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Grape Seed Extract Ameliorate Gamma Radiation Induced Suppression of Delayed Type Hypersensitivity to Oxazolone in Mice

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ABSTRACT

The main aim of the study was to assess the role of grape seed extract in gamma radiation-induced impact on delayed type hypersensitivity (DTH) response to oxazolone in Swiss albino mice. Eight groups each of ten have been used. Four groups have exploited to assess the effect of grape seed, gamma radiation and both on the induction of contact sensitivity and four other groups have been utilized to evaluate the effect of grape seed, gamma radiation and both on the elicitation of contact hypersensitivity. In each group, one was used as a control, the second received 2 Gy gamma irradiation 24 hr before the induction or the elicitation phase using the mouse ear swelling test (MEST). Third group was maintained on oral grape seed extract (0.1%), and a fourth was maintained on grape seed extract (0.1%) and received with total body irradiation (2Gy). At the end of the experiment sera from all groups were tested for total antioxidant and dismutase activities and gamma interferon level. Data showed that exposure to gamma irradiation had a negative impact on DTH response to oxazolone whether the exposure was before the elicitation or the induction phase. Data also showed that radiation was associated with a significant mounting of serum total antioxidant, dismutase activity, gamma interferon. Grape seed extract was able to restore the DTH response of irradiated mice to the control value. This was associated with a decline in serum total antioxidant, dismutase activity and gamma interferon in comparison to gamma irradiated animals.

Keywords: ionizing radiation- delayed type hypersensitivity- grape seed extract – interferon gamma- oxazolone**.**

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INTRODUCTION

Ionizing radiation causes a wide array of biological responses [1]. It targets, through free radical attack, highly dividing cells of the hemopoietic system, gastrointestinal mucosa and gonadal organs [2]. The ability of ionizing radiation to retard cell proliferation made it a useful modality in the treatment of large number of neoplasms [3]. Ionizing radiation, however, causes immunosuppression[4] that may deprive the host from an important mechanism of tumor rejection. This made the search for immunomodulatory agents that preserve the immune function under the negative influence of ionizing radiation an urgent demand. The immunosuppressive effects of ionizing radiation, particularly gamma rays, are well recognized. One of these effects is most clearly evident in term of the negative influence of gamma radiation on the delayed type hypersensitivity (DTH) response to contact sensitizers, which is considered to be a prototypic T-cell-mediated immune response [5]. Experimental evidence in mice and in clinical practice suggest that exposure to gamma radiation is a risk factor in the susceptibility to opportunistic infection [6]. Collectively, these observations suggest that protection from gamma radiation-induced immunosuppression may be an important strategy in the management of cancer by radiotherapy. Interferon gamma (IFNγ)-producing T cells are important effector cells in the DTH response and also are involved in reducing the development of ultra violet B (UVB)-induced skin tumors[7]. The role of (IFNγ) in DTH induced by the haptens, oxazolone and 2, 4, 6-trinitrochlorobenzene (TNCB), has also been examined in mice. The 24-hour ear-swelling response to oxazolone or TNCB in sensitized animals was not considerably reduced by the disruption of IFNγ signalling. This implicated that oxazolone- and TNCB-induced contact hypersensitivity is marginally dependent on a functional IFNγ system [8]. The mechanism of gamma irradiation-induced immuosuppression has been extensively studied. It has been demonstrated that gamma radiation-induced free radicals induce DNA damage and lymphocyte apoptosis. So exposure to gamma radiation is one of the mechanisms that trigger the molecular cascades of apoptosis in lymphocytes [9]. This concept has been supported by the finding that pretreatment of lymphocytes with antioxidants reduced their susceptibility to gamma irradiation-induced immuosuppression [10]. Oral supplementation with antioxidants ameliorated the negative effect of gamma radiation on the immune response in experimental animals [11]. Another possible mechanism for radiation-induced immune suppression is the generation of regulatory T lymphocytes [12]. It has been shown that regulatory T lymphocytes modulate DTH reaction through gamma interferon dependent mechanism. Neutralization of gamma interferon activity from regulatory T lymphocytes abolished

their negative impact on the DTH [13]. Grapes (Vitis vinifera) are rich in polyphenols with nearly 60-70% of these polyphenols being found in the grape seeds. The seeds include a larger fraction of proanthocyanidins, which are composed of dimers, trimers, tetramers and oligomers of monomeric catechins or epicatechins[14]. These grape seed proanthocyanidins (GSPs) have been shown to have anti-oxidant [15], anti-inflammatory and anti-skin carcinogenic activities[16]. Oral administration of GSPs ameliorated UV-induced skin tumor progress in terms of tumor occurrence, tumor proliferation and tumor growth in mice. Recently, supplementation with GSPs has been established to inhibit the UVB-induced suppression of the DTH response to the contact sensitizer, 2,4-dinitrofluorobenzene (DNFB) in C3H/HeN mice. Dietary supplementation of GSPs activated CD8+ T cells and enhanced their ability to produce higher levels of IFNγ (>5-fold, $P<0.001$) and IL-2 (8-fold, $P<0.001$) than CD8+ T cells from UVB-exposed mice. These alterations in cytokine profile of CD8+ T cells under the influence of GSPs have been suggested to play a role in the rescuing the immune response under the negative influence of UVB radiation [7]. This work has been conducted to study the effect of oral supplementation with Grape seed extract (GSE) on gamma irradiation-induced inhibition of DTH response to oxazolone in mice. The effect of (GSE) on the antioxidant status and interferon production has been also been looked at.

MATERIALS AND METHOD

Animals

Adult male Swiss albino mice were used though out this study. Eight weeks old with average weight 25±2 gm. Mice were purchased from the Institute of Serum and Vaccines, Dokki, Giza and they were maintained in the animal facility of National Center for Radiation Research & Technology, Atomic Energy Authority.

Gamma-radiation

Animals positioned in their metal cages were mechanically transported to the irradiation chamber. The exposure time was adjusted in accordance with the radiation dose emitted per second from the source to achieve the prescribed total dose, i.e since the dose rate was 7.85 mGy/sec., animals were exposed for 4 minute, 24 second.

Contact sensitizer

Oxazolone (4- ethoxymethylene -2-phenyl- oxazol -5-one) was purchased from Sigma-Aldrich.

Antioxidants

The purified Grape seed dry extract was supplied by MEPACO-MEDIFOOD. Briefly, Botanical Source is vitis vininfera L, Batch Number is HHG110516. GSE contain approximately 95.0% proanthocyanidin , and GSE are stable for at least 2 years.

Experimental Design

In order to study the effect of gamma irradiation and GSE on (DTH) response to oxazolone, mice were divided into four almost identical groups each of ten for evaluation of each parameter. They were classified as follows: 1) Control group receiving neither irradiation nor Grape seed dry extract and represented as (A). 2) Irradiated group: Animals were subjected to one shot of whole body γ-rays (2 Gy) and represented as (B). 3) Group receiving receive 0.1% Grape seed dry extract in drinking water (as a sole source) one week before the sensitization and throughout the experiment and represented as (C) 4) Groups exposed to 2Gy gamma rays receive 0.1% Grape seed dry extract 1 week before irradiation and throughout the experiment and represented as (D).

Assessment of DTH response, Mouse Ear Swelling Test (MEST)

The mouse ear-swelling test was performed as described by Blaylock, et al., 1993[17]. On day (0) mice were sensitized on their shaved abdomen with 25µ of 1% Oxazolone in acetone: olive oil (4:1). On day (5) mice were challenged with 10µ of 2% Oxazolone on the ventral and dorsal ear surface of both ears. Ear thickness was measured immediately before and 24 hours after challenge using a micrometer. The increase in ear thickness was calculated for each ear and the mean of the increase was expressed as units of 10^{-2} mm. To test the effect of gamma irradiation on the induction phase of contact sensitivity, animals were exposed to gamma irradiation to total dose of 2Gy 24 hr before sensitization. To test the effect of gamma radiation on the elicitation phase, mice were sensitized with 25µ of 1% Oxazolone in acetone: olive oil (4:1) on their shaved abdomen on day (0) and exposed to gamma radiation on day (4) i.e 24 hr before the elicitation of contact sensitivity. Determination of serum mouse interferon gamma level was done utilizing ELIZA kit purchased from Sigma-Aldrich. Determination of serum total antioxidant Capacity was done according to the method described by Koracevic, et al., 2001[18] Determination of serum superoxide dismutase (SOD) level was done utilizing the use of nitroblue tetrazolium and NADH mix as described by Nishikimi, et al., 1972[19].

Statistical Analysis

One way ANOVA is a statistical technique that is used to compare the means of more than two groups. A p value <0.05 was considered significant.

RESULTS AND DISCUSSION

Effect of gamma radiation, grape seed dry extract and both on the induction of delayed type hypersensitivity to oxazolone in mice.

Gamma radiation-induced immunosuppression has been implicated in a number of disorders. Patients undergoing radiotherapy experience an increased incidence of opportunistic infection [20]. Occupational exposure to low dose radiation is also associated with increased risk of infection and decreased production of cytokines in response to antigens [21]. Atomic bomb survivors showed overall decreases in naïve T cell subsets, with normal CD4+, and increased CD8+ memory T cell populations [22]. In bulk culture, T cell responses to mitogens and alloantigens including IL-2 production and proliferation were reduced [23] and this was attributable to the decreased proportion of CD4+ naïve T cells. We examined the effects of oral supplementation of 0.1% GSE on gamma radiation-induced suppression of DTH to 1% oxazolone in mice. The DTH reaction is complex and involves intricate interaction of wide array of cells and soluble factors (cytokines). It is initiated by a sensitization phase which is triggered by haptenation of cellular proteins which lead to modification in their structure. The modified self protein appears to be foreign to the host and undergo further uptake by professional antigen presenting cells (APC). APCs migrate to local lymph node where they engage with receptor specific bearing T lymphocyte in series of signal transduction pathways that end in their activation and proliferation (induction phase). The main aim of proliferation is to overcome the low precursor frequency of antigen specific T lymphocytes and to increase the antigen specific T cell pool. Sensitized T lymphocytes migrate to different tissue where they become more vulnerable to antigen stimulation to produce immunomodulatory cytokines that recruit and activate monocyte/macrophages (elicitation phase)[24]. Data demonstrating the contact sensitivity response to 1% oxazolone in the four tested groups are shown in Figure 1. Bars represent the difference between the ear thickness before and after the elicitation reaction. Animals exposed to gamma radiation (group B) experienced significant decrease of the ear swelling response by 42% ($p \leq 0.05$) of the control level, while administration of grape seed dry extract (group C) caused significant decrease (24%, $p \leq 0.05$) in comparison to control animals. Grape seed extract, however was able to restore the DTH response to oxazalone in gamma-irradiated animals almost to the control level (Figure. 1)

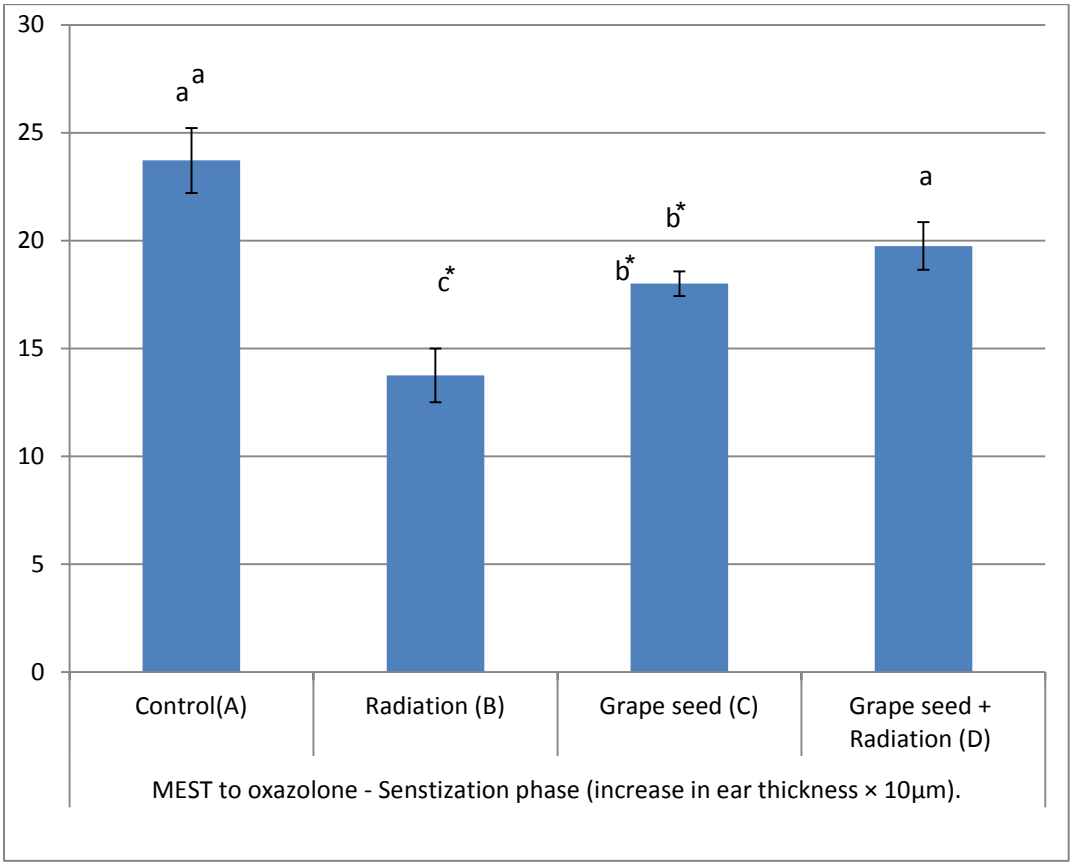

Figure.1: Effect of gamma radiation, grape seed dry extract and both on the mouse ear swelling response to oxazolone in Swiss albino mice (Sensitization Phase).

Effect of gamma radiation, grape seed dry extract and both on the elicitation of delayed type hypersensitivity to oxazolone in mice.

Animals exposed to 2 Gy gamma radiation before the elicitation of DTH experienced a significant decrease of the ear swelling response to oxazolone ($p \leq 0.05$). On the other hand, it caused a significant decrease by 22% in group (C) in comparison to control animal. Grape seed extract was able to regain the DTH response to oxazolone in gamma radiation exposed mice (non significant response in mouse ear swelling test in group D). Data from the present study showed that the percentage of decline in DTH caused by 2 Gy gamma radiation was higher when applied before the induction than the elicitation phase (42% versus 30%) (Figure.1, 2). This could be explained by the differential sensitivity of the elements engaged in either mechanism to the effect of gamma irradiation. For example, it has been shown that exposure to gamma irradiation retards hapten induced migration of Langerhans cells (LCs) [25]. This might have reduced the number of LCs involved in the induction phase and increased the number of LCs engaged in the elicitation phase [26]. This may also be due to relative resistance of memory T cells to radiation in comparison to naïve T cells [27].

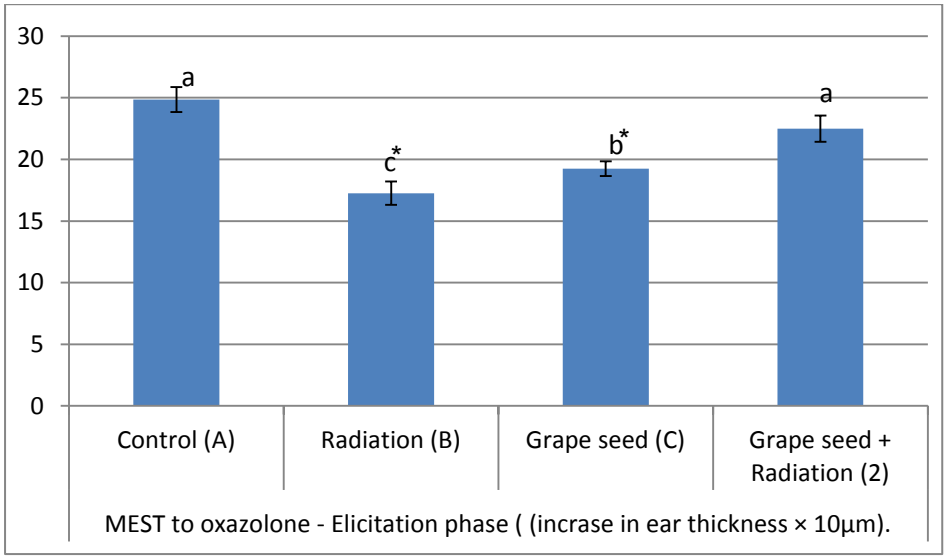

Figure.2: Effect of gamma radiation, grape seed dry extract and both on the mouse ear swelling response to oxazolone in Swiss albino mice (Elicitation Phase).

Effect of gamma radiation, grape seed dry extract and both on serum interferon gamma concentration and antioxidant parameters.

The augmentation of serum interferon level in response to gamma irradiation along with the decline in DTH response seemed to be inconsistent. However, it may suggest that the enhanced response does not reflect an effector but rather a regulatory function. This assumption is favored by many findings which support the important role of enhancement of regulatory T cells in response to gamma irradiation. For example, an enhancement in T regulatory (CD4+CD25+) cells in individuals exposed to irradiation at Chernobyl has been described. Also, in mice primed to an major histocompatibility class I alloantigen, the augmented graft rejection T memory response is exactly lost several weeks following total body gamma irradiation (TBI), whereas identically treated naïve mice at the same time point had totally improved normal rejection kinetics. Reduction *in vivo* with anti-CD4 or anti-CD25 antibodies showed that the involved cells are with a regulatory T cell (T reg) phenotype. The loss of the T memory response following TBI was coupled with a comparative increase of CD4+CD25+ Foxp3+ expressing T regs, as compared to the CD8+ T effector cells necessary for skin graft rejection [28]. It has been recently reported that Foxp3+ Treg cells readily produced interferon gamma (IFNγ) *in vivo* in the inflammatory model of graft-versus-host disease (GVHD) and throughout a Th1-dominated immune response to intracellular microbes. Also it has been shown that stimulation in vitro via TCR in the occurrence of IL-12 alone was adequate to induce IFNγ production by Treg cells in a dose-dependent manner. Transfer of donor Treg cells could avoid fatal GVHD. Interestingly, the allogeneic donor, but

not recipient Foxp3+ Treg cells produced IFNγ after transplantation, suggesting that this cytokine production was alloantigen specific. Blocking of IFNγ with specific mAb totally abolished the useful effect of donor Treg cells. In the mean time, only wild-type Treg cells, but not Treg cells from IFNγ –deficient donor mice, prevented GVHD indicating that Treg cell-intrinsic IFNγ production was required for their function[29]. In this study, we have evaluated the IFN-γ response to whole body exposure to γ-radiation (2Gy). Animals exposed to gamma radiation (group B) experienced significant increase of serum interferon gamma concentration by 141% ($p \leq 0.05$) of the control level. Administration of grape seed dry extract (group C) showed no significant effect on basal serum interferon level. On the other hand, mice in group (D) which were both irradiated with 2Gy gamma irradiation and supplemented with grape seed extract experienced a significant restoration of DTH response when compared to control, however, the serum gamma interferone level was significantly less than that of irradiated animal (Figure. 3). There is no evidence so far supporting the assumption that Treg cells producing gamma interferon is a key player in DTH reaction, however, data from the present study support this assumption. Not only the increased interferon response to radiation was associated with declined DTH but also the decline in irradiation-induced production of interferon by GSE was associated with concomitant improvement of DTH response to oxazolone (Figure. 1, 2 and 3). It should be noted, however, that antibody-mediated depletion of CD4+ T cells previous to sensitization of BALB/c mice with 2,4-dinitrofluorobenzene (DNFB) or oxazolone (Ox) resulted in augmented and prolonged DTH responses, while depletion of CD8+ T cells, resulted in low or abrogated responses, demonstrating CD8+ T cells as the effector cells in CHS. Sensitization with DNFB or Ox induced lymph node cell populations of CD8+ T cells to produce interferon gamma and no interleukin (IL-4 or IL-10), and CD4+ T cells to produce Il-4 and Il-10 and no or little detectable IFN gamma[7]. The role of IFNγ in contact hypersensitivity induced by the haptens, oxazolone and 2, 4, 6-trinitrochlorobenzene (TNCB), has also been explored in mice with a targeted interference of the IFNγ receptor (IFNγ-R-/-). The 24-h ear-swelling response to oxazolone or TNCB in sensitized animals was not considerably reduced by the interference of IFNγ signalling. Dermal mononuclear infiltrates (MN) and epidermal microabscesses, in spite of this, were clearly diminished in the mutant mice. The hapten-induced upregulation of intercellular-adhesion molecule-1 (ICAM-1) and major histocompatibility complex (MHC) class I in IFNγ-R-/-mice was less significant when compared to wild-type mice. This indicated that oxazolone- and TNCB-induced contact hypersensitivity is partially dependent on a functional IFNγ system [8]. In view of the previous data, it seems that our assumption of a possible regulatory role of gamma interferon in response to

gamma irradiation is not a physiological role. In other words, it only works under non physiological conditions such as exposure to ionizing radiation. This is favored by the finding of a positive correlation between serum gamma interferon level and MEST values in gamma irradiation unexposed animals and this correlation was lost in gamma irradiation exposed animal either alone or with GSE intake (data not shown). It is worth mentioning that Vaid and Singh et al, (2011) [7] documented the ability of GSPs to restore DTH suppressed by exposure to UVB and that the immunopreventive effect of GSPs against UVB-induced immunosuppression was mediated, at least in part, during the inhibition of the progress and/or inactivation of CD4+ regulatory T cells. In contrast to our data, they observed that the levels of Th1 (Tc1) cytokine (IFNγ) was much elevated in CD8+ T cells from GSPs-treated mice, than UVB exposed animals.

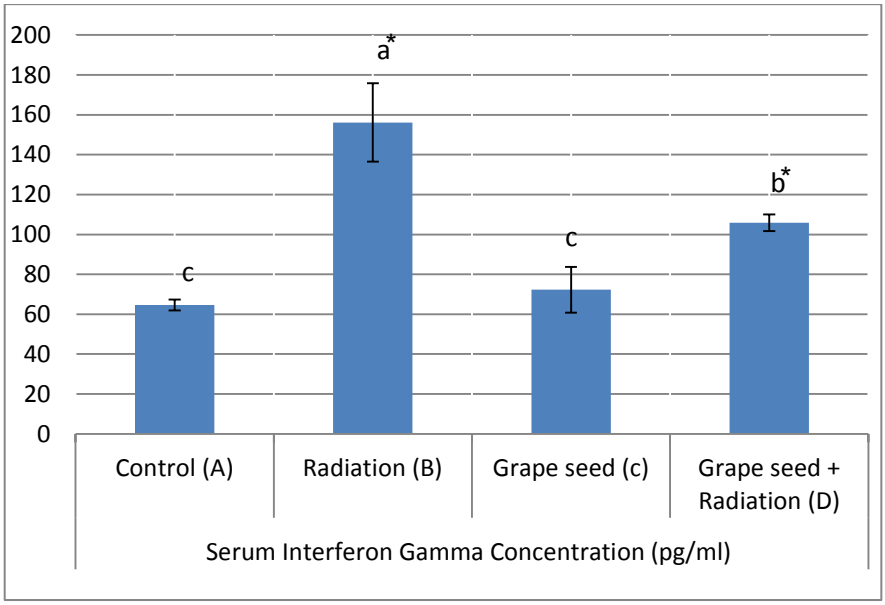

Figure.3: Effect of gamma radiation, grape seed dry extracts and both on the serum interferon gamma concentration in Swiss albino mice.

The serum total antioxidant capacity and superoxide dismutase level in irradiated Swiss albino mice increased significantly 604%, 396% ($p \leq 0.05$) respectively relative to control in mice exposed to a dose of 2Gy. However, they were non significantly changed in control and in animals treated with orally Grape seed extract. In the mean time, mice in group (D) experienced significant increase of serum total antioxidants capacity and superoxide dismutase concentrate 394% and 217% ($p \leq 0.05$) of the control level. Surprisingly, the serum total antioxidant and superoxide dismutase (SOD) activity were significantly mounted in animals exposed to 2 Gy gamma irradiation. The increase in antioxidants parameters levels may be due to the activation of protective response to counteract the excessive formation of ROS as SOD is the first endogenous

antioxidant defense mechanisms as shown by pavil, (2003) [30]. The inability of GSE administration to add to serum antioxidant and superoxide dismutase activities in gamma irradiated animals was unexpected in view of the well known antioxidant potential of GSE (Figure. 4, 5). However, this might have resulted from temporary impairment in absorption induced by gamma irradiation. This also precludes a major role of the antioxidant activity of GSE in rescuing gamma irradiation-induced suppression in DTH. The present data suggest that radiation induced-immunosuppression may not entirely be mediated through free radical preponderance. Gamma irradiation-induced suppression of DTH to oxazolone was associated with enhanced total antioxidant and superoxide dismutase activities. In the mean time, supplementation of GSE reduced these activities but still was able to augment DTH over powered by gamma irradiation.

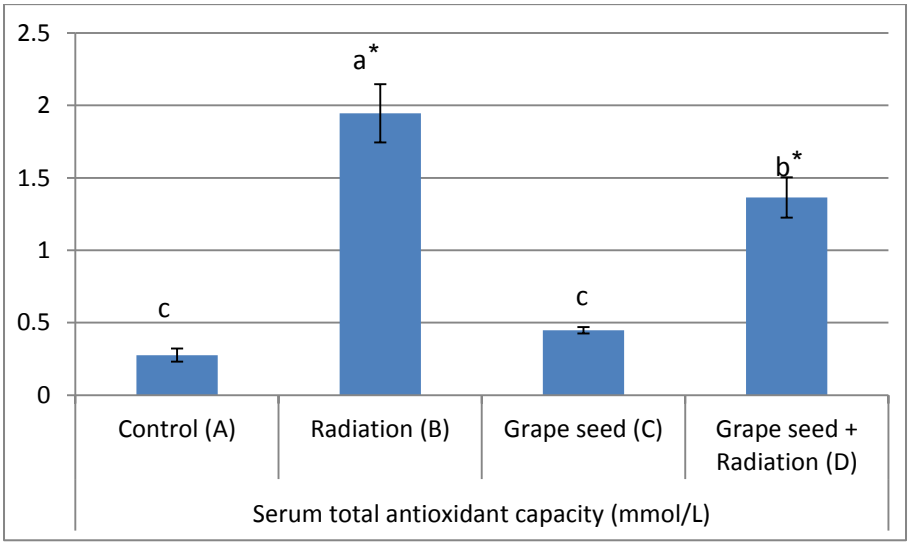

Figure.4: Effect of gamma radiation, grape seed dry extracts and both on the serum total antioxidant capacity in Swiss albino mice.

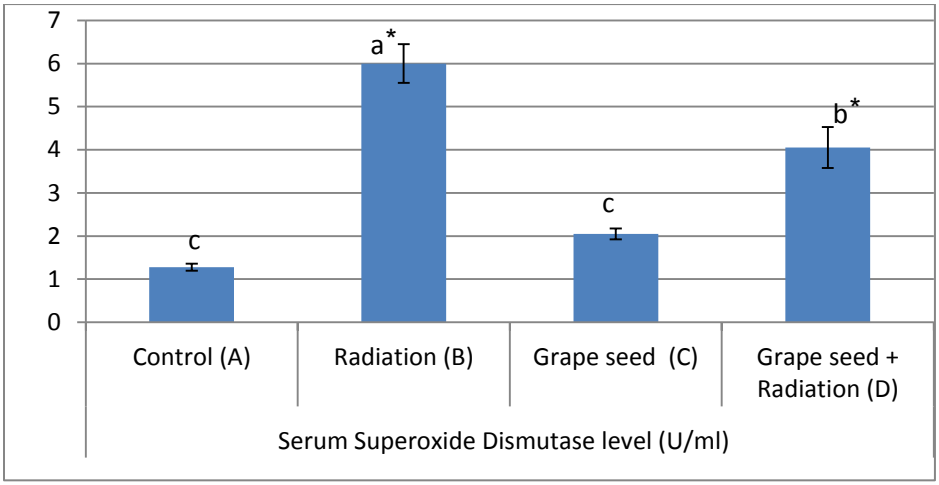

Figure.5: Effect of gamma radiation, grape seed dry extracts and both on the serum superoxide dismutase level in Swiss albino mice.

CONCLUSION

The present study documents the ability of GSE administration as an immune radio protector in mice exposed to total body irradiation at a dose of 2 Gy using DTH response to 1% oxazolone as a model. This was promenent whether the exposure of gamma irradiation was executed 24 hr before the induction or the elicitation of DTH. The study also diversifies the serum antioxidant status and gamma interferon level from gamma irradiation- induced suppression of DTH to oxazolone in mice or its amelioration by GSE.

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