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Ellagic Acid and Zinc Aspartate Ameliorate Gamma Radiation Induced Biochemical Alterations in Male Rats

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THIS STUDY was designed to investigate the protective effect of oral administration of ellagic acid (EA), a natural polyphenol (50mg/kg body wt) and/or zinc aspartate (ZA) (50mg/kg body wt) against the cellular damage induced by whole body gamma irradiation (6.5Gy as a single dose) in male albino rats. The results of the current study revealed that exposure to γ-radiation exhibited a potential elevation of serum iron, total iron binding capacity (TIBC) and transferrin; as well as lipid peroxidation (LPO) and metallothioneins (MTs) in liver and kidney. In addition, there were significant decrease in superoxide dismutase (SOD) and reduced glutathione (GSH). In addition, tissues of liver and kidney displayed changes in some trace elements concentrations. Rats treated with EA and/or ZA before and after whole body γ-irradiation revealed significant modulation of the biochemical parameters and improvement in the antioxidant status, which might be effective in minimizing the radiation-induced increase in LPO as well as changes in essential trace elements in liver and kidney tissues reflecting a synergistic effect.

***Keywords**:* Ellagic acid, zinc aspartate, γ-radiation, rats.

All living organisms are continuously, exposed to background ionizing radiation coming from natural sources or manmade ionizing radiation (Kaczmarska *et al.*, 2011). Radiation therapy is considered one of the most popular and important tools to cure cancer but the radio sensitivity of normal tissues away from the tumour sites are suggested to limit the therapeutic gain (Agrawal *et al.*, 2001). It is well known that high doses of ionizing radiation result in overproduction of oxygen-derived free radicals, which cause peroxidation of membrane proteins and lipids; further products of peroxidation are mutagenic and

carcinogenic (Lowenthal and Airey, 1997). Biological effects of these highly reactive compounds are controlled *in vivo* by a wide spectrum of antioxidant mechanisms (Guney *et al.*, 2004).

There is growing interest in understanding the role and mechanism of the phytochemicals. Among all phytochemicals, EA acid is a naturally occurring polyphenol compound; it has been receiving the most attention because of its wide array of biological properties, such as radical scavenging, chemo preventive, anticancer, antiviral and antibacterial properties (Özkaya *et al.*, 2010). It is mostly abundant in berries, walnuts, pecans, mango kernel, pomegranate and grapes (Wang *et al.*, 2009).

Zinc is an essential trace element with its catalytic and structural role in many enzymes (Maret, 2006). It also plays an essential role in cell viability due to its antioxidant, anti-apoptotic, anti-inflammatory effects, and it acts as a growth cofactor, an important immune regulator, and a cyto-protector (Ozkaya *et al.*, 2011). ZA was found to be more effective than GSH and cysteine as a remarkable radio protector (Sorenson, 2002). The focus of this study was to evaluate the radio protective effect of EA and/or ZA.

Material and Methods

Maintenance of animals

Adult male albino rats (120-150g) were obtained from Ucmma Company for Chemical Industry, Cairo, Egypt, were kept for 7 days for laboratory acclimatization and fed on commercial pellets and provided with tap water. All animal procedures are in accordance with the general guidelines of animal care and recommendations of the Canadian Committee for Care and Use of Animals (Canadian Council of Animal Care, 1993). All efforts were made to minimize the number of animals used and their suffering.

Irradiation

Whole body gamma-irradiation was carried out using a Canadian ^{137}Cesium source, Gamma Cell-40 biological irradiator, located at the NCRRT, Egypt. It is characterized by a uniform distribution of rays for small biological materials with no external hazards for the operating persons. Rats were exposed to a single dose level of 6.5Gy with a dose rate of 0.42 Gy/ min.

Treatments

EA; 97% was purchased from Sigma-Aldrich Co. (St Louis, Mo, USA). It was dissolved in corn oil and administered at a dose of (50 mg/kg body wt), orally (p.o.) by gavagesaccording to Pari and Sivasankari (2008).

ZA was purchased from Atos Pharma, Cairo, Egypt. It was dissolved in distilled water and supplied p.o. by gavages to rats at a dose of (50mg/ kg body wt) equivalent to 10mg elemental zinc according to Turut *et al.* (2009).The animals were categorized into eight groups (n=8). Group 1: control (non-treated& non-irradiated). Group 2: exposed to γ-radiation. Group 3: received EA. Group 4: γ-irradiation+ EA. Group 5: received ZA. Group 6: γ-irradiation+ ZA. Group 7: EA+ ZA. Group 8: γ-irradiation+ EA+ ZA. EA and ZA were supplied to the irradiated groups for 21 days before irradiation and 7 days after irradiation as triple dose per week. At the end of the experimental period of 28 days, the rats were subjected to diethyl ether anaesthesia then, sacrificed. Serum and tissue samples (liver & kidney) were collected.

Biochemical analysis

Serum iron was determined according to Dreux (1977), TIBC was determined according to Piccardi *et al.* (1972) and transferrin was calculated theoretically from TIBC according to Tsung *et al.* (1975). In liver and kidney tissues; SOD activity was determined according to Minami and Yoshikawa (1979), LPO products were estimated as TBARS using the method of Yoshioka *et al.* (1979), GSH content was estimated according to Ellman (1959) and MTs were determined by Ag-saturation hemolysate method according to Onosaka and Cherian (1982).The biochemical assay was achieved using Herios UV/VIS Spectrophotometers, Japan. Trace elements were measured by using Atomic Absorption Spectrometer, SOLAR System Unicam 939, England, after digestion of tissues in nitric acid and H_2O_2 (5:1) by using microwave digestor MLS-1200 Mega, Italy (Kingston and Jassei, 1988).

Statistical analysis

Analysis of data was performed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer test using Prism Dimo-4 program. Results were expressed as mean± S.E. Value of $P< 0.05$ was considered statistically significant.

Results

Results in Table 1. revealed that, γ-irradiation led to a significant increase in serum iron, TIBC and transferrin compared to control group. Administration of EA before and after γ-irradiation returned serum iron to the normal range. Administration of ZA alone pre & post irradiation significantly reduced serum TIBC & transferrin; while together with EA revealed significant reduction in serum iron, TIBC and transferring compared with the irradiated group.

TABLE 1. Effect of ellagic acid and/ or zinc aspartate on serum iron, TIBC and transferrin.

Groups	Iron (µg/dl)	TIBC (µmol/l)	Transferrin (g/l)
Control	103.0± 8.5	219.0± 21.0	8.7± 0.8
IRR	175.0± 15.0[a]	508.0± 42.0[a]	20.2± 1.7[a]
EA	104.3± 7.0	227.3± 15.0	8.9± 0.6
EA+ IRR	148.2± 7.0	499.5± 29.0[a]	19.9± 1.2[a]
ZA	119.4± 11.0	248.5± 8.2	9.9± 0.3
ZA+ IRR	132.8± 13.0	341.0± 29.0[b]	13.6± 1.2[b]
EA+ ZA	115.5± 6.0	221.4± 12.2	8.8± 0.5
EA+ ZA+ IRR	116.8± 8.9 [b]	327.0± 11.0[b]	13± 0.4[b]

[(a)]Significant difference from control at $P<0.05$.[(b)]Significant difference from irradiated group at $P<0.05$. Each group represent 8 animals, mean± S.E.

Table 2. showed that γ-irradiation caused a significant decrease of liver SOD and GSH and a significant increase in MTs and LPO when compared to control group. Supplementation of rats with EA to non-irradiated animals induced significant drop of GSH when compared with control group, while EA administration pre & post exposure to γ-radiation showed a significant elevation in hepatic GSH and a decrease in LPO and MTs compared to the irradiated group. ZA administration before and after exposure to γ-radiation showed a significant elevation in SOD and GSH and a significant drop in LPO. Co-administration of EA and ZA pre & post irradiation showed significant differences compared to the irradiated group in all oxidative stress parameters. Table 3. revealed that γ-irradiation resulted in a significant decrease in SOD and GSH of kidney and a significant increase in LPO and MTs as compared to control group. Supplementation of rats with EA before and after exposure to γ-radiation showed a significant drop in LPO. ZA administration to non-irradiated animals significantly elevated liver and kidney GSH when compared with control group, while ZA pre & post γ-irradiation exhibited a significant

drop in LPO & MTs and significant elevation in SOD & GSH when compared to irradiated group. Both treatments with irradiation dropped LPO and MTs and elevated SOD significantly compared to irradiated group.

TABLE 2. Effect of ellagic acid and/ or zinc aspartate on some liveroxidative stress biomarkers.

Groups	SOD (U/ml)	LPO (nmol/ mg)	GSH (mg/ g tissue)	MTs (mg/g tissue)
Control	58± 1.7	63.5± 2.7	62.0± 2.9	27.5± 1.0
IRR	28± 2.0[a]	168.0± 10.7[a]	14.0± 0.8[a]	40.5± 2.4[a]
EA	53± 2.5	76.1± 2.3	49.0± 0.3[a]	22.0± 1.9
EA+ IRR	37± 2.9[a]	137.0± 10.0[a,b]	29.4± 1.6[a,b]	30.3± 2.0[b]
ZA	65± 2.3	60.0± 2.6	88.9± 2.2[a]	34.3± 2.9
ZA+ IRR	52± 3.3[b]	106.0± 7.0[a,b]	39.0± 1.1[a,b]	44.8± 4.0[a]
EA+ ZA	59± 4.5	41.0± 4.0	91.0± 3.2[a]	31.0± 3.0
EA+ ZA+ IRR	48± 3.0[b]	138.0± 5.3[a,b]	41.0± 3.4[a,b]	20.4± 1.5[b]

Legends are as in Table 1.

TABLE 3. Effect of ellagic acid and/ or zinc aspartate on some kidney oxidative stress biomarkers.

Groups	SOD (U/ ml)	LPO (nmol/ mg)	GSH (mg/ g tissue)	MTs (mg/ g tissue)
Control	48.6± 1.5	138± 6.0	42.0± 3.0	9.0± 0.5
IRR	33.4± 1.4[a]	289± 11.5[a]	13± 1.1[a]	17.0± 1.6[a]
EA	52.0± 1.9	152± 1.5	33± 2.9	8.2± 0.8
EA+ IRR	36.0± 1.9[a]	224± 14.0[a,b]	16± 1.6[a]	15.0± 1.3
ZA	55.7± 3.0	159± 3.6	93± 1.6[a]	12.5± 1.2
ZA+ IRR	54.8± 4.0[b]	216± 15.0[a,b]	32± 2.8[a,b]	14.0± 1.2[b]
EA+ ZA	60.0± 3.0	143.2± 5.6	89± 3.3[b]	7.4± 0.5
EA+ ZA+ IRR	53.7± 1.0[b]	233± 18.0[a,b]	19± 0.5[a]	9.1± 0.8[b]

Legends are as in Table 1.

Table 4. revealed that whole body γ-irradiation caused a significant decrease in the liver Zn, Mg & Mn levels and a significant increase in Fe & Ca levels compared with the control group. Supplementation of non-irradiated rats with EA induced significant elevation of liver Fe when compared with control group, while EA administration before and after exposure to γ-radiation could significantly elevate Zn and Mn levels, Whereas supplementation of rats with ZA pre and post exposure to γ-radiation significantly elevated Zn and Mg levels compared to irradiated group. While co-administration of EA and ZA to the irradiated rats showed significant difference in Zn, Fe and Mg compared to irradiated group.

TABLE 4. Effect of ellagic acid and/ or zinc aspartate on some liver trace elements content.

Groups	Zn (mg/g)	Fe (mg/g)	Ca (mg/g)	Mg (mg/g)	Mn (mg/g)
Control	32.6± 1.1	176.2± 6.0	92.4± 4.0	448.0± 12.0	1.4± 0.08
IRR	23.3± 0.2[a]	307.0± 16.0[a]	124.0± 5.4[a]	404.0± 4[a]	1.0± 0.06[a]
EA	28.6± 1.5	265.0± 7.2[a]	118.5± 9.7	453.0±6.8	1.8± 0.10[a]
EA+ IRR	32.3± 1.5[b]	311.0± 1.4[a]	131.0± 6.5[a]	417.0± 8.0	1.6± 0.10[b]
ZA	28.7± 1.8	283.6± 8.4[b]	125.0± 8.0	452.0± 14.0	1.5± 0.09
ZA+ IRR	28.3± 1.7[b]	267.0± 20.0[a]	126.0± 11[a]	439.0± 9.3[b]	1.2± 0.10
EA+ ZA	32.0± 2.8	267.0± 11.0[b]	118.4± 6.0	428.0± 5.3	1.4± 0.10
EA+ ZA+ IRR	31.0± 1.1[b]	254.0± 5.6[a,b]	120.6± 5.0	437.0± 11[b]	1.3± 0.10

Legends are as in Table 1.

Comparinged with the control group, Table 5. illustrated that whole body γ-irradiation caused significant increase in the kidney Ca & Mn concentrations, while EA and/or ZA showed non-significant effect in all estimated elements in the kidney tissues. Administration of EA and/or ZA pre and post irradiation caused a significant drop of kidney Ca while both treatments with irradiation dropped Ca & Mn concentration compared to irradiated group.

TABLE 5. Effect of EA and/ or ZA on some kidney trace elements content.

Groups	Zn (mg/ g)	Fe (mg/ g)	Ca (mg/ g)	Mg (mg/ g)	Mn (mg/ g)
Control	22.7± 0.6	292± 11	215.5± 14.5	372.7± 18.6	1.3± 0.08
IRR	21.0± 0.5	286± 22	293.4± 17[a]	421.0± 20.4	2.2± 0.20[a]
EA	20.0± 0.7	316± 28	211.0± 13.5	394.0± 30.4	1.5± 0.10
EA+ IRR	22.8± 0.6	271± 20	226.0± 8.0[b]	391.0± 26.3	2.0± 0.20[a]
ZA	21.5± 1.1	305± 26	202.0± 17.0	398.0± 17.8	1.8± 0.18
ZA+ IRR	21.6± 1.7	275± 20	224.4± 17[b]	332.0± 15.5	1.6± 0.10
EA+ ZA	20.7± 0.9	248± 14	223.0± 11.4	364.0± 15.0	1.8± 0.10
EA+ ZA+ IRR	21.0± 1.0	258± 10	237.0± 18.0[b]	368.0± 30.0	1.5± 0.09[b]

Legends are as in Table 1.

Discussion

In the current study-irradiation caused an elevation in the level of serum iron, TIBC and transferrin from the control group. Maiti *et al.* (2001) explained the elevation in transferrin after γ-irradiation by the incensement of its mRNA levels. However the elevated serum iron might be a compensatory response to provide iron to irradiation-damaged tissues or it might also be due to radiation

induced changes in erythrocyte membrane which might contributed to the eventual leakage of haemoglobin out of the cells and release of iron from ferritin (Nassar, 2011). Determination of TIBC of the plasma can give a direct measurement of transferrin (Tsung *et al.,* 1975), so the elevation of transferrin in response to gamma irradiation may reflect an increase in TIBC. In the present study administration of EA before and after γ-irradiation ameliorated serum iron. As it is a polyphenol compound (carrying four OH groups) it could scavenge iron raised by irradiation. EA at dose of 15 mg/kg returned serum iron and TIBC near to normal levels in myocardial infracted rats (Kannan *et al.,* 2012). Moreover, supplementation of irradiated rats with ZA ameliorated the changes in serum iron, TIBC and transferrin disturbed by γ-irradiation. Similar results was obtained by Kandaz *et al.* (2009) who found that the increase in iron level after γ-irradiation was reduced by zinc sulphate administration. Accordingly, it was suggested that an increase in zinc-iron ratio in some organs may confer protection from iron catalysed free radicals-induced damage (Sorenson, 1989).

In addition, γ-irradiation (6.5Gy) led to an increase in hepatic and renal LPO and MTs levels, in parallel with depletion in hepatic and renal SOD and GSH. The decreased antioxidant status in irradiated rats might be due to the increased utilization of these antioxidants to counteract the ROS generated by irradiation (Oidovsambuu *et al.,* 2013). Ping *et al.* (2012) showed that irradiation markedly decreased activities of SOD. GSH decrease upon radiation might be due to the oxidation of sulfhydryl group and diminished activity of glutathione reductase (Sarkar *et al.,* 1998) or due to the deficiency of NADPH which is necessary to change oxidized glutathione to its reduced form (Said *et al.,* 2005). MTs are small, cysteine-rich zinc binding proteins that are powerful antioxidants (Chung *et al.,* 2006). Induction of MTs by irradiation appears to be due to an increased synthesis of liver MTs (Goossens, 2011). On the other hand, LPO has been suggested as one of the molecular mechanisms involved in radiation-induced toxicity (Adaramoye *et al.,* 2008). Bhatia and Jain (2004), observed a significant depletion in the antioxidant system, and an increase in lipid peroxides after γ-radiation exposure. According to Mansour and El-Kabany (2009), the generation of lipid peroxide in the rat's liver and kidney might be due to the free radical attacked cell membrane phospholipids and circulating lipids.

The co-administration of EA and ZA pre and post irradiation in the present study modulated the hepatic and renal oxidative stress.Thresiamma *et al.*, (1998) stated that liver SOD increased upon EA supplementation compared with the irradiated ones. Also, EA treatment significantly induced total thiol and glutathione levels, and enhanced MT protein biosynthesis through selectively up-regulation of MT-mRNA expression (Gamal-Eldeen, 1997). EA being a phenolic compound carrying 4 OH groups might have exerted antioxidant-sparing action by scavenging free radicals (Michael, 2011). In addition, EA inhibits peroxidation of lipids thereby preserving the structural integrity of the cell membrane. Lee *et al.* (2010) indicated that EA inhibited NADPH oxidase-induced overproduction of superoxide, enhancing cellular antioxidant defences. On the other hand, ZA has superoxide-scavenging effect produced by NADH oxidation (Amara *et al.*, 2008). Moreover, the activity of Cu/Zn-SOD was increased with increasing organic Zn supplementation (Bun *et al.*, 2011). It has been shown that zinc ions can elevate the glutathione level in cultured cells (Seagrave *et al.*, 1983). The observed antioxidant effect of ZA may be related to its indirect anti-cytotoxic and anti-nitrosative effects and the ability of protecting thiol-dependent antioxidant proteins from oxidative damage (Turut *et al.*, 2009). Raymond *et al.* (2010) observed that, elevated intracellular zinc content caused MT gene expression which result in increased MTs production and increased monocytes resistance to apoptosis. Expression of MTs genes in association with changes in zinc metabolism may represent a common host response to inflamation which may be due to gamma irradiation. Shaheen and el-Fattah (1995) reported that, Zn deficiency caused increased LPO and that this was overcome by Zn supplementation as Zn promote cell integrity. Treatment with ZA (50mg/ kg) in ischemia reperfusion model had equal MDA levels when compared with sham group (Ozdemir and Inanc, 2005).

In the present work, concerning trace elements in liver tissues, whole body γ-irradiation (6.5Gy) caused a decrease in Zn, Mg & Mn concentrations and an increase in Fe & Ca levels. While in kidney tissues, there was a significant increase in Ca and Mn levels when compared with the control group. This finding was in agreement with Abdou *et al.* (2010) who proposed that, enhancement of calcium influx in the presence of oxygen was related to accumulation of ROS. The decreased Zn concentrations after irradiation

indicated enhanced consumption of this antioxidant element and/or antioxidant enzymes as a counterbalance to the effects of oxidants; this might be due to *de novo* synthesis of Cu/Zn-SODs and catalase which prevent the formation of O_2 and hydroxyl radical associated with irradiation (Fee and Valentine, 1977). According to Hampton and Mayerson (1950), the kidney is capable of forming ferritin from iron released from haemoglobin. While in liver the oxidative stress induced by irradiation causes damage resulting in ferritin degeneration and increase in the intracellular free iron levels (Atkinson *et al.,* 2005) and this explains why radiation increase the level of iron in liver but not in kidney. The disturbances in calcium and magnesium metabolism might be due to the insufficient renal function after irradiation (Kotb *et al.,* 1990).

Supplementation of EA with ZA to the irradiated rats showed a synergistic ameliorative effect in almost tested trace elements of liver and kidney tissues. Kandaz *et al.* (2009) stated that, radiation caused elevation of Fe &Ca level while upon treatment of irradiated rats with zinc sulphatethe elevated iron level start to decrease. On the other hand, Devipriya *et al.* (2007) reported that EA could normalize Zn levels during alcohol-induced toxicity in experimental rats. In addition, EA may have modulator effects on Cu and Zn levels in obstructive jaundice (Gumus *et al.,* 2011). EA by its effective antioxidant property might have decreased the utility of zinc thioniens and other Zn-containing enzymes like SOD and matrix metalloproteinases and thus maintained the levels of Zn as recorded in this study. EA plays an important role in the reduction of intracellular calcium (Gamal-Eldeen, 1997).In a study conducted to evaluates the potential of EA as an enhancer of radiation-induced apoptosis in cancer cells; they found that EA and radiation increased intracellular calcium levels of cancer cells (Bhosle *et al.,* 2010).

In conclusion, we have demonstrated that, EA and/ or ZA may protect against the damage produced by radiation by the up-regulation of the antioxidant status and by ameliorating the alterations in some biochemical parameters as well as some trace elements. Therefore, these results should contribute to future studies that will examine the ability of EA and ZA to limit radiation toxicity and free radical pathologies in such cases of radiotherapy and radiation workers.

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حمـــض الإلجيـــك و أســـبارتات الزنـــك يحســـنان التغيـــرات البيوكيميائية المحدثة بأشعة جاما في ذكور الجرذان

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قسم الكيمياء الحيوية ، كلية الصيدلة (بنات) ، جامعة الأزهر. *قسم البحوث الدوائية الاشعاعية ، المركز القومي لبحوث وتكنولوجيا الإشعاع ، ص. ب. ٢٩ مدينة نصر ، القاهرة ، مصر.

صممت هذه الدراسة لتقييم الدور الوقائي لحمض الالجيك (٥٠ مللي جرام/ كجم من وزن الجسم) ، و هو مركب بوليفينولي طبيعي و/ أو أسبارتات الزنك (٥٠ مللي جرام/ كجم من وزن الجسم) عن طريق الفم في التصدي للأضرار الخلوية الناجمة عن تعرض ذكور الجرذان البيضاء لإشعة جاما (٦.٥ جراى كجرعة واحدة للجسم كله). و كشفت نتائج الدراسة الحالية أن التعرض لإشعة جاما أحدث ارتفاع في نسبة الحديد ، السعة الكلية للحديد المرتبط ، الترانسفيرين ، ميتالوثيونين و مستوي الدهون الفوق مؤكسدة. و بالإضافة إلى ذلك ، كان هناك انخفاض إحصائى في الجلوتاثيون و السوبر اكسيد ديسميوتاز. أيضا أظهرت أنسجة بعض الأعضاء بعض التغيرات في تركيزات العناصر الشحيحة التي قد تكون بسبب قدرة الإشعاع على حث عمليات الأكسدة. بينما أظهرت النتائج أن الفئران المعالجة بحمض الالجيك و/ أو أسبارتات الزنك قبل و بعد التشعيع الجسم كله تحسن ذو دلالة إحصائية في الحديد ، السعة الكلية للحديد المرتبط و الترانسفيرين المقاسة في مصل الدم. و كذلك حالة مضادات الأكسدة ؛ كما أظهرت النتائج فعالية في تقليل الزيادة في مستوي الدهون الفوق مؤكسدة الناجمة عن الإشعاع و كذلك التغيرات في العناصر الشحيحة الأساسية في الكبد و الكلى. و من هذه النتائج تم استنتاج أن إعطاء حمض الإلجيك مع أسبارتات الزنك كان لهما دلالة إحصائية عالية لمعظم القياسات التي تم اختبارها مما عكس وجود تأثيرت ذرى.