ORIGINAL ARTICLE

Protective Role of Vitamin C on Weight & Length of Irradiated Young Albino Rats

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ABSTRACT

Objective: To observe the role of Vitamin C in preventing the side effects of Radiotherapy on weight and length of Young Albino Rats.

Methods: This experimental study was completed in six months duration at BMSI, JPMC Karachi, on30 Litters (Albino rats) of 10 days age. They were grouped in 3 categories: A(Control), B was given 5Gy gamma radiation and C was given radiation and inj. Vitamin C (Ascorbic Acid). Each category was further divided into two subgroups according to their respective period of studys i-e., 2 and 4 weeks respectively. At the end of their respective period of study the animals were weighed and measured.

Result: A highly significant decrease in weight and Crown Ramp Length (CRL), Forelimb and hind limb lengths were noted in irradiated subgroupscompared with control. And highly significant increase in weight and length was noted inVitamin C treated subgroups as compared to irradiated subgroups.

Conclusion: Irradiation causes decrease in weight and length. Vitamin C restores the damage.

Key words: Albino rats, Radiation, Vitamin C, Weight, Forelimb and Hind limb measurements

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INTRODUCTION:

Radiation therapy involves treating the cancer with ionizing radiation; for certain localized causes it may be curative. Ionizing radiation can be delivered by radiation emitted from decay of radio isotopes or high energy radiation beams[1]. On the human body its effects vary from local tissue necrosis to genetic damage, cancer and death[2]. Radiation therapy involves treating the cancer with ionizing radiation; for certain localized causes it may be curative[3]. The use of radiation in medicine has always been rationalized on basis of risk versus benefit[4]. 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 & disturbances in skeletal development within the irradiated field[5].Irradiation of growing bone typically results in retardation of longitudinal growth[6]. Damage to the underlying bone can be a major complication of radiation therapy, whether alone or combined with chemotherapy[7]. Exposure of animals to ionizing radiation causes a series of physiological changes known as acute radiation syndrome, which is dependent on the exposure dose and may lead to death[8]. Irradiation of the epiphyseal plate of a growing bone produces limb shortening, and bowing of the irradiated bone[9]. Furstman[10] in 1971, observed weight loss and destructive changes in epiphyseal growth plate in irradiated rats. 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[11].

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Vitamins are organic substances with key roles in certain metabolic pathways, and are categorized into those that are fat soluble (vitamin A, D, E and K) and those that are water soluble (vitamins of B-complex group and vitamin C)[3].Ascorbic Acid (vitamin C) is the most active reducing agent in aqueous phase of living tissues and is involved in intracellular electron transfer[3]. Vitamin C and vitamin E administered in a single dose before irradiation reduced the level of DNA damage to normal cells[4].

In the light of above facts, this researchis designed to study the effects of vitamin C, on weight, CRL and length of forelimbs and hind limbs in irradiated young animals.

METHODS:

This study was conducted at Department of Anatomy BMSI, JPMC Karachi. 30 newborn litters of Albino rats were obtained from Animal house BMSI, JPMC Karachi. The animals (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 of treatment. 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, for acclimatization to the experimental conditions. Animals were watched daily for their health status.

On 10th post natal day animals were weighed and treated for their respective period of study.In the present study animals were treated as under:

Group-A control.

Group-B received irradiation at the dose of 5 Gy for 2.02 min. from 60-unit cobalt chamber[12,13] at the Department of Radiotherapy JPMC Karachi, at the commencement of study.

Group-C received Radiation and Injection vitamin C (Ascorbic acid) by insulin syringe at the dose of 0.4mg/gm body weight intra-peritoneally[14] daily for their respective period of study.

After treatment, all the animals were watched daily for their health status. All the animals were weighed weekly on 17th, 24th, 31st and 38th post natal day. On completion of their respective period of treatment animals were weighed and their Crown Rump Length (CRL), forelimbs and hind limbs were measured (cm) by a measuring scale and recorded.

Statistical analysis

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

OBSERVATIONS AND RESULTS

This research study was designed to evaluate the effect of radiation and radioprotective effect of vitamin C on weight and length of young albino rats. 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 (Figure-1), and other gross changes in this group were observed as under:

Body Weight

The body weight of animals in both subgroups A1and A2 was increased during their respective period of time. The mean initial body weight in subgroup A1 and A2 was 9.177±0.02gm and 13.88±0.15gm respectively. The mean final body weight of subgroup A1 and A2 was 23.99±0.27gm and 41.39±0.62 gm respectively. There was a highly significant ($P<0.001$) weight gain in the final weight of both subgroups as compared to their initial weight (Table-I).

Measurement of crown rump, forelimb and hind limbs

The mean crown rump length (CRL) recorded in subgroup A1and A2 was as: 10.06±0.10cm and11.68±0.13cm respectively, mean forelimb length was 4.40±0.13cm and 5.5±0.13cm respectively and mean hind limb length was 5.58±0.14cm and 6.86±0.19cm respectively (Table-II).

Figure-1: Photograph of 2 weeks control rats, looking healthy and hair evenly distributed on body

Figure-2: Photograph of 2 weeks irradiated rats, looking ill, weak and scanty hair on the body.

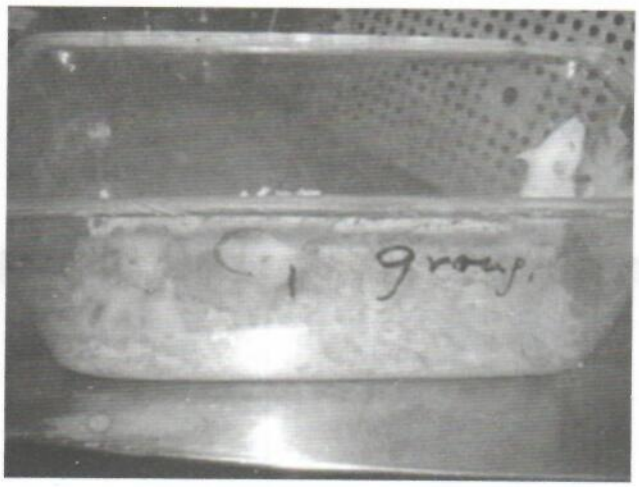

Figure-3 Photograph of 2 weeks irradiated & Vitamin C treated group rats, looking healthy & hair evenly distributed on body

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 (Figure-2).

Body Weight

The mean initial body weight in B1 and B2 subgroups was 9.24±0.3gm and 10.59±0.14gm respectively. The mean final body weight was 14.0±0.56gm and 26.89±0.26gm respectively. There was a highly significant ($P<0.001$) weight gain in both subgroups. But there was a significant ($P<0.001$) decrease in both subgroups compared to control of same duration (Table-I).

Measurement of crown rump, forelimb and hind limbs

Mean crown rump length (CRL) in sub group B1 and B2 was 7.26±0.17cm and 9.36±0.11cm respectively, which showed a moderately significant ($P<0.01$) decrease in length in B1 subgroup, when compared to control A1. There was a highly significant ($P<0.001$) decrease in length of subgroup B2, when compared with control A2 (Table-2). Mean forelimb length in subgroup B1 and B2 was 3.5±0.12cm and 4.17±0.10 cm respectively, which showed a moderately significant ($P<0.01$) decrease in both subgroups B1 and B2 as compared to control A1 and A2 respectively (Table-II). Mean hind limb length in both subgroups B1 and B2 was 4.36±0.10cm and 5.62±0.11 cm respectively, which showed a significant decreasein subgroups B1 and B2 as compared to control A1 and A2 (Table-II).

OBSERVATIONS ON IRRADIATED AND VITAMIN C TREATED GROUP-C

The animals in this group also were weak initially but later on after treatment they became active, looking healthy and they were taking breast feed regularly, hairs were evenly distributed on the body (Figure-3).

Body Weight

The mean initial body weight in subgroups C1 and C2 was 12.69±0.53gm and 12.68±0.15gm respectively. The mean final body weight was 22.77±0.73gm and 35.84±1.22gm respectively. There was a highly significant gain in the final

weight of both subgroups as compared to their initial weight. There was a significant increase in subgroups in comparison to irradiated subgroups. There was insignificant decrease in weight in subgroup C1, significant decrease in weight in subgroup C2, when compared to control (Table-I).

Measurement of crown rump, forelimb and hind limbs :

Mean crown rump length (CRL) in sub group C1 and C2 was 9.16±0.04cm and 12.2±0.03cm respectively, which showed significant increase In subgroups C, when compared with irradiated subgroups. There was significant decrease in subgroup C1 and significant increase in subgroup C2, when compared with control subgroups A1 and A2 respectively (Table-II).

Mean forelimb length in subgroup C1 and C2 was 4.46±0.10cm and 5.36±0.07 cm respectively, which showed a significant increase in subgroup C1 andC2 in comparasion to irradiated Subgroups B1 and B2 respectively. There was insignificant increase in subgroup C1 and insignificant decrease in subgroup C2, when compared with control subgroups A1 and A2 respectively (Table-II).

Mean hind limb measurement in both subgroups C1 and C2 was 5.64±0.07cm and 6.78±0.00cm respectively, which showed a significant increase in both subgroups, when compared to irradiated subgroups. There was insignificant increase of hindlimb length in both subgroups when compared with control subgroups (Table-II).

Table-I: Body Weight (gm) of Animals in DifferentGroups at Variable Time Interval

Groups	Sub groups	Treatment given	Initial weight	Final body weight at sacrificial time	
				2nd week	4th week
A (n=10)	A1	Control	9.177±0.02	23.99±0.27	-
	A2		13.88±0.15	-	41.39±0.62
B (n=10)	B1	Radiation	9.24±0.3	14±0.56	-
	B2		10.59±0.14	-	26.89±0.26
C (n=10)	C1	Radiation+ Vitamin C	12.69±0.53	22.77±0.73	-
	C2		12.68±0.15	-	35.84±1.22

Differences in Body Weight in Same and in Different Groups

Statistical Comparison	P – value
Initial vs. Final wt.at 2nd week in group-A1	<0.001
Initial vs. final wt. at 4th week in group-A2	<0.001
Initial vs. Final wt.at 2nd week in group-B1	<0.001
Initial vs. final wt. at 4th week in group-B2	<0.001
Initial vs. Final wt.at 2nd week in group-C1	<0.001
Initial vs. Final wt.at 2nd week in group-C2	<0.001*

Statistical comparison	P -value
B1vs A1	<0.001
B2vs A2	<0.001
C1vs B1	<0.01
C1vsA1	>0.05
C2vsB2	<0.01
C2vsA2	<0.05

Key: *non significant *significant*moderately significant ****highly significant**

Table-II: Mean length of CR, Forelimb and Hind limb (cm) in different Groups at variable period of Albino rat

Groups	Sub-groups	Treatment given	2 weeks			4 weeks		
			CRL	Forelimb	Hindlimb	CRL	Forelimb	Hindlimb
A	A1(n=5)	Control	10.06±0.01	4.4±0.13	5.58±0.14	-	-	-
	A2(n=5)		-	-	-	11.68±0.13	5.5±0.13	6.68±0.19
B	B1(n=5)	Radiation	7.26±0.17	3.5±0.12	4.36±0.10	-	-	-
	B2(n=5)		-	-	-	9.36±0.11	4.17±0.10	5.62±0.11
C	C1(n=5)	Radiation +Vitamin C	9.16±0.04	4.46±0.10	5.64±0.07	-	-	-
	C2(n=5)					12.2±0.03	5.36±0.07	6.78±0.00

Differences in Mean Lengths Between Different Groups at Variable Time Intervals

Statistical comparison	CRL P-value	Fore limb P-value	Hind limb P-value	Statistical comparison	CRL P-value	Fore limb P-value	Hind limb P-value
B1vsA1	P<0.01***	P<0.01***	P<0.01****	B2vsA2	P<0.001***	P<0.01****	P<0.02***
C1vsB1	P<0.01***	P<0.01***	P<0.001***	C2vsB2	P<0.001***	P<0.001***	P<0.001***
C1vsA1	P<0.01***	P>0.05*	P>0.05*	C2vsA2	P<0.04**	P>0.05*	P>0.05*

Key: *non significant *significant ***moderately significant ****highly significant

DISCUSSION:

Ionizing radiation is indispensable in medical practice but this application constitutes a two-edged sword. Radiation is therefore used in the treatment of cancer, in diagnostic imaging and as therapeutic or diagnostic radioisotopes[15]. Radiations are used in medical treatment and diagnostic procedures. Radiation therapy can be used in combination with surgery and/or chemotherapy to provide permanent control or death of a tumor[13]. 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. Nuniaetal[8], had used Swiss albino mice for whole body gamma irradiation. Lloyd et al.[16]used C57BL/6 mice and observed the effects of ionizing radiation on cortical bone.

Akureket al.[17], observed the radiation induced morphological changes in intestine of male wistar rats. These animal studies describe the radiation injuries in experimental animals. Institute of Medicine US National Academy of Sciences considers only vitamins-E and C and the mineral selenium to be dietary antioxidants[18]. Vitamin-C was also used as a radio-protective agent by Sertet al.[14].In the present study vitamin-C is used as radioprotective agent.

In the present study irradiated animals of group B, appeared ill looking, inactive with sluggish movements, not taking breast milk and hairs were irregularly distributed on the surface of body. It might be because of injurious effects of radiation, which disturb the gastrointestinal epithelium, because gastrointestinal epithelium is highly susceptible to radiation injuries and free radicals are generated, which may produce oxygen

Stress, as suggested by Kumar et al.[5] Animals in group C, which were protected with vitamin-C, were healthy, active and they were taking breast feed and the hairs were evenly distributed on body. As reported by Sertet al.[14], vitamin-C has a protective role in the prevention of gastrointestinal cancers. The body weight of all the animals was changed. The animals of group A, gained the body weight, throughout the experimental period of study. The body weight was lost in the animals of group B, from 2nd to 4th week, because they were not taking interest in breast feed that might be due to destruction of gastrointestinal epithelium and also damaging of other tissues of body. Guyton and Hall[19], also described that vitamin-C is essential for growth and strength of fibers in subcutaneous tissues, cartilage, bone and teeth.

In the present study Crown Rump, forelimb and hindlimb length, was measured.In group A, Crown rump, forelimb and hindlimb length, was normal according to age but length was decreased in group B as compared to control. It might be due to effect of radiation in epiphyseal bone growth. Eifel et al.[20], and Pappas and Cohen[11],observed that whole body irradiation causes growth retardation. In the present study, the group C animals were protected by vitamin-C. The length of crown rump, forelimb, hindlimb was increased at different time periods. It might be due to direct effect of radiation on epiphysis, which results in growth plate architecture disturbances and results in impaired growth as reported by Bakker et al.[21].It was in agreement of Bakker et al., who observed that radiation resulted in persistent growth delay in tissue of the irradiate tibiae, with a difference in length of more than 10% between the irradiated and non irradiated tibiae 15 weeks or more after irradiation. This is also in agreement with Larue et al.[6], who reported irradiation of long bones typically results in retardation of longitudinal growth. Prentice et al.[22], explained the role of nutrition in bone growth. The growth and development requires an additional supply of many different factors such as bone abnormalities occur in deficiency of vitamin-C. 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.

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