Irradiated β-glucan enhances immune response to bacterial infection through CD4 and CD8 T-lymphocytes

S.M. El-Sonbaty[1*], A.F.M. Ismail[2], A.I. Nabeel[3]

[1]Microbiology Department, National Center for Radiation Research and Technology, Cairo, Egypt
[2]Drug Radiation Research Department, National Center for Radiation Research and Technology, Cairo, Egypt
[3]Chemistry Department, Faculty of Education, Ain Shams University, Cairo, Egypt

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**** Corresponding author:***
Dr. Sawsan El-Sonbaty,
E-mail:
sawsansonbaty@yahoo.com

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ABSTRACT

Background: β-glucans are glucose polymers with a variety of stimulatory effects on the immune system. The objective of this study was to evaluate the immune-enhancing activities of low molecular weight gamma irradiated β -glucan (Iβ-g) extracted from *Pleurotus ostreatus* in response to *Pseudomonas aeruginosa* infection in rats. ***Materials and Methods:*** β-glucan (β-g) powder was exposed to 50 kGy of gamma radiation. For experimental study, healthy male rats were divided in six groups. Group I: did not receive any treatment. Group 2: infected group was injected once with *P. aeruginosa*. Group 3: were orally administrated with β-g for 15 days at a dose of 65 mg/Kg body weight/ day. Group 4: were orally administrated with Iβ-g for 15 days at a dose of 65 mg/Kg body weight/day. Group 5: rats were treated with β-g and injected once with *P. aeruginosa*. Group 6: rats were treated with Iβ-g and injected once with *P. aeruginosa*. Rats were sacrificed 24 h post bacterial infection. ***Results:*** β-g and Iβ-g were characterized using FTIR spectra and SEM, showed breakdown of the glycosidic bonds with deformation and splitting of β-g molecule. Oral ingestion of β-g markedly enhanced the production of lymphocytes and leucocytes and CD8 compared to that of Iβ-g, although Iβ-g was more effective in stimulating CD4 count compared to that of β-g and ameliorated CD4/CD8 ratio. Iβ-g enhanced GPx and CAT activity, elevated zinc concentration in the blood compared to that of β-g, although both Iβ-g and β-g elevated GSH and caused no effect on MDA level in post infected groups. ***Conclusion:*** In conclusion Iβ-g improves antioxidant state and enhances immune system in particular CD4 count against bacterial infections which is an important marker of immune system.

Keywords: *β-glucan, gamma irradiation, CD4, CD8, antioxidant, Pseudomonas aeruginosa, zinc, copper.*

INTRODUCTION

Sugars are bioactive components of many plants and microorganisms. Polysaccharides and oligosaccharides of various origins (fungi, bacteria, plants, etc.) can be recognized by surface receptors of host cells; in particular macrophages and dendritic cells, and trigger

host innate immune reactions. Some polysaccharides and oligosaccharides such as β-glucan (β-g) extracted from fungi, as mushrooms and yeasts. Oral intake of β-g enters the proximal small intestine, captured by the macrophages and activates macrophages and neutrophils [1, 2].

CD4 cells (T-helper cells) and CD8 cells (killer T cells) are types of white blood cell that fights infection, made in the spleen, lymph nodes and thymus gland, which are part of the lymph or infection-fighting system. They identify bacteria and viruses via the antigen then a sequence of signals is triggered which causes it to kill the cell attached to it. The CD4 count, CD4 percent, or a CD4/CD8 ratio can tell how strong the immune system. Various disease conditions appear to affect the levels of CD8 and CD4 cells. In most cases, the rise in the percentage of CD8 and CD4 cells appears to be a direct result of cellular activation [3].

Pseudomonas aeruginoa is an antibiotic resistant, widespread bacteria in nature, its infections are complicated and casn be life threatening. It is an important cause of pneumonia, urinary tract infections, and bacteremia infections, especially in patients with compromised host defense mechanisms [4].

The objectives of the present investigation were to evaluate β-g and Iβ-g as immune-stimulators against *Pseudomonas aeruginosa* infection.

MATERIALS AND METHODS

Chemicals

Pleurotus ostreatus mushroom was obtained from the local market. All chemicals used in this study were obtained from (Sigma-Aldrich).

Extraction of β-glucan from Pleurotus ostreatus

β-glucan (β-g) was extracted from edible mushroom *Pleurotus ostreatus* fungi according to the method published by Hunter *et al.* [5].

Gamma irradiation

β-g powder was irradiated using Co-60 Gamma chamber 4000–A– India, irradiation facility, at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt. Irradiation was performed using 60Co gamma rays at a dose rate 0.461 Gy/min., at the time of the experiment. The dose applied in this work was 50 kGy at room temperature (25±2 ˚C).

Fourier transform infrared, scanning and transmission electron microscopy

FT-IR spectra of KBr disk of β-g and Iβ-g, were determined using JASCO FTIR 6300. The micro-structural changes of irradiated β-glucan induced by gamma irradiation were observed by the method of Sokhey and Hanna (1993) [6] using scanning electron microscopy (SEM), JEOL model JSM 5400.

Pseudomonas aeruginosa inoculums

P. aeruginosa (*P.a.*) (ATCC 10145) was grown overnight in nutrient broth, harvested by centrifugation and suspended in phosphate-buffered saline (PBS) at approximately 1 ×10^6 CFU per ml.

Animals and experimental design

Male Wistar rats weighing 100-120 g were purchased from Nile pharmaceutical co. then, housed in accordance with institutional animal care policies had free access to water and standard diet.

Animals were divided randomly into the following groups (6 animals / group): 1- normal control group, 2- *P. aeruginosa* (*P.a.*) infected group, 3- β-g treated group (β-g), 4- irradiated β-g treated group(Iβ-g), 5- β-g + P.a. infected group(β-g/P.a), 6- Iβ-g+P.a. infected group(Iβ-g/ P.a). β-g and Iβ-g were orally administrated for 15 days at a dose of 65 mg/Kg body weight/day. Rats were infected by intra-peritoneal (i.p.) injection of *P. aeruginosa* at a dose of 0.04 ml of bacterial suspension (1.0 x 10^6 CFU/ ml) in phosphate-buffered saline (PBS). Rats were sacrificed 24 h post bacterial infection and heart blood was collected in a vial containing 0.5 M

EDTA. Blood was used for total leukocytes count and for CD4 and CD8 count. Spleen tissues homogenates were prepared in distilled water (10% w/v).

Antioxidant parameters

Lipid peroxides content (MDA) was determined according to the method of Yoshioka *et al.* (1979) [7] using 1,1,3,3-tetraethoxypropane as a standard. GSH content was determined according to the method of Beutler *et al.* (1963) [8]. Glutathione peroxidase determined according to the method of Paglia and Valentine (1967) [9]. Catalase concentration was determined according to the method of Sinha (1972) [10]. Total protein was determined according to the method of Lowry *et al.* [11].

CD4 and CD8 count by flow Cytometry

The flow cytometer used for enumeration of T-cell subsets (CD4 and CD8) is FACS caliber flow cytometer (Becton Dickinson, Sunnyvale, CA, USA) equipped with a compact air cooked low power 15 m watt argon iron laser beam (48nm). The average number of evaluated nuclei per specimen 20.000 and the number of nuclei scanned was 120/ second. CD4 and CD8 histograms were obtained with a computer program for Dean and Jett mathematical analysis (Dean and Jett 1974) [12].

Determination of zinc and copper concentrations

Blood samples were digested in a boiling mixture of H_2NO_3 and H_2O_2 (1:1) until complete digestion of the organic materials. Zinc and copper concentrations were measured in the prepared blood samples using Atomic absorption spectrophotometer, UNICAM 939, USA.

Statistical Analysis

The results were statistically analyzed by one-way ANOVA using SPSS (statistical package for social sciences, 1999; ver.10.0). The significance among the samples was compared at $p<0.05$.

RESULTS AND DISCUSSION

Characterization of β-glucan and irradiated β-glucan

In the present study, verification of the 1,3-β-D-glucan structure was performed using FT-IR spectra. FT-IR spectra of gamma-irradiated (50 kGy) and non-irradiated extracted solid fraction obtained from fruiting body and stems of *P. ostreatus* are shown in figure 1. In the region of functional group of IR- spectra, there are noteworthy absorptions at 3356 (-OH), 2925 (-CH), 1647 (-C=O) and 818 cm^{-1} that corresponding to the stretching absorption bands of poly–OH, C-H, C=O and B configuration, respectively. The co-vibration ranging at 1250 – 950 cm^{-1} as well as in 960-748 cm^{-1} distinguishes β–D-glucan from α-D-glucan spectra. This result showed that the non-irradiated and irradiated β-g both had a similar pattern of

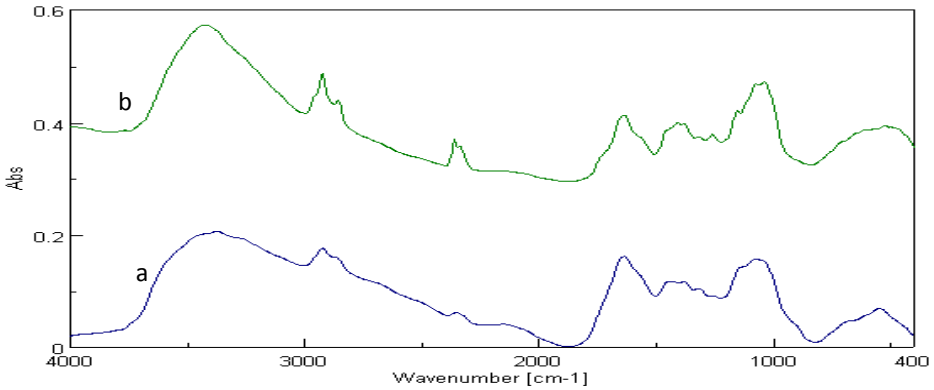

Figure 1. FT-IR spectra of (a): β-glucan and (b): gamma irradiated β-glucan.

FTIR spectra, without any notable changes in the functional group status.

Broad band at 1647 cm^{-1} (amide I), which is composed from two components at 1673 and 1631 cm^{-1} according to second derivative, together with the band at 1502 cm^{-1} (amide II) indicate the presence of α-chitin. Small amount of chitin was found as a component of cell wall chitin–glucan complex.

Microphotography of Iβ-g granules obtained from 50 kGy gamma-irradiated and non-irradiated β–g granules using scanning electron microscopy (SEM) at 1500 magnification are represented in figure 2b, showed deformation and splitting occurs in Iβ-g granules compared to Iβ-g in figure 2a.

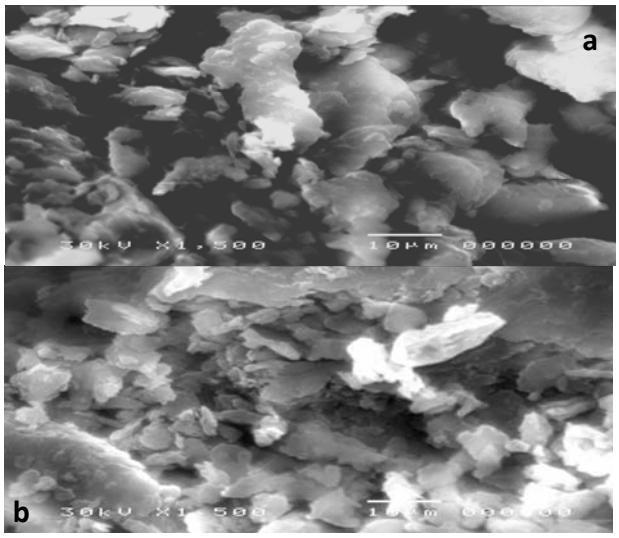

Figure 2. SEM images of β–glucan (a): non-irradiated and (b): irradiated at 50 kGy. β –glucan granules showed deformities after gamma irradiation.

Effect on lymphocytes, segmented and leucocytes count

In the present work, the total leukocyteic count (TLC) and WBCs differentiation are illustrated in table 1. The results showed that, lymphocytes and leukocytic count were significantly increased in β-g and Iβ-g treated groups compared to the control. Segmented cell number was significantly increased only by β-g compared to control, Leucocytes cell number was significantly increased by Iβ-g β-g also administration of Iβ-g significantly increased Leucocytes cell number. Infection with *P. aeruginosa* significantly increased lymphocytes, leucocytes and reduced segmented cells, β-g infected group showed marked immune response represented by lymphocyte, segmented and leukocyte cell number. From the above, we may say that in case of infection β-g exhibited a stronger immune response compared to I β-g.

Effect on CD4 and CD8 count

Results demonstrated in table 2 shows that, infection with *P. aeruginosa* enhanced CD4 and CD8 number with slight decrease in the CD4/ CD8 ratio (1.08+ 0.03). β-g significantly reduced the CD4/CD8 ratios, while, Iβ-g treatment significantly increased CD4/CD8 ratio (1.4+0.028).

The present study results showed that, both β -g and Iβ-g improved immune system through proliferation of lymphocytes, and leuckocytes. β-g was more efficient compared to Iβ-g in inducing cell proliferation of lymphocytes, and leuckocytes. Although, β-g had significant

Table 1. Effect of β-glucan and Iβ-glucan on lymphocytes, segmented and leucocytes count.

Group	Lymphocytes mean±SE	Segmented mean±SE	Leucocytes mean±SE
Control	40.2±1.7	55.2±1.9	4720±73.5
***P. aeruginosa* (P.a.)**	45.0±1.6[a]	47.2±1.2	6160±57.9[ac]
β-g	56.6±1.2[ac]	64.2±1.7[ac]	8160±92.7[ac]
Iβ-g	47.8±0.9[a]	52.4±0.9	5170±53.9[a]
β-g + P.a	50.0±1.7[ac]	56.6±1.1[c]	6770±53.9[ac]
Iβ-g +P.a	45.6±1.2[a]	51.2±1.0	3780±46.4[abc]

***a:** compared to control, b: compared to β-glucan, c: compared to Iβ-glucan

influence on CD8 cell count percentage, Iβ-g stimulated cell count percentage of CD4 and CD8.

Effect on antioxidant parameters

The results obtained in table 3 showed that Iβ -g enhanced antioxidant parameters of GPx and GSH compared to control and β-g groups. *P. aeruginosa* infection changed antioxidant markers via increasing GPx, CAT activity and MDA level compared to control. Pretreatment with β-g or Iβ-g prior to infection significantly stimulated antioxidant parameters of GPx and GSH, Results showed that Iβ-g markedly enhanced GPx and CAT activity compared to that of β-g in most treated groups.

Zinc and copper concentration in the blood

Results in table 4 revealed that, while zinc was significantly increased with Iβ-g with infection than β-g with significant decrease in copper concentration in the blood.

Table 2. Effect of β-glucan and Iβ-glucan on CD4 and CD8 T cell count in the blood represented by mean ± SE.

Group	CD4	CD8	CD4/CD8
Control	11.5±0.4	10.3±0.5	1.15±0.03
P.a.	13.2±0.3^{ac}	12.4±0.7^{bc}	1.8±0.02^{bc}
β-g	12.2±0.4^{bc}	33.4±0.6^{abc}	0.37±0.02^{abc}
Iβ- g	21.7±0.5^{ab}	27.9±0.4^{abc}	0.8±0.02^{abc}
β- g/P.a	8.4±0.5^{abc}	15.3±1.0^{abc}	1.4±0.03^{abc}
Iβ-g/P.a	20.9±0.5^{ab}	10.8±0.4^{bc}	0.8±0.02^{ab}

***a:** compared to control, b: compared to β-glucan, c: compared to Iβ-glucan

Table 3. Effect of β-glucan and Iβ-glucan on GPx, GSH, MDA level, and CAT activity in the spleen represented by mean±SE.

Group	Gpx µg oxidized GSH/ min/ mg protein	CAT mmol (H_2O_2)/ mg protein	GSH mg/ mg protein	MDA mg/ mg protein
Control	4.0±0.7	67.0±3.0	56.0±3.5	49.3±3.3
P.a.	5.0±0.5^{ac}	57.8±4.4^{abc}	53.6±4.0^{bc}	55.8±2.6^{abc}
β-g	4.5±0.2^{c}	71.5±4.5	62.9±4.6^{abc}	48.0±4.3
Iβ-g	7.0±0.4^{abc}	66.7±6.6	75.8±1.9^{abc}	48.8±1.5
β-g/P.a	5.5.0±0.4^{abc}	39.3±5.7^{abc}	71.5±3.0^{abc}	52.2±5.6
Iβ-g/P.a	7.6±0.3^{abc}	51.2±4.8^{abc}	70.7±2.7^{abc}	54.7±4.0^{abc}

***a:** compared to control, b: compared to β-glucan, c: compared to Iβ-glucan

Table 4. Effect of β-glucan, Iβ-glucan on zinc and copper level in the blood.

Group	Zinc (µg/ml) mean±SE	Copper (µg/ml) mean±SE
Control	13.0±1.4	0.82±0.04
P.a.	15.6±0.9^{bc}	0.5±0.04^{ac}
β-g	23.0±0.7^{a}	0.54±0.10^{ac}
Iβ-g	23.8±1.4^{a}	0.29±0.01^{ab}
β-g/*P.a*	17.1±1.6^{abc}	0.65±0.04^{ac}
Iβ-g/*P.a*	23.2±1.1^{a}	0.32±0.02^{ab}

***a:** compared to control, b: compared to β-glucan, c: compared to Iβ-glucan

DISCUSSION

There is an increasing interest in finding more effective and safe biological agents for immune-based therapies that might be integrated into current multimodal regimens. Importantly, resistance to conventional therapies does not appear to confer resistance to immune-based therapies [13]. Bacterial infection releases toxins cause leukocytes to release pro-inflammatory cytokines which stimulate a series of biochemical events that ends in septic shock. Enhancing immune response by β-g is based on reducing the production of pro-inflammatory cytokines, via its interaction with dectin-1 receptors on cell surface expressed mainly on subpopulation of T-lymphocytes [14, 15]. The adaptive immune response is based on two groups of lymphocytes: B cells, which differentiate into immunoglobulin secreting plasma cells and hereby induce humoral immunity, and T cells, which mediate cytotoxic effects and helper cell functions of cell mediated immunity. Both responses depend on the clonal expansion of cells after recognition of their specific antigen. While B cells depend on zinc for proliferation, they do so to a lesser extent than T cells [17, 16].

Degradation of large molecules of polysaccharides using ionizing radiation by the cleavage of the glycosidic bonds can be used as an effective method to produce β-g with high solubility and low viscosity, easily absorbed by intestinal mucosa without causing any notable changes in the functional-group status, without the introduction of chemical reagents and without the need to control the temperature. This technology is simple and more environmentally friendly than conventional methods [18, 19]. In the present study, structural and characterizations of β-g isolated from Pleurotus ostreatus fungi and Iβ-g granules were performed. Comparing FTIR spectra of β-g and Iβ-g showed that, irradiation of β-g did not cause changes in the structure especially the functional groups. Similar results were obtained for β-g extracted from Beakr's yeast [19]. Although our results showed a deformation of β-g granules morphology by irradiation in SEM photos, might be due to the breakdown of glycosidic bonds by gamma radiation, as irradiation of starch granules causes morphology deformation [20, 21].

The results obtained showed that, oral ingestion of β-g and Iβ-g improved the pattern of immune system in experimental animal represented by lymphocytes and luckocytes count when compared to control, although β-g induced greater stimulatory effect compared to Iβ-g. By determining CD4 and CD8 count, results showed that β-g and Iβ-g markedly improved CD4 and CD8 count compared to control with a greater stimulatory effect caused by Iβ-g on CD4 count which is important parameter for immune system. Generally, introduction of P. aeruginosa to Iβ-g group showed increased resistance to infection represented in CD4 and CD8 count percentage compared to that of β-g. Stimulation of anti-microbial immune response and stimulation of lymphocytes and luckocytes count is a result of glucans [22, 23]. Treatment of glucans improved immune parameters attributed to the effect of its constituents since glucans was reported to have the ability to scavenge free radicals mainly OH radicals [24], β-g can stimulate adaptive immunity by enhancing T-cells, activation of CD8 and CD4 T cells and increase the number of specific CD8 via receptors on macrophages and neutrophils [2]. Stimulation of CD4 count by Iβ-g is important and can reduce complications of serious bacterial infections (HIV disease) and extend life span [14].

The present results of antioxidant determination showed that oral gavage of ß-g improved GSH level, while Iß-g markedly increased GPx activity and GSH level. p.a infection reduced CAT activity and elevated lipid peroxidation level. Bacterial infections reduced oxidative metabolism and required less activity of CAT and SOD as consequence of their little production of oxidative radicals. Also cumulative free radicals induce oxidative damage on mice lymphocytes [25]. The modulator action of the supplement might be attributed to the effect of Iß-g by

scavenging free radicals and inhibit lipid peroxidation, since glucans supplementation improve immune activity by regulating antioxidant state [26]. Biological enhancement activity of Iß-g compared to ß-g could be referred to its low molecular weight and higher solubility [27]. Urao *et al.* (1999) [28] suggested that oral administration of glucan allowed the beneficial microorganisms e.g. bifidobacterium to quickly reproduce in the animal intestine. These microorganisms can synthesize vitamins and amino acids, stimulate immunoglobulin activity and improve immune function.

The trace element zinc is essential for growth and development of all organisms and the high rate of proliferation and differentiation of immune cells. Determination of zinc concentration and copper concentrations in the present study showed a significant increase in zinc with a decrease in copper levels compared to control in all treated groups, while zinc concentration was higher in infected group treated with Iß-g compared to ß-g which explain the enhancement of GPx and CAT activity in the same group [29, 30]. The lymphocyte protein tyrosine kinase, a Src-family tyrosine kinase, is an example for a different mechanism by which zinc acts on signal transduction. Zinc ions promote activation of Lck and its recruitment to the T cell receptor complex by linking two protein interface sites. The N-terminal region of Lck is recruited to the intracellular domains of the membrane proteins CD4 or CD8 by a 'zinc clasp' structure [31, 32]. These results suggest that, during acute inflammation, the organism increases its requirement for copper and zinc. Endogenous copper and zinc involved in the regulation of the inflammatory process and may exert a protective role in inflammation during infections pro-inflammatory cytokines mediate changes in hepatic zinc homeostasis, leading to sequestration of zinc into liver cells and subsequently to hypozincemia affect zinc-dependent functions in virtually all tissues, and in particular in the immune system [33].

Zinc is a cofactor for thymulin which regulates the differentiation of immature T cells in the thymus [34]. Copper is an essential cofactor in many enzymatic reactions vital to the normal function of the hematologic, vascular, skeletal, antioxidant, and neurologic systems. Copper deficiency has been described in the setting of zinc supplementation in humans [35].

CONCLUSION

From the results it is concluded that β-g, Iβ-g and Zn-Ps supplement markedly enhanced the lymphocytes and leukocytes, and also have positive relation with antioxidant state in rats' spleen. Iβ-g induced specifically CD4 percentage while β-g and Zn-NPs specifically increased CD8 percentage.

REFERENCES

1. Chan GCF, Chan WK, Sze DMY (2009) The effects of β-glucan on human immune and cancer cells. *J Hematol Oncol,* ***2:*** *25.*
2. Wang J, Dong S, Liu C, Wang W, Sun S, Gu J, Wang Y, Boraschi D, Qu D (2010) β-Glucan oligosaccharide enhances CD8+ T-cellsimmune response induced by a DNA vaccine encoding hepatitis B virus core antigen. *J Biomed Biotechnol, 2010: 645213.*
3. Kitchen GS, Whitmire KJ, Jones RN, Galic Z, Kitchen MRC, Ahmed R, Zack AJ (2005) The CD4 molecule on CD8+ T lymphocytes directly enhances the immune response to viral and cellular antigens. *PNAS,* ***102:*** *10379.*
4. Pollack M (2000) Pseudomonas aeruginosa. In: Principles and Practice of Infectious Diseases, Mandell *et al.* (eds.). Philadelphia, PA: Churchill Livingstone. pp. 2310–2327.
5. Hunter JR KW, Gault RA, Berner MD (2002) Preparation of microparticulate b-glucan from Saccharomyces cerevisiae for use in immune potentiation. *Lett Appl Microbiol,* ***35:*** *267.*
6. Sokhey AS and Hanna MA (1993) Properties of irradiated starches. *Food Struct,* ***12:*** *397.*
7. Yoshioka T, Kawada K, Shimada T, 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.*
8. Beutler E, Duron O, Kelly BM (1963) Improved method of the determination of blood glutathione. *J. Lab & Clin Med,*

61: *882.*
9. Paglia DE and Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med,* ***70:*** *158.*
10. Sinha AK (1972) Colorimetric assay of catalase. *Anal Biochem,* ***47:*** *389.*
11. Lowry OH, Roseberough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. *J Biol Chem,* ***193:*** *265.*
12. Dean PN and Jett JH (1974) Brief note: mathematical analysis of DNA distributions derived from flow microfluorometry. *J Cell Biol,* ***60:*** *523.*
13. Christian M, Capitini MD, Crystal L, Mackall MD, Alan S, Wayne MD (2010) Immune-based Therapeutics for Pediatric. *Cancer Expert Opin Biol Ther,* ***10:*** *163.*
14. Demir G, Klein HO, Mandol-Molinas N, Tuzuner N (2007) Beta glucan induces proliferation and activation of monocytes in peripheral blood of patients with advanced breast cancer. *Int immunopharmacol,* ***7:*** *113.*
15. Vetvicka VB (2001) Glucans as immunomodulators. *JANA, 3: 31.*
16. Zanzonico P, Fernandes G, Good RA (1981) The differential sensitivity of T-cell and B-cell mitogenesis to *in-vitro* zinc deficiency. *Cell Immunol,* ***60:****203-211.*
17. Flynn A (1984) Control of *in-vitro* lymphocyte proliferation by copper, magnesium and zinc deficiency. *J Nutr,* ***114:*** *2034-2042.*
18. Charlesby A (1981) Crosslinking and degradation of polymers. *Radiat Phys Chem,* ***18:*** *59.*
19. Byun E, Kim J, Sung N, Choi J, Lim S, Kim K, Yook H, Byun M, Lee J (2008) Effects of gamma irradiation on the physical and structural properties of β –glucan. *Rad Phys & Chem,* ***77:*** *781.*
20. Von Sonntag C (1980) Free-radicals reactions of carbohydrates as studied by radiation techniques. *Adv Carbohydr Chem Biol,* ***37:*** *7.*
21. Wu D, Shu Q, Wang Z, Xia Y (2002) Effect of gamma irradiation on starch viscosity and physicochemical properties of different rice. *Radiat Phys Chem,* ***65:*** *79.*
22. Reid DM, Gow NA, Brown GD (2009) Pattern recognition: recent insights from dectin-1. *Curr Opin Immunol,* ***21:*** *30.*
23. El-Boshy ME, El-Ashram AM, Abdel Hamid FM, Gadalla HA (2010) Immunomodulatory effect of dietary Saccharomyces cerevisiae, b-glucan and laminaran in mercuric chloride treated Nile tilapia (Oreochromis niloticus) and experimentally infected with Aeromonas hydrophila. *Fish & Shellfish Immunology,* ***28:*** *802.*
24. Azab KHSH and El-Dawi HA (2005) The modifying effect of beta 1,3-D-glucan on gamma radiation induced oxidative stress and genotoxicity in male mice. *Egypt J Rad Sci Applic,* ***18:*** *39–52.*
25. Sklavos MM, Tse HT, Piganelli JD (2008) Redox modulation inhibits CD8 T cell effector function. *Free Radic Biol Med,* ***45:*** *1477.*
26. El-Batal AI, Azab KHSH, Saada HN, Rezk RG, El-Tahawy NA (2008) Ameliorating effects of yeast glucan with zinc bisglycinate on histolog-ical and biochemical changes in g-irradiated. *Rats Int J Agri Biol,* ***10:*** *361.*
27. Chandra R K (1992) Effects of vitamin and trace-element supplementation on immune responses and infections in the elderly. *Lancet,* ***340:*** *1124.*
28. Urao M, Fujimoto T, Lane GJ, Seo G, Miyano T (1999) Does probiotics administration decrease serum endotoxin levels in infants? *J Pediat Surg,* ***34:*** *273.*
29. David JT, Jr. Michael VM, David AN (1997) Zinc induces catalase expression in cultured fetal human retinal pigment epithelial cells. *Curr Eye Res,* ***16:*** *1017-1023.*
30. Hideki F, Masami H, Yu M, Yasohiro Y (1984) Effect of Zinc Pretreatment on Mercuric Chloride-Induced Lipid Peroxidation in the Rat Kidney. *Toxicol Appl Pharmacol,* ***73:****395-401.*
31. Huse M, Eck MJ, Harrison SC (1998) A Zn2+ ion links the cytoplasmic tail of CD4 and the N-terminal region of Lck. *J Biol Chem,* ***273:****18729-18733.*
32. Lin RS, Rodriguez C, Veillette A, Lodish HF (1998) Zinc is essential for binding of p56(lck) to CD4 and CD8 alpha. *J Biol Chem,* ***273:****32878-32882.*
33. Milanino R, Moretti U, Concari E, Marrella M, Velo GP (1988) Copper and zinc status in adjuvant-arthritic rat: studies on blood, liver, kidneys, spleen and inflamed paws. *Agents Actions,* ***24:*** *365-76.*
34. Prasad AS (2008) Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. *Exp Gerontol,* ***43:*** *370-*
35. Griffith DP, Liff D, Ziegler TR, Esper GJ, Winton EF (2009) Acquired copper deficiency: a potentially serious and preventable complication following gastric bypass surgery. *Obesity,* ***17:*** *827.*