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Use of Onion and Curcumin as Radioprotectors against Ionizing Radiation Induced Hepato-Testicular Alterations in Rats

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ABSTRACT

Background: 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. Onion, Allium cepa linn, is a major source of dietary flavonoids and has used since ancient times as a food plants. Curcumin is a yellow pigment from curcuma longa, is a major component of turmeric and has commonly used as a spice and food coloring materials**. Aim:** the aim of the present study is to evaluate the radioprotective role of both onion and curcumin extracted as antioxidant against gamma irradiation that induced some biochemical alterations in rats.

Materials and Methods: animals were pretreated with onion or curcumin by orally administration using suitable stomach tube for two weeks prior to radiation exposure. The levels of malondialdhyde (MDA), glutathione content (GSH), superoxide dismutase (SOD), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and testosterone were estimated in both serum and tissues. **Results:** the results revealed that exposure to ionizing radiation resulted in significant elevation in the levels of MDA, GOT and GPT activities, meanwhile, showed significant depletion in GSH content and SOD activity and testosterone concentration. **Conclusion:** administration of onion or curcumin by using suitable stomach tube pre-irradiation has significantly ameliorated the radiation induced disturbances in all of the investigated parameters.

Keywords: *Ionizing Radiation, Onion, Curcumin and Antioxidants.*

INTRODUCTION

Ionizing radiation has a diversity of beneficial uses in medicine including radiotherapy as an important treatment modality for a wide variety of tumors, radiographs for screening, diagnosis and staging of diseases and malignancies, but its acute side effects on the normal tissues limit the effectiveness of therapy[1].

Scientific and technological advancements have further increased the radiation burden in humans, because exposure to low levels of radiation has become common during space or air travel, cosmic radiation and using certain electronic gadgets. Other sources of radiation include radon in houses, contamination from weapons testing sites and nuclear accidents[2].

It is well known that ionizing radiation induce oxidative stress on target tissues, mainly through the generation of reactive oxygen species (ROS) resulting in imbalance of the pro-oxidant and antioxidant in the cells, attack diverse cellular macromolecules such as DNA, lipids and proteins, eventually inducing cell death[3].The range of antioxidant defense available within the cell and in the extracellular fluid should be adequate to protect oxidative damage. Free radicals are believed to play a role in more than sixty different health conditions, including the ageing process, cancer, radiation damage, atherosclerosis[4].

Owing to the harmful effects of ionizing radiation, radiobiologists have long been paying attention in identifying novel, nontoxic, effective and convenient compound to protect humans against radiation-induced normal tissue injuries.

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 ongoing for several decades[5].

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[6].

The fresh flavors of onions and other members of the *Allium* genus are produced by enzymatic decomposition of S-alkyl- and S-alkenyl-L-cysteine S-oxides from which the primary products are thiolsulfinates containing alkyl and alkenyl substituents (alkyl- and alkenyl-disulfide S-oxides). The flavor precursors of the onion are (+)S-methyl-, (+)S-propyl-, and (+)S-(l-propenyl)-L-cysteine S-oxide, with the last amino acid predominant. Enzymatic decomposition of the propenyl derivative yields the characteristic transitory

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lachrymatory substance. The lachrymator has the unusual structure propanethial S-oxide. On standing, or more rapidly on heating, the thiolsulfinates decompose to yield a mixture of disulfides and trisulfides containing methyl, propyl, and 1-propenyl groups, and smaller quantities of thiophene derivatives and other cyclic sulfur-containing compounds[7].

Onion, Allium cepa linn, is a major source of dietary flavonoids and have used since ancient times. Onion is one of the best agents of preventive medicine and the least expensive of all medication. It contains a powerful active antiseptic element it is recommended in case of rheumatism, hydropsy and disorders of the liver, kidney and heart for intestinal yeast infections, diabetes, bronchial catarrh and tuberculosis[8].

Curcumin, a yellow pigment from *Curcuma longa*, is a major component of turmeric and is commonly used as a spice and food-coloring material. It exhibits anti-inflammatory[9], antitumor, and antioxidant properties[10].

It is known that curcumin prevents the formation of ROS and scavenges free radicals and it protects cells from peroxidative stress. Curcumin not only exhibits antioxidative and free radical scavenging properties, but also enhances the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px)[11].

The aim of the present study is to evaluate the radioprotective role of both onion and curcumin extracted as antioxidant against gamma irradiation that induced some biochemical alterations both in serum and tissues of rats.

MATERIALS AND METHODS

Chemicals:

All chemicals were obtained from Sigma Chemicsl (USA). Kits used in this experiments were purchased from Bio-Diagnostics (UK).

Radiation sources:

Single dose whole body irradiation (10 Gy) was per-formed with rats at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt using the gamma Cell-40 biological irradiator furnished with a Caesium-137 source with the dose rate of 0.912 rad/sec. The radiation process of animals has been carried out at the with a polyethylene cover.

Experimental design:

Seventy-two male Swiss albino rats (120-140 g) were used. Animals were housed in stainless steel 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 (10 Gy) of whole body gamma irradiation.
3- **Onion group:** rats received (200 mg/5ml/kg body weight) of onion for two weeks by gastric gavages, five times weekly[7].
4- **Curcumin group:** rats received (150mg/5ml/kg body weight) of curcumin dissolved in corn oil for two weeks by gastric gavages, five times weekly[12].
5- **Irradiated onion group:** rats received (200 mg/kg/day) of onion for two weeks before exposure to a single dose of whole body gamma irradiation (10 Gy).
6- **Irradiated curcumin group:** rats received (150 mg/5 ml/kg body weight) of curcumin for two weeks before exposure to a single dose of whole body gamma irradiation (10 Gy).

Biochemical analysis:

After the experimentation period, rats were then decapitated, blood samples were collected from heart by using disposable syringes and transferred to dry sterile test tube, thiobarbituric acid (MDA)[13], reduced glutathione content (GSH)[14], super oxide dismutase (SOD)[15], Glutamic oxalacetic transaminase (GOT)[16], Glutamic pyrovic transaminase (GPT)[17] activities and testosterone concentration[18] were assayed.

Liver and Testis homogenate were obtained using a tissue homogenizer. The homogenates (1:10 w/v) were prepared using a 100 mM KCl buffer (7:00 pH) containing EDTA 0.3 mM. All homogenates were centrifuged at 1500 rpm for 30 min at 4ºC and the supernatants were used for assays of MDA level[19], GSH content[20] and SOD activity[21].

Statistical analysis:

Data are expressed as Mean SE. Data were assessed by paired t-test[22].

RESULTS

Table (1) presented MDA level, GSH content and SOD activity of rats exposed to single dose of radiation (10 Gy) with and without oral administration of oinoin or curcumin. Irradiated group recorded very high significant elevation ($P<0.001$) in MDA level, as compared with the corresponding non-irradiated group. In addition, it is evident from

table (1) that irradiation caused very high significant depletion (P<0.001) in both GSH content and SOD activity. Groups treated with onion pre-irradiation turned the value of MDA level, GSH content and SOD activity to its normal value, meanwhile, groups treated with curcumin pre-irradiation caused some amelioration in tested parameters, where the results revealed significant increase (P<0.05) in MDA level and significant decrease (P<0.05) in GSH content.

The present study in table (2) represent, AST, ALT levels and testosterone concentration of rats exposed to single dose of radiation (10 Gy) with and without oral administration of onion or curcumin. Irradiated group recorded very high significant elevation (P<0.001) in GOT and GPT levels, as compared with the corresponding non-irradiated group. In addition, it is evident from table (2) that irradiation caused very high significant depletion (P<0.001) in testosterone concentration.

Groups treated with onion pre-irradiation turned the levels of AST, ALT and testosterone concentration to its normal value, meanwhile, groups treated with curcumin pre-irradiation caused some amelioration in tested parameters, where the results revealed significant increase (P<0.05) in AST and ALT levels and significant decrease (P<0.05) in testosterone concentration.

Irradiated group recorded very high significant elevation (P<0.001) in MDA level both in liver and testis, as compared with the corresponding non-irradiated group. In addition, it is evident from table (3) that irradiation caused very high significant depletion (P<0.001) in GSH and SOD both in liver and testis. Groups treated with onion pre-irradiation turned the level of MDA, GSH content and SOD activity both in liver and testis to its normal value, meanwhile, groups treated with curcumin pre-irradiation caused some amelioration in tested parameters, where the results revealed significant increase (P0.05) in MDA level both in liver and testis and significant decrease (P<0.05) in GSH content and SOD activity both in liver and testis (table 3).

DISCUSSION

A major interest in radiation biology and chemistry is identification of chemical agents that are able to protect humans from ionizing radiation. Hence, the study and use of plants and natural products that may be beneficial in protection against these radiation induced damage are of significant; they are less toxic or in most cases, practically nontoxic compared to synthetic compounds.

Changes in LPO, GSH and SOD have often been used as an index of oxidative stress. These parameters were studied in view of the free radical generating capacity of radiations. The results obtained indicate that the content of GSH and the activity of SOD of rats treated with gamma radiation was significantly (P<0.001) decreased relative to control. This decrease may be due to the effect of gamma radiation.

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)[23].

Our results revealed that, whole body gamma irradiation of male albino rats at 10 Gy produced a significant increase in the level of MDA both in serum and tissue, these results were in agreement with **Sener**[24]. They reported that this elevation might be due to inhibition of antioxidant enzyme activities.

Whole body gamma irradiation (10 Gy) caused a decrease in the levels of GSH both in serum and tissue. 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[25].

After applying 10 Gy gamma irradiation, the activity of SOD dropped significantly when compared with control group. In our observation, the significant decrease in SOD activity after 10 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[26].

Present study investigated the beneficial radioprotective effect of both onion and curcumin, It is known that both of them prevent the formation of ROS and scavenges free radicals and protect cells from peroxidative stress. They not only exhibit antioxidative and free radical scavenging properties, but also enhance the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px)[27]. Serum transaminase activity is the most widely used parameter as a measure of hepatic injury, due to its ease of measurement and high degree of

sensitivity. It is useful for the detection of early damage of hepatic tissue and requires less effect than that for a histological analysis[28].

Whole body gamma irradiation (10 Gy) caused significant increase in the transaminase activities compared to control group. The changes in the enzymatic activity after irradiation are related to either the release of enzymes from radiosensitive tissues or to the extensive breakdown of liver parenchyma. Furthermore, the change in tissues permeability due to irradiation could enhances the release of transaminase enzymes from their subcellular sites of production to extracellular process and consequently to blood circulation[29].

The amelioration resulted from both onion and curcumin pre-irradiation may be due to their beneficial effects on membrane permeability leading to the maintenance of a higher level in serum. These may be due to that both of them have potential antioxidant properties, these results were agreement with **Khaki**[27] ,that reported that they exert their antioxidant activity via scavenging some radical species by acting as chain-breaking antioxidant.

Ionized radiation, being one of the environmental cytotoxic factors, causes death of the germinal cells and therefore, sterility[30].

In this study, a marked significant decrease of testosterone concentration both in serum and tissue was observed in irradiated rats.

The testis of seminiferous tubules, which form the sperm and the interstitial laying cells, which secrete testosterone. The function of the testis is controlled by the hypothalamic pituitary mechanism. In the present study, the disturbed testosterone level might be attributed to hypothalamic and pituitary gland dysfunction, which interferes with hormone production. The decrease of testosterone might also be attributed to the production of free radicals and increase of LPO in testis tissue which attack the testicular parenchyma causing damage to the seminiferous tubules and laying cell[31].

Groups that orally administrated by onion (200 mg/5ml/kg/day) for two weeks prior radiation exposure ameliorated the alterations induced in the antioxidant defense systems. These are in agreement with **Norrish**[32]. They reported that, onion 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 and cancer. They found that, the antioxidant property of onion is to neutralize free radical and may reduce or even help to prevent some of the damage they cause over time.

Curcumin is known to protect biomembranes against peroxidative damage. Peroxidation of lipids is known to be a free-radical-mediated chain reaction, leading to the damage of the cell membranes and the inhibition of peroxidation by curcumin is mainly attributed to the scavenging of the reactive free radicals involved in the peroxidation. Most of the antioxidants have either a phenolic functional group or a diketone group. Curcumin is an unique antioxidant, which contains a variety of functional groups, including the B-diketo group, carbon–carbon double bonds, and phenyl rings containing varying amounts of hydroxyl and methoxy substituents[33].

The central argument is whether the phenolic or the central methylenic hydrogen in the heptadienone moiety is responsible for its antioxidant activity. **Jovanovic**[34] concluded that curcumin is a super H-atom donor by donating the H-atom from the central methylenic group rather than from the phenolic group in acidic and neutral aqueous and acetonitrile solutions. On the other hand, **Eybl**[35] proposed that curcumin is a classical phenolic chain-breaking antioxidant, donating H-atoms from the phenolic group. **Priyadarisini**[36] have also claimed that the phenolic group is essential for the free-radical-scavenging activity and that the presence of the methoxy group increased the activity.

In conclusion, the present results showed that, treating irradiated rats with onion or curcumin significantly protected the antioxidants defense system as compared to the controls. These observations were confirmed by insignificant alterations in the levels of LPO, GSH, SOD activity, and testosterone concentrations.

This study therefore suggests that onion and curcumin may be useful preventive agents against the effect of the studied radiation exposure at least partly due to their antioxidant properties.

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Table (1): effect of radiation on serum MDA level, GSH content and SOD activity and/or administration of (onion or curcumin) in rats.

Parameters / Groups	MDA (μmol/l)	GSH (mg/ml)	SOD (μg/ml)
Control	86.6 ± 4.7	45.5 ± 3.1	61.0 ± 3.6
Onion group	90.0 ± 6.0 (+3.3%)	46.0 ± 3.5 (+1.0%)	62.2 ± 4.3 (+1.9%)
Curcumin group	87.0 ± 6.0 (+0.4%)	46.0±3.7 (+1.0%)	62.0 ±4.6 (+1.6%)
Irradiated groups	141.0 ± 11.2 *** (+62.8%)	28.2 ± 1.7*** (-38.0%)	30.0 ± 1.9*** (-50.8%)
onion Irradiated group	102.6 ± 8.8 (+18.4%)	37.0 ± 2.0 (-18.6 %)	55.0 ±4.2 (-9.8%)
curcumin Irradiated group	108.0±9.3* (+24.7%)	33.0 ± 1.9* (-27.4%)	50.0 ±4.0 (-18.0%)

- Each value represents the mean of 6 rats ± *SE*.
- Significant different from the corresponding control at *P<0.01**, *P<0.01*** and *P<0.01****.
- % change from control.

Table (2): Effect of radiation on serum AST, ALT and Testosterone and/or administration of (onion or curcumin) in rats.

Parameters / Groups	AST (U/L)	ALT (U/L)	Testosterone (ng/ml)
Control group	56.2±3.9	41.0±3.3	1.4±0.1
Onion group	54.4±4.0 (-3.2%)	41.2±2.9 (+0.48%)	1.55±0.11 (10.7%)
Curcumin group	55.0±4.2 (-2.1%)	40.3±3.2 (-1.7%)	1.47±0.1 (+5.0%)
Irradiated group	90.3±6.4*** (+60.6%)	69.2±5.8*** (+68.7%)	0.7±0.05*** (-50.0%)
onion Irradiated group	66.3±5.3 (+17.9%)	50.2±4.2 (+22.4%)	1.1±0.08 (-21.5%)
curcumin Irradiated group	69.2±4.9 (+23.1%)	51.0±4.0* (+24.3%)	1.0±0.09* (-28.5%)

Table (3): Effect of radiation on MDA level, GSH content and SOD activity both in liver and testis tissues and/or administration of (onion or curcumin) in rats.

Parameters / Groups	Liver MDA (nmol/g. tissue)	Testis MDA (nmol/tissue)	Liver GSH (mg/g. tissue)	Testis GSH (μmol/mg protein)	Liver SOD (u/mg protein)	Testis SOD (u/mg protein
Control group	191.6±16.8	90.5±7.1	33.2±2.8	9.7±0.88	11.5±0.9	142.3±12
Onion group	190.0± (-0.8%)	91±6.8 (+0.5%)	31.2±2.0 (-6.0%)	10.0±0.9 (+3.4%)	11.7±1.0 (+1.7%)	149.0±2.0 (+4.7%)
Curcumin group	189.2± (-1.2%)	89.4±7.0 (-1.2%)	32±2.4 (-3.6%)	9.9±0.84 (+2.0%)	11.6±0.9 (+0.8%)	146.0±12.3 (+2.8%)
Irradiated group	322±22*** (+68%)	211±18*** (+133%)	18.6±1.5*** (-43.9%)	6.0±0.52*** (-38.0%)	6.7±0.49*** (-41.7%)	100.9±8.4* (-29.0%)
onion Irradiated group	241±20* (+25.7%)	132±10.3** (+45.8%)	27.1±2.0 (-18.3%)	8.28±0.75 (-14.6%)	10.2±0.98 (-11.3%)	126.0±10.9 (-11.4%)
curcumin Irradiated group	250±21.3** (+30.4%)	120±9.8* (+32.5%)	26.3±1.7 (-20.7%)	8.00± 0.71 (-17.5%)	9.8±0.75 (-14.7%)	120.0±11.2 (-15.6%)