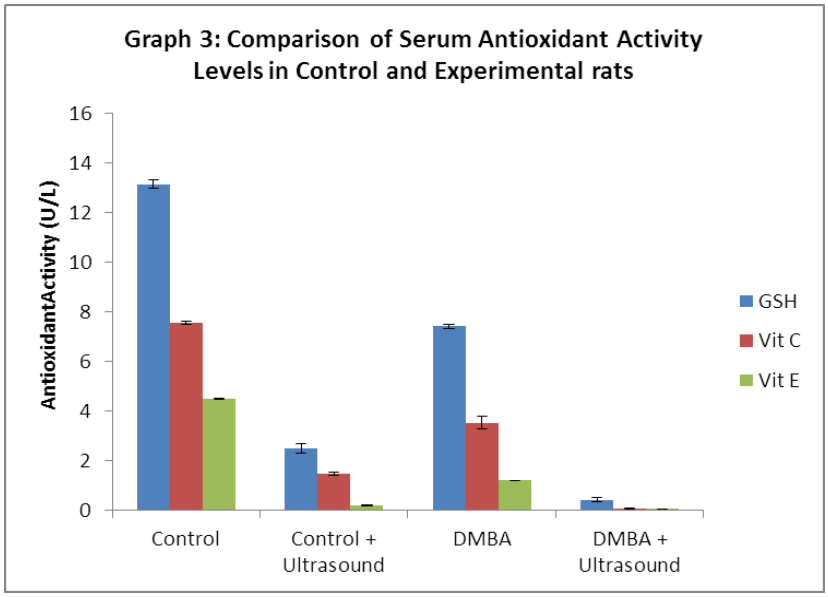

Table 3 & Graph 3 shows the level of non-enzymatic anti-oxidants GSH, Vit C, Vit E in control and experimental rats in serum. The serum level of anti-oxidants in control Group II rats following ultrasound therapy treatment was significantly lower as compared to control group I rats ($p<0.05$). Ultrasound therapy treatment to DMBA induced Group IV rats significantly lowered the level of 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 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 ($p<0.05$).

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.e generalized weight loss.[7] 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.[8],[9]

In our study there was a marked increase in serum 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.[10] 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.[11]

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.[12],[13],[14]

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.[15],[16]

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.[17]

Ultrasound therapy to cancer cells caused an exorbitant rise in the level of oxidative enzymes and leads to cell death.[18] 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.[19] 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.[20],[21] (see Table 2 & Fig 2).

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.[21] Sonication can trigger apoptosis in both normal and malignant cells.[22] Our findings revealed that low intensity ultrasound markedly kills cells by damaging the ultrastructure and morphology.[23]

In our study the sarcoma tumor was particularly susceptible to ultrasonic therapy. This correlated well with other in vitro studies.[24],[25] 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.[26] Cellular necrosis may be due to autophagy in the tumors.[27] The induction of apoptosis by ultrasound therapy may lead to a substantial improvement in antitumor therapy.

In conclusion, the results of the present study clearly establish the anticancer efficacy of ultrasound therapy against DMBA induced sarcoma in rats. Also, the alteration in the levels of tumor biomarker 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 exact molecular mechanism underlying the action of ultrasound in reducing the toxic effects of DMBA in sarcoma cancer.

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