ORIGINAL ARTICLE

Protective Effect of Vitamin C on Length and Width of Irradiated Long Bones in Young Albino Rats

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ABSTRACT

Objective: To observe the Vitamin C protecting, harmful effects of gamma radiation on length and width of long bones in young albino rats.

Methods: Experimental work was completed in six months duration at BMSI, JPMC Karachi.

30 Litters of 10 days age of albino rats were taken for this study. They were divided into three groups: Group A (Control), Group B, 5Gy gamma radiation and Group C, radiation and inj. Vitamin C (Ascorbic Acid). Each group further divided into two subgroups according to their respective duration of treatment i-e., 2 and 4 weeks respectively. At the end of their respective period of study the animals were anaesthetized by ether, dissected and their long bones i-e., humerus, ulna, femur and tibia were taken out, and measured in length and width by electronic digital caliper.

Results: Highly significant decrease in length and width of bones were noted out in irradiated subgroups compared with control. And highly significant increase, length and width of bones were noted out Vitamin C treated groups as compared to irradiated groups.

Conclusion: Irradiation causes decrease in length and width of growing long bones. Vitamin C restores the damage.

Key words: Albino rats, Radiation, Vitamin C, Length and Width of long bones, Electronic digital Caliper.

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INTRODUCTION:

Radiation therapy involves treating the cancer with ionizing radiation; for certain localized causes it may be curative[1]. The use of radiation in medicine has always been rationalized on basis of risk versus benefit[2]. Radiation therapy plays an important role as part of multimodality treatment for a number of childhood malignancies. Dose limiting complications of radiotherapy include skeletal abnormalities and disturbances in skeletal development within the irradiated field[2].

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In infants and young children exposed to radiation, bone growth and maturation may be retarded[3]. Irradiation of growing bone typically results in retardation of longitudinal growth[4]. Whole body irradiation has been reported to retard growth in man after exposure to irradiation from atomic bomb explosion, and in animals after varying doses of X-ray during fetal life or infancy[5]. Free radical scavengers and antioxidants protect against radiation injury[3]. Radiobiological studies have identified several radioprotective compounds some of which are non- toxic to humans. Vitamin C and vitamin E administered in a single dose before irradiation reduced the level of DNA damage to normal cells[2]. Vitamin C, E and â-carotenes have been assumed to have anti-carcinogen effects, because of their antioxidant properties[6]. A major function of ascorbic acid is related to the synthesis of collagen, proteoglycans,

and other organic constituents of the intercellular matrix in such diverse tissues as tooth, bone and capillary endothelium[7]. It has also been considered possible that radiation therapy for cancer patients could be improved by the use of radioprotectors to protect normal tissue[8].

In the light of above facts, this study is designed to evaluate the possible effects of Ascorbic acid (vitamin C), protecting the harmful effects of radiations in growing bones of young albino rats.

METHODS:

This study was conducted at BMSI, JPMC Karachi. 30 litters of Albino rats were obtained from Animal house BMSI, JPMC Karachi. The litters were weighed and marked on 1st post natal day and divided into 3 groups, i-e, A, B and C, each comprising of 10 animals. Each group was further divided into two subgroups, i-e, A1and A2; B1 and B2; C1and C2, according to their time period i-e, 2 and 4 weeks respectively. Each subgroup comprised of 5 animals, and was kept in separate cages along with mothers for milk feeding. The mothers were given laboratory feed and water ad libitum. Animals were kept in experimental room for 10 days prior to commencement of study. Animals were watched daily for their health status. On 10th post-natal day litters were weighed, treated and allowed to survive for their respective period of study. In the present study animals were treated as under:

Group A (A1 and A2), control.

Group-B (B1 and B2), animals received irradiation at the dose of 5 Gy for 2.02 min. from 60-unit cobalt chamber[9,10], at the Department of Radiotherapy JPMC Karachi, at the commencement of study.

Group-C(C1 and C2), animals received Radiation and injection vitamin C by insulin syringe at the dose of 0.4mg/gm body weight intraperitoneally daily[11] for their respective period of study.

After treatment, all the animals were watched daily for their health status on the basis of their activity and weight gain or loss. On completion of their respective period of treatment animals were sacrificed. All the animals were anaesthetized, dissected, and the right sided long bones from forelimbs (Humerus and Ulna) and hind limbs (Femur and Tibia) were taken out and measured (mm) by electronic digital caliper.

Statistical analysis

The statistical analysis was done by student "t" test and p-value less than 0.05 was considered as significant.

RESULTS:

This experiment was designed to observe the effect of radiation and radioprotective effect of vitamin C on growing long bones of young albino rats. Gross observations were made, in all groups. The following observations and results were recorded for statistical analysis.

Observations on Control Group-A:

The animals in this group were looking healthy, active, taking breast feed regularly, hair were evenly distributed on the body.

Length of bones

The mean length of Humerus in subgroup A1 and A2 was12.62±0.38mm and15.08±0.41mm respectively, mean length of ulna in subgroup A1 and A2 recorded was15.76±0.27mm and 19.26±0.19mm respectively, mean length of femur 13.62±0.33mm and 18.25±0.19mm respectively and the mean length of tibia in subgroup A1 and A2 was found to be 19.17±0.28 mm and 24.47±0.27 mm respectively (Table-1, 2).

Width of bones :

The mean width of Humerus in subgroup A1 and A2 recorded was 1.432±0.05mm and 1.57± 0.047mm respectively, mean width of ulna was 1.108±0.04mm and 1.174±0.04mm, mean width of femur was 1.82±0.10mm and 2.188±0.04mm and mean width of tibia in subgroup A1 and A2 was1.40±0.01mm and 1.496±0.02 mm respectively (Table-3 and 4).

Observations on Irradiated Group-B:

The animals in both subgroups B1 and B2 were inactive, looking ill, weak, sluggish movements, not taking breast feed regularly; hair were irregularly distributed on the body .

Length of bones:

The mean length of Humerus in B1 and B2 subgroup was found to be 10.59±0.25mm and 13.45±0.15mm respectively, which showed a moderately significant decrease, and significant decrease in subgroup B1 and B2 respectively as compared to control subgroups A1 and A2 respectively. The mean length of ulna in subgroup B1 and B2 was 13.39±0.36mm and 16.81± 0.23mm respectively, which showed a significant, and highly significant decrease in length of ulna in subgroup B1 and B2 as compared to control subgroups A1 and A2 respectively. The mean length of femur in subgroup B1 and B2 was 11.21±0.17mm and 14.39±0.41mm respectively, which showed a moderately significant decrease, and highly significant decrease in the length of femur in subgroup B1 and B2 as compared to control subgroups A1 and A2 respectively. The mean length of tibia in subgroup B1 and B2 was 16.44±0.16 mm and 21.02±0.72 mm respectively, which showed a moderately significant decrease and significant decrease in the length of tibia in subgroup B1 and B2 as compared to control subgroups A1 and A2 respectively (Table-1& 2).

Width of bones:

The mean width of Humerus in subgroup B1 and B2 was found to be 1.15±0.006mm and 1.31±0.08mm respectively, which showed a significant decrease in both subgroup B1 and B2 as compared to control subgroups A1 and A2 respectively. The mean width of ulna in subgroup B1 and B2 was 0.75±0.04mm and 0.866± 0.002mm, which showed a moderately significant and highly significant decrease in width of ulna in subgroup B1 and B2 as compared to control subgroups A1 and A2 respectively. The mean width of femur in subgroup B1 and B2 was1.40 ± 0.09mmand1.796±0.08mm respectively, which showed a moderately significant decrease in the width of femur in subgroup B1 and B2 as compared to control subgroups A1 and A2 respectively. The mean width of tibia in subgroup B1 and B2 was 1.046±0.05mm and1.34±0.009mm respectively, which showed a significant decrease in the length of tibia in subgroup B1 and B2 as compared to control subgroups A1 and A2 respectively (Table- 3 and4).

OBSERVATIONS ON IRRADIATED AND VITAMIN C TREATED GROUP- C:

The animals in this group also were weak initially but later on they became active, looking healthy and they were taking breast feed regularly, hairs were evenly distributed on the body.

Length of bones:

The mean length of Humerus in subgroups C1 and C2 was found to be 12.63±0.11mm and 15.32± 0.06mm respectively, which showed a moderately significant increase in subgroup C1 and highly significant increase in C2 subgroup as compared to irradiated subgroups B1 and B2 respectively. There was insignificant increase in both subgroups C1and C2, when compared with control subgroups A1 and A2 respectively (Table-1, 2).

The mean length of ulna in subgroup C1 and C2 was 15.53±0.05mm and 18.39±0.43mm respectively, which showed a moderately significant increase in subgroup C1, and significant increase in length of ulna in subgroup D2 as compared to irradiated subgroups B1 and B2 respectively. There was insignificant decrease in length of ulna in both subgroups C1 and C2, when compared with control subgroups A1 and A2 respectively (Table-1&2).

The mean length of femur in subgroup C1 and C2 was 14.17±0.19mm and 18.43±0.18mm respectively, which showed a moderately significant increase in subgroup C1 and C2, when compared to B1 and B2 respectively. There was insignificant increase in both subgroups C1 and C2, when compared with control subgroups (Table-1 &2). The mean length of tibia in subgroup C1 and C2 was 19.12±0.22 mm and 23.97±0.25 mm respectively, which showed a moderately significant increase in subgroup C1, and significant increase in the length of tibia in subgroup C2 as compared to irradiated subgroups B1 and B2 respectively. There was insignificant decrease in both subgroups subgroup C1 and C2, when compared with control subgroups A1 and A2 respectively (Table- 1&2).

Width of bones:

The mean width of Humerus in subgroup C1 and C2 was found to be 1.352±0.07mm and 1.58±0.07mm respectively, which showed

Table – 1

Mean length of long bones (mm) in sub-groups at 2 weeks period of Albino rat

Groups	Treatment given	Sub groups	2 weeks			
			Humerus	Ulna	Femur	Tibia
A	Control	A1 (n=5)	12.62±0.38	15.76±0.27	13.62±0.33	19.17±0.28
B	Radiation	B1 (n=5)	10.59±0.25	13.39±0.36	11.21±0.17	16.44±0.16
C	Radiation+ Vitamin C	C1 (n=5)	12.63±0.11	15.53±0.05	14.17±0.19	19.12±0.22

Statistical analysis of differences in length of bones between sub-groups at 2 weeks

Statistical comparison	Humerus P-value	Ulna P-value	Femur P-value	Tibia P-value
B1vsA1	<0.01***	<0.02**	<0.01***	<0.01***
C1vsB1	<0.01***	<0.01***	<0.01***	<0.01***
C1vs A1	>0.05*	>0.05*	>0.05*	s>0.05*

*non-significant, **significant, ***moderately significant, ****highly significant

Table – 2

Mean length of long bones (mm) in sub-groups at 4 weeks period of Albino rat

Group	Treatment given	Sub groups	2 weeks			
			Humerus	Ulna	Femur	Tibia
A	Control	A2 (n=5)	15.08±0.41	19.26±0.19	18.25±0.19	24.47±0.27
B	Radiation	B2 (n=5)	13.45±0.15	16.81±0.23	14.39±0.41	21.02±0.72
C	Radiation+ Vitamin C	C2 (n=5)	15.32±0.06	18.39±0.43	18.43±0.18	23.97±0.25

Statistical analysis of differences in mean length of bones between sub- groups at 4 weeks

Statistical comparison	Humerus P-value	Ulna P-value	Femur P-value	Tibia P-value
B2vsA2	<0.02**	<0.001****	<0.001****	<0.02**
C2vsB2	<0.001****	<0.02**	<0.01***	<0.04**
C2vs A2	>0.05*	>0.05*	>0.05*	>0.05*

*non-significant, **significant, ***moderately significant, ****highly significant

Table – 3

Mean width of long bones (mm) in sub-groups at 2 weeks period of Albino rats

Group	Treatment given	Sub group	2 weeks			
			Humerus	Ulna	Femur	Tibia
A	Control	A1 (n=5)	1.432±0.05	1.108±0.04	1.82±0.1	1.40±0.01
B	Radiation	B1 (n=5)	1.15±0.006	0.75±0.04	1.40±0.09	1.046± 0.05
C	Radiation + Vitamin C	C1 (n=5)	1.352±0.07	0.874±0.03	1.682±0.06	1.448± 0.05

Statistical analysis of differences in width of bones between sub-groups at 2 weeks' time period

Statistical comparison	Humerus P-value	Ulna P-value	Femur P-value	Tibia P-value
B1vsA1	<0.03**	<0.01***	<0.01***	<0.03**
C1vsB1	>0.05*	>0.05*	<0.01***	<0.01***
C1vsA1	>0.05*	<0.01***	>0.05*	>0.05*

*non significant, **significant, ***moderately significant, ****highly significant

Table – 4

Mean width of long bones (mm) in different groups at 4 weeks period of Albino rats

Group	Treatment given	Sub group	4 weeks			
			Humerus	Ulna	Femur	Tibia
A	Control	A2 (n=5)	1.57±0.047	1.174±0.04	2.188±0.04	1.496±0.02
B	Radiation	B2 (n=5)	1.31±0.08	0.866±0.002	1.796±0.08	1.34±0.009
C	Radiation + Vitamin C	C2 (n=5)	1.58±0.07	0.96±0.04	2.054±0.13	1.68±0.01

Statistical analysis of differences in mean width of bones between different groups at 4 weeks time period

Statistical comparison	Humerus P-value	Ulna P-value	Femur P-value	Tibia P-value
B2vsA2	<0.03**	<0.001****	<0.01***	<0.03**
C2vsB2	<0.03**	>0.05*	>0.05*	<0.01***
C2vsA2	>0.05*	<0.03**	>0.05*	>0.05*

*non-significant, **significant, ***moderately significant, ****highly significant

insignificant) increase in subgroup C1, but there was significant increase in subgroup C2, when compared to irradiated subgroups B1 and B2 respectively. There was insignificant decrease in subgroup C1 and insignificant increase in subgroup C2, when compared with control subgroup A1 and A2 respectively (Table- 3 and 4). The mean width of ulna in subgroup C1 and C2 was 0.874±0.03mm and 0.96±0.04 mm, which showed insignificant increase in width of ulna in both subgroups C1 and C2, as compared to irradiated subgroups B1 and B2 respectively. There was moderately significant decrease in subgroup C1 and significant decrease in subgroup C2, when compared with control subgroups A1 and A2 respectively (Table- 3 and 4).

The mean width of femur in subgroup C1 and C2 was1.682±0.06mm and 2.054±0.13mm respectively, which showed moderately significant increase in subgroup C1 and insignificant increase in the width of femur in subgroup C2 as compared to irradiated subgroups B1 and B2 respectively. There was insignificant decrease in subgroup C1 and C2, when compared with control subgroups A1 and A2 respectively (Table-3and4).The mean width of tibia in subgroup C1 and C2 was 1.448±0.05mm and 1.68±0.01mm respectively, which showed moderately significant increase in the length of tibia in subgroup C1 and C2, as compared to irradiated subgroups B1 and B2 respectively. There was insignificant increase in both subgroups C1 and C2, when compared with control subgroups A1 and A2 respectively (Table-3 and4).

DISCUSSION:

Ionizing radiation is a double-edged sword. It is indispensable in medical practice, being used in the treatment of cancer, in diagnostic imaging and in therapeutic or diagnostic imaging radioisotopes, but it also produces adverse short and long term effectss[6]. In radiation therapy high energy rays are used. The gamma radiation produces anatomical and pathological alterations in bone growth. Several investigators have used experimental gamma radiations in animals. Nunia et al[12], had used Swiss albino mice for whole body gamma irradiation. These animal studies describe the radiation injuries in experimental animals. In this regard many naturally occurring, anti-oxidants exhibit protection against irradiation injuries. The potential of antioxidants to reduce cellular damage induced by ionizing radiation has been studied in animal models, for more than 50 years. Institute of Medicine of US National Academy of Sciences considers only vitamins-E and C and the mineral selenium to be dietary antioxidants[8]. According to Kumar et al[6], vitamin C functions in a variety of biosynthetic pathways by accelerating hydroxylation reaction. Vitamin C also has anti-oxidant properties, it can scavenge free radicals directly or indirectly by regenerating the antioxidant form of vitamin E. In the present study vitamin-C is used as radio protective agent. This study was designed to observe the radio-protective effects of vitamin-C on the growing long bones at variable time interval. In the present study, animals were given gamma radiation at the dose of 5Gy[13].Vitamin-C was also used as a radioprotective agent in a dose of 0.4mg/gm body wt., as Sert et al., 2000, used same dose on the study of small intestine and thyroid gland. Guyton and Hall[14], also described that vitamin-C is essential for growth and strength of fibers in cartilage and bone. In the present study width of long bones was measured at different periods. In irradiated group the width was less than the control. It might be due to injurious effect of radiation on cartilage and bone at the diaphysis level. This is in agreement with Larue et al.[4] who reported irradiation of long bones typically results in retardation of longitudinal growth. In group C, the width of long bones was protected by vitamin C and width was similar to control. Guyton and Hall[14], reported that lack of vitamin-C causes cessation of bone growth, the cells and osteoblasts cannot form new bone matrix.

In the light of above considerations the net result suggest that injurious effect of radiation occur more frequently at a dose of 5 Gy in growing bones of young albino rats. Irradiation can cause cellular damage, but the vitamin-C, restores the growth. The present study suggest that adverse effects of irradiation need special cautions for

human subjects and the study may act as a base line for the extension of project for humans.

CONCLUSION:

This study concludes that gamma radiation reduces the weight and length in rats, which can be minimized by Vitamin-C. The result of present study is suggestive for further studies on animals and trial on human subjects.

REFERENCES:

1. Walker BR, Colledge NR, Ralston SH, Penman ID. Oncology in: Davidson's Principles & Practice of Medicine, 22nd edition, Churchil Living stone, Elsvier, Haryana India, 2014;259-282.
2. Prasad KN, Cole WC, Hasse GM., Radiation Protection In Humans: Extending the concept of as low as achievable from dose to biological damage. Bri J Radiol. 2004; 77: 97-9.
3. Pateder DB, Eliseev RA, O'Keefe RJ, Schwarz EM, Okunieff F, Constine LS, et al. Role of autocrine growth factors in radiation damage to epiphyseal growth plate. Radiat Res. 2001; 155(6):847-7.
4. Kumar V, Abbas A, Fausto N. Environmental and Nutritional Pathology in: Robbins and Kotran Pathologic Basis of Disease. 7th edition, Philadelphia Pennsylvania, Saunders Elsevier, 2004; 436-439.
5. Larue SM, Wrigley RH, Powers B. A review of the effects of radiation therapy on bone. Vet Radiol Ultrasound. 1987; 28(1): 17-22.
6. Pappas AM, Cohen J. The abscopal effect of x-irradiation on bone growth in rats. J Bone Joint Surg Am. 2008; 45:765-72.
7. Akyurek S, Atahan L, Cengiz M, Sokmensuer C, Haberal I, Yildiz F, et al. Effect of ticlopidine in the prevention of radiation enteropathy. Bri J Radiol. 2006; 79:409-14.
8. Hardman JG, Limbird LE, Gilman AG. Pituitary hormones and their hypothalamic releasing factors, Vitamins. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics, Tenth ed. McGraw-Hill, New York. 2001; 1541-1549, 1767-70.
9. Weiss JF and Landauer MR. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicol. 2003; 189:1-20.
10. Koc M, Tayasi S, Buyukokuroglu ME_Bakan N. Melatonin protects rat liver against irradiation-induced oxidative injury. J Radiat Res (Tokyo). 2003; 44(3): 211-5.
11. Nash H. Radiation therapy in Dogs, Cats, and other small animals. Veterinary Services Department, 2008; 1-4.
12. Sert C, Celik MS, Akdag Z, Ketani MA, Mergiz Y. The Radioprotective effect of vit. C, E and vitamin E+ Glutathione on small intestine and Thyroid Gland in Rats irradiated with X-rays. Turk J Med Sci. 2000; 30(5): 417-26.
13. Nunia V, Sancheti G and Goyal PK. Protection of Swiss albino mice against whole-body gamma irradiation by diltiazem. Bri J Radiol. 2007; 80:77-84.
14. Engstrom H. Effects of radiation on growing bones. Swed Dent J Suppl.1987; 45:1-47
15. Guyton AC and Hall JE. Dietary Balances, in: Text book of Medical Physiology, 12th edition, Saunders Elsevier, Philadelphia, Pennsylvania, 2011; 843-8.