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Effect of Green Tea Extract on T cell Mediated Hypersensitivity Reaction in BALB/c Mice Exposed to Gamma Irradiation

A. M. Hashim, M. M. Ismail Al-kadey, M. H. Shabon, S. M. Hussien

Faculty of Pharmacy, Cairo University, Atomic Energy Authority.
E-mail: soha_hussien@hotmail.com
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ABSTRACT

Gamma radiation is widely used in the treatment of malignant neoplasms. However, it deprives the host immune function which may retard tumor rejection by the immune response. The main purpose of the present study is to test the ability of green tea dry extract to restore the T cell hypersensitivity reaction in gamma irradiated BALB/c mice. It aims also to elucidate the possible mechanism of action of ionizing radiation and green tea dry extract in the immune function. Four groups of BALB/c mice, each of ten, have been used in each experiment. The first group served as a control, the second group received green tea dry extract and the third group was exposed to 2Gy gamma irradiation, while the fourth group received green tea dry extract before and after gamma irradiation. The following parameters were determined, the contact sensitivity reaction by the mouse ear swelling response, local dendritic cell migration, local lymph node weight, lymphocyte proliferation, spleen and thymus weight with their lymphocyte count. The effect of gamma irradiation and green tea dry extract on the elicitation phase of contact sensitivity was also determined.

Data from the present study showed that gamma irradiation caused a significant decrease of the mouse ear swelling response and retarded dendritic cell migration. They also showed a significant decline in the lymphocytes proliferation in lymph node draining the contact sensitizer application. Total body exposure to 2 Gy gamma irradiation induced marked decline of thymus weight and thymocyte count, while it reduced spleen weight and spleenocyte count to a lesser extent. Exposure to gamma irradiation enhanced the elicitation phase of contact sensitivity. Administration of green tea dry extract partially preserved the contact sensitivity response to oxazolone in gamma irradiated BALB/c mice. It markedly minimized the enhancement of the elicitation phase of ear swelling. In conclusion, the present study heralds a beneficial role of green tea dry extract in combating the negative effect of gamma irradiation on the T cell function.

Key words: Green tea- ionizing radiation- T cell hypersensitivity reaction- dendritic cells.

INTRODUCTION

Ionizing radiation causes a wide array of biological responses. It targets, through free radical attack, highly dividing cells of the hemopoietic system, gastrointestinal mucosa and gonadal organs**.** The ability of ionizing radiation to retard cell proliferation made it a useful modality in the treatment of large number of neoplasms**.** Ionizing radiation, however, causes immunosuppression that may deprive the host from an important mechanism of tumor rejection through an integrated immune function. This made the search for immunoreactive agents that preserve the immune function under the influence of ionizing radiation an urgent demand [(1)].

The T cell plays a pivotal role in protection against malignant transformation. Substantial advances in understanding the T cell function have been derived from the study of contact sensitivity reaction. These studies revealed a complex sequential series of events that involve engagement of accessory cells and modulation by a variety of endogenous chemical mediators [(2)]**.** Small molecules, called haptens, first bind covalently to cellular or extracellular proteins These complex molecules are taken by antigen presenting cells (APCs) by pinocytosis or receptor mediated–endocytosis. The APCs involve epidermal Langerhans cells and dermal dendritic cells (DCs) which upregulate the expression of the major histocompatability complex (MHC) and co-stimulatory molecules. They migrate to the regional lymph nodes carrying the haptens in the context of MHC and present then to the responsive T cells**.** The T cells undergo activation, proliferation and differentiation into immunoeffector cells**.** They circulate and reach different tissues, this is called the sensitization phase of contact sensitivity**.** Once they encounter the haptens presented by APCs at a distant site, they mediate the elicitation phase characterized the release of array of cytokines that cause inflammatory reaction and recruit inflammatory cells at the challenge site [(3)]**.**

Green tea is one of the most potent antioxidant in the world**.** Epidemiologic research has revealed that individuals who drink a lot of green tea are less likely to develop cancer [(4)]. Green tea contains many ingredients considered to promote health such as polyphenolic flavonoids, of which epigallocatechin galiate (EGCG) is the major constituent. Evidence is mounting that EGCG has anticarcinogenic activity in vitro**.** Supporting the results of the epidemiologic research on the correlation between drinking green tea and the reduced risk of morbidity from cancer. Beside the potent antioxidant function of

green tea, it has been demonstrated that green tea has immunomodulatory potentials [5]. The latter showed that in Lewis lung carcinoma bearing mice given green tea as drinking water, all aspect of the immune functions were improved, along with inhibition of tumor growth. [6] recently showed that the combination of EGCG and DNA vaccination led to an enhanced tumor-specific T-cell immune response and enhanced antitumor effects, resulting in a higher cure rate than either immunotherapy or EGCG alone.

The aim of the present study is to investigate the ability of green tea to rescue the T cell function under the influence of gamma irradiation. The T cell function will be exemplified in the present study by the contact sensitivity reaction to oxazolone in BALB/c mice. It also aims to evaluate the possible role of green tea dry extract in enhancing the immunological reaction under the effect of gamma radiation.

MATERIALS AND METHODS

Reagents:

Green tea extract:

Green tea dry extract was purchased from MEPACO-MEDIFOOD (Arab company for pharmaceutical and medicinal plants).

Oxazolone:

Oxazolone is a chemical allergen used for immunological experiments, particularly for experiments on delayed type hypersensitivity. Its long chemical name is 4-ethoxymethylene–2–phenyl–2–oxazolin–5–on(Oxazolone)[7]. Oxazolone was purchased from Sigma-Aldrich chemical company.

Fluorescien isothiocynate (FITC):

Fluorescien isothiocyanate was purchased from Fluka Analytical Company.

Animals:

Forty adult male BALB/c, six to eight weeks old mice were used through out this study. They were watered and feeded with balanced diet. Mice were purchased from Institute of Serum and Vaccines and were divided into four identical groups, each group comprised of ten mice.

1-Control group receiving neither irradiation nor green tea dry extracts and represented in tables of results as (A).

2-Group receiving green tea dry extracts (dose 0.1% green tea dry extracts in drinking water) one week before the sensitization and throughout the experiment lasted for a week and represented as (B).

3-Irradiated group with 2 Gy gamma rays from cesium 137 source at National Center for Radiation Research & Technology. The dose rate of the source at time of experiment was 0.462 Gy/min and represented as (C).

4-Group exposed to 2Gy gamma rays and receiving green tea dry extracts (dose 0.1% green tea dry extracts in drinking water) 1 week before irradiation then throughout the experiment time and represented as (D).

Mouse Ear Swelling Test (MEST).

The mouse ear swelling test was performed as described by [8]. On day (0) mice were sensitized on their shaved abdomen with 25µl of 1% oxazolone in acetone: olive oil (4:1). On day (5) mice were challenged with 10µl 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 at 24 hr before sensitization. To test the effect of gamma radiation on the elicitation phase, mice were sensitized with oxazolone on day (0) and exposed to gamma radiation on day (4) i.e 24 hr before the elicitation of contact sensitivity.

Dendrtic cell migration to local lymph node.

The effect of gamma radiation on antigen-induced DCs migration was examined using the method described by [9]**.** The method utilized the ability of Fluorescien Isothiocyanate (FITC) to act as a contact sensitizer and the availability to be traced in DCs migrating to local lymph node by fluorescien microscopy. Briefly animals were sensitized on their dorsal surface of both ears with 25µl of 0.5% (FITC) in acetone: dibutylphathalate (1:1). Fourty eight hours later, animals were scarified by cervical dislocation. Right and left auricular lymph nodes were dissected individually. A single cell suspension was prepared by teasing the lymph node with two needles in Petri dish contain phosphate-buffer saline. The suspension was filtered in a nylon mesh to remove tissue debris and washed in PBS by centrifugation. The cell pellet was reconstituted in fixed volume of 1% paraformaldehyde. The cell suspension was

examined and counted using Neuber's chamber by fluorescence microscopy. Migrating DCs were identified by their morphology and their highly bright localized fluorescence uptake.

Local lymphocyte proliferation assay

Local lymph node proliferation response to contact sensitization was determined by subtraction of total lymphocyte from auricular lymph node draining vehicle application from total lymphocytes from lymph nodes draining the application of contact sensitizer [9, 10]. Briefly on day (0) animals were sensitized on their right ear with 10 µl of 2% Oxazolone in acetone: olive oil (4:1) and treated on their left ears with 10 µl of acetone: olive oil (4:1). On day (4), animals were sacrificed by cervical dislocation and right and left auricular lymph nodes were individually dissected. A single cell suspension was prepared by teasing the lymph node with two needles in Petri dish containing PBS. The suspension was filtered in nylon mesh to remove tissue debris and washed in PBS by centrifugation. The cell pellet was reconstituted in fixed volume of 1% par-formaldehyde. The cell suspension was examined and lymphocytes were counted from individual nodes using Neuber's chamber.

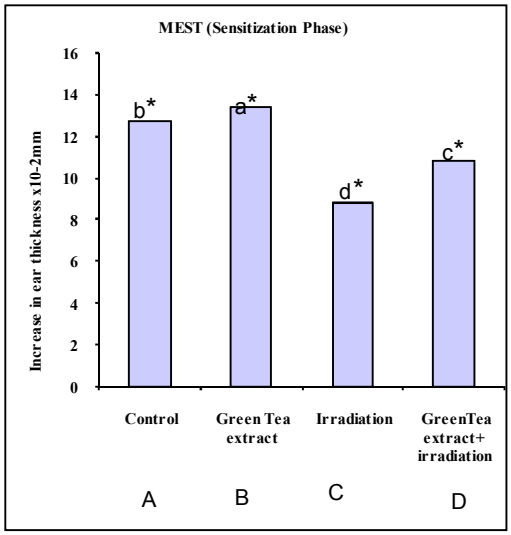
Statistical analysis

Statistical analysis was performed by using ANOVA (F) test to compare between values as described by [11].

RESULTS AND DISCUSSION

The present study was conducted to explore the ability of green tea extract to preserve the T cell mediated function under the influence of gamma irradiation. The T cell mediated function was exemplified in the present study by the contact sensitivity reaction and utilized the mouse ear swelling test. The test measures the inflammatory reaction elicited in the mouse ear after sensitization with the contact sensitizer oxazolone. The test has proven to be reliable, sensitive and widely used to detect the effect of different agents on the T cell mediated hypersensitivity reaction [12].

Data shown in Fig.1 demonstrate the contact sensitivity response to 1% oxazolone in the four tested groups. Administration of green tea extract caused significant increase in the ear swelling response in comparison to control animals. Animals exposed to gamma radiation experienced significant decrease of the ear swelling response to 69% of the control level. This was consistent with data from[13]. The latter used C57BL/6 mice that were exposed to a single dose of 3Gy



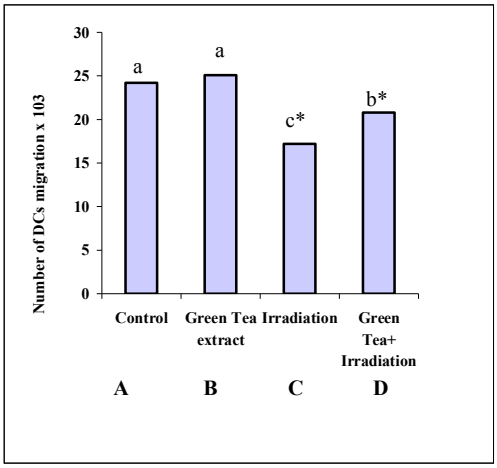
Groups	SD	% of change
A	0.340	–
B	1.039	6%
C	0.323	-31%
D	0.383	-15%

Fig.1: Effect of gamma radiation, green tea extract and both on mouse ear swelling response in BALB/c mice. Bars represent mean ± SD. where values bearing different superscript are significant at ($p<0.01$)

protons or gamma-rays and intrapertioneally injected 1 day later with sheep red blood cells (sRBC).Data from their study concluded that whole-body irradiation with protons or gamma-rays, at the dose employed, resulted in marked, but transient immunosuppression of all parameters associated with T cell mediated immunity. Data from fig.1 showed that administration of green tea extract at a dose of 0.1% in drinking water caused partial preservation on the T cell mediated reaction to 87.4% the control level. This indicated that the green tea extract is a beneficial tool in partial combating of the deleterious effect of gamma irradiation on the T cell mediated reaction.

The contact sensitivity 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) which migrate to local lymph nodes where they present the processed protein to antigen-specific T cells [(14)]. Data from Fig.2 showed that ionizing radiation significantly reduced the accumulation of hapten induced dentritic cell migration to local lymph nodes. It is unlikely that the decrease in dendritic cell accumulation in local lymph node following irradiation was due to radiation-induced Langerhan cells (LC) depletion. Gamma radiation at a dose of 750 rads caused no effect on LC count as assessed by staining of cell membrane

ATPase activity and la antigen immediately after radiation[15]. In the meantime, quantitative analysis of LC density in the anterior chest skin autopsy specimens including patients treated with radiation therapy showed reduction in LC compared with age matched control only one month after completion of treatment [16]. In CBA/H mice, local ionizing radiation caused decline of LC density nineteen month post irradiation. No effect was observed two to fifteen month after local irradiation [17].



Groups	SD	% of change
A	1.135	-
B	0.994	4%
C	0.788	-29%
D	1.2292	-14%

Fig.2: Effect of gamma radiation, green tea extract and both on dendrites migration to local lymph nodes in BALB/c mice. Bars represent mean ± SD. where values bearing different superscript are significant at ($p<0.01$).

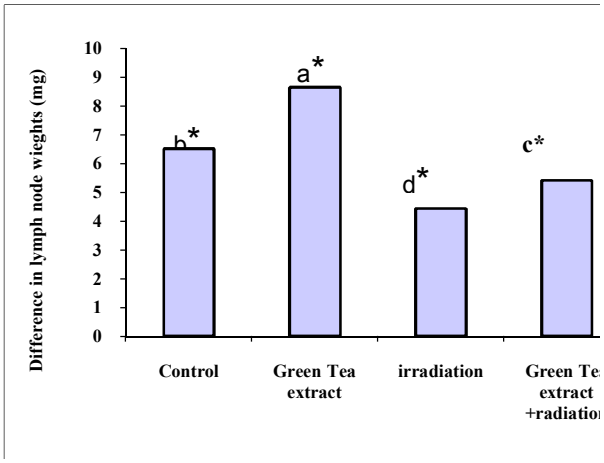
The direct effect of gamma radiation on antigen-induced DCs migration has not been previously studied. However, there have been a lot of information about the effect of gamma radiation on the factors that mediate the DCs migration response. The mechanism of antigen-induced LC migration is complex and is initiated by the effect of cytokines such (TNFα) and IL-1β [18]. Migration of LC also involves the reduction of the expression of adhesion molecules such as integrin-α6 [19], E-cadherin [20] and intracellular adhesion molecule -1(I-CAM-1) [21]. These adhesion molecules had to be down regulated in the course of LCs migration to allow their detachment from the basal layer of the epidermis. DCs migration also depends on an intact cellular cytoskeleton. The integrity of this system is necessary for cell locomotion. A key element in DCs migration is the creation of a path to the draining lymph nodes by digesting collagen in connective tissues, basement membranes and dermal extra cellular matrix. This has been found to be accomplished by the action of the membrane bound matrix metalloproteinase's MMP-2 and MMP-9. Inhibition of the activity of

these enzymes was found to be accompanied by retardation of DC migration [22].

The effect of radiation on the factors affecting DC migration as inconsistent. Data from some studies gave the impression that may impair LCs migration by retarding their detachment from the basal layer by the induction of ICAM-1[23] and Ecadherin [24] molecules. Ionizing radiation was also found to down regulate genes involved in cellular cytoskeleton and cell movement [25] which may also be responsible for the delay in DCs migration. On the other hand, data from other studies suggested that gamma radiation may accelerate DCs migration by its ability to enhance the release of IL-1β [26] and TNF-α [27] and its ability to induce the activity of the metalproteinases MMP-2 and MMP-9 genes [28]. The negative effect of gamma radiation on antigen-induced DCs migration reported here implies that radiation may have a more potent impact on the factors that negatively regulate DCs migration than those which positively regulate DCs migration. The ability of green tea extract to improve the migration of DCs fig-2 is in line with its reported ability to improve DC cluster formation of head and neck cancer patients [29].

The decline of lymphocyte proliferation in local lymph nodes caused by gamma radiation shown in Fig.3 may reflect a reduced antigen presenting ability of DCs or a direct negative impact of radiation on the T cell proliferation. Radiation has been found to have a divergent effect on antigen presentation by dendritic cells depending on whether peptides are endogenously processed and loaded into MHC molecules or exogenously added. Ionizing radiation was found to reduce the antigen presenting function of DC to endogenously processed molecules and increase antigen presentation to pulsed peptides[30]. This means that oxazolone-haptenated DCs, as in the present study are expected to be positively affected in their presentation ability to oxazolone by irradiation. On the contrary to what is expected, local T cell proliferation was reduced in the lymph nodes draining contact sensitization (Fig-3). The reduction of DC migration shown in Fig.2 may be the possible reason of the reduced proliferation of T cells as it reduces the effector stimulator ratio in the regional lymph node.

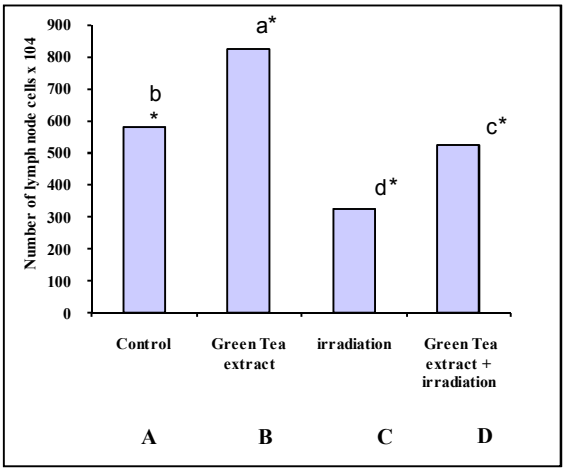
The contact sensitizer oxazolone has been known to induce the T lymphocytes proliferation in vivo[31]. The ability of gamma irradiation to reduce T cell proliferation is in line with data obtained from atomic bomb survivors. The immune systems of the atomic-bomb survivors were proportionately damaged to



Groups	SD	% of change
A	0.813	-
B	0.683	33%
C	0.440	-32%
D	0.596	-16%

Fig.3: Effect of gamma radiation, green tea extract and both on difference of lymph node weights in BALB/c mice. Bars represent mean ± SD. where values bearing different superscript are significant at (p<0.01)

irradiation levels at the time of the bombing over 60 years ago. Although the survivor's immune system repaired and regenerated as the hematopoietic system has recovered, significant residual injury persisted, as manifested by abnormalities in lymphoid cell composition and function. These included attrition of T-cell functions, as (i) reductions in mitogen-dependent proliferation and interleukin-2 (IL-2) production; (ii) decrease in helper T-cell populations; and (iii) increase in blood inflammatory cytokine levels [32].

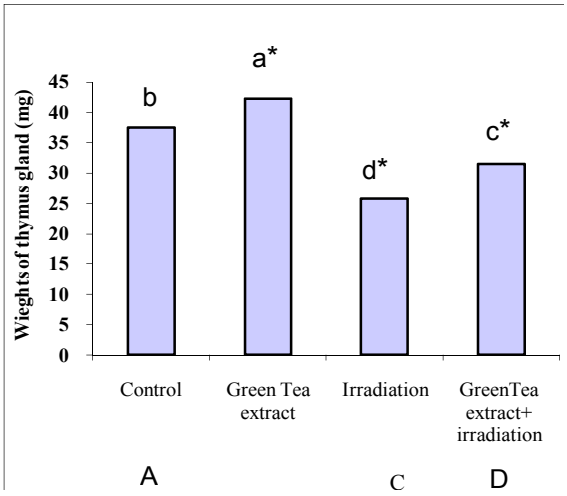


Groups	SD	% of change
A	38.759	-
B	44.045	42%
C	6.3499	-44%
D	19.293	-10%

Fig.4: Effect of gamma radiation, green tea extract and both on proliferation of T lymphocyte in regional lymph node in BALB/c mice. Bars represent mean ± SD. where values bearing different superscript are significant at (p<0.01)

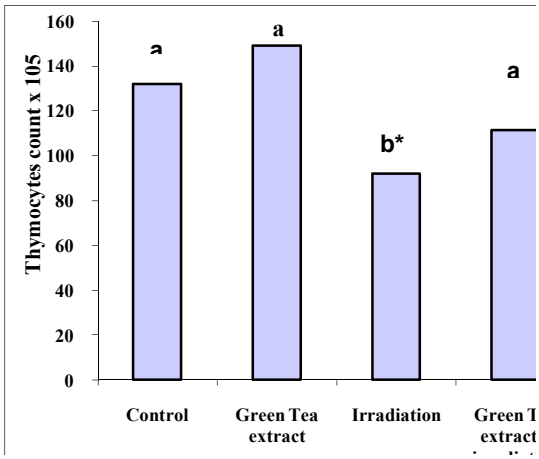
The present data are also consistent with those obtained from radiation

workers. Studies performed on radiation workers exposed to ionizing radiation demonstrated that these workers experienced reduction in the number and function of CD4 helper T lymphocytes [33]. The ability of green tea to partially restore the T cell proliferation under the effect of gamma irradiation is supported by [34] findings.



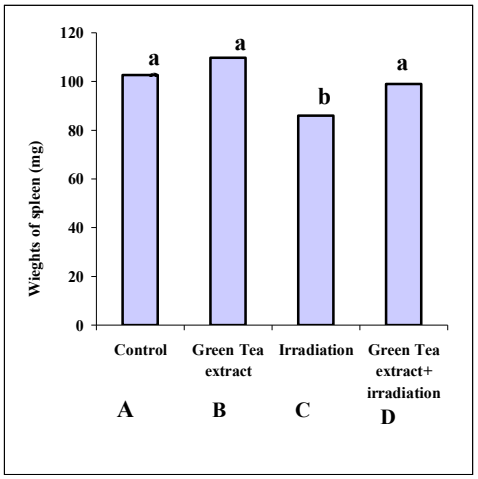
Groups	SD	% of change
A	1.490	-
B	3.791	13%
C	2.258	-32%
D	2.756	-16%

Fig.5: Effect of gamma radiation, green tea extract and both on thymus weight in BALB/c mice. Bars represent mean ± SD. where values bearing different superscript are significant at (p<0.01).



Groups	SD	% of change
A	26.69	-
B	20.03	13%
C	18.12	-33%
D	19.67	-13%

Fig.6: Effect of gamma radiation, green tea extract and both on thymocytes count in BALB/c mice. Bars represent mean ± SD. where values bearing different superscript are significant at (p<0.01).



Groups	SD	% of change
A	3.317	-
B	3.433	7%
C	4.276	-16%
D	6.522	-4%

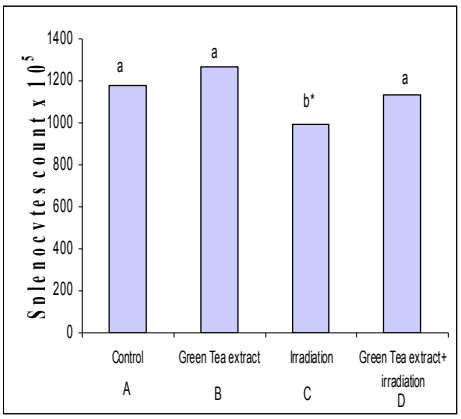
Fig.7: Effect of gamma radiation, green tea extract and both on spleen weight in BALB/c mice. Bars represent mean ± SD. where values bearing different superscript are significant at ($p<0.01$).

They showed that administration of green tea extract potentiated the stimulatory effect of Trichinella spirallis on the number of CD4 and CD8 cells in CFW mice. Green tea extract also restored the cellular immunity in lung cancer immunocompromized patients [(35)].

Data from the present study supported the notion that resting lymphocytes are more resistant to the effect of radiation than dividing lymphocytes. This was obvious when comparing the percentage in the reduction of spleen weight (Fig. 7) (16% of the control) and splenocytes count (Fig. 8) (16% of the control) and percentage in the reduction in thymus weight (Fig. 5) (more than 32% of the control) and thymocytes count (Fig 6) (30% of the control) in response to gamma radiation. The radio resistant ability of spleen cells has been previously reported. It has been shown that the spleen cells remained viable for 3 days following a dose of (600 rad) before latent radiation damage occur and manifested by reduced ability to survive and impairment in function [(36)]. At a higher dose (1000 rad), complete recovery from of splenocyte number and function occurred 100 days post irradiation [(37)].

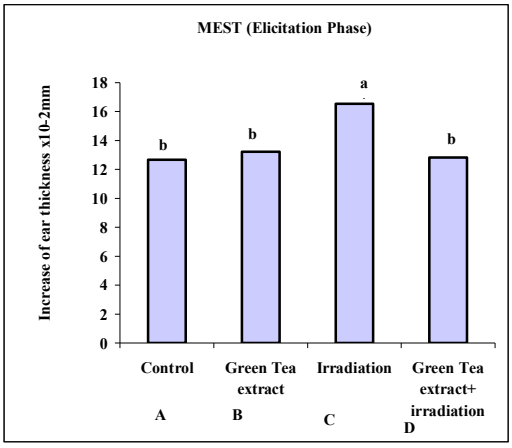
The thymus contains a large number of dividing immature lymphocytes particularly in the cortical region [(38)]. The reduction in thymus weight induced by gamma irradiation is consistent with data from [(39)] who demonstrated thyums involution after a single dose of 3 Gy. The ability of Green tea extract to

preserve the thymus weight following radiation indicates that radiation deprives thymocyte's ability to produce factors necessary for integrity of the gland and these factors are readily induced by the extract. It also adds to data from previous studies showing that Green tea extract improved thymocytes counts of mice bearing Dalton's lymphoma [40] and in mice exposed to restrain stress [41].



Groups	SD	% of change
A	45.129	-
B	100.42	7%
C	82.042	-16%
D	74.812	-4%

Fig.8: Effect of gamma radiation, green tea extract and both on splenocytes count in BALB/c mice. Bars represent mean ± SD . Where values bearing different superscript are significant at (p<0.01).



Groups	SD	% of change
A	0.323	-
B	1.083	5%
C	1.280	31%
D	0.209	2%

Fig.9: Effect of gamma radiation, green tea extract and both on elicitation phase of contact sensitivity in BALB/c mice. Bars represent mean ± SD. where values bearing different superscript are significant at (p<0.01).

The effect of radiation, green tea dry extract and both on the elicitation

phase of contact sensitivity is shown in Fig.9. Surprisingly, gamma irradiation enhanced the elicitation phase of contact sensitivity. In our opinion, there are three possible mechanisms for such enhancement. First, the ability of gamma irradiation to impair LC hapten-induced migration (Fig-2). This might have increased the stimulatory effector ratio at the elicitation site. Second, the ability of gamma irradiation to induce the release of inflammatory cytokines such as IL-1β and TNFα **(18).** Third, the release of reactive oxygen species (ROS) along the course of gamma irradiation. It has been previously demonstrated that ROS play an important in the inflammatory response that associates the elicitation phase **(42).** Adminstration of green extract minimized the increase in ear thickness in animals exposed to gamma rays one day before the elicitation phase (Fig.9). This may be attributed to its ability to scavenge ROS **(5)** or to counteract the negative effect of gamma radiation on DCs migration (Fig.2). Finally, we may conclude that the present study heralds a unique role of gamma radiation in the course of delayed hypersensitivity reaction. Radiation had a negative impact on the overall delayed type hypersensitivity reaction as measured by the mouse ear swelling test. Gamma rays had a discrepant effect on the induction and elicitation phases of contact sensitivity. It decreased the induction phase by reducing hapten-induced DCs migration to the local lymph node, minimizing the local proliferation of T lymphocytes. It boosted the elicitation phase of contact sensitivity reaction possibly by enhancing the release of inflammatory cytokines, ROS and /or impending the migration of DCs. Administration of green tea extract partially conserved the contact sensitivity reaction to oxazolone in BALB/c mice exposed to 2Gy gamma irradiation. It counteracted the effect of gamma irradiation on the DCs migration, T cell proliferation. It also reduced the exaggeration of elicitation phase caused by gamma irradiation, when applied before the elicitation phase.

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مجلة البحوث الإشعاعية
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تأثير مستخلص الشاي الأخضر على تفاعل الحساسية الزائد المحدث بواسطة خلايا (ت) فى الجرذان المعرضة لاشعه جاما(BALB/c)

عبد الجواد هاشم, محسن إسماعيل, منال القاضي, محمود شابون, سها حسين

كلية الصيدلة جامعة القاهرة ــ هيئة الطاقة الذرية

يصحب تعرض الجسم إلى الإشعاع المؤين رد فعل بيولوجي واسع النطاق. يحمل الإشعاع المؤين القدرة على وقف انقسام الخلايا مما يجعله من الوسائل الأساسية في علاج الأورام ولكن من سلبياته انه يتسبب في ضعف مناعة الجسم, ولذلك يعمل على استخدام مواد أخرى مصاحبة للإشعاع لتحسين مناعة الجسم والمحافظة عليها, وتلعب الخلايا (ت) ووظائفها البيولوجية دور أساسي في حماية الجسم ضد التحول السرطاني وعند دراسة اختبار الحساسية التلامسية نستطيع أن نتعرف على كثير من المعلومات التي تخص الوظائف البيولوجية لخلايا (ت) اللمفاوية. في الأعوام القليلة الماضية تنامى الاهتمام بالتطبيقات الطبية لخلاصة الشاي الأخضر وأوضحت الدراسات أن خلاصة الشاي الأخضر لها القدرة على تغيير المناعة. الهدف الأساسي لهذه الدراسة هو معرفة تأثير أشعة جاما (2 جراي) على اختبار الحساسية التلامسية في الخلايا المستحثة بمادة الأوكسازولون (مثير للحساسية الجلدية) في مراحله المختلفة. حيث نستخدم أربع مجموعات من الحيوانات، وكل مجموعة تتكون من عشرة فئران حيث تستخدم المجموعة الأولى كمجموعة ضابطة ، بينما عولجت المجموعة الثانية بتناول جرعة قدرها 0.1 ٪ خلاصة الشاي الأخضر في ماء الشرب ، بينما تعرضت المجموعة الثالثة لجرعة قدرها (2 جراي) من أشعة جاما، وقد تناولت المجموعة الرابعة خلاصة الشاي الأخضر قبل التشعيع. تم إجراء القياسات التالية: اختبار الحساسية التلامسي بواسطة تورم الأذن للفئران وهجرة الخلايا المتشجرة، وزن الغدد اللمفاوية وتكاثر الخلايا اللمفاوية ، وزن الطحال وعدد الخلايا الطحالية ووزن الغدة الثيموسية وعدد الخلايا الثيموسية. كما لوحظ تأثير أشعة جاما وخلاصة الشاي الأخضر على مرحلة الإظهار أيضا. وقد أظهرت نتائج هذه الدراسة إن تناول خلاصة الشاي الأخضر يحدث زيادة نسبية في مقدار تورم الأذن ويلاحظ في الفئران المعرضة لأشعة جاما نقصان نوعى في مقدار تورم الأذن 31٪ من المجموعة الضابطة. في حالة تناول خلاصة الشاي الأخضر يحدث محافظة نوعية على وظائف الخلايا (ت) بمقدار 85٪ من المجموعة الضابطة وهذا يوضح فائدة خلاصة الشاي الأخضر في إصلاح الآثار الضارة لأشعة جاما على الخلايا (ت). وتوضح النتائج أن الأشعة المؤينة تقلل من تراكم الخلايا المتشجرة المستحثة بالأوكسازولون في العقد اللمفاوية المختصة. كما أظهرت النتائج أيضا نقص معنوي في تكاثر الخلايا الليمفاوية وأيضا نقص معنوي في وزن الغدة الثيموسية، كما أدى أيضا التعرض للإشعاع إلى نقص كبير في عدد الخلايا الليمفاوية بالدم وزيادة تورم الأذن عند تعرض الحيوانات في مرحلة الإظهار. وقد أدى تناول الفئران بواسطة مستخلص الشاي الأخضر إلى تحسين الاستجابة في تفاعل الحساسية التلامسية في الحيوانات المعرضة لأشعه جاما كما أدى إلى تحسين هجره الخلايا المتشجرة ووزن الغدد اللمفاوية ووزن الغدد الثيموسية و تكاثر الخلايا الليمفاوية في العقد الليمفاوية وفي عدد الخلايا الليمفاوية في الدم. وقد أدى تناول مستخلص الشاي الأخضر إلى نقص الزيادة الاستجابة في اختبار تورم الإذن عندما تم تناوله قبل مرحلة الإظهار.

وقد خلصت الدراسة إلى أهمية تناول مستخلص الشاي الأخضر لتعويض القصور في تفاعل الحساسية الجلدية التلامسي المحدث بواسطة خلايا (ت) و ذلك في مراحله المختلفة.