

Physiological and Histological Studies on Glucan as Modulator of Hazardous Effects in Rats Treated with Cyclophosphamide and Exposed to γ -Radiation

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Abstract

Aim of the work: β -glucan which is known as biological response modifiers and immunostimulator was investigated in this study to minimize the toxicity of chemo-and radiotherapy induced by cyclophosphamide (CYP) and radiation exposure in rats. **Materials and methods:** β -glucan was orally administered in a dose of 10 mg/kg b.wt. daily against the hazardous impacts of γ -irradiation (1 Gy daily up to 10 Gy) and/or CYP (50 mg/kg b.wt. every other day for 2 weeks). Determination of some biochemical analysis was carried out including calcium, alkaline phosphatase, creatine kinase, lactate dehydrogenase. Hematological analysis was performed on leucocytes and platelets counts. Additionally, histological study was also done on both lung and testis tissues. The experimental rats being sacrificed 1, 7 and 21 days post-treatment. **Results:** Treatment with CYP induced significant decrease in WBCs, platelets counts and alkaline phosphatase activity and significant increase of creatine kinase and LDH activities accompanied by a significant increase in Ca level only at the end of experimental period. Similar results were recorded with irradiated group accompanied with a non significant change in Ca level allover the experimental periods. Combined treatment with both γ -radiation and CYP intensified the effect of each other on most of the investigated parameters.

Microscopic examination of the lung revealed that both γ -irradiation and CYP showed disturbed structure of bronchioles, thickened alveolar walls together with scattered haemorrhagic areas. Signs of pneumonia and compensatory emphysema were also seen. Meanwhile, in testis, irregular seminiferous tubules with reduction in their sizes were observed. Besides, the intertubular spaces were fibrotic and devoid of Leyding cells. Bizarre-shaped spermatogenic cells were also prominent. In combined treatment, these lesions became much more progressed. However, glucan administration prior to treatment with CYP or γ -radiation led to an improvement in most of biochemical, hematological and histological parameters under investigation.

Conclusion: β -glucan has a good protective role against the toxic effects associated with chemo-and radiotherapy.

Introduction

Chemotherapy of cancer has opened new possibilities and chances for improving the quality and life span. Despite this successful trend, treatment with some of the most effective anticancer drugs caused many of toxic symptoms to normal cells. Combination of CYP and fractionated body irradiation was the most conditioning regimen in cancer therapy. These regimens are responsible for late and early complications and may provide higher toxicity rate (Goldberg and Lidsky, 1985).

One of the approaches to deal with this problem is to search for suitable naturally occurring agents that are able to stimulate defense mechanisms of the organisms. Among these agents are glucans, which belongs to the class of drugs, known as biological response modifiers.

β -1,3 D glucan, which is formed of a long-chained polysaccharide, is an effective antioxidant and free radical scavenger (Bobeck and Calbavy, 2001) and effective in preventing coronary heart disease by

significantly lowering low density lipoprotein (LDL) blood cholesterol and increasing high density lipoprotein (HDL) cholesterol levels (Kerckhoffs *et al.*, 2003). Glucan also possesses antitumor activity by enhancing body's capacity to destroy cancer cells (Seljelid and Raa, 2002). β -1,3-glucans are present in variety of living systems including fungi, yeast, algae, various bacteria, and cereal.

Pulmonary drug toxicity is increasingly being diagnosed as a cause of acute and chronic lung disease. Cyclophosphamide is one of the chemotherapeutic agents generally regarded as potentially hazardous to the lungs. In 1975, Gould and Miller reported that cyclophosphamide (20 mg/100 gm b.wt. daily for two weeks) induced alveolar septal thickening. Single therapeutic dose of CYP caused moderate to severe interstitial pneumonitis, pulmonary haemorrhages and oedema together with alveolar wall metaplasia (Cohen and Mathews, 1983). Lymphoid lung infiltration was detected following i.p. injection of CYP at a dose of 50 mg/kg in rats (Lurie *et al.*, 1991). Synergistic effects of CYP and irradiation dose rate was studied by Safwat *et al.* (1996), they stated that combination of CYP with γ -rays at a high dose rate (0.8Gy/min) was more toxic to the lungs than low dose rate (0.7 Gy/min). Distorted bronchioles with thickened interalveolar septa, interstitial fibrosis and oedema were also noted post-CYP treatment by Rossi *et al.* (2000) and Field *et al.* (2002). Testicular atrophy could be seen following CYP treatment (Buchanan *et al.*, 1975). CYP also produced disturbance of the normal spermatogenic architecture that was dose and time dependent (Trasler *et al.*, 1988 and Matsumoto *et al.*, 2000).

Radiation damage of the lung was studied by Abu El-Naga (1989) who noticed narrow alveolar sacs with severe degenerative changes in alveolar cells in the lungs of fetuses maternally exposed to 2 Gy γ -rays on day 12 of gestation. In 2004, Osterreicher *et al.* noticed depletion of type II pneumocytes and increased alveolar neutrophils post-irradiation at a dose of 1 Gy. Also, inflammatory response was

observed in mice lung after whole body γ -irradiation at a dose of 15 Gy, while with a dose of 20 Gy, severe lung fibrosis was seen (Van der Meeren *et al.*, 2005).

Fertility and sterility have been always a matter of arguing as to their response to radiation exposure. Destroyed seminiferous tubules and depletion of spermatogonial cells were noted by Kumar *et al.* (1981) at a dose of 9.5 Gy of ^{60}Co external irradiation. Kamtchouing *et al.* (1988) observed degenerated germ cells after low-dose γ -irradiation of mature rats. Also, destroyed spermatogonia and elongated spermatids were seen after 9Gy of γ -irradiation (Pinon-Lataillade *et al.*, 1991). Guitton *et al.* (2000) reported that γ -irradiation reduced sperm production, testis weight, tubule diameter and the sperm head count. In 2004, Colpi *et al.* reported that spermatozoa cannot tolerate irradiation doses higher than 6 Gy and Leydig cells are damaged by doses higher than 15 Gy. Furthermore, Sayed *et al.* (2005) noticed destruction of the spermatogonia with absence of mature sperms post-exposure to 6 Gy X-ray.

In order to simulate the conditions of cancer patients who undergo chemo- and radiotherapy, as well as the conditions commonly practiced in either occupational or radiological medical exposures encountering intermittent radiation exposure, this study was planned to investigate the protective effect of β -glucan as natural antioxidant against the toxic effects of fractionated doses of γ -irradiation and / or cyclophosphamide treatment. The antioxidant effect of β -glucan was assessed by some hematological and biochemical analysis. Furthermore, histological study on lung and testis tissues was also carried out.

Materials and Methods

1-Gamma-irradiation procedure:

Irradiation process was performed using Gamma Cell-40 achieved by Egypt's National Center for Radiation Research and Technology (NCRRT), Cairo. The dose rate was 0.667 Gy/min. at the time of experimentation.

2- Experiment design: 160 Albino rats weighing 130-150 g were divided into 8 groups, each of 20 rats:

- **Control group**
- **Cyclophosphamide (CYP) group:** CYP was administered i. p. to rats at a dose of 50 mg/kg b.wt. (Paget and Barnes, 1964) every other day for 2 weeks.
- **Irradiated group:** Irradiation was delivered at fractionated doses of 1 Gy daily up to 10 Gy.
- **Cyclophosphamide-Irradiated group:** Animals were i.p. injected with CYP every other day for 2 weeks and then, they were irradiated at the end of the two weeks to 1 Gy daily up to 10 Gy.
- **Glucan treated group:** Animals were i.p. injected with β -1, 3 glucan at a dose level of 10 mg/kg b.wt. for 2 weeks. β -1, 3 glucan was purchased from Fluke Chemical Company. The used glucan was isolated from *Euglena gracilis*.
- **Glucan-Cyclophosphamide treated group:** Animals were treated with glucan daily for 2 weeks and one hr prior to cyclophosphamide injection.
- **Glucan-Irradiated group:** Glucan was administered at a dose of 10 mg/kg b.wt. one hr before exposure to γ -radiation at the same previous doses.
- **Glucan-Cyclophosphamide and Irradiated group:** Animals were treated with glucan before cyclophosphamide injection for 2 weeks and continuing for another 2 weeks parallel with exposure to fractionated doses of γ -radiation.

The experimental animals had been sacrificed 1, 7 and 21 days post-treatment.

3- Hematological and physiological studies: Fresh blood samples were collected for WBCs and platelets counting (Dacie and Lewis, 1991). Clear serum was separated for the determination of alkaline

phosphatase (ALP) (Babson *et al.*, 1966). Calcium, lactate dehydrogenase (LDH) and creatine kinase were also estimated (Biggs and Moorehead, 1974, Buhl and Jackson, 1987 and Rosalki, 1997) respectively. Results were expressed as the mean \pm standard error (S.E.). Differences between the groups were determined by student's t-test (Snedecor and Cochran, 1980).

4- Histological preparations: Following rats sacrifice, suitable pieces of lung and testis were removed quickly and fixed in 10% neutral formalin, then dehydrated, cleared and embedded in paraffin wax. Sections of 5 μ thick were cut and stained with hematoxylin and eosin (Drury and Wallington, 1980)

Results

A. Hematological and Physiological Results:

Treatment with CYP induced significant decrease in WBCs, platelets counts and alkaline phosphatase activity and significant increase of creatine kinase and LDH activities accompanied with significant increase in Ca level only at the end of experimental period. Similar to the effect of CYP treatment, exposure to γ -radiation resulted in the same results in the investigated parameters, accompanied with non significant change in Ca level all over the experimental periods. Combined treatment with γ -radiation and CYP intensified the effect of each other in most of investigated parameters. Glucan treatment alone induced non-significant changes in all studied parameters, except the decreased level of ALP and increased level of platelets which were observed. Glucan administration pre-CYP treatment or irradiation produced more or less improvement of most of the biochemical parameters in the different intervals of experimentation. (Figs. 1-6).

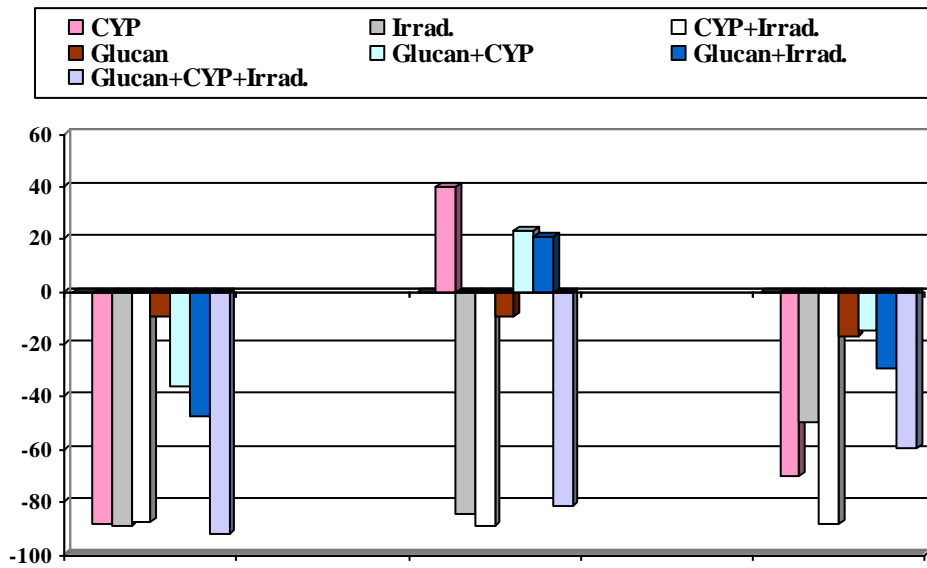


Fig.(1): Effect of cyclophosphamide and/or fractionated doses of γ -irradiation on WBCs ($\times 10^3/\mu\text{l}$) of rats pretreated with β -1, 3glucan

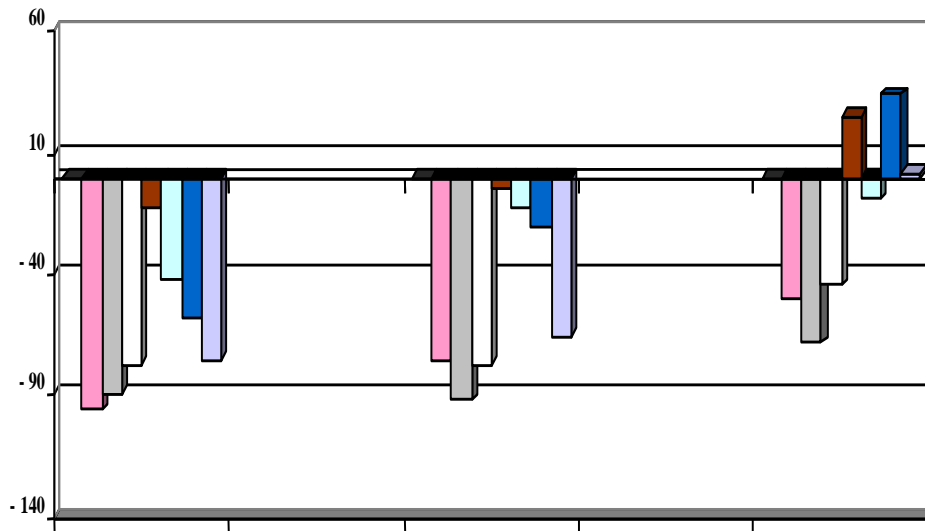


Fig. (2): Effect of cyclophosphamide and/or fractionated doses of γ -irradiation on platelets ($\times 10^3/\mu\text{l}$) of rats pretreated with β -1,3 glucan.

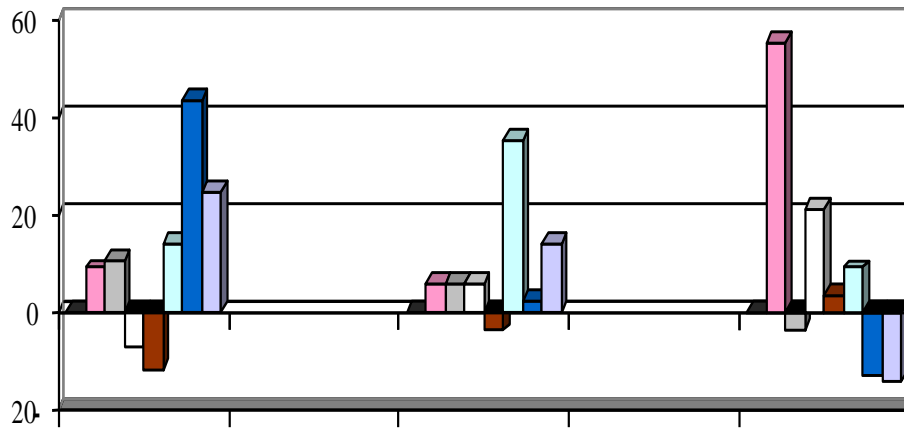


Fig.(3): Effect of cyclophosphamide and/or fractionated doses of γ -irradiation on serum calcium (mg/dl) of rats pretreated with β -1,3 glucan.

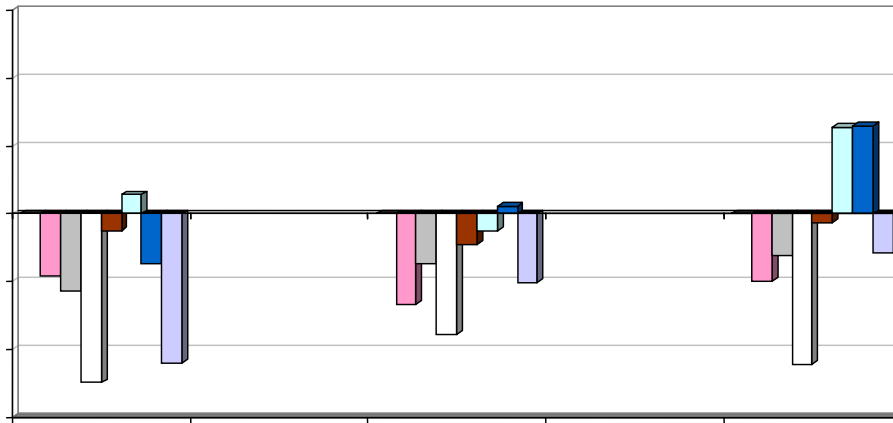


Fig.(4): Effect of cyclophosphamide and/or fractionated doses of γ -irradiation on serum alkaline phosphatase (U/L) of rats pretreated with β -1,3 glucan.

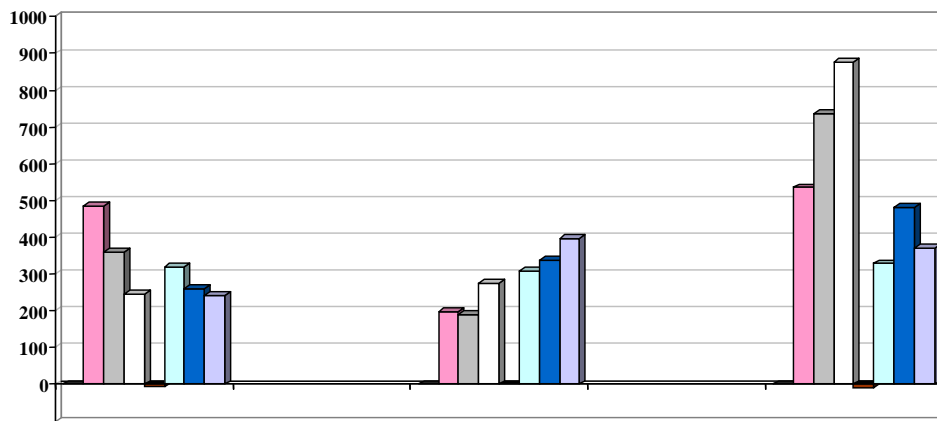


Fig.(5): Effect of cyclophosphamide and/or fractionated doses of γ -irradiation on serum LDH (U/L) of rats pretreated with β -1,3 glucan.

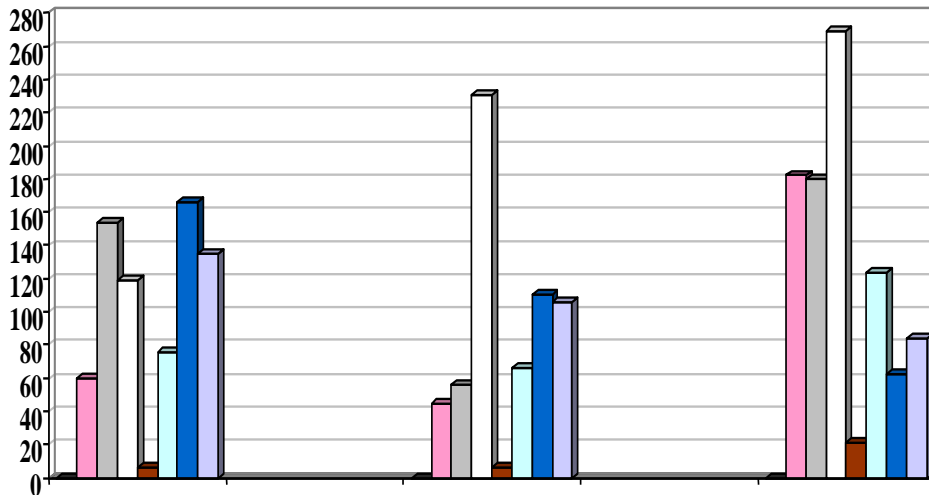


Fig. (6): Effect of cyclophosphamide and/or fractionated doses of γ -irradiation on serum creatine kinase (U/l) of rats pretreated with β -1, 3 glucan.

B. Histological Results:

1-Lung

-Control group: Normal structure of control rat's lung is observed in Fig. (7).

-CYP treated group: Sections of lung taken from rats treated with CYP at a dose of 50 mg/kg, i.p., every other day for 2 weeks and excised 1 & 7 days post-treatment showed dilated bronchioles with thickened epithelium and intrabronchiolar exudate. There were also swelling and vacuolar degeneration of bronchiolar epithelial lining. Most alveoli were obstructed by proliferation of pneumocytes which lost their normal shape. Moreover, thickened wall of the arteries together with haemorrhagic areas were observed (Fig.8). By the day 21, most bronchioles contained thickened wall and accompanied with compact mass of pneumocytes and congested blood vessels (Fig.9).

-Irradiated group: Lungs of rats exposed to fractionated doses of γ -radiation at a dose of 1 Gy daily 5 times weekly for 2 weeks and examined 1, 7 & 21 days later, exhibited disrupted wall of the bronchioles which had shed destroyed epithelial lining.

Some alveoli appeared with thickened wall in which some lipoid droplets have been trapped. Signs of emphysema could also be seen. Besides, elongated and folded bronchiolar and arterial walls were observed. Haemolysis and hyalinosis were also prominent in the lumen of the affected artery (Figs. 10 & 11).

-CYP-Irradiated group: Examination of sections obtained from lungs of rats treated with CYP and then exposed to γ -radiation showed following 1, 7 & 21 days of treatment highly affected bronchioles with thickened wall and desquamated epithelial lining. Most alveolar walls were thickened by mixed cellular infiltrates, however emphysema could also be noted. Furthermore, some arteries exhibited highly thickened tunica media and increased signs of haemolysis (Figs. 12 & 13).

-Glucan treated groups: Lungs of rats treated with glucan at a dose of 10 mg/kg, i.p., daily for 2 weeks and examined 1, 7 & 21 days later appeared almost normal. Moreover, the use of glucan concomitantly with CYP treatment or γ -irradiation induced marked improvement in the pulmonary tissue. Many alveoli and bronchioles

retained their normal pattern with restoration of their epithelial lining. However, some alveolar septa were slightly thickened with inflammatory cellular infiltrate admixed with hyaline material. In addition, compensatory emphysema as well as congested blood vessels were still detected. Using glucan against the combined treatment of CYP and γ -radiation showed partial reparative influence. Some alveolar septa appeared normal, while obvious degenerative changes were still observed in alveoli, bronchioles and even in blood vessels (Figs. 14, 15 & 16).

2- Testis

-Control group: Normal structure of rat testis is illustrated in Fig. (17).

-CYP treated group: The histological picture of rat testis examined one day post-treatment with CYP showed reduced size of some seminiferous tubules (S.T) with irregular basal lamina. Most of them were devoid of sperms, while others had abnormal ones. Besides, diffusely stained patches were quite discerned within the interstitial tissue (Fig.18). By the end of 7 days, the intertubular spaces were highly thickened and appeared fibrotic with nearly absent Leyding cells. Some tubules contained undifferentiated mass of germ cells as well as multinucleated giant cells (Fig.19). Following 21 days, disrupted basement membranes of some S.T with increased incidence of necrotic changes were noticed. There were disintegrated sperms in the testicular lumen with abundant pyknotic nuclei within the primary spermatocytes. Furthermore, thickened and congested blood vessels with moderately oedematous interstitial tissue were also seen (Fig. 20).

-Irradiated group: Examination of the testes of rats subjected to γ -radiation and examined 1, 7 & 21 days later showed markedly atrophied S.T, most of them exhibited depletion of the germinal cells discerned mainly at the spermatocytes and spermatid stages of spermatogenesis. Other

tubules were distorted and faintly stained contained a number of karyolytic nuclei and fatty vacuoles. In addition, highly dilated intertubular spaces with few abnormal Leyding cells were also prominent (Fig. 21).

-CYP-Irradiated group: Testes sections of rats treated with CYP and then exposed to γ -radiation revealed extensive deleterious responses in the testicular tissues. After 1 & 7 days of treatment, complete destruction of the tubular architecture was noticed represented by disruption of the basement membranes, necrotic germinal cells with pyknosis and karyorrhexis of the nuclei and exfoliated spermatogenic cells. Some tubules were also distorted and had acidophilic hyalinization in their lumina. Moreover, the interstitium showed signs of oedema and fibrosis (Fig. 22). By the end of 21 days, the produced lesions were represented by malformation of the outer surface of the S.T which exhibited irregular shape with disrupted basement membranes. Therefore, the tubules have lost their characteristic configuration revealing vacuolar degeneration as well as necrotic features of their lining cells. Furthermore, some tubules contained acidophilic hyalinization and multiple giant cells. Also, the interstitial tissue was moderately widened exhibiting oedematous features (Fig. 23).

-Glucan treated groups: When glucan was administered only to rats, the testicular tissue appeared nearly similar to that of control group during the whole experimental period. On the other hand, using glucan prior to CYP and/or γ -irradiation produced an obvious recovery from the testicular damage. The S.T appeared regular in outline with disappearance of acidophilic hyalinization and gradual appearance of the normal cellular arrangement within the tubules. Triangular shape of some S.T could be seen with vacuolated cells and abnormal shape of spermatocytes. Besides, the interstitial tissue showed some lymphatic oedema and vacuolization (Figs. 24, 25 & 26).

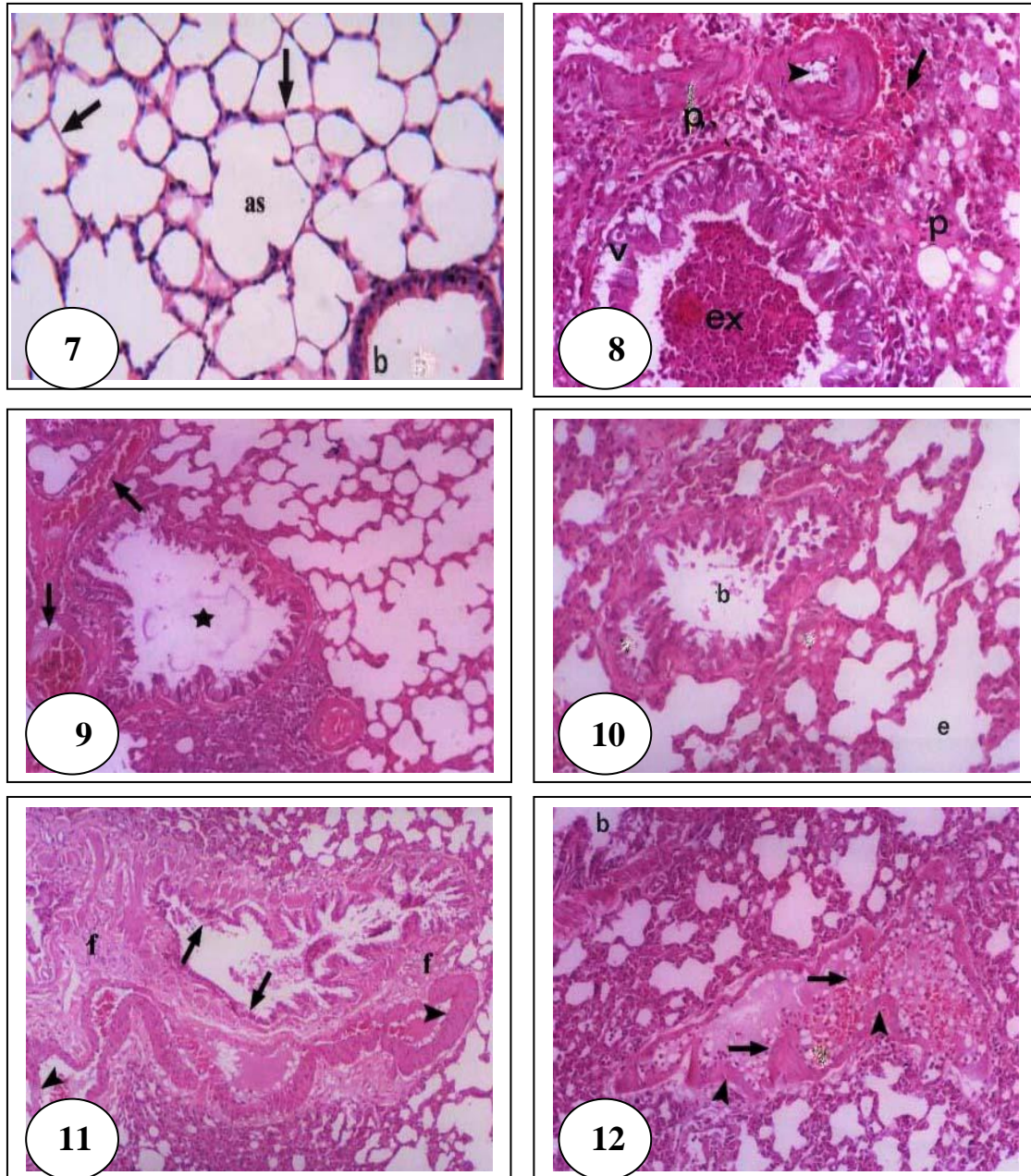


Fig.7: Section in the lung of a control rat showing the bronchiolar (b) wall and interalveolar septum (→). The alveoli are polyhedral sacs (as) lined with squamous and cuboidal cells (H & E, X 200).

Fig.8: Section in the lung of a rat treated with CYP and examined 7 days post-treatment displaying dilated bronchiole with swelling and vacuolar degeneration (V) of the epithelial lining. Intrabronchiolar exudate (ex) is noted. Most alveolar sacs are obstructed by proliferation of pneumocytes (P). Also, thickened walls (▶) of the arteries and foci of pulmonary haemorrhage (→) are seen (H & E, X 200).

Fig.9: Section in the rat lung obtained 21 days after treatment with CYP revealing partial desquamation of bronchiolar wall (*) which appeared surrounded by compact mass of pneumocytes. Congested blood vessels (→) with irregular walls could also be noticed (H & E, X 200).

Fig.10: Section in the lung of a rat subjected to γ -radiation and examined one day later showing dilated bronchiole (b) with shed destroyed epithelial lining in its lumen. Some alveoli appeared with thickened wall. Note also compensatory emphysema (e) (H & E, X 200).

Fig.11: Section in the rat lung subjected to γ -radiation and inspected after 7 days showing elongated and folded walls of the bronchiole and artery which encircled by dense fibrous tissue (f). Ruptured bronchiolar epithelial lining (→) and arterial wall (▶) are observed. Note haemolysis and hyalinosis of the affected artery (H & E, X 200).

Fig.12: Section in the lung of a rat treated with CYP pre-radiation exposure and examined 7 days later exhibiting thickened bronchiolar (b) wall with partial desquamated epithelial lining. Also, thickened interalveolar septa is seen. Note haemolysis (→) of the artery which has corrugated wall (▶) (H & E, X 200).

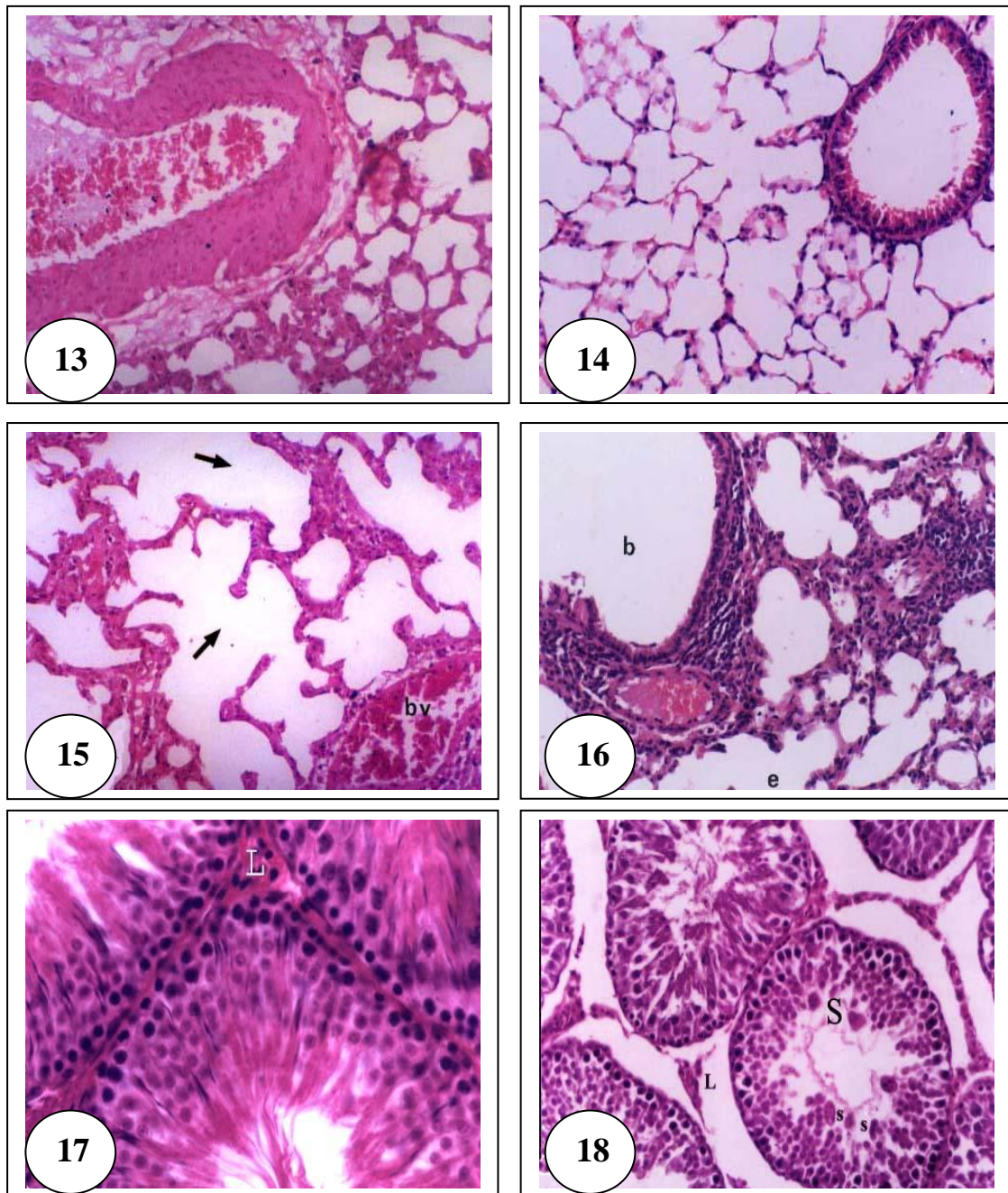


Fig.13: Section in the rat lung 21 days post-treatment with CYP and γ -radiation revealing highly dilated artery with thickened tunica media. Haemolysis is seen in its lumen and fibrosis is common around it. Note thickening of some alveolar septa (H & E, X 200).

Fig.14: Section in the lung of a rat injected with glucan prior to CYP treatment and excised following the 7th day showing an almost normal appearance of the alveolar wall, alveolar spaces and a bronchiole (H & E, X 200).

Fig.15: Section in the rat lung 21 days post-treatment with glucan and CYP displaying that some alveolar septa appeared thickened by infiltrating inflammatory cells admixed with hyaline material. Compensatory emphysema (\rightarrow) and congested blood vessel (bv) were still noticed (H & E, X 200).

Fig.16: Section in the rat lung treated with glucan, CYP and γ -radiation and excised 21 days later showing dilated bronchiole (b), vascular congestion with thickened wall and aggregates of cellular infiltrate. Note also signs of emphysema (e) (H & E, X 200).

Fig.17: Section in the testis of a control rat showing seminiferous tubules lined with spermatogenic cells. Interstitial Leyding cells (L) are also visible (H & E, X 400).

Fig.18: Section in the rat testis one day post-treatment with CYP showing reduced size of some S.T with irregular basal lamina. Some tubules appeared devoid of sperms, others contained debris of sperms (s) with enlarged head. Decreased Leyding cells (L) and diffusely stained patches are quite discerned within the interstitial tissue (H & E, X 200).

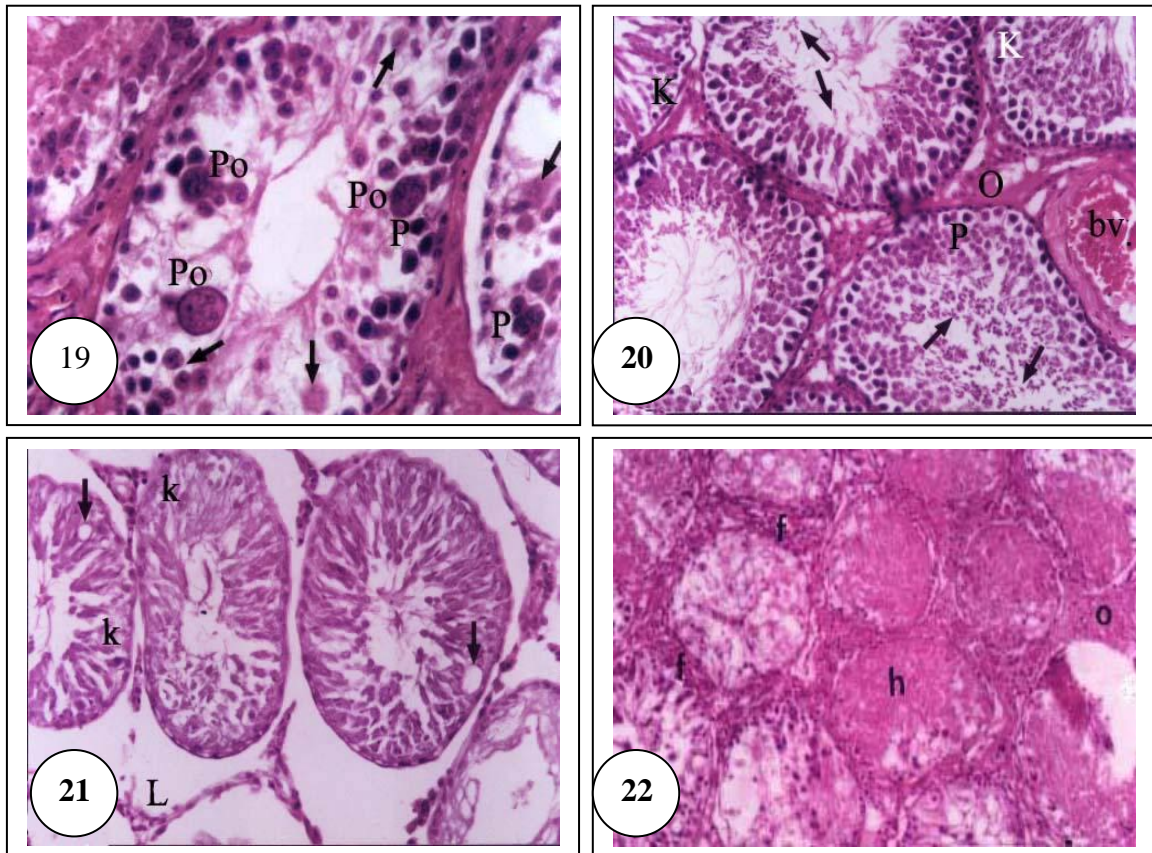
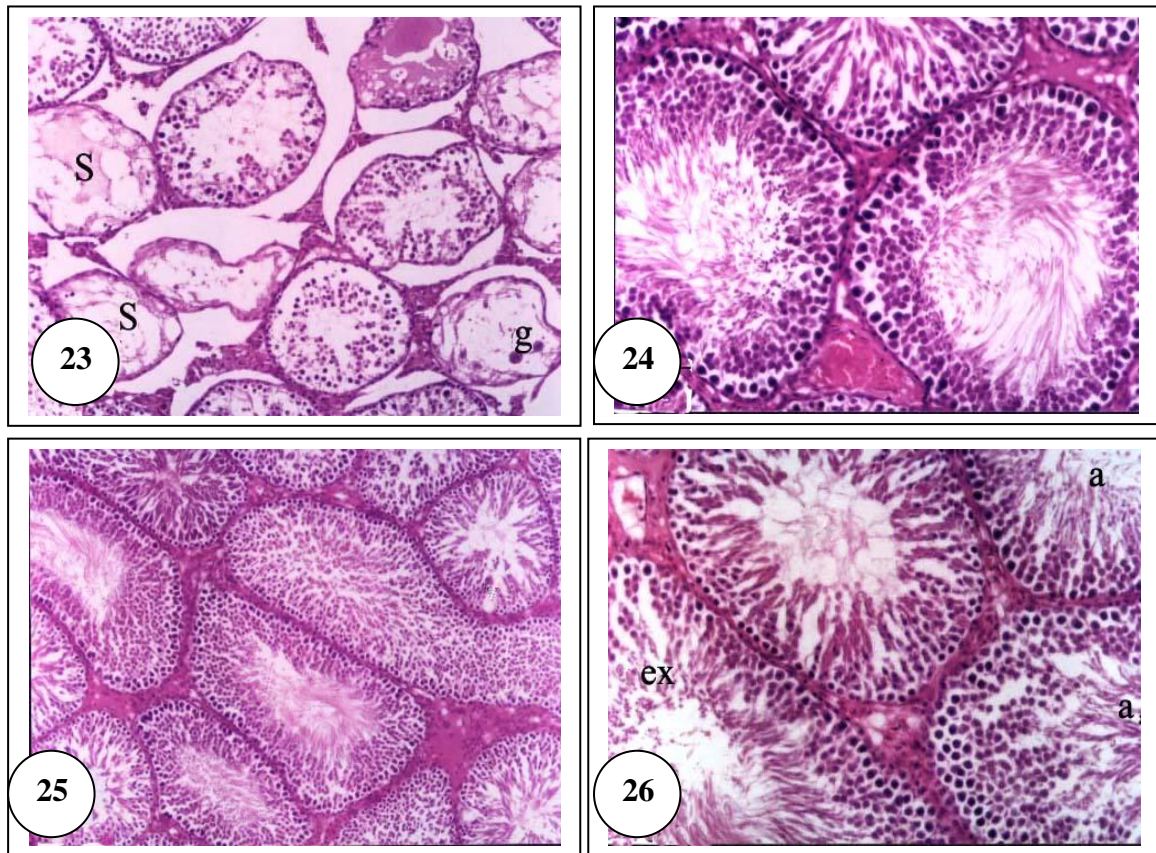


Fig.19: Section in the rat testis 7 days post-treatment with CYP showing S.T with fibrotic intertubular spaces and polynucleated germinal cells (Po). Some cells contained pyknotic nuclei (P), others contained fragmented chromatin (→) (H & E, X 400).

Fig. 20: Section in the rat testis treated with CYP and examined 21 days later showing S.T with disrupted basement membrane, necrotic germinal cells with pyknosis (P) and karyorrhexis (K) of the nuclei and exfoliated spermatogenic cells (→). Thickened and congested blood vessel (bv) together with moderately oedematous (O) interstitial tissue are also seen (H & E, X 200).

Fig.21: Section in the rat testis 7 days post-exposure to γ -radiation showing atrophied S.T, most of them appeared devoid of germinal cells and contained faintly stained cytoplasm, karyolytic nuclei (K) and fatty vacuoles (→). Dilated intertubular spaces and abnormal Leydig cells (L) could also be detected (H & E, X 200).

Fig.22: Section in the rat testis one day post-treatment with CYP and γ -radiation showing complete destruction of the tubular architecture. Some tubules were distorted and had acidophilic hyalinization (h) in their lumina. Also, the interstitium shows mild oedema (O) and fibrosis (f) (H & E, X 100).



- Fig.23:** Section in the rat testis 21 days post-treatment with CYP and γ -radiation showing distorted S.T with depletion of its lining germinal cells (s). Other tubules contained multiple gaint cells (g) and acidophilic hyalinization in their lumina. Also, diffusely stained interstitial areas were observed (H & E, x 100).
- Fig.24:** Section in the rat testis 7 days post-treatment with glucan and CYP showing gradual appearance of normal cellular arrangement within some S. T. However, the interstitial tissue showed some lymphatic oedema and vacuolization (H & E, X 200).
- Fig.25:** Section in the rat testis treated with glucan pre-irradiation and examined 7 days later showing increased regenerative changes in most S.T, few of them were still affected (H & E, X 100).
- Fig.26:** Section in the rat testis 21 days post-treatment with glucan, CYP and γ -radiation showing that some S.T regained their normal appearance with increased number of sperms (a), while others show exfoliated spermatogenic cells (ex) (H & E, X 200).

Discussion

The results of the present work revealed that administration of cyclophosphamide induced a dramatic and remarkable decrease in leucocytes and platelets count in the blood of rats all over the experimental periods. The hematopoietic system is very susceptible to the effects of alkylating agents. Within 8 hours after administration of the sublethal dose of cyclophosphamide, cessation of mitosis and disintegration of formed elements may be evident in the marrow and lymphoid tissues. Lymphocytes were more sensitive to the destructive action of alkylating agents, particularly cyclophosphamide which was more toxic to granulocytes (Calabresi and Chabner, 1991). Cyclophosphamide is well absorbed and initially undergoes metabolic activation by the cytochrome P450 mixed-function oxidase system of the liver, with subsequent transport of the activated intermediate to sites of action (Pass *et al.*, 2005). Cyclophosphamide induced leukopenia and thrombocytopenia which may be referred to its physicochemical properties such as lipophilicity, capacity to cross biological membranes and stability in aqueous solutions. In patients treated with cyclophosphamide, lymphocytopenia was apparent and severe within 24 hours extended for many days. Variable depression of platelet and erythrocyte counts may occur for 3 weeks after therapy (Fisher *et al.*, 1993). In the present study the elevation of WBCs at 7 days after cyclophosphamide administration was unexpected. It could be suggested that mature cells in peripheral blood have limited life spans and the replacement of their functional cell types is dependent on the proliferation of the hematopoietic element of the bone marrow. It has been reported that maximal concentrations in plasma are achieved 1 hour after oral administration and the half-life of cyclophosphamide in plasma is about 7 hours. The temporary rise of WBCs which was noticed 7 days post-treatment of CYP may be due to the rapid proliferation of

bone marrow cells to compensate the initial or immediate destruction of WBCs 1 day post-treatment.

The data of the present study demonstrated a significant increase in the level of calcium, creatine kinase and lactate dehydrogenase as well as a decrease in alkaline phosphatase activity in cyclophosphamide treated rats all over the experimental intervals. Cyclophosphamide induced cardio-and hepatotoxicity might be the result of increased permeability of heart and liver inner mitochondrial membrane (Al-Nasser, 1998). It is strongly suggested that the thrombocytopenia in irradiated rats might be related to platelets production defect and irradiated rats have a lower percent of reticulated platelet (RP), young platelets (Soliman *et al.*, 2005). In addition, irradiated animals had detectable platelet antibodies, which confirm the assumption that immune mechanism through the apoptotic process may play a role in the observed changes of platelets parameters. It has been reported that in liver disease, serum γ -GT activity correlates well with serum alkaline phosphatase levels. Consequently, the changes of alkaline phosphatase in patients exposed to radiation during radiotherapy could be related to the disturbance in γ -GT activity (Abdel-Gawad and Ahmed, 2005). It has been also suggested that radiation-induced oxidative stress may accelerate glycolysis leading to the increase of LDH activity. The increased level of LDH could be due to the irradiation enhancement of enzyme movement from its subcellular production sites to the extracellular fluid and consequently to blood (Ramadan *et al.*, 2003). Lactate dehydrogenase, creatine kinase and alkaline phosphatase are now frequently detected to estimate the degree of liver dysfunction due to radiotherapy and prognosis of advanced liver cirrhosis as well as expectation of heart failure development.

The changes in serum Ca^{2+} level may be due to radiation effect on the active potential of cell membranes and/or to the high energy of gamma-radiation that

penetrate the cell and changes its ionic levels (Abdel-Gawad and Ahmed, 2005). The present results revealed that the combined treatment of rats with radiation and cyclophosphamide exaggerated the disturbances of the investigated parameters all over the experimental intervals. Cyclophosphamide and total body irradiation seem to be the most used conditioning regimen in patients with acute myeloid leukemia (AML), especially in patients with advanced disease (Ferry and Socie, 2003). However, the early toxicity and high incidence of late side effects are the most important problems in patients undergoing this regimen.

An immunomodulating substance, a biological response modifier (BRM) or biotherapy is important for successful treatment of cancer.

The present data also revealed that treatment of animals received cyclophosphamide and/or γ -irradiation with β 1, 3 glucan partially improved most of the investigated parameters (WBCs, platelets, alkaline phosphatase, creatine kinase and LDH) within the intervals of the experiment. It has been reported that administration of yeast β -glucan enhanced the production of peripheral blood progenitor cells (PBPC) in mice (Patchen *et al.*, 1998). Furthermore, glucan is a broad spectrum enhancer of host defense mechanism stimulating humoral and cell-mediated immunity and stimulation of hematopoiesis is the most important mechanism of glucan's radioprotective effects (Chertkov *et al.*, 1999). These conclusions confirmed the findings of the present study, since the leucocytic and platelets counts were effectively improved in the groups of animals received cyclophosphamide and / or exposed to γ -radiation pretreated with glucan. The radioprotective effects of glucan could be explained by its ability to trap OH radicals and consequently decrease the clastogenic effect of irradiation (Chorvatovicova 1991), or its ability to stimulate the granulocyte-macrophage progenitor cell (GM-CFC) recovery in mice receiving whole body γ -irradiation (Patchen *et al.*, 1993). Besides the radioprotective effect of glucan, it also

has a protective role against cyclophosphamide induced leukopenia and immunosuppression (Czop *et al.*, 1989).

A specific receptor was identified on the cells of macrophages origin that binds to the beta 1, 3 D-glucan molecule (Tohamy *et al.*, 2003). This receptor is a protein complex that appears to be present throughout the whole differentiation cycle of macrophages, starting in the bone marrow. When a macrophage encounters β 1,3 D-glucan, it becomes activated. All the functions, including phagocytosis, release of certain cytokines and the processing of antigens are improved. This process needs the particle size of beta-glucan as fine as possible. The particles having around 2 micron diameter in major part of beta-glucan can be considered "fine" and could be easily engulfed by macrophages. However, glucan was proved to have unknown toxicity or reverse action and its safety is closely related with quality, source, structure and purity (Saito *et al.*, 2003). It is worthy to mention that the dose of glucan chosen in the present study (10mg/kg b.wt.) was recommended (Kondo *et al.*, 1992). The source of glucan is an important factor. In the present study the tested glucan was isolated from *Euglena gracilis*, it is linear chain of glucose units without any branching and its molecular weight is approximately 500.000 g/mol (paramylon).

It is well established that CYP can cause pulmonary toxicity that develops soon after drug exposure (Siemann *et al.*, 1986). The present histological results on the lung revealed that CYP intraperitoneal injections at the small therapeutic dose (50 mg/kg every other day for 2 weeks) induced marked dilatation of the bronchioles with vacuolar degeneration of their epithelial lining. There were intrabronchiolar exudation and diffused mononuclear cellular infiltration. Also, some of bronchiolar walls were ruptured and accompanied with excess fibrous tissue. Meanwhile, the interalveolar walls were thickened by numerous pneumocytes. Congested blood vessels and oedematous interstitial tissue were also seen.

Similar observations were recorded in rats (Cohen and Mathews, 1983) and in

mice (Collis *et al.*, 1980). According to Alla-Lunis *et al.* (1988), CYP treatment induced pulmonary damage but with a sparing if it was given in fractionated doses than one single dose. Furthermore, Kumar *et al.* (1988) reported that there was a correlation between functional and morphological responses of type II pneumocytes following CYP treatment (300 mg/kg) in mice. They suggested that the secretory activity of type II pneumocytes is increased in response to the injury induced by CYP. Also, Siemann *et al.* (1986) noticed that pulmonary pneumonitis in mice after CYP injection was associated with impaired lung function. This may be attributed to the exudation of plasma proteins associated with interstitial pneumonitis leading to elevated alveolar proteins levels.

Regarding the effect of whole body γ -irradiation at a dose of 1 Gy daily, 5 times weekly for 2 weeks up to 10 Gy, the lung exhibited thickened bronchiolar walls with intrabronchiolar epithelium fragments. Besides, some alveoli appeared with thickened walls, while others showed signs of emphysema. Diffused pulmonary fibrosis was also well marked. As reported by Cormack (1987), the permanent fibrosis had led to permanent pulmonary hypertension with hypertrophy of the walls of blood vessels which appeared engorged with blood. This was followed by extravasation of RBCs in the interalveolar septa, leading to more destruction of the interalveolar septa with emphysematous changes in the lung.

These results were partially agreed with those of Jinping and Lizhen (1996) who reported that γ -irradiation (2.5 & 5 Gy) disturbed the normal cellular function and this fact is possibly one of the most important links in the development of radiation-induced lung injury. Similar results were obtained by Van der Meeren *et al.* (2005) who noticed severe inflammatory reaction in the lung of mice after whole body γ -irradiation at a dose of 15 Gy. Also, Su *et al.* (2005) stated that radiation induced lung pneumonitis.

The combined treatment with CYP and γ -radiation had induced more

progressive pulmonary lesions. Fibrosis was common around the bronchioles and blood vessels which showed great evidence of destruction and haemolysis. In addition, thickened alveoli by mixed cellular infiltrates and hyaline material were obviously seen. However, alveolar destruction and compensatory emphysema was also noted. Confirming these results, Rossi *et al.* (2000) reported that the treatment with CYP and γ -radiation potentiate the pulmonary toxicity.

The present results also revealed histological changes in the testis that were induced by the therapeutic dose of CYP, where alteration of spermatogenesis was well marked. Reduced size of S.T with deterioration in the basal lamina, pyknosis of the nuclei of the spermatogonia and spermatocytes in addition to the disappearance of spermatogenic cells were noted in this study. The present observations also denoted a marked interstitial vacuolation. In this respect, Flickinger and Loving (1976) stated that lipid accumulation is a well known pathological change that occurs in cells under a variety of pathologic conditions. Moreover, the oedematous vacuoles together with the abnormal aggregation of some late spermatids were explained by Abd El-Hafez (1980) to be due to the disturbance in the spermiogenesis and the tubule itself has lost its power of contraction to push the mature spermatids into the lumen. Besides, the formation of multinucleated giant cells was also detected in the testicular lumen. The rise in these cells was indicative of retarded cell maturation and differentiation at some stages of spermatogenesis (Mamina and Sheiko, 2001). These results agreed with Katsifis and Tzioufas (2004) who demonstrated the serious side effects of CYP on the gonads.

Concerning the influence of γ -irradiation, many authors noticed various anomalies regarding the deleterious action of radiation exposure on the testis (Guitton *et al.*, 2000, Rooij *et al.*, 2002 and Sayed *et al.*, 2005). In the present study, distorted S.T with depletion of germinal cells were prominent. These findings had received further confirmation with Gupta and Bawa

(1977) who reported that there was a lack of differentiation of spermatids to spermatozoa in the testes of irradiated rats, with the disappearance of spermatogenic cells in some tubules to the extent that cellular debris only persisted in the lumens. Furthermore, Roushdy *et al.* (1984) showed marked hypocellularity in the tubules of rats following γ -irradiation. Moreover, spermatocytes showed progressive hypertrophy which was attributed as indicated by the authors to the increased rate of protein synthesis. Degenerated interstitial tissue detected in this study was similarly reported by Mansour *et al.* (1986), who noticed degeneration and necrosis of the interstitial tissue of the testes of irradiated rats. Also, Dellic *et al.* (1986) noted a dysfunction of the Leyding cell population following γ -irradiation.

However, the combined treatment displayed the highest destructive effect of S.T. It can thus be noted that there is some possible interaction between CYP and γ -radiation. Similar findings were recorded by Matsumoto *et al.* (2000).

On the other hand, the intraperitoneal injection of glucan at a dose of 10 mg/kg daily for 2 weeks did not show any histological alterations of the pulmonary and testicular tissues from normal during the whole experimental period. These results were confirmed with the findings of Diluzio (1970) who reported that glucan had no toxicity or side effects as well as it had the ability to activate the macrophage immune cells and in turns the entire immune response. Furthermore, using glucan prior to CYP treatment and / or γ -irradiation had induced an obvious improvement in the pulmonary tissue, where most alveoli and bronchioles retained their normal pattern. However, some alveolar septa were still thickened by infiltrating inflammatory cells admixed with hyaline material. Also, compensatory emphysema and congested blood vessels were observed. It is worthy to mention that glucan have beneficial effects in the therapy of experimental bacterial, viral and fungal diseases (Browder *et al.*, 1990). Chertkov *et al.* (1999) deduced that glucan have antira-

diation therapeutic efficiency following its administration within 24 hours after radiation exposure at doses that cause acute radiation sickness. Also, Heltand and Sandven (2002) reported that glucan reduced the incidence of hospital pneumonitis.

Additionally, glucan produced considerable recovery from the testicular damage. The S.T had more regular outline than the CYP or γ -irradiated groups. Most tubules showed normal architecture with the appearance of the germinal cell population including spermatogonia and spermatocytes. The spermatids were quite evident and spermatozoa filled the lumen of the tubules. However, some tubules were still affected and the interstitial tissue also showed some lymphatic oedema and vacuolization.

The free radical scavenging ability of glucan is not interfere with the antitumor activity of CYP. Moreover, glucan helps in reducing side effects of conventional chemotherapy, while at the same time enhancing its effectiveness (Kogan *et al.*, 2002). Also, Allendorf *et al.* (2003) reported that glucan may be a useful therapeutic intervention following radiation exposure to accelerate myeloid (bone marrow) recovery and increased survival rate. Its radioprotection may be due to its ability to resist the oxidizing effect of γ -radiation, it acts as a free radical scavenger and also it can potentiate and activate the immune response (Hunter *et al.*, 2004).

Considering the above mentioned physiological and histological findings as well as those reported in different laboratories, it is possible to conclude that β 1, 3 D-glucan is effective and is potentially to be used as an adjuvant in several important clinical and therapeutic treatments against radio-and chemotherapy of cancer patients.

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دراسات فسيولوجية وهستولوجية للجلوكان كمعدل للأضرار المحدثة في الجرذان المعالجة بالسيكلوفوسفاميد و المعرضة للإشعاع الجامي

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يمكن أن يخضع مريض السرطان للعلاج الكيميائي بجانب العلاج الإشعاعي، مع أنه من المعروف أن كلا منهما له آثار جانبية ربما تكون طفيفة أو شديدة، واستخدامهما معا يزيد من حدة هذه الآثار الضارة. لذلك استهدفت هذه الدراسة استخدام مواد طبيعية أو مواد مستخلصة من مصادر نباتية للتخفيف من حدة هذه الأعراض ولهذا تم إختيار الجلوكان الذي يوجد في العديد من المصادر الطبيعية ولم يظهر له آثار جانبية. وقد تم إجراء هذا البحث على ١٦ من ذكور الجرذان البيضاء يتراوح أوزانها بين 130-150 جم. وتم قياس مستوى الكالسيوم ونشاط كل من CK ، LDH ، ALP و عدد كرات الدم البيضاء والصفائح الدموية بالإضافة الى إجراء الفحص المجهرى لأنسجة الرئة و الخصية. وقد تم التشريح وأخذ العينات بعد يوم و سبعة أيام وواحد وعشرون يوماً من إنتهاء كل تجربة على حدة.

تم تقسيم الجرذان الى ٨ مجموعات : ١- المجموعة الضابطة. ٢- مجموعة تم إعطاؤها عقار السيكلوفوسفاميد بجرعة مقدارها ٥٠ مجم/كجم يوم بعد يوم ولمدة إسبوعين. ٣- مجموعة تم تعريضها لجرعات مجزأة من أشعة جاما بمقدار ١ جراي يومياً وصولاً إلى جرعة مقدارها ١٠ جراي. ٤- مجموعة تم حقنها بعقار السيكلوفوسفاميد بالطريقة السابقة، ثم تم تعريضها لأشعة جاما بالجرعات السابقة. ٥- مجموعة تم إعطاؤها مادة بيتا جلوكان حقناً بالغشاء البريتوني بجرعة مقدارها ١٠ مجم / كجم لمدة أسبوعين. ٦- مجموعة تم حقنها بالجلوكان يومياً وقبل حقنها بعقار السيكلوفوسفاميد . ٧- مجموعة تم حقنها بمادة الجلوكان قبل تعرضها لأشعة جاما. ٨- مجموعة تم حقنها بمادة الجلوكان أثناء المعاملة بالسيكلوفوسفاميد ثم تعريضها لجرعات مجزأة من أشعة جاما. وقد أوضحت نتائج القياسات البيوكيميائية والدموية أن الجرذان المشععة أو المعالجة بالسيكلوفوسفاميد إكتسبت نقصاً معنوياً ملحوظاً في عدد كرات الدم البيضاء والصفائح الدموية، وفي مستوى ALP. مع وجود زيادة ملحوظة في مستويات CK ، LDH في كل فترات التجربة مصحوباً بزيادة في مستوى الكالسيوم في اليوم الحادى والعشرين. وقد زادت حدة هذه التأثيرات عند التعرض المشترك لكل من السيكلوفوسفاميد والإشعاع الجامي ، وظهر التأثير السلبى على كل القياسات السابقة.

كما أظهر الفحص الميكروسكوبى لنسيج الرئة أن المعاملة بالسيكلوفوسفاميد او التعرض لأشعة جاما قد أحدث تغيراً شديداً فى شكل وتركيب الشعبات الهوائية وكذلك فى جدر الحويصلات الهوائية مع زيادة مساحات النزيف الداخلى و احتقان الأوعية الدموية. و بالنسبة لنسيج الخصية فقد ظهرت الأنبيبات المنوية بصورة غير طبيعية و خلت بعضها من الحيوانات المنوية وانتشر التليف فى المناطق المحيطة بها كما لوحظ غياب خلايا لايدج ووجود خلايا عملاقة و مظاهر التحلل الدهنى. وقد زادت حدة هذه التغيرات تحت التأثير المآزر للسيكلوفوسفاميد مع أشعة جاما.

وعلى الجانب الآخر أظهرت المعالجة بالجلوكان تحت تأثير السيكلوفوسفاميد أو أشعة جاما أو كلاهما معا تحسناً ملحوظاً في عدد كرات الدم البيضاء والصفائح الدموية ، كما حدث بعض التحسن في باقى القياسات البيوكيميائية, وأيضا لأنسجة الرئة اما بالنسبة للخصية فقد كان الدور الوقائى لهذه المادة واضحا وملموسا.

وقد خلصت الدراسة إلى أهمية استخدام مواد طبيعية للوقاية من الآثار السلبية أثناء العلاج الكيميائى والإشعاعى و نوصى بان يخضع الجلوكان لمزيد من الاختبارات بجرعات مختلفة و على فترات متفاوتة وصولا إلى أقصى قدرة وقائية ممكنة.