

THE POSSIBLE PROTECTIVE ROLE OF NIGELLA SATIVA OIL AND VITAMIN E AGAINST RADIATION IN ADULT MALE ALBINO RATS

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ABSTRACT

Background: The potential application of ionizing radiation in medical practice and also, the potential accidental exposure to radiation enhanced the development of effective radio modifier. **The aim of this work** was to evaluate the possible protective role of individual and combined administration of Nigella sativa (NS) and vitamin E against the toxic effects of single 6 Gray (Gy) dose of gamma radiation on adult male albino rats **Material and methods:** ninty adult male albino rats were equally divided into 9 groups: Group SI: (-ve control), group SII (+ve control), group SIII: each rat was exposed to a single dose of radiation 6 Gy, group IV: each rat was gavaged orally with 0.6 ml NS dissolved in one ml distilled water once daily 5days/week, group SV: each rat was gavaged 100 mg/kg vitamin E dissolved in one ml corn oil once daily 5days/week, group SVI: each rat received both vitamin E and NS orally in the same doses once daily for 5 days/week, group SVII: each rat received NS oil for 5days/week daily. At the 3rd day, each rat was exposed to a single radiation in the previous doses, group SVIII: each rat received vitamin E daily for 5days/week, at the 3rd day each rat was exposed to a single radiation in the same previous doses, group SIX: each rat received both vitamin E and NS daily for 5days/week. At the 3rd day each rat was exposed to a single radiation in the previously mentioned doses. After 15 days from last exposure, rats of each group were submitted to estimation of complete blood count (CBC) and oxidative stress parameters, then the rats were sacrificed. The spleen and bone marrow of all rats were dissected and subjected to histopathological examination. **Results:** There was a significant deterioration in all measured parameters in irradiated group (group III). Upon supplementation with individual and combined NS and vitamin E before radiation, there were significant improvements in these measured parameters when compared with irradiated group. The histopathological changes in the spleen, in the form of white pulp depletion and multiple areas of hemorrhage. Bone marrow affection was also noticed, denoted by marked depletion of bone marrow tissue. Administration of individual and combined NS and vitamin E to irradiated rats induced an improvement, represented by the regression of the histopathological changes that occurred after radiation exposure. Biochemical and histopathological improvement in combined NS and vitamin E administration was more significant than that of individual NS and vitamin E administration. **Recommendation:** More efforts are needed to explore efficient natural combined radio protectors to limit deleterious effect of radiation. **Key words:** Nigella Sativa oil, Vitamin E, radiation, acute radiation, individual combined.

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INTRODUCTION

Ionizing radiation is one of corner stone's in Cancer therapy. Unfortunately, ionizing radiation causes unwanted side effects depending upon dose of radiation and sensitivity of organ that is irradiated. (Lamberskiand Theon, 2002).

Hematopoietic system is the most radiosensitive tissue in the body due to its high level of cell turnover. So, exposure to ionizing radiation induces a dose dependant decline in circulating haematopoietic cells, not only through reducing bone marrow production, but also by apoptosis of mature stem cells (Hosseinimehr et al., 2006).

In view of these complications, searches continue to find effective, less toxic and easily self administrated radio protector that can reduce and correct damaging effect of radiation (Landouer et

al., 2003). The naturally occurring compounds that function as antioxidants and immunomodulators are novel strategy for development of radioprotector agents (Dattner, 2003).

Nigella sativa (NS), black seed is a promising source for active ingredients, in particular thymoquinone (TQ) that was a potent radical scavenger. It has been extensively studied for its antioxidant, immunomodulator and anticancer properties (Huffman, 2003 and Ali, 2004)

Vitamin E (alpha tocopherol) and related analogues are nutraceutical that can scavenge reactive oxygen species (Ros). It also induces proliferation of cytokines that stimulate proliferation of progenitor cells in neutrophil lineage and thrombocyte (Singh et al., 2006).

Coupling the mainly benefits of Nigella sativa and vitamin E (antioxidant and immunomodulator), they provide a good defense against oxidative damage following exposure to radiation (Seed et al., 2002).

The aim of this work was to study the toxic effects of single (6 Gy) of gamma radiation on adult male albino rats and to evaluate protective role of each of individual and combined administration of Nigella sativa and vitamin E after exposure to radiation through complete blood count and erythrocyte glutathione peroxidase (GPx) and serum malondialdehyde (MDA).

Histopathological examination of spleen and bone marrow were also evaluated.

MATERIAL AND METHODS

(I) Material:

(a) Irradiation Source:

The whole body Gamma-irradiation was performed with a Canadian Gamma-Cell 40 (¹³⁷Cs) at the National Center for Radiation Research and Technology (NCRRT, Nasr city, Cairo, Egypt). The whole body irradiation dose levels were delivered to animals at a rate of 0.667Gy/minute.

(b) Chemicals:

(1) Crude seed oil of Nigella Sativa:

Pure crude of Nigella Sativa oil (NSO), pressed from Egyptian Nigella Sativa seeds was patently obtained and authenticated by Pharco Pharmaceuticals Company, Alexandria, Egypt; according to botanical specifications described by Jansen, 2013.

(2) Vitamin E: Vitamin E (alpha tocopheryl acetate), in the form of oil for laboratory use (no. T3251) was purchased from Sigma/Aldrich Chemical Company, Cairo, Egypt.

Corn oil was obtained in the form of oily solution as solvent to nigella sativa and vitamin E agent from commercial sources.

(c) Experimental Animals:

Adult male albino rats, obtained from the Animal House of the Faculty of Veterinary Medicine, Zagazig University each weighed about 180-200 gm (Ghosh, 1971).

(II) Methods:

(a) Experimental Design:

The study was carried out on 90 adult male albino rats. They were divided into nine equal groups each group consisted of 10 rats

Group I (negative control group): These rats were left without intervention to measure the basic parameters.

Group II (positive control group): These rats were gavaged orally with a daily dose 1 ml of corn oil for 1 week.

Group III (irradiated group):

These rats exposed to a single dose of radiation 6 Gy/rat for 1 week (Wang and Chiang, 1998).

Group IV (Nigella treated group):

Each rat received Nigella Sativa orally in a dose of 0.6 ml (1\10 LD₅₀) in 1ml corn oil once daily for 5days /week, for 1 week (Zaoui et al., 2002).

Group V (vitamin E treated group):

Each rat received vitamin E orally in a dose of 100 mg\ kg (1\10 LD₅₀) in 1 ml corn oil once daily for 5days / w, for 1 week (Satyamitra et al., 2001).

Group VI (Nigella and vitaminE treated group):

Each rat received both Vitamin E and Nigella Sativa orally in the previously used doses once daily for 5days / week, for 1 week.

Group VII (Nigella treated irradiated group):

Each rat received NS oil in the previous oral dose daily for 5days/week at the 3rd day each rat was exposed to a irradiation dose, for one week only.

Group VIII (vitamin E treated irradiated group):

Each rat received Vitamin E orally in the previous oral dose daily for 5days/week, at the 3rd day each rat was exposed irradiation dose, for one week only.

Group IX (Nigella and vitamin E irradiated treated group):

Each rat received both vitamin E and Nigella Sativa in the previous oral dose daily for 5days/week, at the 3rd day each rat was exposed to irradiation dose, for one week only.

After 15 days from the last dose of radiation (Hussien et al., 2007), the rats of all groups were anesthetized for drawing blood samples from retro-orbital plexuses as described by Johnson (2007) to estimate complete blood count Stephen et al., (2008) and Kerr (2002) and oxidative stress parameters [erythrocyte glutathione peroxidase enzyme levels (GPx) Pleban et al., (1982) and serum levels of malondialdehyde (MDA) Yoshioka et al., (1979).

Then the rats were sacrificed. The spleen and bone marrow of the femur of all subgroups were fixed in 10 % formalin saline. After fixation, the organs were embedded in paraffin blocks and processed for serial sections and subjected for Hematoxylin and Eosin stains for histopathological examination by light microscope according to the method described by (Kieran, 2001).

(b) Methods of Statistical Analysis:

SPSS Software program was used. Mean values ± standard deviations (SD) were calculated, ANOVA (F) test followed by a post hoc test (LSD alpha) for multiple comparisons were performed.

P value of less than 0.05 was considered to be significant.

RESULTS

No statistically significant differences were observed in the studied parameters between **Control groups and treated non irradiated groups (Tables 1, 2).**

Biochemical findings:

• **Complete blood count (CBC):**

There were a highly significant differences in mean values of erythrocytic count (RBC), leucocytic count (WBC), platelet count (Pl), hemoglobin concentration (HB), hematocrit volume (HCT), Neutrophil granulocytes and lymphocytes between negative control group, irradiated group and treated irradiated groups with either individual or combined Nigella sativa and Vitamin E by ANOVA test ($p < 0.001$) (**Table 3**).

There was a highly significant decrease in RBC count, WBC count, Pl count, HB concentration, HCT volume, neutrophil, granulocytes and lymphocytes values in irradiated group when compared with the control group (**Tables 4-10**).

Upon administration of individual and combined NS and vitamin E in radiated treated rats, there were a highly significant increase in mean values of erythrocytic count (RBC), leucocytic count (WBC), platelet count (Pl), hemoglobin concentration (HB), hematocrit volume (HCT), Neutrophil granulocytes and lymphocytes when compared with irradiated group (**Tables 4-10**).

• **Oxidative stress parameters:**

There were a highly significant differences in mean values of serum Malondialdehyde (MDA) and erythrocyte glutathione peroxidase (GPx) between negative control group, irradiated group and treated irradiated groups with either individual or combined Nigella sativa and Vitamin E by ANOVA test ($p < 0.001$) (**Table 11**).

There were a highly significant increase in the serum MDA and highly significant decrease in erythrocyte GPx in irradiated group as compared to their corresponding values in the negative control rats (**Tables 12, 13**).

Upon administration of individual and combined NS and vitamin E in radiated treated

rats, there were highly significant decrease in the serum MDA and highly significant elevation in erythrocyte GPx values when compared with irradiated group (**Table 12, 13**).

• **Mortality observation:**

No deaths throughout the observation time 15 days in the following rat groups; Control groups {-ve Control (S1) and (+ve control (S2))} and treated groups [Nigella Sativa treated (S4), vitamin E treated (S5), Vitamin E + Nigella treated (S6) 100% survivors (0% mortalities).

While in radiated groups:

- Radiated non treated group (S3), 5 rats of ten rats died (50%) throughout the observation time. Nigella sativa pretreated radiated groups (S7), 3 rats died (30%) of the ten rats

- Vitamin E pretreated radiated group (S8), rats died (20%) of the ten rats

- Combined Nigella sativa and Vitamin E (S9) pretreated radiated group 1 rats died (10%) of the ten rats (**table 14**).

Histopathological results:

Microscopic examination of the splenic specimens of the male rats of radiated group showed marked distortion of spleen tissue, marked increase in red pulp area over white pulp and multiple areas of hemorrhage.

Upon individual NS and vitamin E administration groups showed a mild improvement in the spleen tissue. Small sized lymphoid nodules in comparison to large red pulp area and scattered areas of hemorrhage were noticed with Nigella Sativa pretreated group and Vitamin E pretreated group. While combined Ns and vitamin E showed marked improvement in the spleen tissue (**Plate I, Figs A, B, C and D**).

Microscopic examination of the bone marrow of radiated group showed marked depletion of bone marrow tissue (red marrow) and displayed by numerous fat cell (yellow marrow).

Upon individual NS and vitamin E administration groups showed slight improvement. Increase of red marrow tissue and decrease fat cell was noticed with Nigella Sativa pretreated group and Vitamin E pretreated group. While combined NS and vitamin E showed marked improvement in the bone marrow tissue (**Plate II Figs A, B, C and D**).

Table (1): Statistical comparison among the control groups (SI andSII) and treated groups (SIV, SV, SVI) as regard complete blood count of peripheral blood (RBC, HB, HCT, Pl, WBC, Neutro, Lymph) by ANOVA test.

Group Parameter of CBC	SI	SII	SIV	SV	SVI	F	P
	Control	Control	(NS)	(E)	(E+NS)		
	(-ve) TN=10	(+ve) TN=10	TN=10	TN=10	TN=10		
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
RBC (millions/ μ L)	5.44 ± 0.26	5.35± 0.32	5.39± 0.34	5.36± 0.32	5.47± 0.27	0.27	0.89 #
HB (grams/ Decilitre)	11.97± 0.45	11.97± 0.33	12.07± 0.36	12.01± 0.38	12.15± 0.39	0.40	0.81 #
HCT (%)	38.6± 2.22	38.1± 2.42	38.9± 2.60	37.5± 2.07	39.7± 2.3	1.27	0.29 #
Pl (thousand/ mm)	321.5± 24.76	322.2± 23.24	321.1± 19.74	325.6± 21.83	327.9± 25.63	0.16	0.96 #
WBC (thousand/ cmm)	6.27 ± 0.35	6.25± 0.35	6.03± 0.41	6.27± 0.39	6.17± 0.45	0.69	0.61 #
Neutrophil (10^9 /L)	70.2± 2.66	70.3 ± 2.49	71. 0± 2.67	69.8± 2.35	71.9± 2.42	1.07	0.38 #
Lymphocytes (10^9 /L)	40.6± 2.72	41.0± 2.11	39.4± 2.37	39.9 ± 2.08	41.1± 2.33	0.98	0.43 #

#: Non-significance (P>0.05)
NS: Nigella Sativa

TN: Total numbers of rats in each group
E: vitamin E

Table (2): Statistical comparison among the control groups (SI andSII) and treated groups (SIV, SV, SVI) as regard serum Malondialdehyde (MDA) and erythrocyte glutathione peroxidase (GPx) values by ANOVA test

Group Antioxidant parameters	SI	SII	SIV	SV	SVI	F	P
	Control	Control	(NS)	(E)	(E+NS)		
	(-ve) TN=10	(+ve) TN=10	TN=10	TN=10	TN=10		
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
MDA	77.56± 4.39	75.82± 4.45	77.50± 3.52	78.46± 4.31	77.14± 4.39	0.51	0.73#
GPx	62.18± 2.89	61.73± 2.69	62.72± 3.24	62.37± 3.04	64.59± 2.59	1.46	0.23#

#: Non-significance (P>0.05)
NS: Nigella Sativa

TN: total numbers of rats in each group
E: vitamin E

Table (3): Statistical comparison among the -ve Control (SI) radiation group (SIII), radiation+Nigella sativa (SVII), radiation+Vitamin E (SVIII) and radiation+Nigella sativa+Vitamin E (SIX) groups as regard complete blood count [erythrocytic count (RBC), hemoglobin concentration (HB), hematocrit volume (HCT), Platelet (PI), leucocytic count (WBC), Neutrophil granulocytes, lymphocytes values] by ANOVA test

Group	SI	SIII	SVII	SVIII	SIX	F	P
	Control (-ve) TN=10	radiated TN=5	NS+radiated TN=7	E+radiated TN=8	E+NS+ radiated TN=9		
Parameter of complete blood count	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
RBC (millions/μL)	5.44± 0.26	2.52± 0.54	5.16± 0.28	5.21± 0.25	5.45± 0.28	101.27	<0.001**
HB (grams/Deciliter)	11.97± 0.45	8.27± 0.19	11.65± 0.24	11.56± 0.35	11.96± 0.32	144.89	<0.001**
HCT (%)	38.6± 2.22	19.67± 1.63	37.13± 1.81	37.38± 1.68	38.56± 2.29	109.22	<0.001**
PI (thousand/ cmm)	321.5± 24.76	109.5± 7.50	312.63± 14.78	301.0± 16.39	322.78± 19.79	159.77	<0.001**
WBC (thousand/ cmm).	6.27± 0.35	3.22± 0.23	6.02± 0.33	5.91± 0.34	6.24± 0.44	88.34	<0.001**
Neutrophil (109/L)	70.2± 2.66	41.67± 1.63	67.0 ± 2.27	65.87± 1.46	72.44± 2.87	188.96	<0.001**
Lymphocytes (109/L)	40.6± 2.72	21.67± 1.21	38.0± 1.85	38.37± 1.41	41.22± 2.54	95.08	<0.001**

** : highly significant (P<0.001)
NS: Nigella Sativa

TN: total numbers of rats in each group
E: vitamin E.

Table (4): Least significant difference test (LSD) for comparison of the changes of the mean values of RBC among -ve Control (S1) radiation group(S3), radiation+ Nigella sativa (S7), radiation+ Vitamin E (S8) and radiation+ Nigella sativa+ Vitamin E (S9) groups along the periods of the study.

RBC (millions/μl)	SIII Radiation	SVII Radiation+NS	SVIII Radiation+E	SIX Radiation+NS+E	SI Control
Mean±SD	2.52±0.54	5.16±0.28	5.21±0.25	5.45±0.28	5.44 ±0.26
Radiation 2.52±0.54		<0.001**	<0.001**	<0.001**	<0.001**
Radiation+NS 5.16±0.28			0.749 #	0.062 #	0.067 #
Radiation+E 5.21±0.25				0.119 #	0.131 #
Radiation+NS+E 5.45±0.28					0.927 #

** : means highly significant (p<0.001)
NS: Nigella Sativa

#: Non-significance (P>0.05)
E: vitamin E.

Table (5): Least significant difference test (LSD) for comparison of the changes of the mean values of HCT among the -ve Control (SI) radiation group (SIII), radiation+Nigella sativa (SVII), radiation+ Vitamin E (S8) and radiation+Nigella sativa+Vitamin E (SIX) groups along the periods of the study.

HCT (%)	SIII Radiation	SVII Radiation+NS	SVIII Radiation+E	SIX Radiation+NS+E	SI Control
Mean±SD	19.67±1.63	37.13±1.81	37.38±1.68	38.56±2.29	38.6±2.22
Radiation 19.67±1.63		<0.001**S	<0.001**S	<0.001**S	<0.001**S
Radiation+NS 37.13±1.81			0.803 NS	0.148 NS	0.127 NS
Radiation+E 37.38±1.68				0.23 NS	0.203 NS
Radiation+NS+E 38.56±2.29					0.962 NS

** : means highly significant (p<0.001)

: Non-significance (P>0.05)

NS: Nigella Sativa

E: vitamin E

Table (6): Least significant difference test (LSD) for comparison of the changes of the mean values of HB among -ve Control (SI) radiation group(SIII), radiation+ Nigella sativa (SVII), radiation+Vitamin E (SVIII) and radiation+Nigella sativa+Vitamin E (SIX) groups along the periods of the study.

HB (grams/Deciliter)	SIII Radiation	SVII Radiation+NS	SVIII Radiation+E	SIX Radiation+NS+E	SI Control
Mean±SD	8.27±0.19	11.65±0.24	11.56±0.35	11.96±0.32	11.97±0.45
Radiation 8.27±0.19		<0.001**	<0.001**	<0.001**	<0.001**
Radiation+NS 11.65±0.24			0.605 #	0.06#	0.052 #
Radiation+E 11.56±0.35				0.018*	0.015*
Radiation+NS+E 11.96±0.32					0.983 #

** : means highly significant (p<0.001)

* : means significant (p< 0.05).

: Non-significance (P>0.05)

NS: Nigella Sativa E: vitamin E.

Table (7): Least significant difference test (LSD) for comparison of the changes of the mean values of PL among the -ve Control (SI) radiation group (SIII), radiation+Nigella sativa (SVII), radiation+Vitamin E (SVIII) and radiation+Nigella sativa+Vitamin E (SIX) groups along the periods of the study.

Pl (thousand/cmm)	SIII Radiation	SVII Radiation+NS	SVIII Radiation+E	SIX Radiation+NS+E	SI Control
Mean±SD	109.5±7.50	312.63±14.78	301.0.±16.39	322.78±19.79	321.5±24.76
Radiation 109.5±7.50		<0.001**	<0.001**	<0.001**	<0.001**
Radiation+NS 312.63±14.78			0.217 #	0.267 #	0.319 #
Radiation+E 301.0.±16.39				0.021 *	0.025*
Radiation+NS+E 322.78±19.79					0.881 #

** : means highly significant (p<0.001)

* : means significant (p< 0.05).

: Non-significance (P>0.05)

NS: Nigella Sativa

E: vitamin E

Table (8): Least significant difference test (LSD) for comparison of the changes of the mean values of WBC among the -ve Control (SI) radiation group (SIII), radiation+Nigella sativa (SVII), radiation+Vitamin E (SVIII) and radiation+Nigella sativa+Vitamin E (SIX) groups along the periods of the study.

WBC (thousand/ cmm)	SIII Radiation	SVII Radiation+NS	SVIII Radiation+E	SIX Radiation+NS+E	SI Control
Mean±SD	3.22±0.23	6.02±0.33	5.91±0.34	6.24±0.44	6.27±0.35
Radiation 3.22±0.23		<0.001**S	<0.001**S	<0.001**S	<0.001**S
Radiation+NS 6.02±0.33			0.575 NS	0.185 NS	0.133 NS
Radiation+E 5.91±0.34				0.061 NS	0.04* S
Radiation+NS+E 6.24±0.44					0.876 NS

** : means highly significant (p<0.001)

* : means significant (p< 0.05).

: Non-significance (P>0.05) NS: Nigella Sativa

E: vitamin E

Table (9): Least significant difference test (LSD) for comparison of the changes of the mean values of Lymphocytes among the -ve Control (SI) radiation group (SIII), radiation+Nigella sativa (SVII), radiation+Vitamin E (SVIII) and radiation+Nigella sativa+Vitamin E (SIX) groups along the periods of the study.

Lymphocytes (109/L)	SIII Radiation	SVII Radiation+NS	SVIII Radiation+E	SIX Radiation+NS+E	SI Control
Mean±SD	21.67± 1.21	38.0± 1.85	38.37±1.41	41.22± 2.54	40.6±2.72
Radiation 21.67± 1.21		<0.001**	<0.001**	<0.001**	<0.001**
Radiation+NS 38.0± 1.85			0.727 #	0.004 *	0.014*
Radiation+E 38.37±1.41				0.009*	0.034 *
Radiation+NS+E 41.22± 2.54					0.529 #

** : means highly significant (p<0.001)

* : means significant (p< 0.05).

: Non-significance (P>0.05) NS: Nigella Sativa

E: vitamin E

Table (10): Least significant difference test (LSD) for comparison of the changes of the mean values of neutrophil among the -ve Control (SI) radiation group(SIII), radiation+Nigella sativa (SVII), radiation+Vitamin E (SVIII) and radiation+Nigella sativa+Vitamin E (SIX) groups along the periods of the study.

Neutrophil (109/L)	SIII Radiation	SVII Radiation+NS	SVIII Radiation+E	SIX Radiation+NS+E	SI Control
Mean±SD	41.67±1.63	67.0 ±2.27	65.87±1.46	72.44±2.87	70.2±2.66
Radiation 41.67±1.63		<0.001**	<0.001**	<0.001**	<0.001**
Radiation+NS 67.0 ±2.27			0.339 #	<0.001**	0.006*
Radiation+E 65.87±1.46				<0.001**	<0.001**
Radiation+NS+E 72.44±2.87					0.042*

** : means highly significant (p<0.001)

* : means significant (p< 0.05).

: Non-significance (P>0.05) NS: Nigella Sativa

E: vitamin E

Table (11): Statistical comparison among -ve control (SI) radiation group (SIII), radiation+Nigella sativa (SVII), radiation+ Vitamin E (SVIII) and radiation+Nigella sativa+Vitamin E (SIX) as regard serum Malondialdehyde (MDA) and erythrocyte glutathione peroxidase (GPx) values by ANOVA test

Group Antioxidant Parameters	SI Control (-ve) TN=10	SIII radiated TN=5	SVII NS+radiated TN=7	SVIII E+radiated TN=8	SIX E + NS+ radiated TN=9	F	P
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
MDA	77.56± 4.39	175.39± 8.73	80.35± 4.52	81.18± 3.43	77.49± 4.13	477.19	<0.001**
GPx	62.18± 2.98	34.85± 2.07	60.83± 3.15	59.06± 3.12	63.02± 3.05	68.83	<0.001**

**: means highly significant (P<0.001)

NS: Nigella Sativa E: vitamin E

Table (12): Least significant difference test (LSD) for comparison of the changes of the mean values of serum MDA among -ve Control (SI) radiation group (SIII), radiation+Nigella sativa (SVII), radiation+Vitamin E (SVIII) and radiation+ Nigella sativa+Vitamin E (SIX) along the periods of the study.

Serum MDA	SIII Radiation	SVII Radiation+NS	SVIII Radiation+E	SIX Radiation+NS+E	SI Control
Mean±SD	175.39±8.73	80.35±4.52	81.18±3.33	77.49±4.13	77.56±4.39
Radiation 175.39±8.73		<0.001**	<0.001**	<0.001**	<0.001**
Radiation+NS 80.35±4.52			0.739 #	0.237 #	0.249 #
Radiation+E 81.18±3.33				0.128 #	0.136 #
Radiation+NS+E 77.49±4.13					0.973 #

**: means highly significant (p<0.001)

#: Non-significance (P>0.05)

NS: Nigella Sativa

E: vitamin E

Table (13): Least significant difference test (LSD) for comparison of the changes of the mean values of Glutathione -ve Control (SI) radiation group (SIII), radiation+Nigella sativa (SVII), radiation+Vitamin E (SVIII) and radiation+Nigella sativa+Vitamin E (SIX) along the periods of the study.

Glutathione	SIII Radiation	SVII Radiation+NS	SVIII Radiation+E	SIX Radiation+NS+E	SI Control
Mean±SD	34.85±2.07	60.83±3.15	59.06±3.12	63.02±3.05	62.18±2.98
Radiation 34.85±2.07		<0.001**	<0.001**	<0.001**	<0.001**
Radiation+NS 60.83±3.15			0.654#	0.806 #	0.430 #
Radiation+E 59.06±3.12				0.721#	0.621#
Radiation+NS+E 63.02±3.05					0.563 #

**: means highly significant (p<0.001)

#= Non-significance (P>0.05)

N: Nigella Sativa E: vitamin E

Table (14): Statistical comparison regarding death rate in Radiation group (SIII), Radiation+NS (SVII), Radiation+E (SVIII) and Radiation+NS+E (SIX):

Death rate	Radiation		Rad+NS		Rad+E		Rad+NS+E	
	No	%	no	%	no	%	No	%
1 wk	5	50	3	30	2	20	1	10

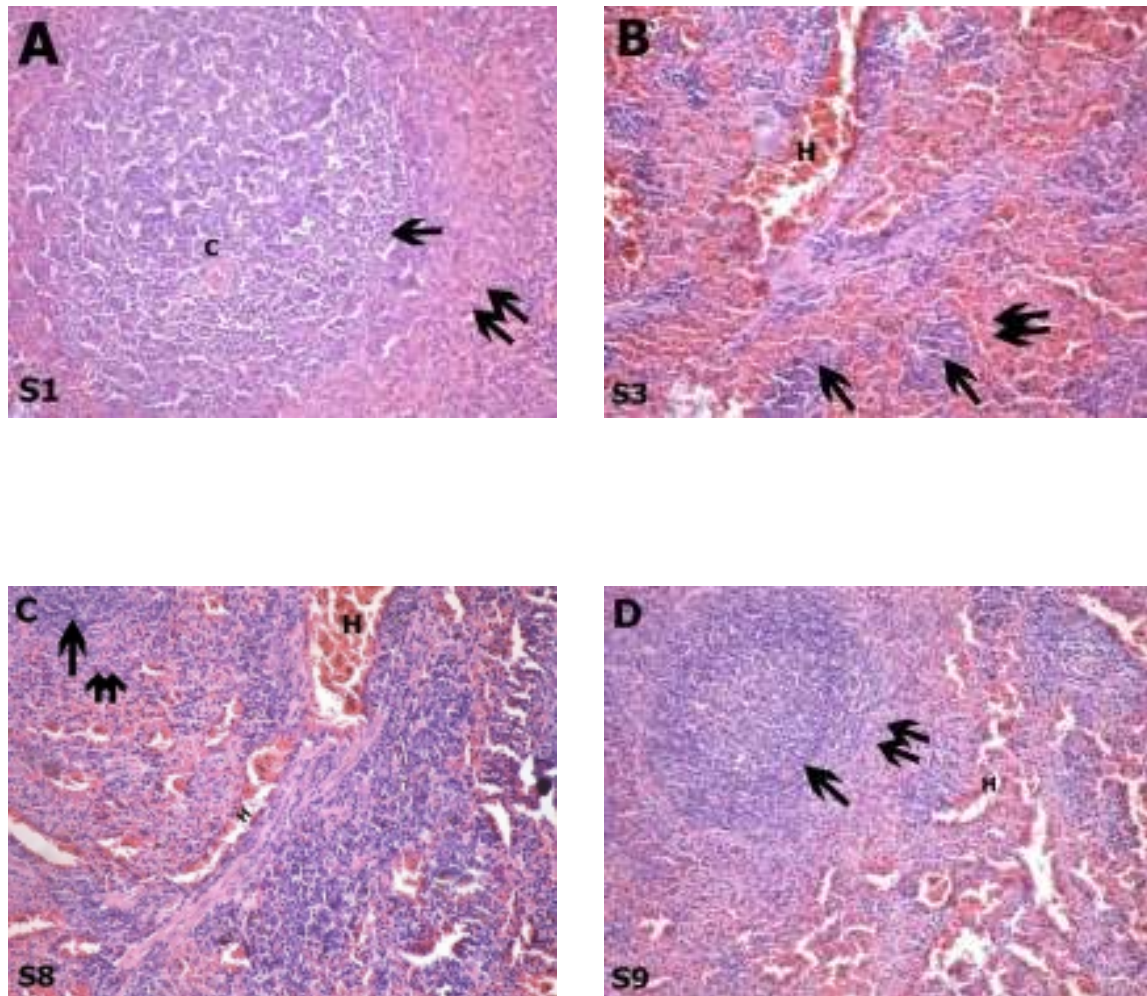


Plate (I) :sections in the spleen of adult male albino rats showing:

Fig. (A): normal mature spleen (control group) (H & E x200).

Fig. (B): absence of lymphoid follicles (white pulp depletion) (arrow) with large areas of red pulp (double arrows) and multiple areas of Hemorrhage (H) (radiated group (6Gy/rat) (H & E x 200).

Fig. (c): mild increase in the size of the lymphoid follicle (arrow) surrounded by large red pulp area (double arrows) with scattered areas of Hemorrhage (H) (vitamin E pretreated irradiated group) (H & E x 200).

Fig. (D): slightly normal lymphoid follicle (arrow) surrounded by red pulp (double arrows). The red pulp composed of sinusoids lying between cells of elongated splenic cords with Scanty areas of Hemorrhage (H) (combined *Nigella Sativa* and vitamin E pretreated irradiated group) (H & E x 200).

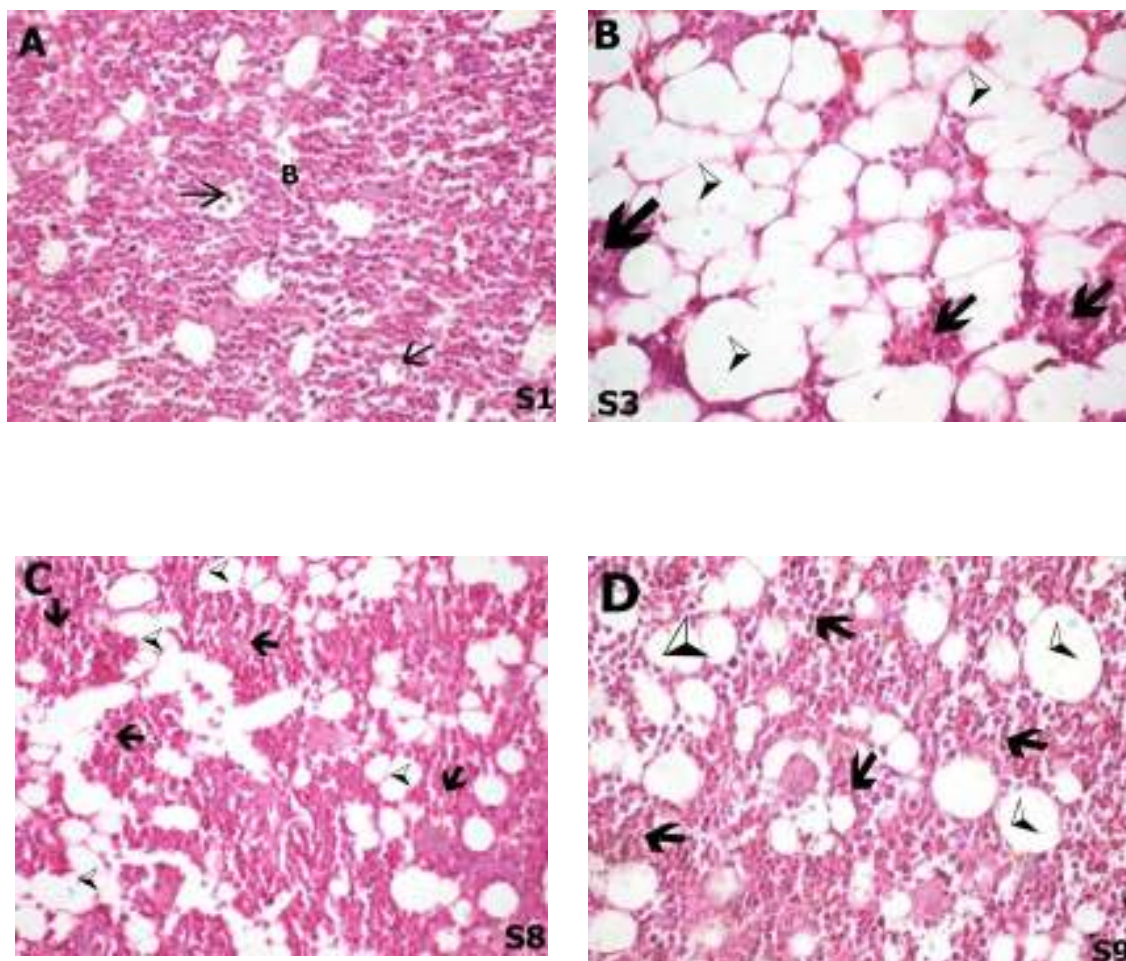


Plate (II): sections in the bone marrow of adult male albino rats showing:

Fig. (A): Normal bone marrow (control group) arrow →bone marrow elements (H & E x200).

Fig. (B): Marked depletion of bone marrow tissue (red marrow) (arrow) and displayed by numerous fat cell (yellow marrow) (arrowhead) (H & E x 200).

Fig. (c): Mild increase of red marrow (arrow) and decrease fat cell (arrowhead) (vitamin E pretreated irradiated) (H & E x 200).

Fig. (D): Increase of red marrow tissue (arrow) in relation to the yellow marrow (arrowhead) (combined *Nigella Sativa* and vitamin E pretreated irradiated group) (H & E x 200).

DISCUSSION

The exposure to ionizing radiation (IR) results in the formation of free radicals in living systems. In the presence of O₂, the free radicals are transformed to reactive oxygen species. These radicals may cause oxidative damage to critical biomolecules such as lipids, protein and nucleic acids of the cell during and following irradiation (Shirazi et al., 2012).

In recent years, an array of natural occurring antioxidants and immunomodulatory radioprotectors specifically antioxidant nutrients (such as vitamin E, C, A and selenium) and phytochemicals (such as *Nigella sativa*, soy products, caffeine, Ginger rhizome, melatonin and genistein) have been examined for their ability to ameliorate radiation induced damage either short

damage like effects on hematopoietic stem cell or long deteriorating effects such as cancer (Baliga and Suresh, 2010).

As regard complete blood count in the present study, there was a significant decrease in erythrocytic count (RBC), total leucocytic count (WBC), neutrophil granulocytes, lymphocytes and remarkable fall in hemoglobin concentration (HB), Ht percentage, hematocrit volume (HCT), mean corpuscular hemoglobin concentration (MCHC) values while a highly significant elevation in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) values when compared with the control group denoting that radiation induced hematotoxic effect throughout the experimentation period.

At the same time, these rats on oral supplementation of Nigella sativa and Vitamin E either individual or combined before irradiation showed a highly significant elevation in the complete blood count of peripheral blood as regard erythrocytic count (RBC), leucocytic count (WBC), platelet count (Pl), hemoglobin concentration (HB), hematocrit volume (HCT), mean corpuscular hemoglobin concentration (MCHC), neutrophil granulocytes, lymphocytes values and highly significant decrease in MCH and MCV values 15 days post irradiation when compared with irradiated group.

The results of the present study were in a harmony with **Ashry and Hussien (2007)** who found a dramatic decrease in RBC, WBC, Hb concentration, Ht percentage, circulating lymphocytes, monocytes and neutrophils after radiation exposure.

El-Mahdy and Ghoneim (2013) stated that the dramatic decrease in erythrocyte count may be related to hemolysis which is induced by radiation promoting the liberation of free radicals, and also they suggested that the underlying cause of the increased RBC lyses by irradiation effect on the production of erythropoietin.

Derval and Sichevskaia (2000) demonstrated that radiation causes retardation in iron in corporation and a decrease in hemoglobin binding to erythrocyte membrane. Also, **Krokosz and Lewandowska (2006)** study recorded that radiation damage of hemoglobin is considered as one of the important mechanisms triggering radiation sickness.

Klebanoff et al. (2005) attributed the reduction in leucocyte count after radiation exposure to the decreased ability of radiated bone marrow to produce mature highly differentiated blood cells and radiation inhibits mitotic division of progenitor cells or induces apoptosis.

The improvement present in the current study after individual or combined supplementation of Nigella sativa and Vitamin E were in a harmony with **Satyamitra et al. (2011)** study which observed hematological recovery after post-irradiation treatment with Vitamin E.

Traber and Stevens (2011) demonstrated that vitamin E is the integral part of cellular membranes whose main role is to defend the cell against oxidation. Within cells and organelles (e.g. mitochondria) vitamin E is the first line of defense against lipid peroxidation induced by radiation. The vitamin E also plays a very important function in lending red blood cells (RBC) flexibility as they make their way through the arterial network.

Also, in a study performed by **Assayed (2010)** recorded a considerable rise in total and differential leukocytic count to normal values in N. sativa pre irradiated rats.

Hosseinimehr (2007) mentioned recent strategies for reducing side effects induced by ionizing radiation; in particular, compounds that can affect hematopoietic stem cell regeneration and increase survival rate: cytokines and immunomodulators represent the bulk of agents in this category. It has been suggested that these agents mediate their radioprotective effects by mechanisms, such as enhancement of the proportion of hematopoietic stem cells into more radioresistant phase of the cell cycle, or induction of bone marrow recovery via stimulating growth, differentiation and proliferation of hematopoietic progenitor and stem cells.

In the current work, the combined treatment with Nigella sativa and vitamin E pre irradiation seemed to produce better effect on biochemical results than individual agents. This represented by non significant changes in the mean values of complete blood count of combined group when compared with control group.

The current study was in a harmony with the study performed by **Hussien et al. (2007)** who informed that combined treatment with vitamin E and coenzyme Q10 pre- irradiation induced more protection against deleterious effect of gamma irradiation in all tested hematological parameters than individual treatment. They also attributed that to their combined antioxidant effects.

As regard oxidative stress parameters in the acute study, there was a highly significant elevation in serum Malondialdehyde (MDA) value and highly significant decreases in glutathione peroxidase (GPx) activity in irradiated group when compared to their corresponding values in control group throughout the experimentation period denoting that radiation induced oxidative stress. At the same time, these rats on oral supplementation of Nigella sativa and Vitamin E either individual or combined before irradiation showed a highly significant decrease in the serum Malondialdehyde (MDA) and highly significant increase in erythrocyte glutathione peroxidase (GPx) activity in male albino rats 15 days post irradiation when compared with irradiated group.

These results were consistent with the work of **Norman and Ashry (2006)** and **Fahmy et al. (2007)** which noticed elevated serum MDA and decreases in glutathione peroxidase (GPx) activity after radiation exposure.

Manda and Bhatia (2003) and **Omran et al. (2007)** demonstrated that MDA is a marker of

lipid peroxide formation, and elevation of it indicates presence of peroxides after radiation exposure.

Moreover, the results were in the same line with **Darwish et al. (2007)** study which found a significant decrease of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activities in female rats after exposure to 6.5 Gy radiation through the experimental period.

Mansour et al. (2006) demonstrated that irradiation can cause extreme oxidative stress which led to degradation of ROS scavenging enzymes as glutathione peroxidase (GPx). The improvement present in the current study after individual or combined supplementation of Nigella sativa and vitamin E were in the line with **Velho-Pereira et al. (2012)** who recorded that macerated extract of Nigella sativa seeds had protective effects against radiation-induced oxidation of protein and peroxidation of membrane lipids in organs such as spleen, liver, brain and biochemical alterations of antioxidant enzymes such as superoxide dismutase, catalase and thiobarbituric acid reactive substances. They referred this effect to the ability of Nigella sativa to scavenge free radicals by its antioxidant properties.

In the present study, the combined treatment with Nigella sativa and Vitamin E pre irradiation seemed to produce more protective effect on oxidative stress parameters of male albino than individual agents. This represented by non significant changes in the mean values of oxidative stress parameters of combined group when compared with control group.

The result of the present study was in the line with **Noaman et al. (2002)** who found that radiation-induced depressions in blood glutathione (GSH), glutathione peroxidase (GSH Px), superoxide dismutase (SOD) and increases in plasma lipid peroxide products were normalized in the irradiated rats that received combined vitamin E and selenium. They mediated that selenium and vitamin E confer radioprotection by inducing or activating cellular free-radical scavenging systems and by enhancing peroxide breakdown.

Based on the present results in the current work, the combined treatment with Nigella sativa and Vitamin E pre irradiation seemed to produce more protective effect on studied parameters. These biochemical results were in parallel with **Prasad (2005) and Alcaraz et al. (2009)** studies which proposed that a combination of dietary antioxidants could be useful in protecting normal tissues against radiation damage no matter how small that damage might be. Hence, it is seemed that pre-treatment with Nigella sativa and

vitamin E combination may augment the function of endogenous free radical scavengers against deleterious effect of gamma irradiation than the individual agents.

On contrary, **Lawenda et al. (2008)** claimed that multiple supplementary antioxidants during chemotherapy and radiation therapy should be discouraged because they may show pro-oxidant activity, increasing the damage induced by ionizing radiation.

As regard histopathological changes, splenic specimens of the male rats in this study revealed marked distortion of splenic tissue, remarkable increase in red pulp area over white pulp and multiple areas of hemorrhage.

The result of the present study was consistent with the findings of **Assayed, (2010)** who found that radiation 4 Gy to male rats caused marked histopathological changes in the spleen in the form of white pulp depletion, sinusoid were filled with erythrocyte and marginal zone around lymphoid nodules and splenic cords were poorly visible.

Upon individual supplementation with Nigella sativa and vitamin E pre-irradiation, there was partial protection against deleterious effect of gamma irradiation in the form of mild improvement in splenic tissue but the effect of radiation were still noticed. While the combined treatment with Nigella sativa and vitamin E pre-irradiation showed marked improvement in splenic tissue than individual treatment.

The results of the current work were consistent with a previous study which indicated that Nigella sativa had a radio protective impact on splenic pulp of irradiated mice (**Assayed, 2010 and Velho-Pereira et al., 2012**).

Bone marrow specimens of the male rats in this study revealed severe distortion of the bone marrow tissue in the form of depletion of red marrow tissue and displayed by numerous fat cell.

These findings were similar in the study developed by **Kiyohara et al, (2003)** who found an expressive increase in fat tissue, with consequent reduction of bone marrow tissue in rat trabecular bone after exposure to external radiotherapy.

Upon individual supplementation with Nigella sativa and vitamin E pre-irradiation, there was mild improvement in bone marrow tissue. Increase of red marrow elements were observed but the effect of radiation was still noticed. While the combined treatment with Nigella sativa and vitamin E pre-irradiation showed marked improvement in bone marrow tissue in the form of

apparently normal ratio of red marrow and yellow marrow.

The current findings were in a harmony with **Anzai et al. (2014)** Study which showed that vitamin E analog, is a strong radiation protector (given before exposure) and also a strong radiation mitigator (given after exposure) acting to prevent bone marrow death in irradiated mice (7.5Gy).

CONCLUSION

From the above mentioned results, it can be concluded that:

- Single sub-lethal (6Gy/rat) dose of gamma radiation induced oxidative stress, hematotoxicity and toxic effects on spleen and bone marrow histopathology of adult male albino rats.
- Individual and combined administration of Nigella Sativa (NS) and vitamin E can ameliorate deleterious effect of gamma radiation as well as histopathological improvement in adult male albino rats. While marked improvement is achieved after combined treatment with NS and vitamin E.

RECOMMENDATIONS

- More studies are needed to investigate the protective effect of Nigella Sativa (NS) and vitamin E on the other organs in experimental animals and humans.
- Other studies are needed to investigate the protective effect of Nigella Sativa (NS) and vitamin E on cancer animals and patients.

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الدور الوقائي المحتمل لزيت حبة البركة وفيتامين (هـ) ضد الاشعاع على ذكور الجرذان البيضاء البالغة

المشتركون في البحث

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مقدمة البحث: لقد ادي انتشار مجالات التطبيقات الإشعاعية وخصوصا في الممارسة الطبية، وكذلك حوادث الإشعاع الى زيادة وتطوير المواد الواقية ضد الاشعاع. **الهدف من البحث:** كان الهدف من هذا البحث تقييم الناثير الوقائي لكل من حبة البركة وفيتامين هـ على حدة او سويا في ذكور الجرذان البيضاء البالغة المعرضة لجرعة من الاشعاع الجامى تبلغ 6 جراي. **طريقة البحث:** تم استخدام تسعون من ذكور الجرذان البيضاء البالغة وقد تم تقسيمهم إلى 9 مجموعات تتكون كل مجموعة من 10 جرذان على النحو التالي: المجموعة الاولى (المجموعة الضابطة السالبة) ، المجموعة الثانية (المجموعة الضابطة الموجبة) ، المجموعة الثالثة (مجموعة الاشعاع). ثم تعريض هذه الجرذان 6 وحدات اشعاع، المجموعة الرابعة: 0.6 مل حبة البركة مذاب في 1 مل زيت ذرة مرة واحدة يوميا لمدة 5 أيام / أسبوع ، المجموعة الخامسة: 100 مجم / كجم فيتامين هـ يوميا لمدة 5 أيام/أسبوع ، المجموعة السادسة: حبة البركة وفيتامين هـ بنفس الجرعات السابقة يوميا لمدة 5 أيام / أسبوع ، المجموعة السابعة : حبة البركة ثم الاشعاع في اليوم الثالث بنفس الجرعات السابقة لمدة اسبوع ، المجموعة التاسعة: حبة البركة و فيتامين هـ ثم الاشعاع في اليوم الثالث بنفس الجرعات السابقة لمدة اسبوع. وبعد 15 يوم تم سحب عينات دم من الجرذان لاجراء صورة دم وقياس مواد الاكسدة الفائقة للدهون ثم ذبح الجرذان لإستئصال الطحال والنخاع العظمى وفحصها بالميكروسكوب الضوئي لتحديد التغيرات الباثولوجية التى حدثت بهما . **النتائج:** من نتائج البحث لوحظ تدهور في جميع القياسات في المجموعة المشعة بينما عند تناول حبة البركة وفيتامين هـ على حدة او سويا قبل التعرض للاشعاع فان النتائج تتحسن وخصوصا عند تناول حبة البركة فيتامين هـ - سويا قبل التعرض للاشعاع . **التوصيات:** توصى هذه الدراسة باجراء المزيد من التجارب على استخدام اكثر من مادة واقية مستخلصة من مصادر طبيعية قبل التعرض للاشعاع.