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Proactive Role of Garlic and Lycopene Extract against Gamma Irradiation-Induced Alterations in Antioxidant Defense Systems in the Brain of Rats

Tamer M. M. Saad
Medical and Radiation Research Dept., Nuclear Materials Authority, Cairo, Egypt

Abstract

Introduction

Radiation protection concepts and philosophy have been evolving over the past several decades. The inadvertent exposure of human from various source of radiation causes Ionization of molecules, setting off potentially damaging reactions via free radicals production. Garlic, Allium sativun, is a member of the lily family that has been cultivated by humans as a food plant for over 10,000 years. Ancient Egyptian used garlic as a remedy for a variety of diseases. Lycopene is a naturally occurring carotenoid found almost exclusively in tomatos and tomato products and the red pigments of the tomato. Lycopene is one of the most potent antioxidants among dietary carotenoids, it exhibits the highest antioxidant activity and singlet oxygen quenching ability of all dietary carotenoids**.**

Aim

The present study aims to investigate the antioxidative activity of garlic and lycopene extract on the oxidative stress in the damaged brain tissue, irradiated with a single dose of 15 Gy.

Material and Methods

Animals were pretreated with garlic or lycopene by orally administration using suitable stomach tube for one month prior to radiation exposure. The levels of malondialdhyde (MDA), glutathione content (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were estimated in brain tissue.

Results

The results revealed that exposure to ionizing radiation resulted in significant elevation in the levels of MDA and (GSH-Px) as well as, significant reduction in SOD, CAT activities and GSH content.

Conclusion

Administration of garlic or lycopene by using suitable stomach tube pre-irradiation has significantly ameliorated the radiation induced disturbances in all of the investigated parameters.

Key words: ionizing radiation, garlic, lycopene and Antioxidants.

Introduction

Radiation protection concepts and philosophy have been evolving over the past several decades. The inadvertent exposure of human from various source of radiation causes ionization of molecules, setting off potentially damaging reactions via free radicals production. Free radicals are believed to play a role in more than sixty different health conditions, including the ageing process, cancer, radiation damage, atherosclerosis, etc **(Laverne, 2000)**. Fortunately, there are many plants derived natural antioxidants that interfere with free radicals before they can damage the body. Antioxidants work in several ways by reducing the energy of the free radicals, stop the free radical from forming in the first place, or interrupt an oxidizing chain reaction to minimize the damage of free radicals **(Krol *et al*., 2002)**.

The development of radioprotective agents has been the subject of intense research in view of their potential for use within a radiation environment; however,

no ideal, safe synthetic radioprotectors are available to date, so the search for alternative sources, including plants, has been on going for several decades **(Song *et al.*, 2003 and Arora *et al.*, 2005)**.

Radiation is known to produce various reactive oxygen species (ROS) in biological systems such as superoxide, hydrogen peroxide and hydroxyl radical reaction **Adler et al., 1999**. The range of antioxidant defense available within the cell and in the extracellular fluid should be adequate to protect oxidative damage.

Radiation therapy (RT) is considered to be one of the most popular and important tools to care cancer **(Brock and Geara, 1995)**. The radio-sensitivity of normal tissues particularly organs away from the tumor sites are suggested to limit the therapeutic gain **(Agrawal *et al.*, 2001)**.

Determintal effect of ionizing radiation occurs mainly due to free radicals generated through the decomposition of cellular water **(Winterbourn, 1993)**. However, organisms have protective systems against free radical reaction, for example, endogenous antioxidants and antioxidative systems.

It has been widely recognized that the brain tumors, such as gliolastome multiforme (GBM) is highly resistant to standard therapies such as chemotherapy and radiotherapy. However, total dose should not be increased in order not cause damage in the surrounding brain tissue in high dose. The effectiveness of RT frequently is limited by the tolerance of normal brain tissue. The pathogenesis of this damage is uncertain and understanding the response of potential target cell population may provide information useful for developing strategies to optimize therapeutic irradiation **(Danan *et al.*, 2007)**.

Recent improvements in RT delivery systems, coupled with a better understanding of the molecular biology of normal tissue injury have resulted in more aggressive attempts to ascalate radiation dose in an afford to improve the outcome for patients with GBM **(Denham and Hauer, 2002)**.

Garlic, Allium sativun, is a member of the lily family that has been cultivated by humans as a food plant for over 10,000 years (Fig. 1). Ancient Egyptain records mentioned that use of garlic as a remedy for a variety of diseases **(Ang-Lee *et al.*, *2001* and Borrelli *et al.*, 2007)**. Recently, it has been found that the sulfur-containing compounds of garlic have anti-mutagenesis and anti-carcinogenesis effects **(Milner, 2001)**. In vivo studies show that garlic and its associated sulfur components suppress the incidence of tumors in rodent models **(Fleischauer *et al*, 2000 and Morihara *et al.*, 2007 and Gullett *et al.*, 2010)**.

Lycopene is a naturally occurring carotenoid found almost exclusively in tomatos and tomato products and the red pigments of the tomato **(Tsen *et al.*, 2006** and **Gitenay *et al.*, 2007)** (Fig. 2).

It is also found in a small amount in few other foods such as guava, water lemon and grape fruits but tomatoes and tomato products are the major sources for such compounds **(Kirsh *et al.*, 2006** and **Srinivasan *et al*, 2007).**

Lycopene is one of the most potent antioxidants among dietary carotenoids, it exhibits the highest antioxidant activity and singlet oxygen quenching ability of all dietary carotenoids **(Bose and Agrawal, 2007** and **Wood *et al.*, 2008).** The aim of the present study is to evaluate the radioprotective role of
garlic and lycopene extracted as antioxidant against gamma irradiation that induce damage in brain tissue of rats.

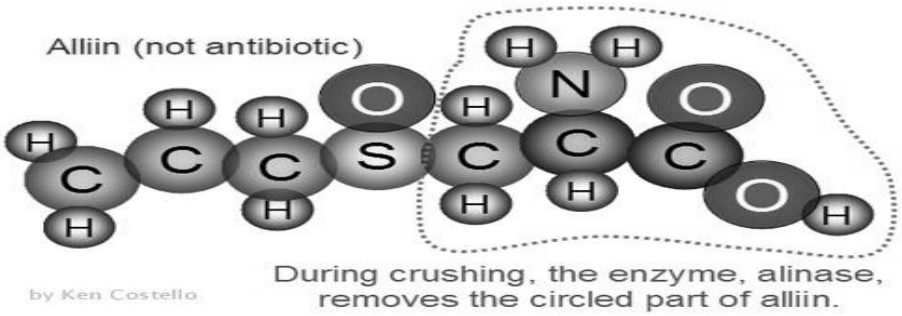

Fig.(1): Structure of garlic.

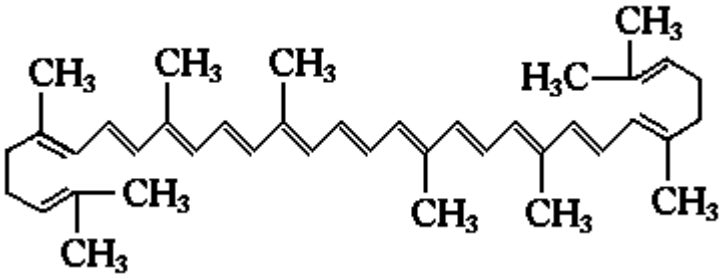

Fig.(2): Structure of lycopene.

Material and Methods

Preparation of garlic extract:

Garlic extract was prepared by homogenizing the required amount of dehusked loves of garlic in an appropriate volume of distilled water to prepare a concentration of 20 mg/ml **(Balasenthil *et al.*, 1999)**.The homogenate was centrifuged at 3000xg for 10 minutes to remove particulate matter and the supernatant fraction was used for the experiment. Garlic extract is composed of different beneficial molecules, especially antioxidants and disulfide. Source and preparation of tomato pomace: Tomato pomace including peels, pulp and seeds were obtained from tomato sauce processing line in the factory of Kaha. The aforementioned pomace was dried by the hybrid solar convective drying system belonging to the Solar Energy Dept.,National Research Centre, Dokki, Egypt at 30-40 °C. The dried tomato pomace then stored at room temperature 25 °C for further use. Extraction and purification of lycopene from tomato pomace: Dried tomato pomace was ground for lycopene extraction according to **Paiva and Russell (1999)** as follow: The tomato pomace powder was well mixed with diethyl ether (1:2 w/w) in a blender for three min. The mixture was then filtered using Bucher funnel. The residue was rinsed with diethyl ether for several times. These rinsing were added to the original extract. The extract was concentrated in a rotary evaporator under vacuum at 50 °C. Saponification was carried out to remove oils extracted with lycopene. The concentrated extract was washed twice with 200 ml portions of methanolic potassium hydroxide solution (100 g potassium hydroxide + 750 ml of methyl alcohol + 250 ml distilled water) in a separating dihydrogen potassium phosphate solution until the PH reached 7.5. The aqueous layer was discarded and the extract was dried over anhydrous sodium sulphate and then was concentrated in a rotary evaporator. After removal of diethylether, the d-limonine was removed from the remaining concentrated crude lycopene by steam distillation, which was carried out at 50 °C and under 10 mm Hg by passing atmospheric steam into the crude lycopene extract. After cooling to room temperature, the mixture was transferred to a separating

funnel and a double volume of fresh diethylether was added. The aqueous layer was discarded and the extraction was dried over anhydrous sodium sulphate. The extract was concentrated using of lycopene reached 95% as assessed by high performance liquid chromatography (HPLC).The extracted lycopene then stored at 4 °C for futher use. **Radiation source:** Irradiation was performed by gamma cell 40 source (Cesium-137) belonging to the National Centre for Radiation Research and Tochnology (NCRRT), Egypt. This Cesium source offers a dose rate 1.3 rad/sec at the time of experiment. **Experimental design and biochemical analysis:** Seventy-two male Swiss albino rats (120-140 g) were used. Animals were housed in stainless steal cages. They were kept under the same controlled laboratory conditions of temperature, lighting and ventilation. All rats were fed on standard casein diet and water *ad libitum*. Rats were categorized into 6 groups each of 12 rats as follows:

1-Control group: maintained on standard diet.

2-Irradiated groups: rats fed standard diet and exposed to single dose (15 Gy) of whole body gamma irradiation.

3-Garlic group: rats received (20 mg/kg/day) of garlic for one month by gavage according to **Samaranayake *et al.*, (2000).**

4-Lycopene group: rats received (1.2 mg/kg/day) of lycopene for one month by gavage according to **Norrish *et al.*, (2001).**

5-Irradiated garlic group: rats received (20 mg/kg/day) of garlic for one month before exposure to a single dose of whole body gamma irradiation (15 Gy).

6-Irradiated lycopene group: rats received (1.2 mg/kg/day) of lycopene for one month before exposure to a single dose of whole body gamma irradiation (15 Gy). At the end of the experimental periods, 15 days post irradiation, rats were anaesthetized. The tissues were briefly washed in ice-cold 0.9% saline (w/v) and frozen in liquid nitrogen. The tissues were stored at -70 °C until the subsequent protein and enzymes assays.

Biochemical assays:

Determination of the levels of malondialdehyde (MDA) were determind in tissue samples homogenized in the ratio of 1/10 (w/v) in 1.5% (w/v) cold Kcl solution, by thiobarbituric acid method **(Ohlawa *et al.*, 1979)** and the results were obtained in nmol/tissue weight. Determination of superoxide dimutase (SOD) activity tissue samples were homogenized in the ratio of 1/10 (w/v) in phosphate buffer (PH 7.4) and centrifuged at 5000 g for 30 min. The supernatant was carefully separated, the 3/5 (v/v) chloroform and ethanol were added. This mixture was centrifuged at 5000Xg for 2 h. The supernatant was used for the determination of SOD. This assay involves Xanthine oxidase used as superoxide generator **(Yi *et al.*, 1988)**. The protein concentration of the same supernatant was measured by the method of **Stadtman and Oliver (1991)** and the results were expressed as unit per mg protein that inhibit the rate nitroblue tetrzolium (NBT) reduction by 50%. Determination of reduced glutathione (GSH) content of tissue samples were determined by the method of **Owen and Butterfeild (2009)**. Tissue samples were homogenized in the metaphosphoric acid solution and colored by DTNB. The results were expressed as micromoles per mg protein. Determination of glutathione peroxidase (GSH-Px) activity**,** tissue samples were homogenized at the ratio of 1/10 (w/v) in phosphate buffer (PH 7.0) containing 0.5 mM EDTA and then centrifugated at 3500 rpm for 15 min. GSH-Px activity was measured by a modificatio n of the coupled assay procedure of **Koller *et al.* (1984)**. The results were expressed as nmoles oxidized NADPH per minute in per mg protein. Determination of Catalase (CAT) activity, tissue CAT activity was measured by the method of **Aebi (1984)**. Tissue samples were homogenized at the ratio of 1/10 (w/v) in phosphate buffer (PH 7.0) and then centrifugated at 3500 rpm for 15 min. H_2O_2 was added to the supernatant and the decrease in absorbance was measured.

Table (1): Lipid peroxides as malondialdehyde (MDA) level, glutathione (GSH) content, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase activities in rats after whole body gamma irradiation and/or (garlic and lycopene) administration .

	MDA (nmol/tissue)	**SOD** (u/mg protein)	**GSH-Px** (nmol oxidized NADPH/min/mg protein)	**CAT** (K/mg protein)	**GSH** (µmol/mg protein)
Control group	90.5±7.1 (100%)	31.4±1.9 (100%)	2.1±0.11 (100%)	0.004± 0.003 (100%)	0.29± 0.04 (100%)
Irradiated groups	*** 211±18 (233%)	*** 20.5±1.6 (65.2%)	*** 3.5±0.22 (166.6%)	*** 0.0027± 0.0003 (67.5%)	*** 0.14± 0.008 (48.2%)
Garlic group	91±6.8 (100.5%)	30.9±2 (98.4%)	2±0.19 (95.2%)	0.004± 0.0008 (100%)	0.3± 0.005 (103.4%)
Lycopene group	89.4±7.0 (98.3%)	32±2.2 (101.9%)	2.4±0.2 (114.2%)	0.0038± 0.0007 (95.0%)	0.31± 0.004 (106.8%)
Irradiated garlic group	** 132±10.3 (145.8%)	* 23.2±1.7 (73.8%)	** 3.1±0.21 (147.6%)	* 0.0031± 0.0008 (77.5%)	* 0.21± 0.0034 (72.4%)
Irradiated lycopene group	* 120±9.8 (132.5%)	25±1.5 (79.6%)	** 2.9±0.19 (138.0%)	0.0034± 0.0006 (85.0%)	0.24± 0.005 (82.7%)

Each value represents the mean of 6 rats ± SE.
Significant different from the corresponding control group at P<0.05*, P<0.01** and P<0.001***.

Results

The values of brain MAD, SOD, GSH-Px, CAT activities and GSH content in the six experimental groups are presented at tables (1).

Glutathione (GSH) content, superoxide dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase activities in rats after whole body gamma irradiation and/or MDA contents of brain after 15 Gy gamma irradiation were markedly elevated when compared with control group (P< 0.001). Also, brain GSH-Px activity showed significant increase (P<0.001) compared with control group after 15 Gy gamma irradiation. Meanwhile, brain SOD, CAT activity and GSH content showed significantly depression after radiation exposure by (P<0.001) compared with control group. Both groups that administrated by garlic or lycopene pre-whole body irradiation resulted in sufficient amelioration in all investigated parameters.

Discussion

The use of ionizing radiation to kill tumor or cells is a common treatment of cancer. The tolerance of normal brain tissue to irradiation is the primary factor limiting the dose of 50-60 Gy in 6 weeks prolongs the median life span of patients with malign gliomas **(Miura *et al.*, 1997** and **Graf *et al.*, 2005)**.

Meanwhile, the positive effects are generally for short duration because of the aggressive behavior of the more malignant gliomas, especially GBM and the intolerance of normal brain tissues to higher radiation doses.

One of the major reasons for cellular injury after radiation exposure is the generation of free radicals and the possible increased levels of lipid peroxides in tissue.

In the present study, no acute behavioral changes were observed in rats following irradiation. There were no deaths or obvious neurologic changes in animals that received 15 Gy following irradiation.

Chiang *et al.* (1993) reported that, no acute behavioral changes were observed in mice in the days following brain irradiation, although acute cellular changes were noted, this was in agreement with this findings.

Exposure to ionizing radiation causes radiolysis of water in tissues leading to generation of ROS which are known to affect the antioxidant defense systems and induce lipid peroxidation (LPO) **(Davydov *et al.*, 2000)**.

Our results revealed that, whole body gamma irradiation of male albino rats at 15 Gy produced a significant increase in the level of brain MDA, these results were in agreement with **(Sener *et al.*, 2003** and **Guney *et al.*, 2004)**. They reported that this elevation might be due to inhibition of antioxidant enzyme activities.

After applying 15 Gy gamma irradiation, the activities of SOD and CAT dropped significantly when compared with control group. In our observation, the significant decrease in both SOD and CAT activities after 15 Gy gamma irradiation leads to increase in the formation of $O_2^{\cdot -}$ and H_2O_2 . This decline may be due to inactivation of SOD by ROS **(Wiseman and Halliwell, 1996** and **Weiss *et al.*, 1996)**.

The activity of CAT significantly decrease after 15 Gy whole body gamma irradiation inagreement with previous observations **(De et al., 1995** and **Thresiamma *et al.*, 1996)**. RT, causes enzyme deficiencies which arise as a result of enormous production of free radicals in the system at high concentrations, hydrogen peroxide is converted to oxygen and water by CAT, which is predominantly localized in the peroxidases **(Robbins and Zhao, 2004)**.

Another antioxidant enzyme, GSH-Px, markedly increased after applying 15 Gy gamma irradiation when compared with control group. GSH-Px is a defense enzyme against hydrogen peroxides and another hydroperoxides. In the present study, it may reasonable to speculate that the observed increase in GSH-Px activity in brain after radiation exposure is one of the self-defense mechanisms against lipid peroxidation. These results are in agreement with **(Rekha *et al.*, 2001** and **Klotz *et al.*, 2003)**.

Whole body gamma irradiation (15 Gy) caused a decrease in the levels of GSH. Glutathione content represents a key cellular defense mechanism against oxidative injury and lowered concentrations of GSH resulting from increased formation of ROS. H_2O_2 which is produced during oxidative stress can cause extensive damage and GSH levels are greatly decreased **(Skaper et al., 1997 and Korotkina et al, 2002)**.

Ramadan *et al.* (2001) examined the levels of GSH in the liver and a significant decrease was detected. Therefore, the decline of GSH level in the brain after irradiation may be due to its consumption during the oxidative stress induced by irradiation. High dose gamma irradiation caused to decrease the GSH, which is known to be a free radical scavenging agent.

Groups that orally administrated by garlic (20 mg/kg/day) for one month prior radiation exposure ameliorated the alterations induced in the antioxidant defense systems. These are in agreement with **Ashraf *et al.* (2005)**. They reported that, garlic is rich in antioxidants, which help destroy free radicals particles that can damage cell membrane and DNA and may contribute to the aging process as well as the development of a number of conditions, including heart disease, cancer and ionizing radiation. **Morihara *et al.* (2007)** found that, the antioxidant property of garlic is to neutralize free radical and may reduce or even help prevent some of the damage they cause over time.

Groups that orally administrated by lycopene (1.2gm/kg/day) for one month before irradiation improved the changes induced in the investigated parameters. These results are in agreement with **Tsen *et al.* (2006)**. They reported that, lycopene is phytochemical**,** synthesized by plant and microorganism. It is a cyclic isomer of beta-carotene. This highly unsaturated hydrocarbon contain 11 conjugated and 2 unconjugated double bonds, making it longer than any other carotenoid. Also, **Jacob *et al.* (2008)** suggested that, antioxidant property of lycopene is due to

lycopene´s effective oxygen quenching capabilities and their ability to act as radical scavengers against oxygen species **(Lawenda *et al.*, 2008)**.

As a result, in the present model delivering single dose of ionizing iiradiation (15 Gy) caused the accumulation of ROS and the antioxidants defense systems were not enough to eliminate ROS accumulation.

It could be concluded that, the administration of garlic and lycopene pre-whole body gamma irradiation resulted in sufficient amelioration against radiation effects on the biochemical aspects examined in the present study.

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الدور الوقائى لمستخلص الثوم و الليكوبين ضد التغيرات في أنظمة الدفاع المضادة للأكسدة نتيجة التعرض للإشعاع الجامى فى مخ الجرذان

تامر محمد محمود سعد
قسم البحوث الطبية و الإشعاعية - هيئة المواد النووية ، القاهرة، مصر

الخلاصة

هذة الدراسة تهدف الى دراسة النشاط المضاد للأكسدة لمستخلص الثوم و الليكوبين على الضغط التأكسدى فى الأنسجة التالفة للمخ فى الجرذان المشعة بجرعة فردية مقدارها 15 جراى. تم معالجة الجرذان قبل التشعيع بخلاصة الثوم و الليكوبين عن طريق الفم بإستخدام أنبوبة معديه لمدة شهر.

تم تقدير مستويات أكسدة الدهون (ثيوباربيتيوريك أسيد) و محتوى الجلوتاثيون ونشاط كل من السوبر اكسيد ديسميوتيز و الكتاليز و الجلوتاثيون بير اكسيديز فى أنسجة المخ .

أوضحت النتائج أن التعرض للأشعة المؤينة يسبب إرتفاع معنوى فى مستويات أكسدة الدهون و نشاط الجلوتاثيون بير اكسيديز ، كما يسبب إنخفاض معنوى فى نشاط السوبر اكسيد ديسميوتيز و الكتاليز و محتوى الجلوتاثيون.

النتائج

نستنتج أن تعاطى مستخلص الثوم و الليكوبين بأستخدام أنبوبة معديه قبل التشعيع قد خفف من الإضطرابات المسببة بواسطة التشعيع فى جميع المعايير المقاسة.