R E S E A R C H   A R T I C L E

Khaled M. Sharafeldin

THE PHYSIOLOGICAL IMPACT OF GINGER, *ZINGIBER OFFICINALE* AND BLACK SEED OIL, *NIGELLA SATIVA* L. AS MEDICINAL PLANTS IN GAMMA-IRRADIATED RATS

ABSTRACT:

The study reported the radioprotective effects of some herbal plants in rats exposed to gamma-radiation. Male albino rats were treated orally with the ginger extract, GE (250 mg/kg) and black seed oil, BSO (250 mg/kg) separately and in a combination before and after the whole body-irradiation of 8 Gray γ-radiation dose. Glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), lipid peroxidation (LP) and nitric oxide (NO) levels were estimated in the blood. Haematological analysis was done at all treatments. The exposure of rats to γ-ray decreased the GSH content, CAT, SOD and GPx enzyme activities while increased the LP activity and NO levels. Treatment of irradiated rats with GE or BSO ameliorated the changed levels of antioxidants in comparison with the control levels. Also, the protective effect of mixture of both GE and BSO were observed, particularly for GPx, LP, and NO. The results of the haematological parameters showed that only platelet, lymphocyte, monocyte and neutrophil percent were ameliorated as compared with the control values when animals were given GE or BSO or in combination before and after irradiation. The current study revealed the radioprotective role of ginger extract and black seed oil as an antioxidant defense system inducer.

CORRESPONDENCE:

Khaled M. Sharafeldin

Department of Zoology, Faculty of Science, Benha University, Benha, Egypt

E-mail: keldeen@hotmail.com

INTRODUCTION:

The oxidative stress at a large extent is an evidence of radiation injury to living cells (Wallace, 1998). Antioxidants as a counter act to reactive oxygen species (ROS)-mediated toxicity, protect membranes and cytosolic compounds (Kota *et al.*, 2008). Activation of antioxidant enzymes is essential method before the last step of the antioxidative defence system (Deger *et al.*, 2003). Antioxidation process includes transfer of sensitive material to compartments that provide better protection from the action of reactive species, inhibit vulnerable processes such as DNA replication, repair damaged molecules, initiate apoptosis, and the liability to internal and external modifying factors, and finally use a variety of direct free radical scavengers (Riley, 1994). In this process, particular enzymes are involved in antioxidative defence such as superoxide dismutase (SOD), glutathione peroxidases (GPx) and catalase (CAT) (de Zwart *et al.*, 1999). Lipid peroxidation (LP) product is one of the major biomarkers of oxidative damage and living cells have efficient mechanism to protect against oxidant species (de Zwart *et al.*, 1999). Nitric oxide (NO) is produced in large quantities during host defence and immunologic reactions (Moncada and Higgs, 1993).

Living cells induces a variety of reaction products, and many macromolecules and their degradation products when exposed to ionizing radiation. Radiation induced toxicity in Swiss albino mice were shown previously to be protected by antioxidant agents which reduced lipid peroxidation levels (Jagetia and Reddy, 2005). Previous studies have indicated numerous biochemical compounds such as cysteine, germanium and tocopherol that have been used individually to target oxygen and oxygen free-radicals in attempts to reduce radiation-induced damage (Chow, 1991; Yang and Kim, 1999; Sagrista *et al.*, 2002). Also, some commonly used antioxidants of plant origin include vitamin E, vitamin C, selenium, phenolic compounds, carotenoids and flavonoids (Jagetia and Reddy, 2005). The radioprotective ability of several chemical compounds and their analogues has been screened and their high toxicity at optimum protective doses precluded their clinical use (Jagetia and Reddy, 2005; Hanzha *et al.*, 2008). Disability to provide post-irradiation protection

was the other major drawback of these compounds. Also recent terror attacks throughout the world has strengthened the idea that it is necessary to devise appropriate measures against the nuclear terror attacks by using pharmacological agents that can protect against the ill effects of radiation (Nabil *et al.*, 2009).

Ginger, which is underground stem or rhizome of the plant *Zingiber officinale*, has been used in several medicinal preparations (Warrier *et al.*, 1993). Terpenes and oleoresin which called ginger oil are present in ginger. Ginger contains volatile oils approximately 1-3% and non-volatile pungent components oleoresin (Zick *et al.*, 2008). From terpenes the major identified components are sesquiterpene hydrocarbons and phenolic compounds which are gingerol and shogaol (Hasan *et al.*, 2012). The extracts of lipophilic rhizome yielded potentially active gingerols, which can be converted to shogaols, zingerone, and paradol (Govindarajan, 1982). Its extract contains polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity (Chen *et al.*, 1986; Warrier *et al.*, 1993; Nabil *et al.*, 2009). Constituents of ginger extract such as [6]-gingerol, [6]-paradol, shogaols, zingerone, and galanals A and B showed diseases control via modulation of various biological activities (Rahmani *et al.*, 2014). One of these activities is the antioxidative effect (Al-Tahtawy *et al.*, 2011; Bellik, 2014). Pre-treatment with ginger extract reduces the severity of sickness symptoms and mortality in gamma-irradiated mice and also increases the number of survivors (Okunieff *et al.*, 2008). Five-days oral administration of 250 mg/kg hydroalcoholic extract of ginger extract was found to protect mice against the radiation-sickness and death of due to gastrointestinal syndrome as well as bone marrow syndrome (Jagetia *et al.*, 2003). Utilization of *Z. officinale* as radioprotector in male and female rats was indicated by Haksar *et al.* (2006).

The other promising natural radioprotective agent against immuno-suppressive and oxidative effects of ionizing radiation is *Nigella sativa* (Assayed, 2010). The most important active compounds of black seeds are thymoquinone (30% - 48%), thymohydroquinone, dithymoquinone, p-cymene (7% - 15%), carvacrol (6% - 12%), 4-terpineol (2% - 7%), t-anethol (1% - 4%), sesquiterpene longifolene (1% - 8%) α-pinene and thymol etc. Seeds also contain alkaloids as isoquinoline and pyrazol ring bearing alkaloids. Additionally, *N. sativa* seeds contain alpha-hederin, a water soluble pentacyclic triterpene and saponin (Ahmad *et al.*, 2013). One ml oral administration of *Nigella sativa* oil before irradiation considerably normalized all irradiation effects (Assayed, 2010). Whole-body irradiation in rats caused a significant increase in blood malondialdehyde, nitrate and nitrite levels. The administration of *N. sativa* (1 ml/kg BW) for 30 consecutive days in rats decreased the higher levels of GSH. The blood oxidative stress

marker levels in irradiated rats that were significantly decreased after *N. sativa* pre-treatment. The data concerning the radioprotection of *N. sativa* is very scarce; only those reported by (Cemek *et al.*, 2006, Assayed, 2010) are intended about the role of *N. sativa* during gamma-irradiation.

The present study was undertaken to investigate the possible radioprotection role of ginger and black seeds oil, *in vivo*, against whole body γ-irradiation, via induced alteration in the antioxidant system.

MATERIAL AND METHODS:
Animals:

Male albino rats weighing 100 – 120 g (6 – 8 weeks old) were obtained from the Egyptian Organization for Biological Products and Vaccines, VACSERA (Alagouza, Giza, Egypt). Rats were housed at a constant temperature (24 ± 2 °C) with alternating 12-hours light and dark cycles and fed standard laboratory food and water *ad libitum*. The animals were treated in accordance with the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals (NIH Publication No. 85-23, revised 1985) in accordance with international ethical considerations.

Drugs:

Ginger, *Zingiber officinale* was obtained as tablet (400 mg) from MEPACO Company (Arab Co for Pharmaceuticals and Medicinal Plants, MEPACO, Egypt). Treated rats received oral dose of 250 mg/kg of ginger extract, GE as an optimum oral dose according to Jagetia *et al.* (2004). Black seed oil (BSO) extracted from *Nigella sativa* was obtained as soft gelatin capsules, each of 450 mg (Pharco Pharmaceuticals, Alexandria, Egypt. Same dose of 250 mg/kg of BSO were tested in rats.

γ-Irradiation:

γ-irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Nasr City, Cairo, Egypt, using Caesium-137 in a Canadian Gamma cell-40 Irradiator (Atomic Energy of Canada Limited, Canada). The whole bodies of the animals, in special cages, were exposed to gamma irradiation at an acute single dose level of 8 Gray delivered at a dose rate of 0.46 Gy/min^{-1}.

Experimental design:

Sixty-Four male rats were divided into eight groups, each of 8 animals.

Group I: Animals were served as control group and no treatment was given to rats.

Group II: Rats were treated with successive six doses of 0.5 ml distilled water containing GE (250 mg GE /kg BW).

Group III: Rats were treated with successive six doses of 0.5 ml distilled water containing (250 mg BSO/kg BW).

Group IV: Animals were treated with successive six doses of 0.5 ml distilled water containing GE (250 mg/kg BW) and BSO (250 mg/kg BW).

Group V: Rats were γ - irradiated with a dose of 8 Gy then were left for 4 days before termination.

Group VI: Animals were treated with 2 successive doses of GE (each, 250 mg /kg BW) before irradiation with 8 Gy then followed by 4 successive doses of GE (250 mg /kg BW).

Group VII: Rats were treated with 2 successive doses of 250 mg BSO/kg before irradiation with 8 Gy then followed by 4 successive doses of BSO (250 mg /kg BW).

Group VIII: Animals were treated with successive 2 doses of mixture of 250 mg GE /kg and 250 mg BSO/kg before irradiation with 8 Gy, then animals were given 4 successive doses of GE /BSO mixture.

All previous doses of GE and BSO were orally taken by 18G gauge, ball diameter 2.25 and 2-inch length. Animals were sacrificed 24 hours after the last dose of treatments.

Biochemical analysis:

Glutathione (GSH) was measured according to the colorimetric method of Beutler *et al.* (1963). Superoxide dismutase activity (SOD) was measured according to the method of Minami and Yoshikawa (1979). Catalase (CAT) activity was determined according to the method of Johansson and Borg (1988). Lipid peroxidation (LP) was measured using the method described by Yoshioka *et al.* (2001). Glutathione peroxidase (GPx) activity in the whole blood of rats was estimated according to the method of Belsten and Wright (1995). The nitric oxide (NO) was estimated by oxidation end products of NO production (Totan *et al.*, 2001).

Haematological analysis:

Total number of erythrocytes (RBCs), leukocytes (TLs), differential leukocyte count; lymphocytes (Ls) %, monocytes % (M), and neutrophils % (N), platelets count (PLT), mean platelet volume (MPV), haematocrit (Hct) %, and haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were determined using automated blood cell counter (Cell dine 1700, GMI, USA).

Statistical analysis:

All results were expressed as mean ± SD. The intergroup variation was measured by one-way analysis of variance (ANOVA) followed by LSD Post-Hoc test.

RESULTS:

Biochemical results:

Animals treated with 250 mg/Kg of GE or BSO and those treated with both GE and BSO in combination showed slightly change of antioxidants profile in comparison with the control group (I) (Table 1). The levels of GSH, CAT, SOD, and GPx were lowered significantly (p< 0.05) when animals were exposed to γ-radiation in group (V) (Table 1). On the other hand, LP and NO GE recorded significant elevated levels (p < 0.05) after irradiation (Table 1). Administration of GE in irradiated rats in group (VI) caused mitigation of GSH, GPx, LP, and NO levels when compared to control levels (Table 1). Treatment of irradiated animals with GE showed no significant differences in CAT and SOD activities (Table 1). The treatment with BSO before and after γ-radiation ameliorated significantly the levels of GSH, CAT, GPx, LP, and NO, as compared to the levels in group (V) and almost close to the normal control levels, albeit the SOD level did not reach statistical significance level (Table 1). The combined treatment of irradiated rats with GE and BSO alleviated the levels of GPx, LP, and NO (Table 1). In spite of the alleviation levels of CAT and SOD activities after treatment with GE and BSO, this effect was statistically insignificant when compared with their levels in γ-irradiated animal (Table 1).

Table 1. GSH, CAT, SOD, GPx, LP, and NO activity levels in the blood plasma of normal and irradiated rats treated with GE, BSO or their combination.

Groups		GSH (mg/dl) ± SD	CAT (µmol/min/ml) ± SD	SOD (µg/ml) ± SD	GPx (µg/min/ml) ± SD	LP (µmol MAD/ml) ± SD	NO (µmol/ml) ± SD
Groups I	Control	17.83 ± 1.8 [e]	27.35 ± 1.6 [e]	55.56 ± 9.4 [e]	39.59 ± 3.9 [ec]	79.39 ± 5.4 [e,B]	21.77 ± 2.5 [e]
Groups II	GE	19.23 ± 1.4 [e]	28.26 ± 2.1 [e]	58.805 ± 6.2 [e]	40.48 ± 2.7 [e]	79.99 ± 2.3 [e,A,B]	22.96 ± 4.3
Groups III	BSO	18.81 ± 1.8 [e]	28.37 ± 1.9 [e]	53.21 ± 16.4 [e]	41.47 ± 3.4 [e]	76.88 ± 10.2 [e]	20.79 ± 3.25 [e]
Groups IV	GE + BSO	19.18 ± 1.8 [e]	29.02 ± 1.1	54.84 ± 15.9 [e]	40.83 ± 2.7 [e,B,C]	76.99 ± 15.3 [eAB]	20.53 ± 3.5 [e]
Groups V	Rad*	13.34 ± 1.8 [abcd,ABC]	19.41 ± 5.6 [abc]	40.08 ± 5.3 [abd,B]	29.55 ± 4.3 [abcd,ABC]	116.38 ± 7.3 [abcd, ABC]	26.64 ± 2.3 [acd,ABC]
Groups VI	GE + Rad	18.346 ± 1.4 [e]	25.26 ± 2.9	49.81 ± 10.5	38.46 ± 4.3 [e]	85.14 ± 7.5 [de]	19.95 ± 4.9 [e]
Groups VII	BSO +Rad	17.81 ± 3.0	27.87 ± 2.3 [e]	51.54 ± 11.9	37.63 ± 5.6 [de]	91.04 ± 4.3 [de]	21.29 ± 3.7 [e]
Groups VIII	GE + BSO + Rad	18.39 ± 1.3 [e]	25.50 ± 4.6	52.43 ± 12.5	39.56 ± 1.1 [ade]	87.55 ± 8.7 [e]	19.46 ± 4.2 [e]

n = 8; data are Means ± S.D.;   (a) significant vs. control at p< 0.05. (b): significant vs. GE group at p < 0.05, (c) significant vs. BSO at p< 0.05, (d) significant vs. GE + BSO at p< 0.05, (e) significant vs radiation at p< 0.05, (A) significant vs. GE + radiation at p < 0.05, (B) significant vs BSO + radiation at p< 0.05, and (C) significant vs. GE + BSO + radiation at p< 0.05; * γ -Radiation

Haematological results:

The administration of GE displayed no change in all haematological parameters and cells counts (Tables 2 & 3). BSO administration lowered the values of Hct and MCV significantly (p < 0.05) (Table 2). The association of GE and BSO displayed no change of the same haematological parameters except lowering the MCV values (Table 2). Exposure to γ-radiation lowered the levels of Hct and MCV and elevated MCHC values (Table 2). Haemoglobin, RBCs, and MCH values were not changed in all treated animal groups. The oral administration of GE, BSO and their combination in γ-irradiated animals mitigated the values of Hct, MCV, and MCHC reaching the control values (Table 2).

Table 2. Levels of haemoglobin (HB), RBCs, Hct%, MCV, MCH, and MCHC in the blood of normal and irradiated rats treated with GE, BSO or their combination.

Groups		HB	RBCs	Hct	MCV	MCH	MCHC
		(g/dl) ± SD	(×10^{12}/L) ± SD	(%) ± SD	(fl) ± SD	(pg) ± SD	(g/dl) ± SD
Groups I	Control	12.53 ± 0.4	7.32 ± 0.3	47.60 ± 1.7	65.33 ± 5.0	17.10 ± 0.6	26.30 ± 1.3
Groups II	GE	12.44 ± 0.4	7.09 ± 0.5	46.12 ± 1.7	65.3 ± 3.5	17.56 ± 0.8 [c]	26.92 ± 0.5
Groups III	BSO	11.95 ± 0.6	7.28 ± 0.4	43.6 ± 1.0 [a]	60.08 ± 2.9 [a]	16.38 ± 0.2	27.33 ± 1.1
Groups IV	GE + BSO	12.44 ± 0.6	7.35 ± 0.4	45.32 ± 3.4	61.32 ± 5.4 [a]	16.90 ± 1.0	27.68 ± 0.8
Groups V	Rad*	12.03 ± 0.4	6.93 ± 0.3	41.45 ± 1.8 [abd]	59.91 ± 1.8 [a]	17.32 ± 0.6 [c]	29.02 ± 0.4 [ac]
Groups VI	GE + Rad	12.80 ± 0.5	7.41 ± 0.4	44.38 ± 2.0 [ad]	60.05 ± 1.7 [a]	17.25 ± 0.4 [c]	28.80 ± 0.5 [ac]
Groups VII	BSO +Rad	12.30 ± 0.8	7.17 ± 0.7	41.55 ± 2.5 [abd]	58.23 ± 3.3 [a]	17.17 ± 0.9	29.53 ± 0.4 [ac]
Groups VIII	GE + BSO + Rad	13.18 ± 0.5	7.52 ± 0.3	43.4 ± 2.7 [a]	57.7 ± 2.5 [a]	17.48 ± 0.8 [c]	30.35 ± 1.1 [ace]

n = 8; data are Means ± S.D.; (a) significant vs. control at p< 0.05. (b): significant vs. GE group at p< 0.05, (c) significant vs. BSO at p< 0.05, (d) significant vs. GE + BSO at p< 0.05, (e) significant vs. radiation at p< 0.05, (A) significant vs. GE + radiation at p< 0.05, (B) significant vs. BSO + radiation at p< 0.05, and (C) significant vs. GE + BSO + radiation at p< 0.05; * γ -Radiation

Exposure of rats to γ-radiation sharply lowered the total leucocytes count (TLs) when compared with the control values (Table 3). Administration of GE has significantly reduced the TLs levels as compared to the data of control animals (Table 3). In contrast, administration of both GE and BSO in combination without irradiation, have raised the TLs twice above the control values (Table 3). Administration of GE, BSO or their combination with radiation increased TLs when compared with its value in irradiated rats only, however the values were non-comparable with the control group (Table 3). The exposure of rats to γ-radiation increased the lymphocytes percentage (Ls) sharply (p < 0.05) when compared to all other groups (Table 3). Percentages of monocytes were deceased and percentages of neutrophils sharply decreased in irradiated animals as compared with all other groups (Table 3). Administration of GE, BSO or both before and after irradiation alleviated monocytes and neutrophils to almost the control levels (Table 3). Ginger extract and BSO administration displayed no changes in the PLT and MPV levels. Gamma-radiation exposure decreased significantly the values of PLT significantly (p < 0.05). This was ameliorated by the administration of GE or BSO after irradiation (Table 3).

Table 3. Total leukocytes (TLs), Ls %, M %, N %, PLT, and MPV in the blood of normal and irradiated rats treated with GE, BSO or their combination.

Groups		TLs	Ls	M	N	PLT	MPV
		(×10^9/L)	(%)	(%)	(%)	(×109/L)	(Fl)
Groups I	Control	5.88 ± 1.6 [bdeAB]	1.6 ± 0.6 [e]	31.4 ± 3.5	66.9 ± 3.9 [e]	376.83 ± 40.8 [e]	7.78 ± 0.2
Groups II	GE	2.82 ± 0.8 [ad]	2.3 ± 1.8 [e]	29.6 ± 5.8	68.0 ± 6.8 [e]	425.80 ± 69.1 [e]	7.68 ± 0.2
Groups III	BSO	4.92 ± 2.0 [de]	2.3 ± 9.0 [e]	30.3 ± 3.4	67.2 ± 4.0 [e]	431.17 ± 120.8 [e]	7.81 ± 0.3
Groups IV	GE + BSO	10.42 ± 5.0 [bceAB]	5.1 ± 2.1 [e]	32.5 ± 7.4 [e]	62.3 ± 6.3 [e]	462.60 ± 153.6 [e]	7.84 ± 0.0
Groups V	Rad*	0.78 ± 0.2 [acd]	70.4 ± 26.4 [abcdABC]	23.2 ± 7.4 [adABC]	11.9 ± 17.4 [abcdABC]	162.17 ± 34.8 [a]	8.00 ± 0.1
Groups VI	GE + Rad	2.98 ± 2.0 [ad]	5.0 ± 3.7 [e]	30.9 ± 8.1 [e]	64.0 ± 8.7 [e]	363.00 ± 195.6 [e]	7.82 ± 0.4
Groups VII	BSO +Rad	2.95 ± 1.7 [ad]	4.5 ± 3.7 [e]	32.8 ± 3.7 [e]	67.1 ± 12.0 [e]	350.83 ± 176.0 [e]	7.97 ± 0.3
Groups VIII	GE + BSO + Rad	1.65 ± 0.9 [acd]	6.9 ± 5.3 [e]	33.6 ± 6.5 [e]	65.3 ± 16.2 [e]	284.25 ± 142.3	8.13 ± 0.1

n = 8; data are Means ± S.D.; (a) significant vs. control at p< 0.05. (b): significant vs. GE group at p < 0.05, (c) significant vs. BSO at p< 0.05, (d) significant vs. GE + BSO at p < 0.05, and (e) significant vs. radiation at p < 0.05, (A) significant vs. GE + radiation at p < 0.05, (B) significant vs. BSO + radiation at p< 0.05 and (C) significant vs. GE + BSO + radiation at p < 0.05; * γ –Radiation

DISCUSSION:

Radiotherapy has great importance as integral part of modern medicine (Maurya *et al.*, 2006; Hogle, 2007). Srinivasan *et al.* (2007) demonstrated that exposure to ionizing radiation produces significant alterations in the oxidant activity of different tissues. Therefore, plants as radio-protector agents as ginger extract and black seed oil are proposed to use against γ-radiation. Previous study used ginger against γ-radiation (Jagetia *et al.*, 2004). The best predictive administration for ginger is giving the animals oral single doses per day for 3 to 7 consecutive days before irradiation to guarantee the release of antioxidants (Jagetia *et al.*, 2004). The administered GE or BSO in the present study was one dose daily for 2 consecutive days before animals' irradiation. This pattern of administration may initiate and release the antioxidants response. Animals were exposed to 8 Gy as sublethal dose. However, the determined $LD_{50/30}$ of γ-radiation was 8.2 Gy which increased by GE to be 9.4 Gy $LD_{50/30}$ (Jagetia *et al.*, 2003).

In the current study the exposure of male rats to 8 Gy γ-rays decreased GSH content, CAT, SOD, and GPx enzyme activities. The mentioned enzymes were involved particularly in antioxidative defense (de Zwart *et al.*, 1999). They exist in cells to protect against oxidant species (Deger *et al.*, 2003). Antioxidant enzymes perform multifunctional activities to attenuate the radiotoxicity (Jagetia *et al.*, 2003). The cells maintained glutathione in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems as well as reacting directly with oxidants (Jagetia *et al.*, 2003). SOD dismutates oxygen radical ($O_2^{\cdot-}$) to H_2O_2 that converted by CAT into H_2O and molecular oxygen (Uslu *et al.*, 2003). The biochemical function of GPx is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water (Haksar *et al.*, 2006). Also, as a result of γ-radiation exposure to rats in the present study, the increasing of lipid peroxidation level was observed. Production of hydroxyl radicals (•OH) may be resulted from the radiolysis of water in the aqueous media of the cells prior to radiation exposure. When ROS was not removed, damage occurs through peroxidation of polyunsaturated fatty acids within membrane phospholipids (Uslu *et al.*, 2003). Increased levels of LP lead to DNA damage and cell death (Leyko and Bartosz, 1986; Reddy andd Lokesh, 1992; Jagetia *et al.*, 2004). Additionally, the exposure of rats to γ-radiation caused severe increased level of NO. One of the most important reactions under

physiological conditions is that of superoxide and NO radicals resulting in peroxynitrite. The protonated form of peroxynitrite is a powerful oxidizing agent that might cause depletion of sulfhydryl groups and react with other components present in high concentrations; such as H_2O_2 or CO_2, to form adduct that might be responsible for many of the deleterious effects seen in biological sites (Kohen and Nyska, 2002). It can also cause DNA damage such as breaks, protein oxidation, and nitration of aromatic amino acid residues in proteins. Repair mechanisms of radiotoxicity depend on the status of endogenous antioxidant enzymes at exposure time and in post irradiation hours (Sun *et al.*, 1998).

The administration of GE before and after γ-radiation ameliorated the changed levels of antioxidants. Gamma-irradiated rats treated with GE returned the levels of GSH, GPx, LP, and NO almost to the control values. The enzymes of CAT and SOD showed insignificant rise in their values when animals were treated with GE in combination with γ-radiation. Other studies revealed depletion of LP and elevation of GSH concentration when mice were treated with GE before irradiation (Ganesh *et al.*, 2003; Jagetia *et al.*, 2003 & 2004; Parihar *et al.*, 2006). The reduction of LP, accompanied by an elevation of GSH, after GE administration was responsible for scavenging of radiation-induced free radicals. GE was found to scavenge OH and O^{-2} radicals in dose dependent manner (Ganesh *et al.*, 2003). Elevated SOD levels in GE pre-treated mice may prevent the hydroxyl radical formation by dismutazing the superoxide radicals (Parihar *et al.*, 2007). Also, GE elevated CAT that reduces H_2O_2 into water which may prevent hydroxyl radical formation (Parihar *et al.*, 2007). The antioxidant action of GE has been proposed as one of the major possible mechanisms for the protective actions of the plant against toxicity and lethality of radiation, (Jagetia *et al.*, 2003; Haksar *et al.*, 2006). So it seems that GE acts on antioxidants response initiated by γ−radiation releasing free radicals. It has been shown that gingerol, as a major bulk of GE, has strong antioxidant action, strong anti-inflammatory and anti-apoptotic actions (Kim *et al.*, 2007). Ginger extract revealed an important role in extract scavenge superoxide anion and hydroxyl radicals. Also lipid per-oxidation was ameliorated by gingerol that inhibited ascorbate/ferrous complex in rat liver microsomes (Bellik, 2014). Ginger extract ingredient's 6-Dehydroshogaol, 6-shogaol and 1-dehydro-6-gingerdione inhibited nitric oxide synthesis in activated macrophages (Li *et al.*, 2011). Another

possible mechanism for antioxidative effect of GE is the role of its phenolic substances in donating electrons to H_2O_2 and neutralizing it to water (Khader *et al.*, 2010).

Regarding the administration of BSO before and after γ-radiation, the levels of CAT, GPx, LP, and NO were alleviated significantly, while levels of GSH and SOD were not changed significantly. Oral administration of BSO before irradiation considerably normalized the levels of CAT, GPx, and NO. It has been shown that both the crude oil of *Nigella sativa* as well as thymoquinone (the main compound of the essential volatile oil), inhibit lipid peroxidation in liposome (Houghton *et al.*, 1995). Another study has shown that compounds isolated from *N. sativa* including thymoquinone, carvacol, tanethole and 4-terpinol have appreciable free radical scavenging properties (Baynes, 1991; Burits and Bucar, 2000; Nagi and Mansour, 2000). Thymoquinone is a potent superoxide radical scavenger which is as effective as SOD against superoxides generated (Nagi and Mansour, 2000). The present study revealed that BSO lowered the levels of LP as compared to its level in irradiated rats only. This lowering effect of BSO may be due to inhibitory effect of thymoquinone on lipid peroxidation induced by Fe^{3+}/ascorbate. Also, *N. sativa* treatment significantly protected the effects of ionizing radiation as reported by Cemek *et al.* (2006). Ganesh *et al.* (2003) found that black seed oil was found to scavenge OH and O^{-2} radicals in dose-dependent manner *in vitro*. Assayed (2010) recommend *N. sativa* as a promising natural radioprotective agent against immunosuppressive and oxidative effects of ionizing radiation.

Certain fluctuations of haematological measurements can be seen in this study. The results in γ-radiation exposed rats showed decreases in levels of Hct, MCV, PLT, TLs M, and N. But, elevation of Ls was observed. The considerable decreases in RBCs, TLs, M, N, platelets count, and haematocrit post-irradiation were supported by the findings of Azab *et al.* (2011). γ- irradiation induced direct destruction of mature circulating cells, loss of cells from the circulation by haemorrhage, or leakage through capillary walls and reduced cell production (Abdelhalim *et al.*, 2015). The decrease in the values of haematological parameters following radiation exposure may be attributed to direct damage caused by a lethal dose of radiation (Heda and Bhatia, 1986). The cellular elements of the blood are particularly sensitive to oxidative stress because their plasma membranes contain a high percentage of polyunsaturated fatty acids (Chew and Park, 2004). Therefore, the decrease in white blood cells differential count recorded in the irradiated rats might be the consequence of radiation-induced lipid peroxidation and damage of their cell membranes which leads to leucopoenia. The increase of WBCs with administration of GE and BSO might be due to activation of proliferation in bone marrow. The increasing was not supported by the other leucocytes of blood parameters. The administration of GE or/with BSO as a complex did not make significant amelioration MCHC.

In conclusion, based on the experimental findings, it was suggested that administration of GE could protect γ-irradiated rats from oxidative stress. The present study also pointed out that treatment with BSO has beneficial effect showing the same range of radioprotection potency. The combination of both plants extracts had no significant effect as radio-protectors.

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التأثير الفسيولوجى للجنزبيل وزيت حبة البركة كنباتات طبية فى الفئران المشععة بجاما

خالد محمد شرف الدين

قسم علم الحيوان ، كلية العلوم ، جامعة بنها ، بنها ، مصر

تهدف الدراسة إلى تقييم التأثير المقاوم للأشعة للنباتات العشبية في الفئران المعرضة لأشعة جاما. تم تجريع ذكور الفئران البيضاء عن طريق الفم بالزنجبيل (250 مجم / كجم من وزن الجسم)، وزيت الحبة السوداء (BSO) ، (250 ملجم / كجم من وزن الجسم)، كل على حدة وكذلك فى خليط قبل وبعد تشعيع الجسم كله بأشعة جاما (8 جراى). تم تقييم مستوى مضادات الأكسدة فى الدم وشملت الجلوتاثيون (GSH) ، الكاتاليز (CAT)، سوبراوكسيد ديس ميوتيز (SOD) ، الجلوتاثيون المختزل (GPx) ، بيروكسيد الدهون (LP) وأكسيد النيتريك (NO).كما تم أيضا تحليل قياسات الدم. بينت النتائج أن تعرض الفئران لأشعة جاما ادى الى انخفاض كلا من مستوى نشاط انزيمات GSH و CAT و GPx ، في حين زاد محتوى LP و NO فى الدم. كذلك بينت النتائج أن استخدام

المستخلص المائي للزنجبيل أو وزيت الحبة السوداء منفردا مع التعرض لأشعة جاما أدى إلى التحسن في المستويات المتغيرة لمضادات الأكسدة بالمقارنة مع المجموعة الضابطة. وتحسنت مستويات مضادات الأكسدة أيضا مع اعطاء خليط المستخلصين في الحيوانات المشععة وخصوصا لمستويات GPx LP ، NO. أظهرت نتائج قياسات الدم أن الصفائح الدموية ، الخلايا اللمفاوية والمونوسايت وكذلك النيتروفيل تأثرت واستعادت نسبتها الطبيعية عند المقارنة بالمجموعة الضابطة وذلك فى وجود الزنجبيل او زيت الحبة السوداء او كلاهما قبل وبعد التشعيع. وقد كشفت الدراسة الحالية الدور المقاوم لمستخلصى الزنجبيل وزيت الحبة السوداء وكنظام محفز لمضادات الأكسدة ضد أضرار الإشعاع.