The Role of Yeast Beta Glucan on Blood Coagulation in Streptozotocin-Induced Diabetes and Irradiated Rats

M.M.A. El-Kashoury1, S.M. Abdel Fattah1, L.A. Ramadan1 and E.S. El-Denshary2

1Department of Drug Radiation Research, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt
2Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Egypt

Received: 20/2/2015 Accepted: 2/5/2015

ABSTRACT

Clotting abnormalities are observed after exposure to ionizing radiation as well as in diabetes mellitus. The objective of this study is to elucidate the role of yeast beta glucan (YBG) in the modulation of some biochemical variations observed in γ-irradiated, diabetic and diabetic γ-irradiated rats.

Gamma-irradiation was performed through the whole body exposure of rats to 6 Gy administered in four fractions of 1.5 Gy two times per week for two weeks. Diabetes was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg body weight). YBG was given orally to male albino rats (1 g/kg body weight) for two weeks post irradiation and/or induction of diabetes.

Animals were divided into 4 main groups: 1- control, 2- γ-irradiated, 3- diabetic and 4- diabetic-γ-irradiated rats. Each group was subdivided into 2 subgroups (a) untreated and (b) treated. The 3rd and 14th day, after the last dose of radiation in the irradiated groups and after the induction of diabetes in diabetic groups, were chosen to evaluate the effect of oral YBG in irradiated and/or diabetic rats.

The results revealed that the body weight decreased significantly in irradiated, diabetic and diabetic–irradiated rats. The loss of weight was accompanied by a reduction in the pancreas weight. Glucose concentration was significantly increased in diabetic group at the two time intervals. It is worth noting that, radiation ameliorated blood glucose level in diabetic-γ-irradiated group. Radiation exposure and/or diabetes caused an oxidative stress manifested by a significant increase of malondialdehyde (MDA) accompanied by a significant decrease in glutathione (GSH) level. This oxidative stress caused disturbances in the measured clotting parameters by enhancing platelet aggregation (PA) induced by arachidonic acid and increased thrombin level as concluded from the significant shortening of prothrombin time (PT) and activated partial thromboplastin time (APTT). Also, exposure to radiation and/or diabetes disturbed blood counts by decreasing red blood cells (RBCs); white blood celld (WBCs) and platelets and increasing platelet indices platelet mean volume (MPV) and platelet distribution width (PDW) as well as disturbances in lipid profile. The levels of these estimated parameters approached to normal levels by YBG treatment.

The obtained results show that YBG ameliorate hyperglycemia, exhibit antiplatelet activity, acts as immune enhancer by improving blood counts, ameliorate dyslipidemia and atherosclerotic index. It is concluded that YBG minimizes the harmful effects induced by exposure to ionizing radiation and reduces various complications of diabetes

Key Words: Yeast Beta Glucan/ γ-irradiation/ Coagulation/ Diabetes Mellitus.
INTRODUCTION

In recent years, a great deal of research has taken place in the area of free radicals and reactive oxygen species (ROS). It is now believed that they are either initiating or promoting agents in nearly every known disease (1). Aerobic cells have multiple defense mechanisms against free radical attack. There are both enzymatic defense systems and non-enzymatic antioxidants. Free radicals are generated in both the aqueous and lipid portions of the intracellular and extracellular environments. Therefore, it is crucial for the body to have a combination of water-soluble and lipid-soluble antioxidants to provide the full range of protection. Some antioxidants are synthesized by the body, whereas others must be obtained from food or food supplements (2). The oxidative stress that results from excessive free radical formation and limited antioxidant defense leads to changes in proteins, lipids, polysaccharides, and DNA in biological systems (3). Eventually, it results in pathophysiological conditions such as aging, cardiovascular disease, Alzheimer’s disease, and diabetes (4,5). In recent years, much attention has been focused on the role of oxidative stress, and it has been reported that oxidative stress may constitute the key and common event in the pathogenesis of secondary diabetic complications (6,7). So, one of the important goals of DM treatment is to prevent its complications (8).

Radiation injury of blood vessels was originally described more than a century ago and remains a contemporary clinical problem, despite dramatic advances in the field of radiation oncology (9). Ionizing radiation (IR) is associated with an increased risk of thrombotic occlusion of vessels and organ fibrosis (10,11). Also, Secondary vascular complications are frequently observed in patients with diabetes mellitus. The role of diabetes as an independent risk factor for cardiovascular disease has been well established in many studies (12).

Although almost all organisms possess antioxidant defense and repair systems that have evolved to protect them against free radicals, these systems are insufficient to protect them completely against oxidative damage (13). Many of the types of damage observed after irradiation can be ameliorated by antioxidants (14). Restriction on the use of synthetic antioxidants due to their carcinogenic nature has led to a growing interest in recent years in natural antioxidants of plant origin. The potential value of antioxidants has prompted researchers to look for natural antioxidants with low cytotoxicity (15).

Hofer and Pospisil (16) reported that glucan is a stimulator of the function of the reticuloendothelial system and a modulator of cellular and humoral immunity. Glucan was successfully tested from the point of view of stimulation of nonspecific immunity against bacterial, viral, mycotic and microparasitic infections, as well as against malignant cell growth. YBG has beneficial effects on the immune system and are claimed to have no toxic or adverse effects (18).

The aim of the present study is to evaluate the beneficial role of yeast beta glucan in minimizing radiation hazards and diabetic complications.

MATERIAL AND METHODS

Animals

Male Wistar rats purchased from the Research Institute of Ophthalmology (Giza, Egypt) weighing 150-200 g were used in this study. Animals were maintained under standard conditions of ventilation, temperature, humidity, lighting (light/dark: 13h/11h) and fed on standard pellets diet containing all nutritive elements (proteins, fats, carbohydrates, vitamins, salts and minerals). Food and water were available ad libitum. All animal procedures were carried out in accordance to the Ethics Committee of the Faculty of Pharmacy, Cairo University conformed to the “Guide for the care and use of Laboratory Animals” published by the National Institutes of Health (NIH publication No. 85–23, revised 1996).

Irradiation Procedure

Rats were whole body γ-irradiated at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt, using an AECL Gamma cell-40, which is a Cesium-137 irradiation unit manufactured by Atomic Energy of Canada Limited. Animals received 6 Gy administered in four doses of 1.5 Gy two times per week for two weeks at a dose rate of 0.43 Gy/min.
Induction of Diabetes

Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) (55 mg/kg body weight) to animals fasted overnight (17) in 0.1 citrate buffer PH 4.5 freshly prepared before injection. Diabetes was verified 48 h later by measuring tail vein blood glucose, and rats with blood glucose 300 mg/dl or more were considered diabetic (17).

Beta Glucan Treatment

Yeast beta glucan (Shaanxi Sciphar Hi-Tech Industry Co., Ltd, China), was dissolved in distilled water and administered to the experimental animals (1g/kg body weight) by oral intragastric tube daily for two weeks (18).

Experimental design and animal groups: Animals were categorized into four groups each one subdivided in two subgroups (a and b) as follows:

Group 1: Control groups
a- Control group not treated with yeast beta glucan (30 rats): was given a single intraperitoneal (i.p) injection of citrate buffer pH 4.5.

b- Control group treated with yeast beta glucan (30 rats): given a single intraperitoneal (i.p) injection of citrate buffer pH 4.5 and 48 h later received YBG treatment daily for two weeks.

Group 2: Gamma irradiated groups
a- Gamma-irradiated control group (45 rats): given a single intraperitoneal (i.p) injection of citrate buffer pH 4.5 and 48 h later exposed to γ-irradiation regimen.

b- Gamma-irradiated group treated with yeast beta glucan (45 rats): given a single intraperitoneal (i.p) injection of citrate buffer pH 4.5 and 48 h later exposed to the gamma-irradiation regimen then followed by YBG daily for two weeks starting post the last γ-irradiation dose.

Group 3: Diabetic groups
a- Diabetic control group (45 rats): rats with blood glucose 300 mg/dl or more.

b- Diabetic group treated with yeast beta glucan (45 rats): Diabetic rats receiving YBG treatment daily for two weeks starting 48 h after STZ injection.

Group 4: Diabetic gamma irradiated groups
a- Diabetic-γ-irradiated control group (n=45): Diabetic rats exposed to γ-radiation regimen 48 hr after STZ injection.

b- Diabetic-γ-irradiated group treated with yeast beta glucan (n=45): Diabetic rats exposed to γ-radiation regimen followed by YBG treatment daily during 2 weeks starting post the last γ-radiation dose.

Normal control rats and diabetic rats, not subjected to YBG treatment, were sacrificed 3 and 14 days post citrate buffer or STZ injection, respectively.

γ-irradiated and diabetic-γ-irradiated rats not subjected to YBG treatment were sacrificed 3 and 14 days post the last γ-irradiation dose.

Normal control, γ-irradiated, diabetic and diabetic-γ-irradiated rats given YBG were sacrificed 3 and 14 days post YBG treatment.

Blood Sampling and Analysis

Rats were anaesthetized with diethyl ether; then weighed. Samples were withdrawn from the retro-orbital venous plexus. Plasma were separated using non-heparinized capillaries in sterile sodium fluoride tubes for measuring glucose level (Glu), in ethylene diamine tetra acetic acid (EDTA) tubes for measuring the haematological parameters complete blood count (CBC) and in sterile 3.2% sodium citrate tubes for measuring platelet aggregation test (PA), prothrombin time (PT) and activated partial thrombinoplatin time (APTT) and heparinized capillaries for measuring malondialdehyde (MDA) and glutathione (GSH). Serum was separated using non heparinized capillaries in sterile plain tubes for measuring lipid profile.
For the determination of body and pancreas weight: Rats were weighed then killed by cervical dislocation, the pancreas was immediately excised washed with cold saline, blotted and weighed.

Glucose was determined using reagent kit (Stanbio, USA), according to Young (19). Malondialdehyde (MDA) and reduced glutathione (GSH) levels were determined according to Yoshioka et al. (20) and Beutler et al. (21), respectively.

Platelet aggregation test was determined using reagent kit (Bio/Data Corporation, USA) using Apat 4004, platelet aggregometer, Germany according to McCabe-White & Jennings (22) and Bain et al. (23).

Prothrombin time (PT) and activated partial thromboplastin time (APTT) were determined using reagent kit (Biomed, Egypt), as indicated by Hirsh et al. (24) and CRC (25), respectively.

The haematological parameters including haemoglobin (Hb), haematocrit (Hct), red blood cells count (RBCs), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, mean platelet volume (MPV), platelet distribution width (PDW), total leucycytic count (TLC), neutrophils, lymphocytes, monocytes, eosinophils and basophils were determined using Sysmex XE 2100 and XT 2000i operator manual, USA as indicated by Bain et al. (23).

For the determination of lipid profile: Total lipids were determined using reagent kit (Constant Medical Scientific, Egypt), according to the method of Frings et al. (26). Total cholesterol (TC), triglycerides (TG) and high density lipoprotein-cholesterol (HDL-C) were determined using the corresponding reagent kit (Stanbio, USA) as described by Stein (27), Buccolo & David (28), and NIH (29), respectively. Low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and atherogenic index (AI) were determined according to Friedewald et al. (30) equations: LDL-C = TC- HDL-C- (TG/5); VLDL-C = TG/5 and AI = (TC- HDL-C)/ HDL-C. Antiatherogenic index (AAI) was determined according to Guido & Joseph (31) AAI = (HDL-C×100)/(TC-HDL-C). In addition, risk factor TC/HDL-C ratio, TC/LDL-C ratio and HDL-C/LDL-C ratio were determined according to the previous parameters.

All biochemical determination were done using spectro-photometric technique using UNICAM 8625,UV/VIS, England, Spectrophotometer.

Methods of disposal of remaining animals: Animals were disposed though the holocaust of Atomic Energy Authority.

Statistical analysis: Results are given as means ± SD. Comparisons between means were carried out using one-way ANOVA followed by the Tukey-kramer multiple comparisons test using Instat software, version 2 (Graphpad Software, Inc., Diego, USA).

RESULTS

Effect of YBG on Blood Glucose Level, Body Weight and Pancreas Weight in Different Groups of Rats (Fig. 1 A, B and C)

The diabetic control group displayed a highly significant increase in blood glucose level when compared to control, γ-irradiated and diabetic γ-irradiated groups (p <0.01). While, with all other groups including γ-irradiated, diabetic γ-irradiated control and treated groups, a highly significant decrease in blood glucose level occurred gradually during both intervals approaching the normal control level (p <0.01).

There was no significant difference in body weight observed between the different groups on the 3rd day (p >0.05), but on the 14th day, diabetic control group displayed a high significant decrease of body weight when compared with control, γ-irradiated and diabetic γ-irradiated groups (p <0.01). Meanwhile, all other groups including γ-irradiated, both diabetic γ-irradiated control and treated and
diabetic treated groups exhibited a high significant increase in the body weight approximating the normal control level ($p > 0.05$).

There was a non-significant difference between all groups of rats in the pancreas weight recorded the 3rd day ($p > 0.05$) but on the 14th day, diabetic control group showed a high significant decrease in pancreas weight ($p < 0.01$). The reduction of pancreas weight was less significant in diabetic $\gamma$-irradiated group ($p < 0.05$). In addition, both treated $\gamma$-irradiated and diabetic $\gamma$-irradiated groups showed a significant increase in the pancreas weight with some significant differences between groups approaching the normal control level.

Effect of YBG on Plasma MDA and Blood GSH in Different Groups (Fig. 2 A& B)

$\gamma$-irradiated, diabetic, and diabetic-$\gamma$-irradiated control groups displayed a highly significant increase in plasma MDA level ($p < 0.01$). However both treated $\gamma$-irradiated and diabetic $\gamma$-irradiated groups showed a significant decrease in the MDA level occurred especially in 14th day with some significant differences between groups but didn’t approach the normal control level.

$\gamma$-irradiated, diabetic, and diabetic $\gamma$-irradiated control groups displayed a highly significant decrease in blood GSH level ($p < 0.01$). Meanwhile with both treated $\gamma$-irradiated and diabetic $\gamma$-irradiated groups a significant increase in the GSH level occurred especially at the 14th day with some significant differences between groups but didn’t approach the normal control level.

Effect of YBG on Platelet Aggregation (Induced by Arachidonic Acid), Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) in Different Groups (Fig. 3 A, B & C)

$\gamma$-irradiated, diabetic and diabetic $\gamma$-irradiated control groups displayed a highly significant increase in platelet aggregation at the 3rd day which gradually decrease till the 14th day except diabetic control group which exhibited an increased platelet aggregation when compared with other groups ($p < 0.01$). However, all treated groups including $\gamma$-irradiated, diabetic and diabetic $\gamma$-irradiated groups exhibited a gradual highly significant decrease in platelet aggregation with some significant differences between groups at both intervals reaching approximately the normal control level.

$\gamma$-irradiated, diabetic and diabetic $\gamma$-irradiated control groups displayed a highly significant decrease of PT and APTT during both time intervals ($p < 0.01$). On the contrary, all other treated groups including $\gamma$-irradiated, diabetic and diabetic $\gamma$-irradiated groups showed a highly significant gradual increase of PT and APTT with some significant differences between groups during both time intervals but didn’t approach the normal control level.

Effect of YBG on Complete Blood Count In Different Groups (Fig. 4A, B, C, D and E; Fig. 5A, B and C and Fig. 6A, B, C, D and E)

In $\gamma$-irradiated, diabetic and diabetic $\gamma$-irradiated control groups a highly significant decrease in Hb, Hct, RBCs, MCV, MCHC, platelet count, WBCs, neutrophils, lymphocytes, monocytes and eosinophils as well as a high significant increased in MPV and PDW were recorded at both time intervals ($p < 0.01$). On the contrary, all other treated groups including the $\gamma$-irradiated, diabetic and diabetic $\gamma$-irradiated groups showed a highly significant increase in Hb, Hct, RBCs, MCH, platelet count, TLC, WBCs, neutrophils, lymphocytes, monocytes and eosinophils and high significantly decreased MPV and PDW were recorded at both time intervals with some significant differences between groups at both intervals reaching nearly normal control level.

Effect of YBG on on Lipid Profile in Different Groups (Fig. 7A,B, C, D,E and F and Fig. 8A,B, C, D)

$\gamma$-irradiated, diabetic and diabetic-$\gamma$-irradiated control groups showed a highly significant increase of total lipids (TL), TC, TG, LDL-C, VLDL-C, AI, HDL risk factor and TC/LDL-C ratio and a high significant decrease in HDL-C, AAI and HDL-C/LDL-C ratio at both time intervals ($p < 0.01$). On the other hand, all other treated groups including $\gamma$-irradiated, diabetic and diabetic $\gamma$-irradiated groups exhibited a highly significant decrease in the levels of TC, TG, LDL-C, VLDL-C, AI, HDL...
risk factor, TC/LDL-C ratio and TL and a highly significant increased HDL-C, AAI and HDL-C/LDL-C ratio at both time intervals with some significant differences between groups in both intervals approaching the normal control level.

Fig. 1A

Fig. 1B

Fig. 1C

**Fig. (1) A, B & C:** Effect of yeast beta glucan (YBG) on body weight, pancreas weight and blood glucose level in the different groups

G1a: Normal control group; G1b: Normal treated with YBG; G2a: γ-irradiated control group; G2b: γ-irradiated treated with YBG; G3a: Diabetic control group; G3b: Diabetic treated with YBG; G4a: Diabetic-γ-irradiated control group; G4b: Diabetic γ-irradiated treated with YBG. Each value represents a Mean ± SD. a: significantly different from normal control group at $p < 0.05$; b: high
significantly different from normal control group at \( p < 0.01 \) and \( p < 0.001 \); c: significantly different from \( \gamma \)-irradiated control group at \( p < 0.05 \); d: high significantly different from \( \gamma \)-irradiated control group at \( p < 0.01 \) and \( p < 0.001 \); e: significantly different from diabetic control group at \( p < 0.05 \); f: high significantly different from diabetic \( \gamma \)-irradiated control group at \( p < 0.01 \) and \( p < 0.001 \); g: significantly different from diabetic \( \gamma \)-irradiated control group at \( p < 0.05 \); h: high significantly different from diabetic \( \gamma \)-irradiated control group at \( p < 0.01 \) and \( p < 0.001 \).

Fig. (2) A & B: Effect of yeast beta glucan (YBG) on plasma malondialdehyde (MDA) and blood glutathione (GSH) levels in the different groups

G1a: Normal control group; G1b: Normal treated with YBG; G2a: \( \gamma \)-irradiated control group; G2b: \( \gamma \)-irradiated treated with YBG; G3a: Diabetic control group; G3b: Diabetic treated with YBG; G4a: Diabetic-\( \gamma \)-irradiated control group; G4b: Diabetic \( \gamma \)-irradiated treated with YBG. Each value represents a Mean ± SD. a: significantly different from normal control group at \( p < 0.05 \); b: high significantly different from normal control group at \( p < 0.01 \) and \( p < 0.001 \); c: significantly different from \( \gamma \)-irradiated control group at \( p < 0.05 \); d: high significantly different from \( \gamma \)-irradiated control group at \( p < 0.01 \) and \( p < 0.001 \); e: significantly different from diabetic control group at \( p < 0.05 \); f: high.
significantly different from diabetic control group at $p < 0.01$ and $p < 0.001$; g: significantly different from diabetic $\gamma$-irradiated control group at $p < 0.05$; h: high significantly different from diabetic $\gamma$-irradiated control group at $p < 0.01$ and $p < 0.001$

Fig. 3

**Fig. 3A**

**Fig. 3B**

**Fig. 3C**

Fig. (3) A, B & C: Effect of yeast beta glucan (YBG) on platelet aggregation, prothrombin time (PT) and activated partial thromboplastin time (APTT) in the different groups.

G1a: Normal control group; G1b: Normal treated with YBG; G2a: $\gamma$-irradiated control group; G2b: $\gamma$-irradiated treated with YBG; G3a: Diabetic control group; G3b: Diabetic treated with YBG; G4a: Diabetic-$\gamma$-irradiated control group; G4b: Diabetic $\gamma$-irradiated treated with YBG. Each value represents a Mean ± SD. a: significantly different from normal control group at $p < 0.05$; b: high significantly different from normal control group at $p < 0.01$ and $p < 0.001$; c: significantly different from $\gamma$-irradiated control group at $p < 0.05$; d: high significantly different from $\gamma$-irradiated control group at $p < 0.01$ and $p < 0.001$; e: significantly different from diabetic control group at $p < 0.05$; f: high
significantly different from diabetic control group at $p < 0.01$ and $p < 0.001$; g: significantly different from diabetic $\gamma$-irradiated control group at $p < 0.05$; h: high significantly different from diabetic $\gamma$-irradiated control group at $p < 0.01$ and $p < 0.001$

**Fig. 4A**

**Fig. 4B**

**Fig. 4C**

**Fig. 4D**

**Fig. 4 E**

**Fig. (4) A,B, C, D & E.** Effect of yeast beta glucan (YBG) on hemoglobin, hematocrit, RBCs, MCV and MCHC, in the different groups.

G1a: Normal control group; G1b: Normal treated with YBG; G2a: $\gamma$-irradiated control group; G2b: $\gamma$-irradiated treated with YBG; G3a: Diabetic control group; G3b: Diabetic treated with YBG; G4a: Diabetic-$\gamma$-irradiated control group; G4b: Diabetic-$\gamma$-irradiated treated with YBG. Each value represents a Mean ± SD. a: significantly different from normal control group at $p < 0.05$; b: high significantly different from normal control group at $p < 0.01$ and $p < 0.001$; c: significantly different from $\gamma$-irradiated control group at $p < 0.05$; d: high significantly different from $\gamma$-irradiated control group at $p < 0.01$ and $p < 0.001$; e: significantly different from diabetic control group at $p < 0.05$; f: high
significantly different from diabetic control group at $p < 0.01$ and $p < 0.001$; g: significantly different from diabetic $\gamma$-irradiated control group at $p < 0.05$; h: high significantly different from diabetic $\gamma$-irradiated control group at $p < 0.01$ and $p < 0.001$.

Fig. (5) A, B and C. Effect of yeast beta glucan (YBG) on Platelet count, MPV and PDW respectively, respectively in diabetic gamma/irradiated rats.

G1a: Normal control group; G1b: Normal treated with YBG; G2a: $\gamma$-irradiated control group; G2b: $\gamma$-irradiated treated with YBG; G3a: Diabetic control group; G3b: Diabetic treated with YBG; G4a: Diabetic-$\gamma$-irradiated control group; G4b: Diabetic $\gamma$-irradiated treated with YBG. Each value represents a Mean ± SD. a: significantly different from normal control group at $p < 0.05$; b: high significantly different from normal control group at $p < 0.01$ and $p < 0.001$; c: significantly different.
from γ-irradiated control group at $p < 0.05$; d: high significantly different from γ-irradiated control group at $p < 0.01$ and $p < 0.001$; e: significantly different from diabetic control group at $p < 0.05$; f: high significantly different from diabetic control group at $p < 0.01$ and $p < 0.001$; g: significantly different from diabetic γ-irradiated control group at $p < 0.01$ and $p < 0.001$; h: high significantly different from diabetic γ-irradiated control group at $p < 0.01$ and $p < 0.001$

**Fig. 6A**

**Fig. 6B**

**Fig. 6 C**

**Fig. 6 D**

**Fig. 6 E**

**Fig. (6) A,B, C, D & E**. Effect of yeast beta (YBG) on total leucocytic count, neutrophil, lymphocytes, monocytes and eosinophils, in different animal groups.

G1a: Normal control group; G1b: Normal treated with YBG; G2a: γ-irradiated control group; G2b: γ-irradiated treated with YBG; G3a: Diabetic control group; G3b: Diabetic treated with YBG; G4a: Diabetic-γ-irradiated control group; G4b: Diabetic γ-irradiated treated with YBG. Each value represents a Mean ± SD. a: significantly different from normal control group at $p < 0.01$; b: high significantly different from normal control group at $p < 0.05$; c: significantly different from normal control group at $p < 0.05$; d: high significantly different from normal control group at $p < 0.01$ and $p < 0.001$; e: significantly different from diabetic control group at $p < 0.05$; f: high significantly different from diabetic control group at $p < 0.01$ and $p < 0.001$; g: significantly different from diabetic γ-irradiated control group at $p < 0.05$; h: high significantly different from diabetic γ-irradiated control group at $p < 0.01$ and $p < 0.001$.
from $\gamma$-irradiated control group at $p < 0.05$; d: high significantly different from $\gamma$-irradiated control group at $p < 0.01$ and $p < 0.001$; e: significantly different from diabetic control group at $p < 0.05$; f: high significantly different from diabetic $\gamma$-irradiated control group at $p < 0.01$ and $p < 0.001$; g: significantly different from diabetic $\gamma$-irradiated control group at $p < 0.05$; h: high significantly different from diabetic $\gamma$-irradiated control group at $p < 0.01$ and $p < 0.001$

Fig. 7A, 7B, 7C, 7D, 7E, 7F. Effect of yeast beta glucan (YBG) on total lipids, total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol in different animal groups

G1a: Normal control group; G1b: Normal treated with YBG; G2a: $\gamma$-irradiated control group; G2b: $\gamma$-irradiated treated with YBG; G3a: Diabetic control group; G3b: Diabetic treated with YBG; G4a: Diabetic-$\gamma$-irradiated control group; G4b: Diabetic $\gamma$-irradiated treated with YBG. Each value represents a Mean $\pm$ SD; a: significantly different from normal control group at $p < 0.05$; b: high...
significantly different from normal control group at $p < 0.01$ and $p < 0.001$; c: significantly different from $\gamma$-irradiated control group at $p < 0.05$; d: high significantly different from $\gamma$-irradiated control group at $p < 0.01$ and $p <0.001$; e: significantly different from diabetic control group at $p < 0.05$; f: high significantly different from diabetic $\gamma$-irradiated control group at $p < 0.01$ and $p < 0.001$; g: significantly different from diabetic $\gamma$-irradiated control group at $p < 0.01$; h: high significantly different from diabetic $\gamma$-irradiated control group at $p < 0.01$ and $p < 0.001$

**Fig. 8A**

**Fig. 8B**

**Fig. 8C**

**Fig. 8D**

**Fig. 8E**

Fig. (8) A, B, C, D & E. Effect of yeast beta glucan (YBG) on atherogenic index, antiatherogenic index (%), HDL Risk Factor, (TC/HDL-C Ratio), (TC/LDL-C Ratio), and (HDL-C/LDL-C Ratio), in different animal groups.
G1a: Normal control group; G1b: Normal treated with YBG; G2a: γ-irradiated control group; G2b: γ-irradiated treated with YBG; G3a: Diabetic control group; G3b: Diabetic treated with YBG; G4a: Diabetic-γ-irradiated control group; G4b: Diabetic γ-irradiated treated with YBG. Each value represents a Mean ± SD. a: significantly different from normal control group at p <0.05; b: high significantly different from normal control group at p <0.01 and p <0.001; c: significantly different from γ-irradiated control group at p <0.05; d: high significantly different from γ-irradiated control group at p <0.01 and p <0.001; e: significantly different from diabetic control group at p <0.05; f: high significantly different from diabetic control group at p <0.01 and p <0.001; g: significantly different from diabetic γ-irradiated control group at p <0.05; h: high significantly different from diabetic γ-irradiated control group at p <0.01 and p <0.001.

DISCUSSION

Free radicals are important mediators in the complex pathogenesis of acute and chronic inflammatory reactions (2). In biological systems, inactivation and removal of free radicals depend on reactions involving the antioxidant defense system. Natural antioxidants from vegetables and fruits that can reduce the risk of chronic diseases such as diabetes have been the focus of much investigation (32). Beta glucans are polysaccharides of glucose that can be produced by many prokaryotic and eukaryotic organisms. This group of compounds has several beneficial properties and because of that they have found a wide variety of uses in human and in veterinary medicine, pharmaceutical, cosmetic and chemical industries as well as food and feed production (33). Beta glucan activates the immune response through the immune cells, called macrophages, showing various therapeutic effects (34).

In the present study, the body weight and pancreas weight of untreated irradiated, diabetic and diabetic γ-irradiated rat groups were reduced significantly. The results also revealed that exposure of rats to radiation didn’t change glucose concentration in irradiated animal groups, while it caused a hypoglycemic effect in diabetic γ-irradiated groups.

The body weight reduction observed post-irradiation of rats could be due to anorexia and intestinal damage provoking a reduction in gastric secretion associated with a great decrease in acidity. However, absorption would also be impaired as an extensive injury to the gastrointestinal tract following γ-irradiation. The results confirmed that there was a significant decrease in pancreas weight due to irradiation of rats (35). The loss in body weight of diabetic animals agrees with that found by Oyedemi et al. (36) who observed a similar effect. The explanation of this reduction has been possibly linked to degradation of structural proteins and muscle wasting.

The results of the present study showed that the gain of body weight was achieved by oral administration of yeast beta glucan. Urao et al. (37) suggested that the glucan administration allowed the beneficial microorganisms e.g. *bifidobacterium* to quickly reproduce in the animal intestine. These beneficial microorganisms can synthesize vitamins and amino acids, stimulate immunoglobulin activity and improve immune function. The anti-diabetic effect of beta glucans was attributed to its activation of macrophages, the main source of IL-1 in the body, which increases insulin production resulting in lowering of blood glucose level (38). Increasing in the insulin level by yeast beta glucan administration may prevent catabolism of structural protein and increases the peripheral utilization of glucose leading to improvement in body weight and reduction in the blood glucose levels in the YBG treated diabetic rats. Moreover, the effect of yeast beta glucan to reduce blood glucose could be mediated possibly by delaying stomach emptying so that dietary glucose is absorbed more gradually (39). Yeast beta glucan passes the stomach virtually un-changed. In the intestine, there are macrophages that inhabit the intestinal wall and are able to pick yeast beta glucan particles through beta glucan receptors via phagocytic transport mechanism (40). Another possible mechanism for beta-glucans to reduce blood glucose level is mediated by signal pathway through PI3K/Akt activation. Decreased PI3K/Akt activity has been shown to play a key role in the pathogenesis of diabetes. Beta-glucans have been demonstrated to increase PI3K/Akt through several receptors (41).
Radiation exposure induced hypoglycemia in experimental rats. This conclusion could be attributed to inhibition of glucose absorption by damaged intestinal epithelium of irradiated intestine. Also, direct effect of radiation on the pancreatic β-cells of islets of Langerhans, thus stimulating rise in insulin secretion (42). The results of Ashry et al. (42) showed an increase in glucose oxidation subsequent to the increase in oxygen consumption as a result of stimulation of thyroid gland activity by gamma irradiation.

Data of the current study revealed that irradiation of rats and STZ administration caused a significant increase in plasma MDA level accompanied by a significant reduction in blood GSH level.

The interaction of ionizing radiation with biological system results in generations of free radicals, H and OH radicals, H₂ and H₂O₂. Radiations-induced free radicals in turn impair the antioxidant defense mechanism leading to increased membrane lipid peroxidation, which results in the damage of membrane bound enzymes (43,44). Also, it is well known that oxidative stress is a contributor to the development of complications in DM. Previous studies have demonstrated that diabetes exhibits enhanced oxidative stress and high reactive oxygen species in pancreatic islets due to persistent and chronic hyperglycemia, thereby depletes the activity of antioxidative defense system, and thus promotes free radical generation (45).

The oxidative stress due to diabetes which induced complications in body hemostasis agrees with that found by Shukla et al. (46) and Kakadiya et al. (47).

Treated animals with yeast beta glucan showed an improvement of oxidative stress parameters due to the antioxidant properties of yeast beta glucan. Cheng et al. (48) reported that the control of hyperglycemia leads to improvement in oxidative stress profile, and enhancing antioxidant defense mechanisms in pancreatic islets helps them to cope better with oxidative stress.

The present results showed increased platelet aggregation on the 3rd day in irradiated and diabetic irradiated groups which then decreased on the 14th day. Diabetic group exhibited an increased platelet aggregation throughout the experimental periods and shortened PT and APTT in untreated groups at both days.

Thrombosis induced by radiation has been explained in many studies. The vascular endothelium is believed to be a target for radiation-induced injury. Overexpression and increased activity of tissue factor (TF) have been shown to be involved in radiation-induced changes (49). In response to IR, monocytes have been reported to produce inflammatory cytokines such as TNF-α. NFkB, one of the main mediators of cellular responses involved in inflammation, apoptosis and regulation of TF expression, was documented to be activated by IR through a cascade requiring endogenous TNF-α production (50).

Oxidative stress up-regulates numerous pathways pertinent to vascular disease, including matrix metalloproteinases, adhesion molecules, pro-inflammatory cytokines, and smooth muscle cell proliferation and apoptosis, while inactivating vasculoprotective nitric oxide. Importantly, NF-kB is controlled by redox regulation, making it a prime candidate to link chronic oxidative stress to activation of downstream inflammatory pathways in radiation injury (51). The study by Halle et al. (52) provides the first direct evidence that NF-kB is chronically up-regulated in human arteries after radiation exposure.

Platelets functions are significant to understanding the pathophysiology of vascular disease in diabetes. The role of hyperglycemia is not clear in platelet hyperactivity in diabetic patients (53). Increased level of lipid peroxides might activate the release of arachidonic acid from phospholipids and subsequently amplify platelet activation (54).

Platelet dysfunction may develop before vessel wall damage in diabetes (55,56). Platelet dysfunction in diabetes, including altered adhesion and aggregation, is hypersensitivity to agonists (57).

An elevated level of prothrombin has also been associated with thrombosis, and elevated levels of prothrombin lead to increased thrombin generation in an in vitro model of hemostasis. Thus, it
seems likely that elevated prothrombin levels could contribute both to thrombotic risk and to a shortening of the PT and APTT (58).

The in vitro results of Saluk-Juszcak et al. (59,60) demonstrate that antiplatelet activity of beta-glucan from Saccharomyces cerevisiae is dependent on its antioxidative properties. They stated that beta glucan supplementation may be beneficial in the prevention of excessive blood platelet activation-related diseases, such as cardiovascular or inflammatory diseases.

In untreated rat groups it was observed that a significant reduction occurred in hemoglobin level, red blood count and their indices (Hct, MCV, MCH, MCHC). A reduction occurred in platelet count and elevation of platelet indices (MPV & PDW). Finally reduction was observed in total leucocytic count and differential count (neutrophils, eosinophils, monocytes and lymphocytes).

Hematopoietic cells are highly sensitive to radiation damage and relatively low levels of exposure can result in bone marrow failure and potentially lethal hemorrhage or infections. The damaging effects of radiation on hematopoiesis have been well established (61). The observed effects are due to both a decrease in the number of hematopoietic stem cell progenitors and a reduction in the self-renewal capacity of stem cells (62,63).

The current results showed that gamma irradiation of rats caused suppression in RBCs count, Hct value and Hb content as well as total WBCs, lymphocytes and neutrophils counts. These results are in agreement with those of Hanafi et al (64) and Salama (65). This is complicated by thrombocytopenia and concomitant hemorrhages besides effects in adaptive immune system resulting from apoptosis of lymphocytes and deficient lymphopoiesis (66). Ionizing radiation is known to induce oxidative stress. By this oxidative imbalance, irradiation kills or damages the major classes of parenchymal cells of the lymphohaematopoietic system, depresses the number of the highly radioprotective bone marrow cells (the major of haematopoiesis) and causes atrophy of spleen which are clear in the results of Salama (65).

The occurrence of anaemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins (36). Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC (66). Reduction of levels of RBC, Hb, haematocrit and leucocytes in the diabetic animals may be attributed to the infections of the normal body systems (67). The intraperitoneal injection of streptozotocin into rats significantly reduced the WBC count and its differentials such as basophils, monocytes, eosinophils, lymphocytes and neutrophils. The reduction of these parameters could be linked to suppression of leukocytosis from the bone marrow which may account for poor defensive mechanisms against infection (36). Consequently, they might have effects on the immune system and phagocytic activity of the animals (68). Medications decrease PLT counts in two ways, either by suppression of bone marrow or by destruction of PLTs in peripheral blood via immune mechanism (69).

Reduction of platelets levels in diabetic rats induced with streptozotocin was confirmed in this study in relation to the normal control rats. Long term reduction of this parameter may result in internal and external haemorrhage and finally leads to death (39).

The mean platelet volume is increased in type 2 diabetes mellitus. This increase can be due to increased number of younger platelets in diabetics. Younger platelets have increased platelet volume. Increased endothelial damage is seen in diabetes mellitus which reduces the survival of platelets and increases turnover of younger platelets. Moreover MPV is an indicator of the average size and activity of platelets. Large platelets are younger, more reactive and aggregateable as they contain denser granules, secrete more serotonin and β-thromboglobulin, and produce more thromboxane A2 than smaller platelets (70). All these can produce a pro-coagulant effect and cause thrombotic vascular complications. This suggests a relationship between the platelet function especially MPV and diabetic vascular complications thus indicating that changes in MPV reflect the state of thrombogenesis. Function is directly regulated by insulin via a functional insulin receptor (IR) found on human platelets (71). High MPV is emerging as a new risk factor for the vascular complications of DM of
which atherothrombosis plays a major role (72). Thus, DM has been considered as a “prothrombotic state” with increased platelet reactivity (73).

The results of Riahi-Zanjani et al. (74) indicate that long term low dose ionizing radiation may have side effects on thrombocytosis and coagulation function. They measured PDW of the radiation workers and found that it was significantly higher than the control group. The present results showed that oral gavage treatment of beta glucan caused improvement in RBCs count, Hct value and Hb content as well as total WBCs, absolute lymphocytes and neutrophils counts. Zhao et al. (75) and Yuan et al. (76) recorded an enhanced cellular immune response to glucan administration. It is well documented that total body irradiation followed by β-glucan has been shown to raise the erythropoietic activity in both bone marrow and spleen (77).

Beta glucan of different origin has been demonstrated to be potent anti-oxidants, prevent damage by H2O2 and other reactive oxygen species (78). In addition, there are some reports on the immune-antioxidant activity relationship of glucan (79,80), which may result in proliferation of bone marrow stem cells as indicated by increased in bone marrow cell count and its viability in the study performed by Salama (74). This phenomenon was also observed by Pospilil et al. (81) and Patchen et al. (82) who concluded that the improvement effects of beta glucan when given to animals submitted to radiation, was not due only to hematopoietic regeneration, but also the capacity of this substance to inactivate free radicals.

The use of highly purified, orally administered yeast beta glucan accelerated the early recovery of peripheral blood leukocytes following sublethal irradiation. On the other hand, yeast beta glucan could modulate the autoimmune mechanisms directed to pancreatic islets and inhibit the development of diabetes in rats. Furthermore, it could reduce carbohydrate absorption from the gut (83).

Immunomodulation by beta glucan was confirmed both in vitro and in vivo in numerous animal and human studies involving a wide range of tumors, including breast, lung and gastrointestinal cancers (84). The immunomodulating and cancerostatic properties make β-glucan one of the substances with a great potential in the ongoing fight against cancer (33,85). At the same time, only a few drugs have similar advantages as beta glucan.

In the present study, serum lipid profile was altered in irradiated as well as diabetic groups characterized by increasing total lipids, total cholesterol, triglycerides and LDL cholesterol levels accompanied by decrease in HDL cholesterol. These disturbances influence other lipid profile indices. Improvements of lipid profile was achieved by β-glucan treatment as compared with control group.

In case of irradiation effects, free radicals impair liver functions and can be a major reason of hormonal imbalance. This imbalance induces hyperlipidemia through its multiple effects on lipid metabolism, including increased synthesis of cholesterol and triglyceride (86). Results of the present study are in agreement with previous findings demonstrating an increase of plasma level of lipids of rats post irradiation (87,88). They attributed the hypercholesterolemia conditions to the stimulation of cholesterol synthesis in the liver after γ-irradiation. Moreover, Bok et al. (89) attributed the irradiation-induced hypercholesterolemia to the increase of activation of HMG-CoA reductase enzyme, the key regulatory enzyme in the reduction of the overall process of cholesterol synthesis. Sedlakova et al. (90) explained that the increase in serum triglyceride level after irradiation might result from the inhibition of lipoprotein lipase activity, leading to reduction in uptake of triacylglycerols. Mahmoud (91) attributed the hyperlipidemic state under the effect of gamma-irradiation to the stimulation of liver enzymes responsible for the biosynthesis of fatty acids and mobilization of fats from adipose tissue to blood stream.

Diabetes associated dyslipidemia is a major risk factor for CVD (92). The dyslipidemia is caused either by insulin resistance or adipocytokines. In diabetes, adipose cells are insulin resistant, thus, insulin-mediated uptake of free fatty acids in skeletal muscle is impaired. Increased circulating free fatty acids flux to the liver, results in increased triglyceride synthesis and the assembly of very low-density lipoprotein (VLDL) (93). Thus, the characteristics of dyslipidemia in the patients with diabetes is hypertriglyceridemia. Hyperglycemia and low insulin may also contribute to VLDL production (93).
In diabetes, adiponectin is reduced, which increases muscle free fatty acid uptake and reduces plasma free fatty acid level (84). This mechanism is independent of insulin-resistance (85). In addition, high density lipoprotein (HDL) may also decrease (96).

Beta glucan has been shown to decrease LDL cholesterol and increase HDL to alleviate possibly dyslipidemia and reduce CVD (97). This altered serum lipid profile was reversed towards normal after treatment with beta glucan. The possible mechanism through which beta glucan exerts its anti-hyperlipidemic effect might include the changed activity of cholesterol biosynthesis enzymes and/or the changed level of lipolysis which are under the control of insulin (98).

Chen & Huang (99) suggested that beta glucans sequester bile acids in the intestine, reducing their reabsorption and return to the liver. Reducing hepatic bile acid concentrations activates the enzyme CYP7A1, which converts cholesterol into bile acids. Drozdowski et al. (100) concluded that the reduced intestinal fatty acid uptake after beta glucan administration is associated with inhibition of genes regulating intestinal uptake and synthesis of lipids. Salama (67) results strongly indicated that beta glucans may up-regulate low-density lipoprotein receptor gene. Moreover, it has been reported that yeast beta glucan treatment could decrease the capacity of LDL to carry free cholesterol to various tissues without affecting the capacity of HDL to carry cholesterol back to the liver in rats (101).

**CONCLUSION**

From the present results it could be concluded that oral yeast beta glucan administration possesses radioprotective and anti-hyperglycemic properties. In addition, it could prevent various complications of diabetes as well as improving some hematological parameters.

**REFERENCES**


Do platelets have anything to do with diabetic microvascular disease? Diabetes, 32(2), 14-19 (1983).

Diabetes mellitus, hypercholesterolemia, and hypertension but not vascular disease per se are associated with persistent platelet activation in vivo. Evidence derived from the study of peripheral arterial disease. Circulation, 96(1), 69-75 (1997).


(100) A. Drozdowski, R. Reimer, F. Temelli, C. Bell, T. Vasanthan, and A. Thomson, β-Glucan extracts inhibit the in vitro intestinal uptake of long-chain fatty acids and cholesterol and down-