Third International Conference on
Radiation Sciences and Applications
12 – 16 November 2012/ Hurghada, Egypt

Amelioration of radiation induced oxidative stress using water soluble chitosan produced by *Aspergillus niger*

EL-Sonbaty S.M.*, Swailam ,H. M *. and Noaman, E.**

**Radiation Microbiology Dept. and **Radiation Biology Dept.*
National Centre for Radiation Research and Technology (NCRRT)

ABSTRACT

Chitosan is a natural polysaccharide synthesized by a great number of living organisms and considered as a source of potential bioactive material and has many biological applications which are greatly affected by its solubility in neutral pH. In this study low molecular weight water soluble chitosan was prepared by chemical degradation of chitosan produced by *Aspergillus niger* using H_2O_2. Chitosan chemical structure was detected before and after treatment using FTIR spectrum, and its molecular weight was determined by its viscosity using viscometer.

Its antioxidant activity against gamma radiation was evaluated *in vivo* using rats. Rats were divided into 4 groups; group 1: control, group 2: exposed to acute dose of gamma radiation (6 Gy), group 3: received water soluble chitosan, group 4: received water soluble chitosan then exposed to gamma radiation as group 2. Gamma radiation significantly increased malondialdehyde, decreased glutathione concentration, activity of superoxide dismutase, catalase, and glutatione peroxidase, while significantly increase the activity of alanine tranferase, aspartate transeferase, urea and creatinine concentration. Administration of water soluble chitosan has ameliorated induced changes caused by gamma radiation.

It could be concluded that water soluble chitosan by scavenging free radicals directly or indirectly may act as a potent radioprotector against ionizing irradiation.

Key words: Water soluble chitosan, Gamma radiation, Antioxidants, Radioprotector.

INTRODUCTION

Radiation injury to living cells is, to large extent, due to oxidative stress **(Wallace, 1998)**. Reactive oxygen species (ROS) and free radicals induced by partial reduction of oxygen (O_2) react with cellular macromolecules (i.e., nucleic

acids, lipids, proteins, and carbohydrates) **(De-Zwart et al., 1999)** and damage them. The interaction of ionizing radiation with living cells induces a variety of reaction products and a complex chain reaction in which many macromolecules and their degradation products participate. The assumption that destructive processes initiated by ionizing radiation begin exclusively in a single subcellular organelle is questionable. Major biomarkers of oxidative damage to living cells are (i) lipid peroxidation (LPO) products, (ii) DNA-hydroxylation and (iii) protein hydroxylation products **(De-Zwart et al., 1999)**.

The efficient defense and repair mechanisms are exist in living cells to protect against oxidant species (**Deger et al., 2003).** The antioxidative defense system is composed of different methods including active antioxidant enzymes and a variety of direct free radical scavengers **(Riley, 1994).** The enzymes involved in antioxidative defense, particularly well documented are the antioxidative properties of the superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) **(De-Zwart et al., 1999)**.

Since the amount of DNA damage caused by ionizing radiation is correlated with the intensity of oxidative stress (**Sagrista et al., 2002**), with the efficacy of defense mechanisms that metabolize toxic intermediates **(Jagetia and Reddy, 2005)** and with intrinsic repair mechanisms **(Badaloo et al., 2002)**, there are several potential means to reduce macromolecular damage due to ionizing radiation. Since radiation-induced cellular DNA damage is primarily attributed to the damaging effects of free radicals, molecules with direct free radical scavenging properties are particularly promising as radioprotectors. The involvement of free radical scavengers in protecting against radiation damage is emphasized by the observation that whole body irradiation decreases the total antioxidant capacity of the organism and depletes the levels of known antioxidants such as glutathione and nitric oxide as they are used in the scavenging of free radicals **(Yang and Kim, 1999)**.

A number of different substances have been examined for their radioprotective effects against cellular damage caused by ionizing radiation. Several antioxidants have been proven to be efficient protectors against ionizing radiation **(Prasad et al., 2004).**

The development of radioprotective agents with lower toxicity and an extended window of protection have attracted much attention. Natural compounds have been evaluated as radioprotectants and they seem to exert their effect through antioxidant and immunostimulant activities **(Hosseinimehr, 2007**).

Chitosan poly ((1,4)-2-amino-2-deoxy-β-D-glucose) is a natural polysaccharide synthesized by a great number of living organisms and functions as a structural polysaccharide. It has attracted much attention as a biomedical material, owing to its unique biological activities such as antitumor, antiulcer, immunostimulatory and antibacterial also for its diverse application in food, pharmaceutical and cosmetics **(Kumar, 2000).**

Solubility is a crucial characteristic for chitosan which by improvement can facilitate its application in medicine and food. High molecular weight is responsible for the low solubility of chitosan in water, therefore, it is important to improve its water solubility by decreasing the molecular weight **(Feng et al., 2008).**

It has been reported that chitosan can be degraded by acidic hydrolysis or enzymatic treatment. Decreasing its molecular weight can improve its water solubility and some special biological functions. Recently, the effect of molecular weights of chitosan or their bioactivities was studied **(Tao et al., 2004)**.

Chitosan antioxidant activities were primarily obtained on *in vitro* models, which depends on its molecular weight and deacetylation degree.

MATERIALS AND METHODS

Microorganism:

The fungus strain used in this study was *Aspergillus niger*. In order to cultivate *A. niger*, strain potato dextrose agar (PDA) slants were prepared according to the manufacturer's instructions.

Inoculum preparation:

The spore of *A. niger* was inoculated on PDA slants and incubated at 30 º C. After 3 days, the fungus grown on the PDA slants was stored at 4 ºC in a refrigerator. The sterile solution (9 gm/L NaCl) was poured into a tube and mixed well with the spore then, spores were counted and the number was adjusted to 3x 10^6 spores/ml **(Maghsoodi et al., 2009).**

Submerged fermentation:

Sabouro dextrose broth (2% glucose) medium was used for submerged fermentation. The glucose content in the SDB was changed up to 12%. The spores ($3x10^6$) were inoculated into 250 ml sterilized flasks containing 50 ml of sabouro dextrose broth. The culture flasks were incubated at 30 ºC for 6 days at

150 rpm. **(Maghsoodi et al., 2009).**

Chitosan extraction:

The fungal mycelia were harvested and 50 ml of 1 N NaOH solution were added per g (wet weight) of mycelia and homogenized. The content was sterilized at 121ºC for 20 minutes (alkali treatment) and the alkali insoluble materials (AIM) were collected by centrifugation at 6000 rpm for 20 min, then washed several times with distilled water to neutralize them (pH 7). AIMs were dried in an oven at 40 ºC, then treated with acetic acid 2% (v/v), as a chitosan solvent, for 6 hours at 95ºC (1:30 w/v). The acidic insoluble fraction was separated by centrifugation at 6000 rpm for 15-20 min and the supernatant containing the chitosan was isolated. The pH was adjusted with a 2N NaOH solution in order to precipitate the fungal chitosan. Precipitated chitosan was centrifuged at 6000 rpm for 15 min, then isolated chitosan was washed with distilled water to neutralize it. At the same time, ethanol (96%) and acetone were employed to rinse the chitosan and then it was dried in a vacuum oven dryer at 60 ºC. Chitosan obtained was about 0.7 g/L **(Maghsoodi et al., 2009).**

Preparation of water soluble chitosan:

Water soluble chitosan was prepared by the degradation of high molecular weight chitosan according to **Sun et al., (2007)**. Chitosan solution was prepared by dissolving 1g chitosan in 50ml 2% acetic acid solution. In a water bath at 80 °C, 30 % H_2O_2 solution was added into chitosan solution and kept for 1-8 h, then the solution was adjusted to pH 10 then filtrated to get water insoluble parts. The filtrate was precipitated by adding three amounts of ethanol (known volume) then filtrated. The filtrate was dried to get the water soluble chitosan.

Characterization:

The IR spectrum of chitosan was carried out using Fourier transform infrared (FTIR) spectrometer(ATI Mattson, Genesis FTIR^{TM}) using the KBr disc method to compare and characterize the structural changes of chitosan before and after the treatment.

Molecular weight determination:

The molecular weight was determined by a literature viscometric method **(Chen and Hwa, 1996).** Chitosan samples were prepared in 0.2 M acetic acid/0.1 M sodium acetate aqueous solutions. The relative viscosity [η] of chitosan sample was measured using a Brook hield, RVDV III, USA, at

30±0.5ºC. Specific viscosity was determined by:

$$\eta_{sp} = (\eta_{solution} - \eta_{solvent}) / \eta_{solvent}$$

Intrinsic viscosity [η] is defined as reduced viscosity (η_{red}) extrapolated to a chitosan concentration (C) of zero before treatment:

$$[\eta] = (\eta_{sp}/C)_{C0} = (\eta_{red})_{C0}$$

where *C* is chitosan sample concentration in g/ml. Viscosity average molecular weight was calculated according to the Mark-Houwink equation:

$$[\eta] = KM^{a}_{v}$$

with

$$K = 1.64 \times 10^{-30} \times DD^{14}$$

$$a = -1.02 \times 10^{-2} \times DD + 1.82$$

were DD is the degree of deactylation of chitosan expressed as the percentage and M is the molecular weight **(Wang et al., 1991).**

Soluble chitosan was administered at dose of 100 mg/kg body weight according to **Koryagin et al., (2006).**

Experimental animals:

Mature Sprague Dawley male albino rats weighing 150±20 g were obtained from the Egyptian Organization for Biological Product and Vaccines at Giza, Egypt and were used throughout the present study. Rats were housed in cages in a climate controlled room of humidity (55%) and temperature (25°C) with diurnal environment of light and dark (12 hr light/ dark cycle). Rats were freely fed on standard pellets of rat's diet. They were allowed to acclimatize to the environmental conditions for one week before starting the experiment. All rats were cared according to The National Institute of Health (publication number 85-23, USA).

Gamma irradiation procedure:

Irradiation of rats was carried out at the National Centre for Radiation Research and Technology, Cairo, Egypt, with a Gamma cell–40 (cesium–137 irradiation unit, Canada). The irradiation dose rate was 0.61Gy/min. Whole body irradiation was carried out with dose of 6 Gy.

Experimental design:

The male rats were divided into four groups (n=8), namely (1) control:

healthy untreated rats, (2) IR: rats were exposed to 6 Gy of γ-radiation, (3) WSC: rats injected *ip* with water soluble chitosan (100 mg/kg) day after day for 4 weeks and (4) WSC+IR: rats treated with WSC (100 mg/Kg) day after day for 4 weeks then exposed to single dose of 6 Gy of γ-radiation .

Rats of all groups, on the day before sacrificed, were overnight fasted. Blood samples were collected by heart puncture then plasma of each blood sample was separated and kept frozen for biochemical assays.

Antioxidant parameters and lipid peroxidation content:

Lipid peroxide concentrations were determined by measuring the malonaldialdahyde (MDA) end product content in plasma according to the method of **Yoshioka et al., (1979).** Reduced glutathione (GSH) estimated in the whole blood as yellow color which developed when 5, 5 dithiol-bis (2-nitrobenzoic acid) was added to sulfhydryl compounds according to the method described by **Beutler et al., (1963)**.Superoxide dismutase (SOD) activity levels in the whole blood were estimated by the detection of superoxide anions using nitroblue tetrazoluim formazan color development as reported by **Minami and Yoshikawa, (1979)** and catalase (CAT) activity was determined according to **Sinha, (1972).** Glutathione peroxidase (GPx) level was assayed as described by **Paglia, and Valentine , (1967)**. AST and ALT levels in plasma were determined by a colorimetric method as described by **Reitman and Frankel, (1957)** using a diagnostic kits supplied by Diamond (Egypt). Urea in plasma was **Palton and Crouch, (1977)** using a diagnostic kits supplied by Diamond (Egypt). Creatinine in plasma was determined by a colorimetric method as described by **Henry, (1974)** using a diagnostic kit supplied by Diamond (Egypt). Creatinine and urea levels were measured by spectrophotometric methods as described in the assay kits.

Statistical analysis:

Results of biological evaluation of water soluble chitosan were expressed as mean ± standard error (SE), (n=8). Statistical comparisons were performed by analysis of variance (ANOVA) test to determine the level of significance ($P<0.05$).

RESULTS

Characterization of chitosan:

a- FTIR spectra:

Structure changes of initial chitosan and chitosan oligomers were

confirmed by FTIR spectroscopy (figure). The IR spectrum of chitosan showed peaks assigned to the pyranose ring 841, 902, 3008, 3083 glucoside 1076, and amino amides characteristic peak around 1601 cm^{-1} were identifiable. This indicated that the important functional groups were still present after treatment and the main polysaccharide chain structure remained after the degradation process. The band at 1601 which had strong absorption suggested that the carbonyl group form stronger hydrogen bonds. With the decrease of molecular weight, the IR spectra of chitosan oligomers showed many fine absorption peaks as compared with initial chitosan.

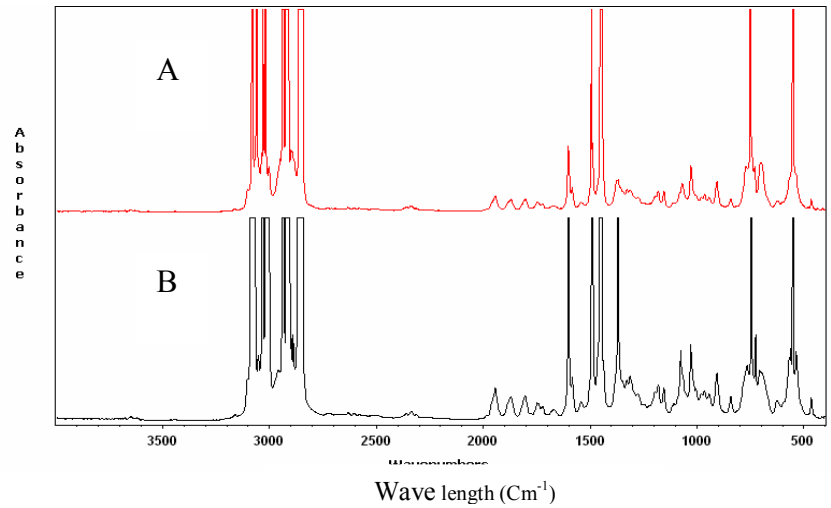

Figure: FTIR spectrum of high molecular weight chitosan (A) and water soluble chitosan (B).

The average molecular weight, the most difficult parameter to be obtained, can be determined by several methods; viscometry is claimed to be the simplest, most rapid and probably the most precise method **(Hua and Steven, 2001).** The viscosity average molecular weight (M_v) of prepared water soluble chitosan was determined by this method. The intrinsic viscosity was obtained which was applied in the Mark-Houwink equation to calculate the M_v using the appropriate Mark-Houwink parameters.

b- Biochemical findings;

As shown in Table (1) the results for lipid peroxidation which were evaluated as TBARS level shows that, rats irradiated group at 6 Gy exhibited a significant increase in TBARS level compared with the control. Administration

of WSC pre-irradiation resulted in significant improvement in the levels of lipid peroxidation when compared to irradiated group.

The level of glutathione (reduced form) in blood showed that γ-irradiation induced significant decrease in GSH level as compared with the control group. Treatment of animals with WSC pre-radiation exposure significantly improved the GSH levels compared to irradiated group.

Table (1): Effect of WSC pre-irradiation on MDA and GSH levels of male rats

Groups	Blood MDA (µg/ml)	Blood GSH (mg/dl)
Control	116.66 ± 1.54^{b}	24.1 ± 1.24^{b}
Radiation	153.16 ± 0.87^{ac}	11.8 ± 1.01^{ac}
WSC	95.00 ± 1.34^{b}	23.0 ± 1.06^{b}
WSC+ Radiation	94.16 ± 1.01^{abc}	20.5 ± 1.52^{b}

Each value represents mean ± SE for eight rats in each group. a: Significant difference from control group. b:Significant difference from irradiated group. c; Significant difference from WSC group.

Table (2) shows the effect of WSC on the blood antioxidants SOD, CAT and GPx activity levels of rats exposed to γ-irradiation. In comparison to the control group, there was a decline in the levels of antioxidants SOD, CAT and GPx in blood when the rats were irradiated with 6 Gy, but WSC pre-treatment normalized the levels of SOD and GPx while CAT levels recorded significant increase when compared to either irradiated or control values.

Table (2): Effect of WSC pre-irradiation on SOD, CAT and GPx of male rats.

Groups	Blood SOD (U/ml)	Blood CAT (µmol/ml)	Blood GPx (oxidized GSH/min/ml)
Control	7.71 ± 0.17^{b}	0.26 ± 0.01^{bc}	124.50 ± 7.04^{b}
Radiation	1.40 ± 0.09^{ac}	0.20 ± 0.01^{ac}	86.33 ± 3.23^{ac}
WSC	7.29 ± 0.10^{b}	0.32 ± 0.01^{ab}	132.16 ± 6.06^{b}
WSC+Radiation	3.45 ± 0.09^{abc}	0.29 ± 0.01^{ab}	117.83 ± 8.06^{b}

Legends as in Table (1).

As shown in Tables 3 & 4, rats irradiation significantly elevated liver enzymes (AST and ALT) compared with control value, also significantly elevated kidney parameters (urea and Creatinine). Pretreatment with wsc before gamma exposure completely restored their normal values.

Table (3): Effect of WSC pre-irradiation on AST and ALT of male rats.

Groups	Blasma AST (U/L)	Blasma ALT (U/L)
Control	16.1 ± 1.07^{bc}	68.1 ± 0.94^{b}
Radiation	23.3 ± 1.14^{a}	89.3 ± 1.11^{ac}
WSC	21.16 ± 1.06^{a}	70.6 ± 1.33^{b}
WSC +Radiation	19.6 ± 1.22^{b}	73.6 ± 0.88^{ab}

Legends as in Table (1).

Table (4). Effect of WSC pre-irradiation on urea and creatinine of male rats.

Groups	Blood urea (mg/dl)	Blood creatinine (mg/dl)
Control	24.1 ± 1.16^{b}	13.6 ± 1.02^{b}
Radiation	32.1 ± 1.47^{ac}	17.3 ± 0.88^{ac}
WSC	27.0 ± 1.23^{b}	14.3 ± 0.88^{b}
WSC +Radiation	24.6 ± 1.28^{b}	14.0 ± 1.06^{b}

Legends as in Table (1).

DISCUSSION:

The increasing use of radiation technologies in medicine, industry, agriculture and scientific research has been paralleled by increasing the potential risk for radiation over exposure. Under normal conditions, the inherent defense system, including glutathione and the antioxidant enzymes, protects against oxidative damage. GSH offers protection against oxygen-derived free radicals and cellular lethality following exposure to ionizing radiation **(Biaglow et al., 1987 and Noaman and El- Kabany, 2002).** There is growing evidence that the effects of ionizing radiation are mediated by the formation of reactive oxygen species and free radicals, which are highly reactive, removing hydrogen atoms from fatty acids, causing lipid peroxidation and consequently cell death **(Felemovicius et al., 1995).**

In the present study, whole body γ-irradiation strongly initiated the process of lipid peroxidation as indicated by the formation of TBARS in blood and reduction in the level of GSH (Table 1). GSH is a versatile protector and executes its radioprotective function through free-radical scavenging, restoration of the damaged molecules by hydrogen donation, reduction of peroxides, and maintenance of protein thiols in the reduced state **(Wambi et al., 2008).** The significant reduction in blood GSH due to radiation could be due to an enhanced utilization of the antioxidant system during detoxification of the

free radicals generated by radiation. Depletion of glutathione enhanced lipid peroxidation **(Jagetia and Reddy, 2005 and Ghorab et al., 2009)** and marked depletion of blood GSH levels in irradiated animals might be due to higher availability of GSH, which enhanced the capability of cells to cope with the free radicals generated by radiation.

The present data revealed a marked depletion in the antioxidant enzymes (CAT, GPx and SOD) in blood of rats exposed to γ-irradiation as compared with control group (Table 2). It is commonly accepted that SOD protects against the free radical injury by converting O^{2-} radical to H_2O_2 and prevent the formation of $OH^{\bullet}$ radical, and the H_2O_2 can be removed by catalase or GPx. Due to the inhibition of SOD, superoxide anion radicals may be combined with nitric oxide to form peroxynitrite anion which initiates lipid peroxidation. The decrease in the levels of antioxidant enzymes are in close relationship with the induction of lipid peroxidation. These results are in accordance with many studies which reported that the inhibition of antioxidant systems in blood and tissues of mice and rats was accompanied by an increase in lipid peroxide products after irradiation exposure **(Noaman and Ashry, 2006 and Zhao et al., 2007).** Also, **Borek, (2004)** mentioned that when the oxidative damage is extreme as a result of irradiation, ROS scavenging enzymes such as SOD and catalase are degraded. In support of these results **Lim et al., (2009)** showed a decline in GPx in liver of mice and rats exposed to irradiation, and hypothesized that the enzyme inactivating action of ROS or lipid peroxides induced by irradiation can overcome enzyme synthesis capacity. **Ueda et al. (1996)** postulated that the decrease in SOD level could be due to mitochondria damage and decrease of mitochondria Mn-SOD activity, which leads to a decrease in total SOD in different tissues of rats exposed to radiation.

The present data demonstrated significant elevation in serum ALT and AST in irradiated group, which reflects detectable changes in liver functions. This elevation in transaminases might be due to drastic physiological effects caused by direct interaction of gamma irradiation with cell membranes or indirectly via excessive production of free radicals and lipid peroxides, which lead to elaboration of intracellular enzymes into the blood stream and may enhance transaminases release from their intracellular sites to extracellular then to blood circulation. Furthermore, such elevation in transaminases may be related to the extensive body tissue breakdown induced by irradiation; such body tissues include liver and kidney parenchyma and heamopoietic tissues **(Sridharan and Shyamaladevi, 2002).**

In an effort to minimize the toxicity of ionizing radiation on normal tissues of exposed rats, chitosan was synthesized by *A. niger* and made susceptible for animal injection.

Water soluble chitosan applied prior to γ-irradiation nearly prevented lipid peroxidation in blood and significantly normalized GSH. This treatment efficiently elevated the levels of GPx, SOD, and CAT up to normal values. In addition, water soluble chitosan applied pre- irradiation to rats, in great extent, ameliorated the levels of ALT and AST in blood. Water soluble chitosan was found to have antioxidant activity and scavenging ability on hydroxyl radicals *in vitro* **(Xie et al., 2001)** and *in vivo* **(Anraku et al., 2009).**

To clarify the mode of the protective effect of water soluble chitosan against oxidative stress-induced cell damage, lipid peroxidation was determined. According to the results obtained, water soluble chitosan significantly inhibited lipid peroxidation. It is generally thought that the inhibition of lipid peroxidation by water soluble chitosan may be due to their free radical-scavenging activities as mentioned by **Mahakunakorn et al., (2003)** who postulated that, superoxide indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and $OH^{\bullet}$ (hydroxyl radicals) which eliminate hydrogen atoms from the membrane lipid and result in lipid peroxidation. It is well known that superoxide anions can cause cellular macromolecules damage directly or indirectly by forming H_2O_2, $OH^{\bullet}$, peroxylnitrile, or singlet oxygen during pathophysiologic events such as radiation exposure injury. It is possible that water soluble chitosan has protective effect against the H_2O_2 induced cell damage. Hydrogen peroxide formed by two-electron reduction of O_2 is not a free radical but it is an oxidizing agent. In the presence of O_2 and transition metal ions, H_2O_2 can generate $OH^{\cdot}$ *via* Fenton reaction. In addition, H_2O_2 can easily cross the cell membrane and exerts injurious effects on tissues through a number of different mechanisms**.** The protective effect of water soluble chitosan on H_2O_2 may at least partly result from its antioxidant and free radical-scavenging properties as mentioned by **Mahakunakornm et al., (2003)**.

The superoxide radical-scavenging activity of water soluble chitosan was also evaluated based on their ability to quench the superoxide radical which can be evaluated through measuring SOD enzyme responsible for quenching these superoxide radicals. The superoxide radical-scavenging activity of chitosan previously mentioned by **Tao et al., (2004),** but **Tian et al., (2009)**

proved that the water soluble chitosan increased the O^{2-} scavenging capability.

The hydroxyl radical is a highly potent oxidant that reacts with almost all biomolecules found in living cells. Determination of catalase level allows view of rate constants of reactions with $OH^{\bullet}$. The present study clearly demonstrated that water soluble chitosan exhibited potent free radical-scavenging activity.

We can be concluded that, water soluble chitosan showed potent antioxidant activity by trapping free radicals, delaying lipid peroxidation by scavenging free radicals directly or indirectly. Among the known antioxidants, water soluble chitosan is an effective scavenger for free radicals and can ameliorate radiation induced stress.

REFERENCES

1. Anraku, M.; Fujii, T.; Furutani, N.; Kadowaki, D.; Maruyama, T.; Otagiri, M.; Gebicki, J.M. and Tomida, H. (2009): Antioxidant effects of a dietary supplement: reduction of indices of oxidative stress in normal subjects by water-soluble chitosan. Food Chem. Toxicol. **47**(1): 104.

2. Badaloo, A.; Reid, M.; Forrester, T.; Heird W.C. and Jahoor, F. (2002): Cysteine supplementation improves the erythrocyte glutathione synthesis rate in children with severe edematous malnutrition. Am. J. Clin. Nutr. **76**: 646.

3. Beutler, E.; Duron, O. and Kelly, B.M. (1963): Improved method of the determination of blood glutathione. J. Lab. Clin. Med. **61**(5): 882.

4. Biaglow, J.E.; Varnes, M.E.; Epp, E.R. and Clark, E.P., (1987). In: Anticarcinogenesis and Radiation Protection. Cerrutti, P.A., Nygaard, O.F., Simic, M.G. (Eds.). Plenum Press, New York, p: 387.

5. Borek, C. (2004): Antioxidants and radiation therapy. J. Nutr, **134**: 3207S**.**

6. Chen, R.H. and Hwa, H.D. (1996): Effect of molecular weight of chitosan with the same degree of deacetylation on the thermal, mechanical, and permeability properties of the prepared membrane. Carbohy. Polym. **29**: 253.

7. Deger, Y.; Dede, S.; Belge, A.; Mert, N.; Kahraman, T. and Alkan, M. (2003): Effects of X-ray radiation on lipid peroxidation and antioxidant systems in rabbits treated with antioxidant compounds. Biol. Trace Elem. Res. **94**: 149.

8. De-Zwart, L.L.; Meerman, J.H.N.; Commandeur J.N.M. and Vermeulen, N.P.E. (1999): Biomarkers of free radical damage applications in experimental animals and in humans. Free Rad. Biol. Med. **26**: 202.

9. Felemovicius, I.; Bonsack, M.E.; Baptista, M.L. and Delaney J.P. (1995): Intestinal radioprotection by vitamin E (alpha-tocopherol). Ann Surg.; **222**(4): 504.

10. Feng, T.; Du. Y.; Li, J.; Hu, Y. and Kennedy, J.F. (2008): Enhancement of antioxidant activity of chitosan by irradiation. Carboh. Polym. **73**: 126.

11. Ghorab, M.M.; Ragab, F.A.; Noaman, E.; Heiba, H.I. and Aboulmagd, S.A. (2009): Synthesis, anticancer and radioprotective activities of some new pyrazolo[3,4-d]pyrimidines containing amino acid moieties. Arzneimittelforschung. **59**(2): 96.

12. Henry, R.J. (1974): Principles and Techniques. Clinical Chemistry, 2nd Ed. Harper and Row. p:525.

13. Hosseinimehr, S.J. (2007): Foundation review: Trends in the development of the radioprotective agents. Drug discovery today. **12**: 794.

14. Hua, Z. and Steven, H. (2001): In vitro degradation of chitosan by a commercial enzyme preparation: effect of molecular weight and degree of deacetylation. Biomaterials. **22**: 1653.

15. Jagetia, G.C. and Reddy, T.K. (2005): Modulation of radiation-induced alteration in the antioxidant status of mice by naringin. Life Sci. **77**: 780.

16. Koryagin, A.S.; Erofeeva, E.A.; Yakimovich, N.O.; Aleksandrova, E. A.; Smirnova, L.A. and Mal'kov, A.V. (2006): Analysis of antioxidant properties of chitosan and its oligomers. Bull.experim. Boil. Medic. , **142**(4): 461.

17. Kumar, M.N. (2000): A review of chitin and chitosan applications. Reactive Funct. Poly. **46**: 1.

18. Lim, W.; Kim, J.H.; Gook, E.; Kim, J.; Ko, Y.; Kim, I.; Kwon, H.; Lim, H.; Jung , B.; Yang, K.; Choi, N.; Kim, M.; Kim, S.; Choi, H. and Kim, O. (2009): Inhibition of mitochondria-dependent apoptosis by 635-nm irradiation in sodium nitroprusside-treated SH-SY5Y cells. Free Rad. Biol. Med. **47**(6): 850.

19. Maghsoodi, V.; Razavi, J. and Yaghmaei, S. (2009); Production of chitosan by submerged fermentation from *Aspergillus niger*. Transactions C: Chemistry and Chemical Engineering, **16**(2): 145.

20. Mahakunakorn, P., Tohda, M., Murakami, Y., Matsumoto, K. and Watanabe, H. (2003): Antioxidant and Free Radical-Scavenging Activity of Choto-san and Its Related Constituents. Biol. Pharm. Bull. **27**(1): 38.

21. Minami, M and Yoshikawa, H. (1979): A simplified assay method of superoxide dismutase. Clinica Chemica Acta, **92**: 337.

22. Noaman E. and El-Kabany, H. (2002): Effect of Novel Copper Compound (Cu-BISDO) and γ-irradiation on Copper and Zinc Concentration, Ceruloplasmin and Superoxide Dismutase activity, in Mice loaded with Ehrlich Tumor. Egypt. J. Biomed Sci. **9**: 209.

23. Noaman, E. and Ashry, O.M. (2006): Protective role of L-Arginine against γ-irradiation induced oxidative stress in rats. Egypt. J. Rad. Sci. Appl. **19**(1): 23.

24. Paglia, D.E. and Valentine, W.N. (1967): Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, J. Lab. Clin. Med. **70**: 158**.**

25. Palton, C.J. and Crouch, S.R. (1977): Enzymatic determination of serum urea by modified Berthelot reaction. Anal Chem. **49**: 464.

26. Prasad, K.N.; Cole, W.C. and Haase, G.M. (2004): Radiation protection in humans: extending the concept of as low as reasonably achievable (ALARA) from dose to biological damage. British J. Radiol. **77**: 97.

27. Reitman S. and Frankel S., (1957): Colorimetric determination of GOT and GPT activity. Amer. J. Clin. Pathol. **28**: 56**.**

28. Riley, P.A. (1994): Free radicals in biology: Oxidative stress and the effects of ionizing radiation. Int. J. Rad. Biol. **65**: 27.

29. Sagrista, M.L.; Garcia, A.E.; De Madariaga A.M. and Mora, M. (2002): Antioxidant and pro-oxidant effect of the thiolic compounds *N*-acetyl-L-cysteine and glutathione against free radical-induced lipid peroxidation. Free Radical Res. **36**: 329.

30. Sinha, A.K., (1972): Colorimetric assay of catalase. Anal. Biochem. **47**: 389.

31. Sridharan, S. and Shyamaladevi, C.S., (2002): Protective effect of N-acetylcysteine against gamma ray induced damages in rats--biochemical evaluations. Indian J. Exp. Biol. **40**(2): 181.

32. Sun, T.; Zhou, D.; Xie, J. and Mao, F., (2007): preparation of chitosan oligomers and their antioxidant activity. Eur. Food Res. Technol. **225**: 451.

33. Tao, S.N.; Xie, W.M. and Xu, P.X. (2004): Superoxide anion scavenging activity of graft CS derivatives. Carbohy. Polym. **58**:379.

34. Tian, X.L.; Tian, D.F.; Wang, Z.Y., and Mo, F.K. (2009): Synthesis and Evaluation of Chitosan-Vitamin C complex. Indian J. Pharm. Sci. **71**(4): 371.

35. Ueda, T.Y.; Toyoshima, T.; Moritani, R.K.; Otsuki, N.; Kushihashi, T.; Yasuhara, H. and Hishida, T. (1996): Protective effect of dipyridamole against lethality and LPx in liver and spleen of the ddY mouse after whole-body irradiation. Int. J. Rad. Biol. **69**: 199.

36. Wallace, S.S., (1998): Enzymatic processing of radiation-induced free radical damage in DNA. Rad. Res. **150** (Suppl. 5) S60.

37. Wambi, C.; Sanzari, J.; Wan, X.S.; Nuth, M.; Davis, J.; Ko, Y.H.; Sayers, C.M.; Baran, M.; Ware, J.H. and Kennedy, A.R. (2008): Dietary antioxidants protect hematopoietic cells and improve animal survival after total-body irradiation. Rad. Res. **169**: 384.

38. Wang, W.; Bo, S.; Li, S. and Qin, W. (1991): Determination of the Mark-Houwink equation for chitosan with different degree of deacetylation. Int. J. Biol. Macromol., **13**: 281.

39. Xie,W.; Xu, P. and Liu, Q. (2001): Antioxidant activity of water soluble chitosan derivatives. Bioorg. Medic. Chem. Lett., **11**(13): 1699.

40. Yang, M.K. and Kim, Y.G. (1999): Protective role of Ge-132 against paraquat-induced oxidative stress in the livers of senescence-accelerated mice. J. Toxicol. Environ. Health. **58**: 289.

Yoshioka, T.; Kawada, K.; Shimada, T. and Mori, M. (1979): Lipid peroxidation in maternal and cord blood and protective mechanism against activated oxygen toxicity in the blood. Am. J. Obstet. Gynecol., **135**: 372.

41. Zhao, W.; Diz, D.I. and Robbins, M.E. (2007): Oxidative damage pathways in relation to normal tissue injury. Br. J. Radiol., **80**: S23–S31

المؤتمر الدولي الثالث
للعلوم الإشعاعية وتطبيقاتها
١٢ – ١٦ نوفمبر ٢٠١٢ – الغردقة - مصر

تحسين الإختلال فى مستوى الأكسدة المحدثة بالإشعاع باستخدام الكيتوزان القابل للذوبان فى الماء و المنتج بواسطة اسبرجلس نيجر

***سوسن محمد السنباطى - *هشام محمود سويلم -** ايمان نعمان على**

*قسم الميكروبيولوجيا الإشعاعية – **قسم البيولوجيا الإشعاعية المركز القومى لبحوث و تكنولوجيا الإشعاع ص.ب. ٢٩ مدينة نصر .*

يعتبر الكيتوزان من المنتجات الطبيعية العديد السكريات والتى يقوم بتصنيعها العديد من الكائنات الحية و الكائنات الدقيقة، و تعتمد التطبيقات البيولوجية للكيتوزان على قابليته للذوبان فى الوسط المتعادل و التى تتناسب عكسيا مع الوزن الجزيئى، لذلك تم التكسير الكيميائى لجزىء الكيتوزان المنتج بواسطة فطر الأسبرجلس نيجر باستخدام فوق اكسيد الهيدروجين و نتج عنه كيتوزان ذو قابلية عالية للذوبان فى الماء. ثم تقدير الوزن الجزيئى بواسطة جهاز مقياس اللزوجة والتعرف عليه بمقارنة نتائج امتصاص الموجات تحت الحمراء قبل و بعد التكسير الكيميائى . ولتقييم دور الكيتوزان الذائب كمضاد للأكسدة الناتجة من الأشعة المؤينة الجامية على الفئران ، تم تقسيم الفئران الى ٤ مجموعات؛ الأولى:المجموعة الضابطة، الثانية: المجموعة الضابطة للإشعاع، تم تعريض الفئران كليا لجرعة ٦ جراى، المجموعة الثالثة:المجموعة المعاملة بالكيتوزان المذاب فى الماء (١٠٠مجم/كجم من وزن الفأر) والمجموعة الرابعة: حقنت بالكيتوزان المذاب فى الماء يوم بعد يوم لمدة ٤ أسابيع ثم تعرضت كليا لجرعة واحدة من أشعة جاما (٦ جراى) .

أوضحت النتائج أن التعرض لأشعة جاما سبب انخفاضاً معنويا فى نشاط إنزيمات السوبر اكسيد ديسميوتيز و الكتاليز و الجلوتاثيون اكسيديز ومحتوى الجلوتاثيون فى الدم وزيادة معنوية فى نشاط انزيمات الكبد فى مصل الدم (الألانين والأسبرتيت الناقلين للأمين) وايضا تركيز اليوريا والكيرياتينين والمالون داى ألدهيد.

بحقن الكيتوزان الذائب ، وجد انه ادى الى تحسين فى مستوى إنزيمات الأكسدة وإنزيمات الكبد وتركيز الجلوتاثيون واليوريا والكيرياتينين والمالون داى ألدهيد مما يؤكد ان له نشاط مضاد للأكسدة وذلك بجمع الشوارد الحرة وتعديل مستوى الليبيدات المتأكسدة بطريقة مباشرة أو غير مباشرة مما يجعله إحدى مضادات الأكسدة القوية كما انه يقلل من التأثير السلبى للإشعاع.