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Ameliorative Role of β-Carotene on Some Adverse Biochemical Effects Induced by γ-Radiation in Rats.

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Abstract

In the current study,120 rats were divided into six equal groups, including; **Group 1:** neither irradiated nor treated with ß- carotene (**control group**), **Group 2:** given ß- carotene in a concentration of 8 mg/kg body weight/day for a period of 14 consecutive days, **Group 3:** irradiated through whole body exposure to γ rays (6.5 Gy) single dose, **Group 4:** given ß- carotene as group 2 then irradiated as group 3, **Group 5:** exposed to γ rays as group 3then given ß- carotene as group 2, **Group 6:** was the same as group 4 but was additionally given ß- carotene for another 14 consecutive days after irradiation. The obtained results revealed that malondialdehyde (MDA), triglycerides, total Cholesterol, and low density lipoproteins (LDL) concentrations were significantly increased. While, reduced glutathione (GSH) and high density lipoproteins (HDL) concentrations as well as superoxide dismutase (SOD) activity were significantly decreased in γ irradiated rats and all β- carotene treated groups(4, 5&6) in comparison with control levels. The above mentioned elevated and reduced parameters were significantly better in all β- carotene treated groups (4, 5&6) than γ irradiated one indicating the ameliorative effect of β- carotene against γ rays-induced oxidative stress.

Introduction

The antioxidants are molecules that can safely interact with free radical and terminate the chain reaction before vital molecules are damaged **(Young and Woodside, 2001).** All antioxidants have chemical elements referred to as a redox potential, which is the measurement of their ability to be oxidized. They are classified as "reducing agents" which are needed to quickly block the chain reaction caused by free radical before cell damage can result (**McCune and Johns, 2002).** Carotenoids are a widespread group of naturally occurring fat-soluble colorants. In developed countries, 80-90% of the carotenoid intake comes from fruit and vegetable consumption. About 50 of the 700 naturally occurring carotenoids, are present in the human diet and can be absorbed and metabolized by the human body **(Maiani et al., 2009).**

β-Carotene is a naturally occurring orange-colored carbon-hydrogen carotenoid, abundant in yellow-orange fruits and vegetables and in dark green, leafy vegetables **(Krinsky and Johnson, 2005).** It is also the most widely distributed carotenoid in foods **(Rodriguez-Amaya et al., 2008).**

β -Carotene has antioxidant properties and may inhibit carcinogenesis by preventing DNA damage induced by free radicals **(Sun, 1990)** or by interfering with the metabolic activation of chemical carcinogens **(Bryla and weyand, 1991).** It also may prevent the binding of carcinogens to DNA **(Salgo et al., 1999).** In addition, β-Carotene is converted to vitamin A in humans. The hormone like effects of vitamin A on epithelial tissue cell growth and differentiation may inhibit the promotional stages of carcinogenesis **(Lippman and Meyskens, 1988).**

Electromagnetic radiation is divided into non ionizing and ionizing radiation according to the energy required for ejecting electrons from molecules. Ionizing radiation, which may exhibit the properties of both waves and particles, has sufficient energy to produce ionization in matter. This irradiation includes alpha and beta particles, besides those that behave more like waves of energy including X- rays and γ-rays **(Sanders, 1986).** Ionizing radiation interacts with living tissues inducing a number of adverse toxicological effects through ionization of the DNA, membrane lipids and proteins **(Jagetia et al., 2005** and **Meade et al., 2010).**

Exposure of animals to ionizing radiation leads to systemic dysfunctions of various organs and system**s (Miesel et al., 1996).** One of the major reasons for cellular injury after radiation exposure is the generation of free radicals and the possible increased levels of lipid peroxides in tissue. This leads to oxidative modification of the cellular molecules (**Romero et al., 1998).** Free radicals are the cause of ageing and certain diseases as cancer, cardiovascular disease, kidney and liver dysfunction **(Florence, 1995).**

The present study was planned to clarifying some of the adverse oxidative stress effects of γ-radiation and to illustrate some of beneficial impacts of β-carotene as anti-oxidant in rats. Those objectives were achieved through measuring of lipid peroxidation in the form of malondialdehyde (MDA), low molecular weight scavenger; reduced glutathione (GSH) ,the antioxidant enzyme; superoxide dismutase (SOD) activity, and lipid profile including; Triglycerides, Cholesterol, High Density Lipoproteins (HDL) and Low Density Lipoproteins(LDL) concentration.

Materials and Methods

1- ß- carotene:

The ß- carotene obtained from Sigma Co. and dissolved in pure corn oil with a concentration of 8 mg/ml and orally offered to the rats by stomach tube with dose of 8 mg/kg body weight/day according to **Maria et al. ,(1998).**

2- γ-Radiation exposure:

Irradiation was carried out as a whole body exposure of gamma ray (6.5 Gy), single dose **(Osman *et al.*, 2001)** using a ventilated Cesium-137 source (γ Cell-40) installed at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority (AEA), Cairo, Egypt.

3- Experimental animals:

One hundred and twenty adult male albino rats weighing 100-120 g were obtained from the laboratory animal house of Atomic Energy Authority, Anshas, Abu Zaabal and kept for one week for acclimatization under standard hygienic conditions. They were fed commercial balanced diet, formulated according to American Institute of Nutrition **(NRC, 1980)**. Diet and drinking water were offered *ad libitum*.

4- Experimental Design.

120 rats were divided into six equal groups, each of twenty rats as following:

Group 1: given 1.0ml saline per os daily for 14 days; (**control group**).

Group 2: given ß- carotene in a concentration of 8 mg/kg body weight/day for a period of 14 consecutive days; (**ß-carotene group**).

Group 3: rats of this group were irradiated through whole body exposure to γ rays (6.5 Gy) single dose; (**Irradiated group**).

Group 4: given ß- carotene as group 2 then irradiated as group 3; (**before irradiation protected group**).

Group 5: exposed to γ rays as group 3then given ß- carotene as group 2; (**after irradiation treated group**).

Group 6: was the same as group 4 but was additionally given ß- carotene for another 14 consecutive days after irradiation; (**before& after irradiation treated group**).

5- Blood samples:-

Blood samples were collected from retro-orbital capillary plexus at 1, 7, 14 and 21 days after irradiation and/or treatment. Blood was centrifuged to obtain serum to be used for determination of malondialdehyde(MDA) according to **Yoshioka *et al.*, (1979),** reduced glutathione(GSH) according to **Beutler et al., (1963),** superoxide dismutase(SOD) activity (EC 1.15.1.1) was assayed by method of **Minami and Yoshikawa (1979).** Triglycerides concentration was measured according to the method described by **Wahlrfeld (1974)**, Cholesterol level was measured by procedures of **Richmond, (1973)**, high density lipoprotein(HDL),and low density lipoprotein **(**LDL) were determined according to of **Assmanm (1979),** and **Bachorik and Ross(1995)** respectively.

6- Statistical analysis:

Data were presented as mean ± standard error (SE).Two way Analysis of variance (ANOVA or F) test was performed to evaluate the difference between groups and the

significance of differences between groups was estimated using least significant difference(LSD) at $P \leq 0.05$ by The Statistical Package for the Social Sciences PC statistical program(**SPSS 14, 2006)**.

Results and Discussion

The results shown in table **(1)** revealed a significant elevation in serum MDA concentration in irradiated rats from the beginning of 1st day, with a direct proportional increasing fashion with time in comparison with control. While, in all β-Carotene treated groups; (4, 5&6), the significant elevation was observed at the beginning of 7th day. The lowest increase was observed in group (6) followed by group (4) then, group (5).

The above mentioned significant increase of Lipid peroxidation (MDA) level in irradiated group compared with control is similar with that reported by **Iman and Ahmed, (2005); Ashry and salama, (2010); Osman and Hamza, (2012)** and **Mohamed and Sherif, (2013).**

Reactive oxygen species (ROS) derived from xenobiotic metabolism or UV/γ-radiation are examples of exogenous sources. Elevated ROS levels are damaging to cellular macromolecules like proteins, lipids, and DNA and will induce a state of oxidative stress and redox imbalance (**Circu** and **Aw, 2010**).

Oxidative stress induced by gamma radiation causes increase in lipid peroxidation and subsequent tissue damage through free radical production **(Morcillo et al., 2000).** The free radical–mediated indirect effect is known to account for approximately 75% of the subsequent damage to cells exposed to ionized radiation **(Murley et al., 2006).**

Zavodnik, (2003), found that, single whole-body gamma irradiation of rats resulted in increase of plasma and liver microsomal membrane lipid peroxidation, and impairment of microsomal membrane structure and function.

β-carotene treated groups either before or after irradiation or both showed significant lower serum MDA concentrations than irradiated group indicating its ameliorative effect against the adverse effect of γ radiation. These results are in agreement with **Manda and Bhatia, (2003)** and **Osman and Hamza, (2012)**.

Carotenoids partially or completely protect intact cells against oxidant-induced lipid peroxidation (**Martin et al., 1996).** Carotenoids act as antioxidants by reacting more rapidly with peroxyl radicals **(Burton and Ingold, 1984**. and **Woodall et al. , 1997).** Prevention of lipid peroxidation by carotenoids has been suggested to be mainly via free radical scavenging and singlet oxygen quenching **(Stahl and Sies, 1996 and Ezejindu et al., 2013)**.

In comparison with control group, it is obvious from the data presented in table **(2)** that the serum GSH concentration was significantly reduced in irradiated and all β-Carotene treated groups; (4, 5&6) after 1st day and 7th day respectively reaching their minimal level at the 21th day. However, the GSH concentration was still higher in groups (4, &6) than that of irradiated group until the 7Th day.

Concerning the effect of γ radiation and β-Carotene on serum superoxide dismutase (SOD) activity, the data presented in table **(3)** showed the same pattern of GSH concentration.

Such observed significant decrease of serum glutathione (GSH) concentration and superoxide dismutase (SOD) activity in irradiated rats compared with control group are coincided with the findings of **Hekmat et al., (2005)** and **Khaled and Hassan, (2005).**

Glutathione (GSH) protects cells against reactive oxygen species (intracellular radical scavenger) as well as, against many toxins, mutagens, ionized radiation and drugs **(Schnelldorfer et al., 2000)**. **Ohta et al., (2004),** indicated that, oxidative stress causes depletion of intracellular GSH; a reducing agent with its sulphydryl group, leading to serious consequences. The decrease could be due to ROS generation, which can in turn impair the antioxidant defense mechanism leading to increase lipid peroxidation.

The reduction of GSH concentration following radiation exposure may be due to the use of GSH to catalyses the reduction of lipid peroxide and hydrogen peroxide to non toxic alcohols or water respectively (**Blum and Fridovich, 1995).** Moreover, the lowering in the GSH level is suggested to be an oxidative type of tissue injury that induced by radiation (**Hasegawa et al., 2009**).

Superoxide dismutase (SOD) is the key antioxidant enzyme in the metabolism of oxygen free radical. It catalyzes the dismutation of superoxide anion radical to oxygen and hydrogen peroxide **(Balin and Altain, 1986**). Decrease in SOD activity may be attributed to inflammatory processes developed during irradiation exposure. Inflammation is characterized by the accumulation of endogenous inflammatory reactions and associated with severe oxidative damage and depleted antioxidant capacity **(Morcillo et al., 2000; Abady et al., 2003).**

β-carotene treated groups (4, 5&6) showed a significant improvement in their serum glutathion (GSH) concentration and superoxide dismutase (SOD) activity compared with irradiated group, this results are concomitant with that reported by **EL-Habit et al., (2000); Manda and Bhatia, (2003)** and **Osman and Hamza , (2012)**.

The biological mechanisms for such protection role of caretenoids may be due to its conversion to retinoids (i.e. have provitamin A activity), modulation of the proinflammatory lipoxygenases enzymatic activities and its antioxidants properties which are above that of vitamin A **(Bendich, 1993).**

The obtained results in table (4) showed a significant increase in triglycerides concentration in irradiated and all β-Carotene treated groups; (4, 5&6), the highest elevation was observed in irradiated group. The increment was observed from the 1^{St} day reaching the maximum at the 21^{Th} day. However in β-Carotene treated groups; (4, 5&6), the relative increase was still lower than that in irradiated group and started at the 7^{Th} day.

It is evidenced from the results recorded in tables **(5&6)** that serum total cholesterol and low density lipoprotein **(**LDL) concentrations in irradiated and all β-Carotene treated groups (4, 5&6) were significantly increased by the same manner of the triglycerides.

The significant increase in serum triglyceride level of irradiated group comparing with control group is in agreement with **Ashry and salama (2010); Rehab and Ibrahim, (2012)** and **Ibrahim, (2013)** The significant increase in serum cholesterol level of irradiated group comparing with control group is concomitant with **Feurgard et al., (1999)**; **Onody et al., (2003); Bhatia and Manda, (2004)** and **Rehab and Ibrahim, (2012)**.

Bowden et al., (1989).reported that irradiation induced hyper-lipidaemia through cell membrane destruction, cholesterol release and triglycerides synthesis.

Whole body gamma irradiation significantly increased liver fatty acids as well as triglycerides. This effect may be related to the oxidative stresses induced by gamma radiation which cause increase in lipid peroxidation and subsequent tissue damage through free radical production **(Morcillo et al., 2000).** Gamma irradiation at dose level 8 Gy induced changes in biliary excretion of cholesterol and total lipids **(Kafafy and Ashry, 2001).**

All groups treated with β-carotene (groups4, 5&6)showed amelioration in the effect of radiation on serum cholesterol concentration. These results are compliant with the findings of **(Rehab and Ibrahim, 2012** and **Yasemin et al., 2013)**. Also,β-carotene treated groups showed amelioration in the effect of radiation on serum triglyceride concentration. Such results are in agreement with **(Godard et al., 2009; Rehab and Ibrahim, 2012** and **Yasemin et al., 2013)**.

β-carotene has hepatic protective properties by improving liver function **(Manda and Bhatia, 2003** and **Zahra et al., 2010).** β-carotene scavenges a variety of free radical species in tissues at low partial pressures of oxygen while others antioxidants are effective only at higher oxygen concentration **(Bender and Mayes , 2006).**

As shown in table **(7)**, serum high density lipoprotein (HDL) concentration was significantly decreased after 7 days of irradiation in irradiated group and β-Carotene after Irradiation group; (group5) and after 21days in both β-Carotene before irradiation and β-Carotene before and after Irradiation groups; (groups4&6). The HDL concentration reached its minimal level after 21days of Irradiation in groups (3&5).

Such significant decrease of serum HDL level in irradiated group comparing with control group appears to go parallel with those obtained by **Hekmat et al., (2005)** and **Ashry and salama (2010).** Where, the significant increase in serum LDL level of irradiated group comparing with control group are consistent with that recorded by **Feurgard et al., (1999); Bhatia and Manda, (2004); El-Deeb et al., (2006)** and **Rehab and Ibrahim ,(2012).**

Onody et al., (2003).indicated that ionizing radiation induced oxidative stress which might alter hepatic lipid metabolism and serum lipoproteins. There is an association between radiation-induced oxidative stress and elevated levels of lipid fractions and LDL

The observed improvement effect of β-carotene against γ radiation on both serum HDL and LDL concentrations was coincided with that reported by **Romanchik et al., (1995); Woodall et al., (1997); (Godard et al., 2009)** and **Yasemin.et al., (2013).**

Oxidative modification of low density lipoprotein (LDL) is protected by the lipoprotein-associated antioxidants such as carotenoid and α- tocopherol molecules **(Romanchik et al., 1995).** β-carotene was unique among the carotenoids being has a significant effect on protecting LDL against oxidation **(Romanchik et al., 1997).**

Conclusion

It was concluded from the present study that whole body exposure to γ rays (6.5 Gy), single dose caused some adverse biochemical effects including; elevation of malondialdehyde (MDA), triglycerides, total Cholesterol, and low density lipoproteins (LDL) concentrations and reduction of reduced glutathione (GSH) and high density lipoproteins (HDL) concentrations as well as superoxide dismutase (SOD) activity.

Oral administration of ß- carotene in a concentration of 8 mg/kg body weight/day for a period of 14 consecutive days before or after irradiation or both before and after irradiation improved the adverse biochemical effects of γ rays.

Table (1); Serum MDA concentration (nmol/ml) in all groups.

Time / Groups	Time after irradiation& or treatment			
	1st Day	7th Day	14th Day	21th Day
1- Control	11.40 ± 0.40 Aa	11.16 ± 0.32 Aa	11.70 ± 081 Aa	11.32 ± 0.42 Aa
2- β-Carotene	11.28 ± 0.40 Aa	11.08 ± 0.28 Aa	11.52 ± 0.46 Aa	11.60 ± 0.42 Aa
3- Irradiated	12.78 ± 0.22 BCa	19.34 ± 0.45 BCb	25.80 ± 0.27 BCb	34.12 ± 0.38 BCb
4- β-Carotene before Irradiation	11.86 ± 0.21 ACa	16.12 ± 0.43 BDb	22.26 ± 0.34 BDb	26.28 ± 0.36 BDb
5- β-Carotene after Irradiation	12.04 ± 0.17 ACa	18.92 ± 0.80 BCb	23.54 ± 0.37 BDb	28 ± 0.49 BDb
6- β-Carotene before& after Irradiation	12.36 ± 0.38 ACa	15.62 ± 0.41 BDa	19.16 ± 0.37 BDb	24.96 ± 0.45 BDb

Data represented as means ± standard error (S.E).

In **columns** presence of the same capital letters **(A)** means non significant while, different capital letter **(B)** means significant variation with **control** group. Presence of the same capital letters **(C)** means non significant while, different capital letter **(D)** means significant variation with **Irradiated** group at ($P<0.05$).

In **rows** presence of the same small letter means non significant while, different small letter means significant variation at ($P<0.05$).

NB: These statistical terms are applied for all the following result tables.

Table (2) [#]**;** Serum glutathione (GSH) concentration (mg/dl) in all groups.

Time / Groups	Time after irradiation& or treatment			
	1st Day	7th Day	14th Day	21th Day
1- Control	16 ± 0.38 Aa	15.96 ± 0.27 Aa	16.22 ± 0.48 Aa	16.16 ± 0.32 Aa
2- β-Carotene	16 ± 0.42 Aa	15.98 ± 0.46 Aa	16.62 ± 0.33 Aa	16.02 ±0.54 Aa
3- Irradiated	14.60 ± 0. 33 BCa	12.82 ±0.23 BCb	11.34 ± 0.31 BCb	8.86 ± 0.21 BCb
4- β-Carotene before Irradiation	15.40 ± 0.33 ACa	14.36 ± 0.13 BDb	12.84 ± 0.28 BDb	11.06 ± 0.31 BDb
5- β-Carotene after Irradiation	14.96 ± 0.15 ACa	13.16 ± 0.12 BCb	12.77 ± 0.27 BDb	10.50± 0.11 BDb
6- β-Carotene before& after Irradiation	15.40 ± 0.32 ACa	14.38 ± 0.88 BDb	12.98 ± 0.14 BDb	11.88 ± 0.11 BDb

Table (3) [#]**;** Serum Superoxide dismutase (SOD) activity (U/ml) in all groups.

Time / Groups	Time after irradiation& or treatment			
	1st Day	7th Day	14th Day	21th Day
1- Control	28.00 ± 0.44 Aa	28.40 ± 1.20 Aa	28.60 ± 0.92 Aa	28.80 ± 0.80 Aa
2- β-Carotene	27.60 ± 1.07 Aa	27.2 ±0.86 Aa	27.60 ± 0.81 Aa	29.00 ± 0.89 Aa
3- Irradiated	26.70 ± 0.66 BCa	24.00 ± 0.44 BCb	20.40 ± 0.50 BCb	16.40 ± 0.74 BCb
4- β-Carotene before Irradiation	27.80 ± 0.80 ACa	26.00 ± 0.50 BDb	23.80 ± 0.37 BDb	19.90 ± 1.07 BDb
5- β-Carotene after Irradiation	27.60 ± 0.82 ACa	24.60± 0.50 BCb	22.40± 0.50 BDb	19.40 ± 0.92 BDb
6- β-Carotene before& after Irradiation	27.40 ± 0.96 AC	26.40 ± 0.54 BDa	24.60 ± 0.50 BDb	21.40 ± 0.50 BDb

#: The same statistical terms of table (1).

Table (4) [#]; Serum triglyceride level (mg/dl) in all groups.

Time / Groups	Time after irradiation& or treatment			
	1st Day	7th Day	14th Day	21th Day
1- Control	40.60 ± 0.73 Aa	41.20 ± 0.37 Aa	40.00 ± 0.70 Aa	40.60 ± 0.74 Aa
2- β-Carotene	40.20 ± 0.48 Aa	41.00 ± 0.44 Aa	40.80 ± 0.66 Aa	41.00 ± 0.83 Aa
3- Irradiated	41.76 ± 0.55 BCa	61.20 ± 1.15 BCb	73.00 ± 1.14 BCb	81.80 ± 1.28 BCb
4- β-Carotene before Irradiation	41.20 ± 0.36 ACa	45.8 ± 0.86 BDb	52.2 ± 0.86 BDb	58.8 ± 0.37 BDb
5- β-Carotene after Irradiation	40.61 ± 0.72 ACa	52.00 ± 0.70 BDb	54.8 ± 0.86 BDb	61.2 ± 0.73 BDb
6- β-Carotene before& after Irradiation	41.00 ± 0.43 ACa	47.6 ± 0.74 BDb	52.2 ± 1.20 BDb	57.00 ± 1.18 BDb

Table (5) [#]; Serum total cholesterol level (mg/dl) in all groups.

Time / Groups	Time after irradiation& or treatment			
	1st Day	7th Day	14th Day	21th Day
1- Control	52.20± 0.35 Aa	54.24 ± 1.43 Aa	54.4 ± 1.52 Aa	53.32 ± 2.59 Aa
2- β-Carotene	53.84 ± 1.89 Aa	53.8 ± 1.24 Aa	54.16 ±1.42 Aa	53.2 ± 0.68 Aa
3- Irradiated	55.78± 1.41 BCa	93.84 ± 5.66 BCb	121.8 ± 2.85 BCb	139.76 ±1.54 BCb
4- β-Carotene before Irradiation	52.3 ± 0.33 ACa	73.96 ± 2.76 BDb	89.92± 1.33 BDb	103.36 ±1.01 BDb
5- β-Carotene after Irradiation	53.31 ± 2.57 ACa	87.44 ± 1.20 BDb	97.96 ± 2.89 BDb	113.8 ± 2.07 BDb
6- β-Carotene before& after Irradiation	53.8 ± 1.22 ACa	78.92 ± 2.58 BDb	94.44 ± 1.41 BDb	100.6 ± 0.83 BDb

#: The same statistical terms of table (1)**.**

Table (6) [#]; Serum low density lipoprotein (LDL) level (mg/dl) in all groups.

Time / Groups	Time after irradiation& or treatment			
	1st Day	7th Day	14th Day	21th Day
1- Control	30.2 ± 0.37 Aa	31± 0.70 Aa	31.6 ± 0.50 Aa	30.6 ± 1.077 Aa
2- β-Carotene	31.8 ± 0.86 Aa	31.6 ± 0.50 Aa	31.2 ± 1.01 Aa	30.8 ± 0.58 Aa
3- Irradiated	32.62 ± 1.02 CAa	68.8 ± 3.57 CBb	98.4 ± 2.13 CBb	114.6 ± 0.67 CBb
4- β-Carotene before Irradiation	30.2 ± 0.35 ACa	50.4 ±1.50 BDb	66 ± 0.54 BDb	79.6 ± 0.74 BDb
5- β-Carotene after Irradiation	30.6 ± 0.07 ACa	65 ± 0.70 BDb	73.8 ± 1.15 BDb	90.4 ± 1.20 BDb
6- β-Carotene before& after Irradiation	31.6 ± 0.50 ACa	54.2 ± 1.49 BDb	70.6 ± 0.87 BDb	78.4 ± 0.50 BDb

Table (7) [#]; Serum high density lipoprotein(HDL) level (mg/dl) in all groups.

Time / Groups	Time after irradiation& or treatment			
	1st Day	7th Day	14th Day	21th Day
1- Control	13.6 ± 0.24 Aa	15.0 ± 0.38 Aa	14.8 ± 1.24 Aa	14.6 ± 1.56 Aa
2- β-Carotene	14.0 ± 1.0 Aa	14.0 ± 0.94 Aa	14.8 ± 0.58 Aa	14.2 ± 0.37 Aa
3- Irradiated	14.80 ± 0.59 ACa	12.80±2.20 BCb	8.8 ± 2.39 BCb	8.8 ± 0.86 BCb
4- β-Carotene before Irradiation	13.6 ± 0.24 ACa	14.8 ±1.63 ADa	13.6 ± 1.07 ADa	12.0 ± 0.44 BDb
5- β-Carotene after Irradiation	14.5 ± 1.51 ACa	12.0 ± 0.83 BCb	11.4 ± 0.92 BDb	11.2 ± 0.96 BDb
6- β-Carotene before& after Irradiation	14.0 ± 0.94 ACa	15.0 ± 1.04 ADa	13.4 ± 0.50 ADa	11.8 ± 0.44 BDb

#: The same statistical terms of table (1).

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