International Invention of Scientific Journal
Online ISSN: 2457-0958
Available Online at http://www.iisj.in Volume 06|Issue 10 (October-Nov.-December)|2022|Page: 01-08
Original Research Paper- Microbiology and Technology

Study of some hematological and Physiological Alterations Induced by Cisplatin and the Possible Protective Role of β-glucan in rats

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Article Received 01-11-2022, Article Revised 15-11-2022 , Article Accepted 03-12-2022

ABSTRACT:
Cisplatin (CDDP) is an anti-cancer DNA alkylating chemotherapeutic agent that acts against a variety of tumors. The present study aimed to evaluate the possible protective effects of β-glucan on the physiological and hematological parameters in rats chronically treated with CDDP. Four groups of rats were examined: control rats with saline PBS solution (group I), rats treated with CDDP (group II), rats treated with CDDP and β-glucan (group III), and normal rats treated with β-glucan (group IV). All animals were treated for successively five days and killed one week after the last treatment. The results recorded that CDDP treatment significantly decreased the levels of white blood cells (WBCs), red blood cell distribution width (RDW) and increased lymphocytes count. Also, CDDP increased the hepatocyte's oxidative stress which is characterized by increasing prooxidants xanthine oxidase (XO), thiobarbituric acid-reactive substances (TBARS), and decreasing antioxidants glutathione peroxidase (GPx). As a result, hepatocyte injury took place that was characterized by serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities. The treatment of rats by β-glucan with CDDP (group III) or rats treated with β-glucan alone (group IV) successfully normalized the physiological parameters in the form of returning WBCs, RDW, and lymphocyte counts to normal levels and decreased the hepatocyte's oxidative stress which characterized by decreasing Prooxidants OX, TBARS and increasing of antioxidants GPx reflected by a significant decrease in the serum activities of AST, ALT and ALP activities.

Keywords: ***Cisplatin, β-glucan, oxidative stress, chemotherapy, antioxidant, Prooxidants, xanthine oxidase.***

INTRODUCTION:
Cisplatin is one of the best and first metal-based chemotherapeutic medication, utilized for treatment of many malignancies like malignant tumors of the testis, ovaries, bladder, lungs, stomach, head and neck [1]. It exerts its anticancer activity by binding with genomic and mitochondrial deoxyribonucleic acid (DNA). This action creates DNA lesions, arrests its replication, production of messenger ribonucleic acid (mRNA) and proteins. In addition, it activates many transduction pathways which in turn induce cell necrosis or apoptosis[2]. The usage of cisplatin for chemotherapeutic purposes is hindered by its numerous side effects which include nephrotoxicity, hepatotoxic, and neurotoxicity[3]. On the other hand, CDDP is high lypmutagenic, inducing chromosome aberrations in peripheral blood lymphocytes in patients and in rats. CDDP causes oxidative stress in human lymphocytes, which might reflect on their life expectancy and induction of apoptosis by ultimately reducing the number of these cells in the blood [4]. On the other hand, the decrease in the leukocyte number could be the consequence of infection and inflammation during CDDP treatment and metabolism. CDDP is a very effective chemotherapeutic agent, used in the treatment of a wide range of malignant diseases. However, it exhibits certain toxic effects on the blood and liver which interfere with Its therapeutic efficiency [5]. β-Glucans are polysaccharides of D-glucose monomers linked by β-glycosidic bonds. β-glucans are a diverse group of molecules that can vary with respect to molecular mass, solubility, viscosity, and three-dimensional configuration. They occur most commonly as cellulose in plants, the bran of cereal grains, the cell wall of baker's yeast, certain fungi, mushrooms, and bacteria [6] For the past 35 years, many yeast-derived β-glucans, either soluble or particulate, isolated from various natural sources and with variable molecular sizes and secondary structures, have been observed to exhibit antitumor in mouse model systems[7-10]. Naturally occurring β -glucans are β -glycosidic polymers of D-glucose with varying

tissue) = (At/0.156) × 10, where At is the absorbance of the test sample and ε = 0.156 is the extinction coefficient. The liver XO activity (µmole/h/g tissue) was estimated as follows: (C) ×10/ (0.284 × xanthine M. Wt), where 0.284 is a constant and C is the concentration in the test sample. The activity of the antioxidant enzyme glutathione peroxidase (GPx) was measured according to Paglia and Valentine [19]. The enzyme activity was calculated by using the following equation ; GPx activity (U/g wet tissue) = (At ×6.2 × 10 × 10)/(13.1 × 0.05 × 10), where ε 1= 6.2 and ε2 = 13.1 are extinction coefficients for H2O2 and DTNB (5,50-dithiobis-(2-nitrobenzoic acid).

Statistical analysis:

Data obtained from each experiment were analyzed using Microsoft Excel (Seattle, WA). The significance of the difference between the parameters of different experimental groups and their corresponding controls were assessed using the Mann_Whitney U-Test. The Z score, p-value and U value were calculated and the results were considered significantly different at $p < 0.05$ by using the free Mann_Whitney U-Test calculator online (http://www. socscistatistics.com). Values in figures and tables represent means with standard deviation (±S.D.).

RESULTS:

Table 1 showed that CDDP treatment significantly decreased the total numbers of WBC and coincided with decreases in the number of RDW and increases in the number of lymphocytes when compared to the normal group. The co-treatment with CDDP + β-glucan returned the WBCs to their normal coinciding with the recovery of the relative numbers of lymphocytes and higher numbers of RDW as compared to control values (PBS group). treatment with β-glucan alone increased the numbers of WBC and RDW counts as compared to PBS. .

Table 2 shows that CDDP increased the level of TBARS and XO activity that was associated with decreases in GPx activity compared to control one ($p<0.001$). Co-administration of β-glucan with CDDP decreased the XO and GPx activities, respectively with no effect on the TBARS level as compared to the CDDP group. However, treatment with β-glucan alone treatment decreased the pro-oxidants parameters (TBARS and XO) and increased the anti-oxidant ones to their normal level. As shown in Table 3, CDDP treatment significantly increased the activities of AST and ALT in sera ($p<0.001$) with no effect on the serum ALP level as compared to the control group. Co-administration of CDDP with β-glucan combination post CDDP significantly decreased AST and ALT activities more than those in CDDP alone groups.

molecular mass. The soluble forms of these compounds have been used for tumor immunotherapy[11]. They protect against hepatotoxicity from radiation and chemotherapy [12]. and enhance the recovery of bone marrow cellularity and stem cell mobilization [13] by increasing the levels of stromal cell-derived factor alpha (SDF-1 alpha) in plasma [14]. Moreover, glucan was found to directly enhance hematopoietic progenitor cell (HPC) expansion in mouse models [15,16].

Animals:

Female rats, from 7 to 8 weeks old with an average of 140–180 g obtained from VACSERA Institute (Cairo, Egypt). were acclimatized for one week to make them adaptive to the environment. The rearing conditions were as the following: temperature = 23 ± 1 ºC, humidity = 60 ± 10%, photoperiod = 12:12 h light: dark, keeping ventilation. During the feeding process, the rats were allowed to drink water and eat food freely, and the water and food were renewed daily.

Treatment injection:

rats were treated with subcutaneous injection of β-glucan dissolved in PBS at a dose of 200 µg/rat for 5 consecutive days. CDDP was injected intraperitoneally at 10 mg/rat. Rats were divided into four groups; untreated "rats injected with PBS," naïve rats treated with β-glucan for 5 consecutive days, naïve rats treated with chemotherapy CDDP and naïve rats treated with β-glucan combined with CDDP, (n = 5 rats/group).

Preparation and counting of peripheral blood mononuclear:

cells. rats were anesthetized by inhalation of isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether; Hospira, Inc. Lake Forest, IL, USA) and blood samples were collected from the orbital sinus using heparinized microhematocrit tubes into 1.5 mL Eppendorf_ tubes. Samples were analyzed for the total number of leukocytes, using an automated instrument for complete blood counts (Vet-Scan HM2TM Hematology System, Abaxis, Union City, CA) to determine white blood cells (WBCs), red blood cell distribution width and number of lymphocytes.

Serum Biochemical Analysis:

The serum liver enzymes aspartate transaminase (AST) and alanine transaminase (ALT) as well as alkaline phosphatase (ALP) were determined according to the manufacturer's instructions (Biosystem, Egypt). The level of thiobarbituric acid reactive substances (TBARS) and xanthine oxidase (XO) as prooxidants indicator were measured according to Tappel and Zalkin [17] and Litwack et al. [18], respectively. The level of liver TBARS was calculated with the following equation (nmol/g wet

Table: 1. Effect of different treatments on blood cell counts

Groups	WBCs ($\times 10^3$)	Lymphocytes/cmm	RDW ($\times 10^4$)
PBS	5.40±0.32	2606.23±39.89	60.21±0.59
CDDP	1.71±0.30***	2930.25±16.88**	43.08±0.57*
CDDP+β-glucan	4.61±1.48	2282.17±37.40	55.48±1.23
β-glucan	5.35±0.39	2268.83±27.64	54.98±0.63

CDDP, Cisplatin; PBS, Phosphate buffer saline; β-glucan; White blood cells (WBCs) ; lymphocytes and, Red blood cell distribution width RDW; ns, non-significant; *,**, *** significant difference compared to PBS group at P≤ 0.05,0.01and 0.001.

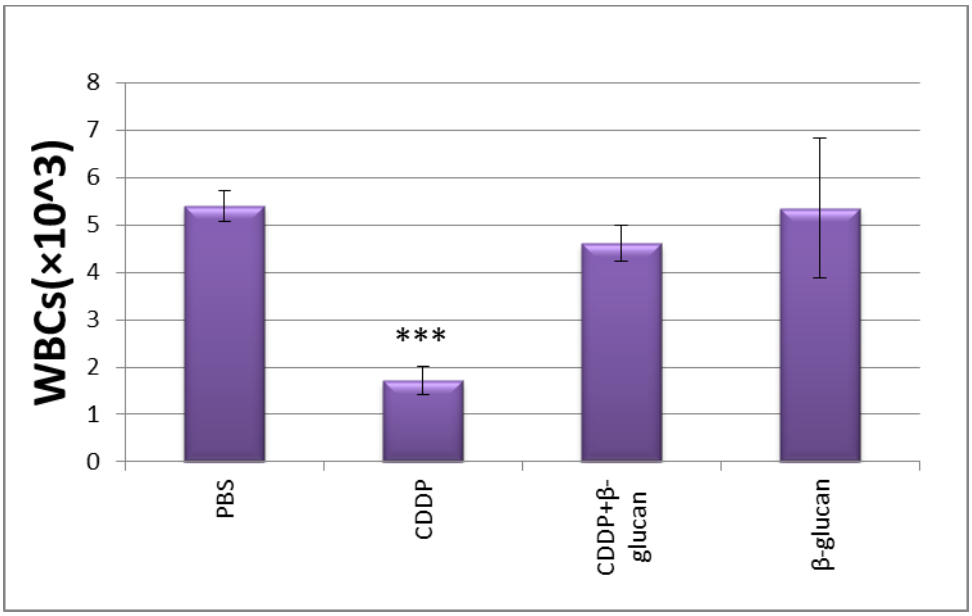

Figure:1 Statistical changes in the number of WBCs after different treatment.

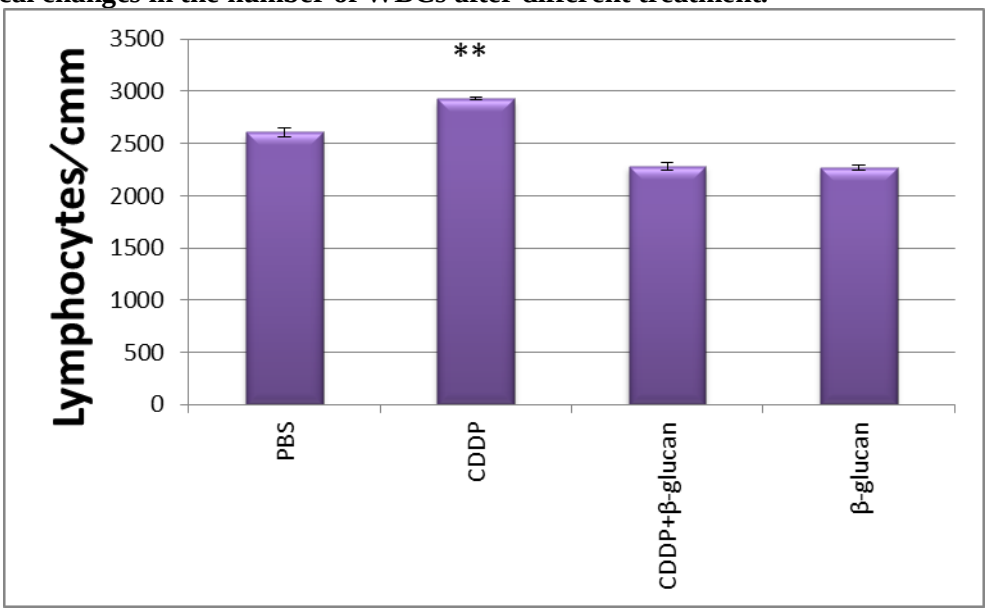

Figure:2 Statistical changes in the number of lymphocytes after different treatment.

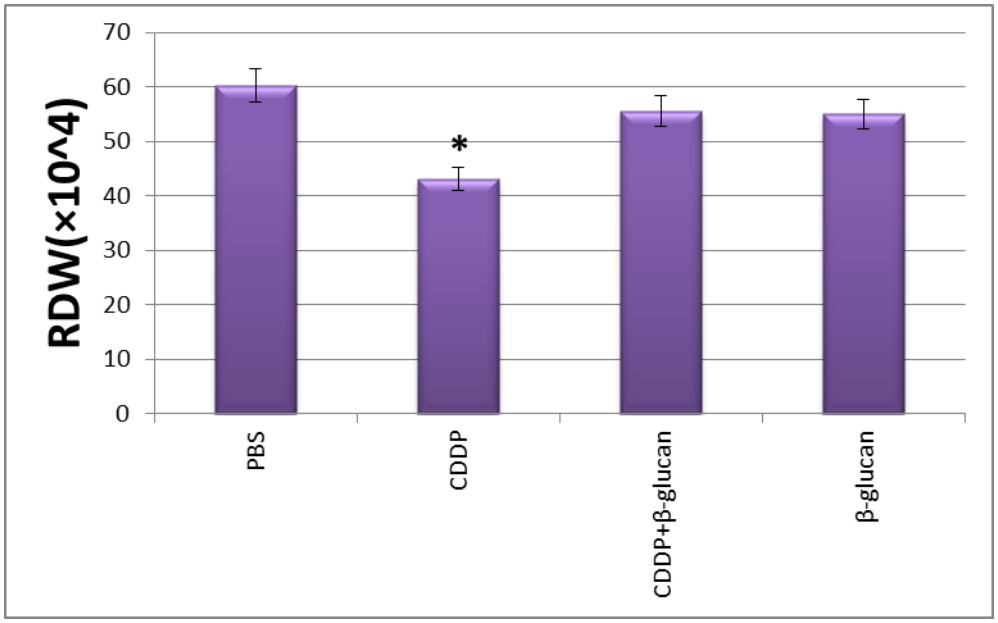

Figure: 3. Statistical changes in RDW after different treatment.

Table: 2. Effect of different treatments on hepatic pro-oxidant/anti-oxidant status.

Groups	GPx (U/g protein/g tissue)	Xanthine oxidase (IU)	TBARS (nmol/ g wet tissue)
PBS	1.64 ± 0.81	3.69 ± 0.97	1.36 ± 0.31
CDDP	1.14 ± 0.28***	6.14 ± 0.44***	2.06 ± 0.18***
CDDP+β-glucan	2.62 ± 0.28	4.33 ± 0.56	1.81 ± 0.03
β-glucan	2.30± 0.03	3.64 ± 0.90	1.19 ± 0.21*

CDDP, Cisplatin; PBS, Phosphate buffer saline; β-glucan; Glutathione Peroxidase (GPx); xanthine oxidase (XO) and TBARS, Thiobarbituric acid-reactive substances;ns, non-significant; *, ***significant difference compare to PBS group at P≤ 0.05 and 0.001.

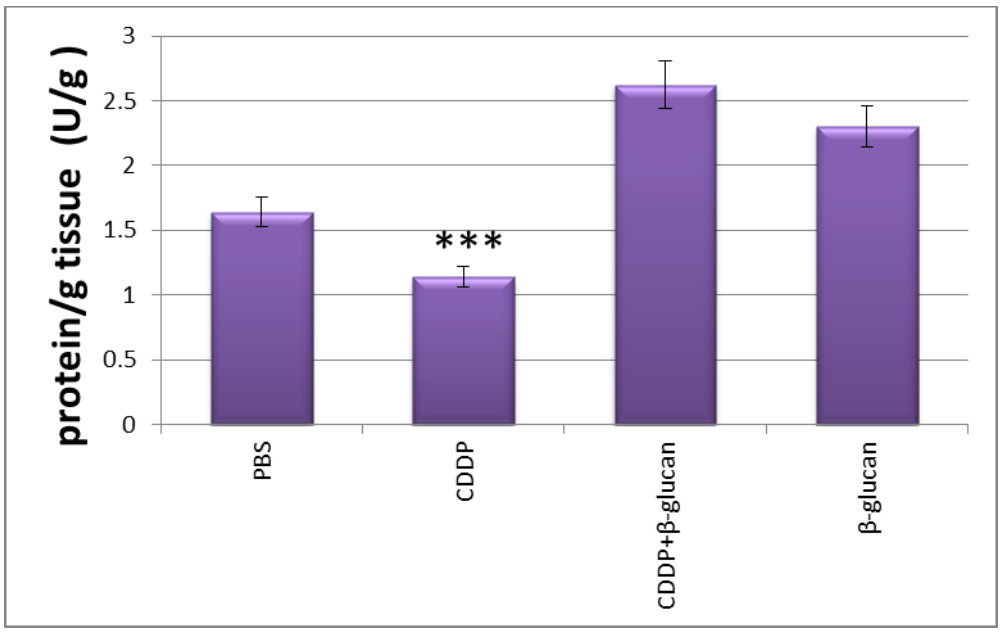

Figure: 4. Statistical changes in liver GPx activity after different treatment.

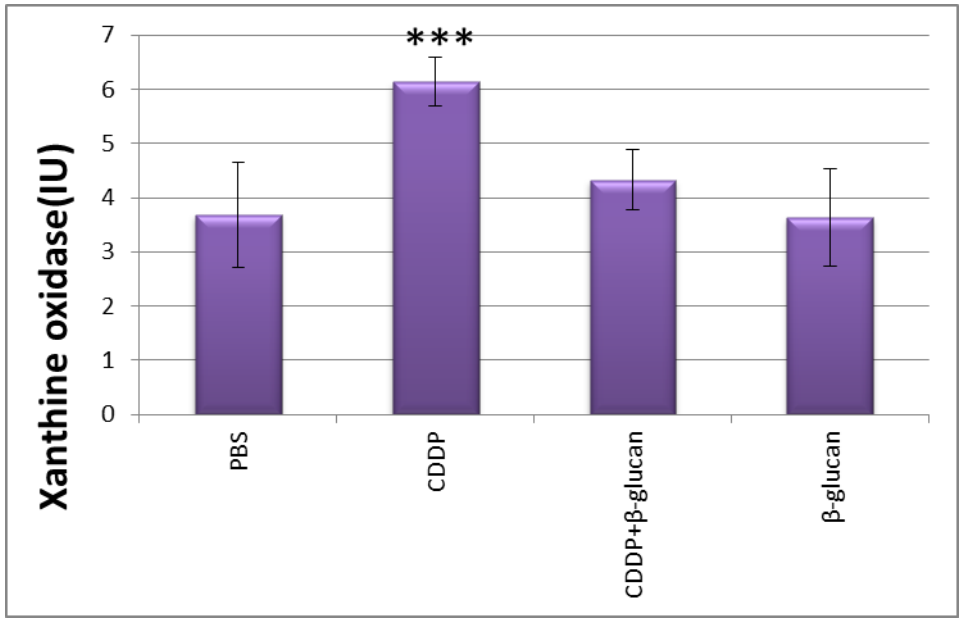

Figure: 5. Statistical changes in liver Xanthine oxidase activity after different

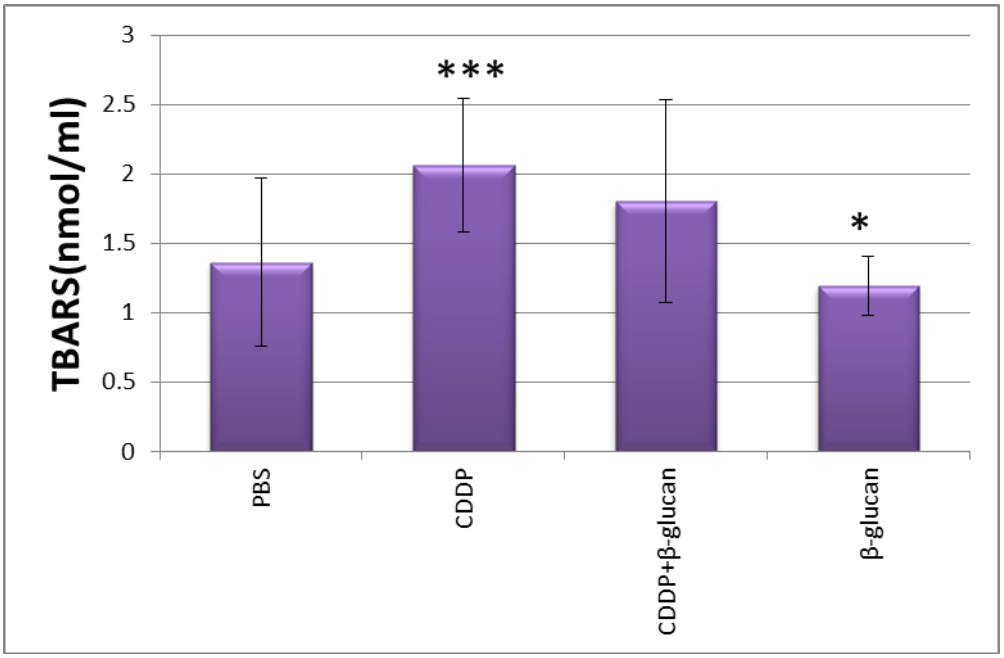

Figure: 6. Statistical changes in liver TBARs level after different treatment.

Table3. Effect of different treatments on serum liver function parameters.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
PBS	39.44±0.21	71.43±1.17	45.01 ±0.91
CDDP	55.48±1.70***	79.38±2.24***	45.76 ±1.10
CDDP+β-glucan	38.06±1.58	65.00 ± 2.87	43.09 ±1.98
β-glucan	35.81±1.95*	70.25 ± 0.69*	45.31 ±2.74

CDDP, Cisplatin; PBS, Phosphate buffer saline; β-glucan; alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities, non-significant; *, ***significant difference compare to PBS group at P≤ 0.05 and 0.001.

the number of lymphocytes. These data are consistent with data reported by [20] on the effect of CDDP in rats also, the results showed that the numbers of leukocytes and RDW increased during β-glucan injection alone or in combination with CDDP treatment. Consistent with these results

DISCUSSION:

First of all, we found that rats who received treatment with CDDP induced a sharp decrease in both the total number of leukocytes as well as in the number of RDW. This treatment, however, induced increases in

detoxifies hydrogen peroxide to water. Data showed that treatment with CDDP decreases the concentration of GPx in the liver while treatment of CDDP in combination with β-glucan, or β-glucan alone increases it to normal levels. Also, GPx concentration in the liver decreased after treatment with CDDP and increased in combination with β-glucan Our results are in agreement with studies of [27&28]. Also in this study, a single dose of CDDP (10 mg/kg) induced hepatotoxicity. The hepatocellular damage was demonstrated by a significant increase in liver enzymatic markers such as serum ALT, AST, and ALP. Our serum biochemical results showed that β-glucan had a protective effect on liver tissue and reduced the amount of these enzymes by reducing oxidative stress [29&9].

CONCLUSION:

β-glucan has the best antioxidant effect, demonstrated in the return of the number of white blood cells, RDW, and lymphocyte count of the CDDP group to the normal range compared to the control PBS group, and also it returned the changes in liver enzymes to a normal level. Therefore, the present study recommends using β-glucan as a natural product for such patients put on cisplatin therapy to reduce its toxicity.

[21&22]reported that the administration of β-glucan promoted the generation of the granulocytic lineage, mainly leukocytes, and mobilize hematopoietic stem cells from the bone marrow into the peripheral circulation [23]. Our results showed a reduction in the number of leukocytes and RDW in the blood of rats treated with acute doses of CDDP. In support of the above discussion [24]. have shown that CDDP causes oxidative stress in human RDW and leukocytes, which might affect their life expectancy and induction of apoptosis, thereby reducing the number of these cells in the blood. According to the presented results, high, acute doses of CDDP did affect leukocyte maturation in rats. Oxidative stress and ROS accumulation are one of the main mechanisms of CDDP -induced hematotoxicity [25]. The results showed that treatment after CDDP in combination with β-glucan normalized the levels of TBARS and Xanthine oxidase in the liver as compared to untreated rats [26]. Also, studies confirmed that a protective effect of β-glucan against the CDDP -induced oxidative effects was observed. In fact, the TBARS concentration in the liver of rats that received β-glucan at the same time was lower by 44%. The major intracellular antioxidant enzyme is glutathione peroxidase (GPx) which catalyzes the reaction of hydroperoxides with reduced glutathione (GSH) to form glutathione disulfide (GSSG) and

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