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RESEARCH ARTICLE

Influence of Erythropoietin and Platelet-Rich Plasma on Cyclophosphamide-Induced Hepatotoxicity in Male Albino Rats

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Abstract

The present study was conducted to investigate the protective effects of erythropoietin (EPO) and platelet-rich plasma (PRP) against cyclophosphamide (CP)-induced hepatotoxicity. Thirty six male albino rats were divided into six equal groups. The first group (control).The 2nd group was injected with a single dose of EPO (3000 IU/Kg, i.p.). The 3rd group was injected with PRP extract (150 µl/Rat, s.c.) on days (0,5 and 10). The 4th group was injected with a single dose of CP (150 mg/Kg, i.p.). The 5th group was injected with (EPO+CP) and the 6th group was injected with (PRP+CP). At the end of the experiment (day 15) sera were collected for assessment of liver function enzymes (AST, ALT and ALP) and protein levels (total protein, albumin and globulin). The CP-induced hepatotoxicity was evidenced by increased serum AST, ALT, and ALP accompanied by reduction in the levels of total protein, albumin and globulin in the serum. Administration of EPO or PRP significantly reduced the liver toxicity. The results revealed that EPO and PRP have ameliorative effect against CP-induced hepatotoxicity in male albino rats.

Keywords: *Cyclophosphamide, Erythropoietin, Platelet-Rich Plasma, Hepatotoxicity*

Introduction

Cyclophosphamide (CP) is the most widely used drug in autoimmune disease, lymphomas, breast cancer and leukemia [1]. Unfortunately the clinical use of CP has been limited due to its ability to damage normal tissues which resulted in much organ toxicity mainly in the liver, reproductive system, and urinary bladder [2].Hepatotoxicity is a major side effect of CP [3]. The toxic effects of CP are linked with two active metabolites, phosphoramide and acrolein [4]. These metabolites are responsible to induce the oxidative stress which is considered the major cause of CP-induced hepatoxicity [5].

Erythropoietin (EPO) is widely known as the major growth factor that controls erythropoiesis [6]. It is a hypoxia-inducible glycoprotein hormone, which is predominantly secreted by interstitial fibroblasts in the kidney [7]. Platelet-rich plasma (PRP) has been of great concern in regenerative medicine which deals with repairing and replacing damaged cells and tissues [8]. It has been defined as an autologous concentration of platelets that is

three to five times over the physiologic concentration of platelets in circulation [9]. Many studies indicated that antioxidants could protect cells against CP toxicity [10, 11, 12,]. The present study was carried out to investigate the protective effect of erythropoietin and platelet-rich plasma on hepatotoxicity induced by cyclophosphamide in rats.

Materials and Methods

Drugs

Cyclophosphamide was purchased from Baxter (Germany). Erythropoietin was purchased from Espogen (Korea).

Preparation of PRP

PRP was prepared using the double centrifugation method [13]. Twelve male rats were anesthetized with diethyl ether, and 8-

10 ml blood was drawn from each rat by cardiac puncture. 0.5 ml of each blood sample was transferred to EDTA tube for the initial platelet count, and the remaining sample was collected into acid citrate dextrose (ACD) PRP tube.

The blood was centrifuged at 2000 rpm for 5 min. Supernatant was transferred to another tube, and was centrifuged again at 4000 rpm for 15 min. The top 2/3 layer which consisted of platelet-poor plasma (PPP) was removed. The remaining layer (1/3) was considered as platelet-rich plasma (PRP). 0.75 µl of PRP was separated for final platelet count (platelet count was carried out with a Mindary BC-5000 automated analyzer, china).

The remaining PRP was activated with 10% $CaCl_2$ (0.8 ml PRP + 0.2 ml $CaCl_2$) for 1 h at room temperature to promote growth factor release, and then centrifuged at 4000 rpm for 5 min. The supernatant was filtered through a 0.22 µm pore filter to eliminate the membrane fragments of platelets.

Experimental Animals

Thirty six male albino rats weighing 300-350 g were randomly assigned to cages (6 rats per cage), acclimatized for 14 days at a temperature of 25 ± 4 and accessed libitum to fresh water and rodent pelleted food.

Rats were divided into six groups, each group included six rats:

Group 1: (Control group) rats intraperitoneally received normal saline.

Group 2: Rats treated intraperitoneally with a single dose of 3000 IU/kg EPO on day (0)

Group 3: Rats treated subcutaneously with 150 µl/rat PRP extract on days (0, 5, and 10).

Group 4: Rats treated intraperitoneally with a single dose of 150 mg/kg CP (dissolved in normal saline) on day 1.

Group 5: Rats treated intraperitoneally with a single dose of 3000 IU/kg EPO + a single dose of 150 mg/kg CP {EPO was administrated 1 day prior to CP treatment (day 0)}

Group 6: Rats treated intraperitoneally with 150 µl of PRP extract + 150 mg/kg of CP {PRP extract was administrated on days (0, 5 and 10).

The initial and final body weights of the animals were recorded.

Blood Sampling

At the end of the experiment (day 15), The rats were anaesthetized with diethyl ether, and blood samples obtained by heart puncture were collected into gel/ clot activator tube for 30 min, then centrifuged at 3000 rpm for 10 min to separate serum.

Biochemical Analysis

Liver function enzyme and protein levels were carried out with a Mindary BS-200 Automated Analyzer, (China). The following parameters were estimated: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, and globulin.

Statistical Analysis

Data were analysed using Prism statistical analysis software (Graph Pad version 244.1.0.8) for the year 2019 and expressed as mean ± SEM. Analysis of variance (one way ANOVA) Bonferroni's multiple comparison *post tests* for *post hoc* analysis was used for finding the statistical significance between groups at the significance level of $P < 0.05$. Unpaired two tailed *t-tests* were performed for the comparison among treatments.

Results

Platelet Yield

Final platelet concentration (in prp) was approximately 4 fold over the baseline concentration (in whole blood).

Body Weight

The present study showed that final body weight in control, EPO, and PRP groups were significantly ($p<0.05$) increased as compared to initial weight.

Administration of single intraperitoneal dose of CP (150 mg/Kg) induced a significant ($p<0.05$) reduction in body weight. Treatment with EPO or PRP potentially alleviated the reduction in body weight (Fig.1).

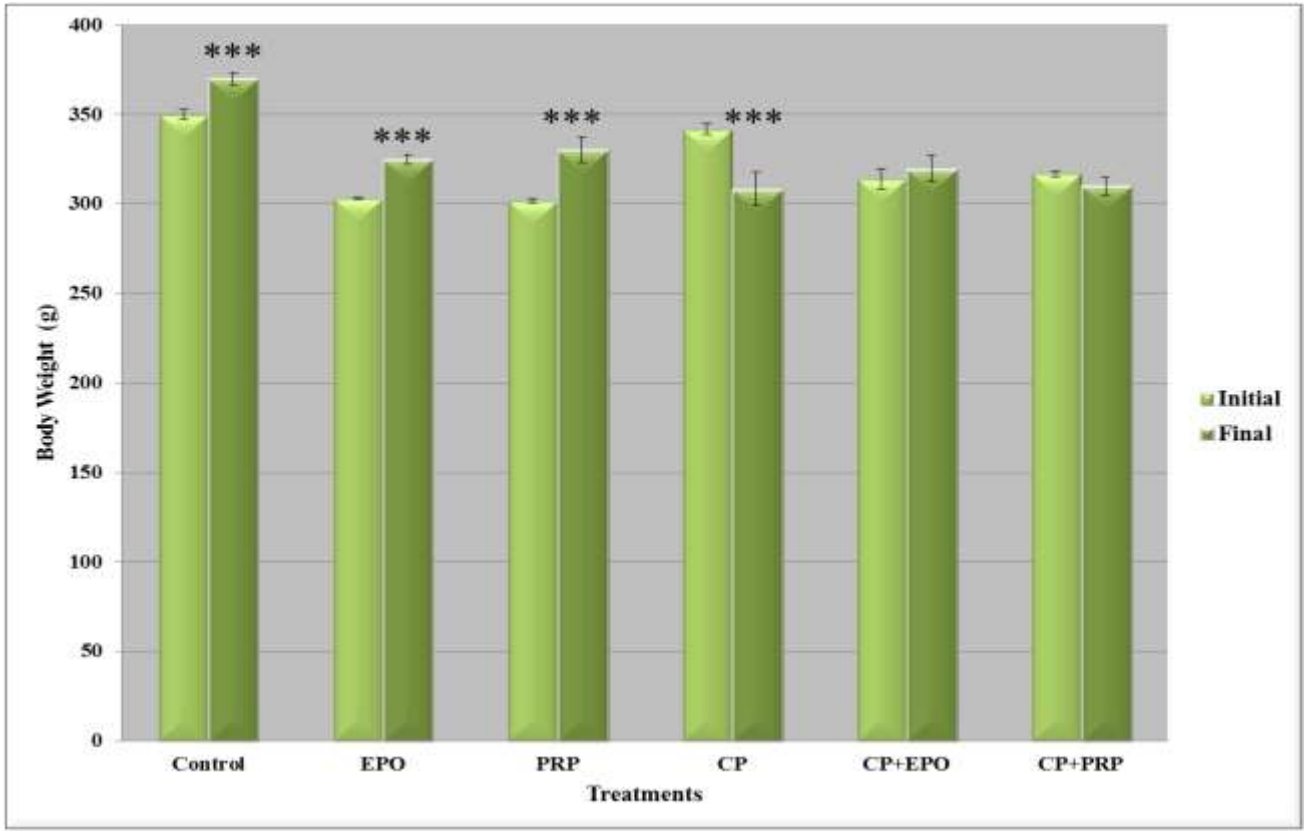

Figure1: Effect of Erythropoietin (EPO) and Platelete-Rich Plasma (PRP) on body weight of Cyclophosphamide-Treated rats . Values are expressed as mean ± SEM. *: Referring to the significant differences between final and initial body weight**

Liver Function Enzymes

The results of the present study showed that the administration of single intraperitoneal dose of CP (150 mg/kg) induced a significant ($p<0.05$) increase in AST, ALT and ALP activity in serum as compared to control. Treatment with EPO or PRP showed a significant ($p<0.05$) decrease in serum AST, ALT and ALP activity induced by CP. The effect of PRP was higher compared to EPO (Fig. 2, 3 and 4).

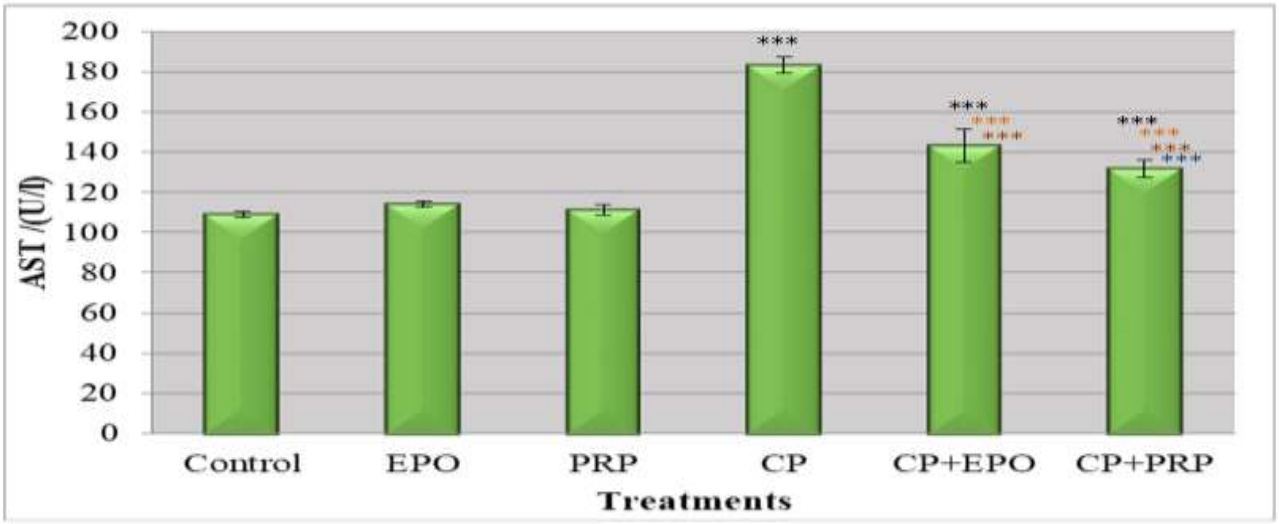

Figure 2: Effect of Erythropoietin (EPO) and Platelete-Rich Plasma (PRP) on the level of AST of Cyclophosphamide-Treated rats. Values are expressed as mean ± SEM

- **Referring to the significant differences between control group and each treated group separately.**
- **Referring to the significant differences between CP treated group and the groups**
- **that treated with the combination of CP +EPO /or CP+PRP.**
- **Referring to the significant differences between EPO treated group and the group that treated with the combination of CP +EPO.**
- **Referring to the significant differences between PRP treated group and the group that treated with the combination of CP+PRP.**

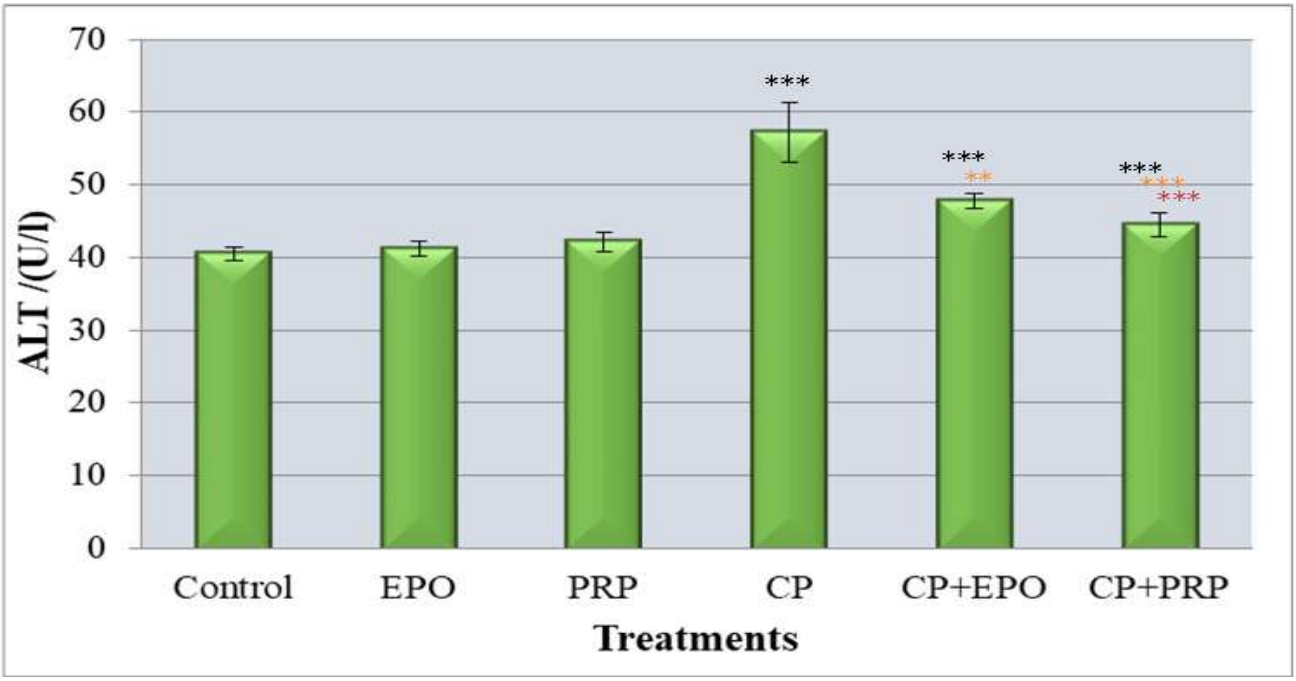

Figure 3: Effect of Erythropoietin (EPO) and Platelete-Rich Plasma (PRP) on the level of ALT of Cyclophosphamide-Treated rats. Values are expressed as mean ± SEM

- **Referring to the significant differences between control group and each treated group separately.**
- **Referring to the significant differences between CP treated group and the groups**
- **that treated with the combination of CP +EPO /or CP+PRP.**
- **Referring to the significant differences between EPO treated group and the group that treated with the combination of CP +EPO.**

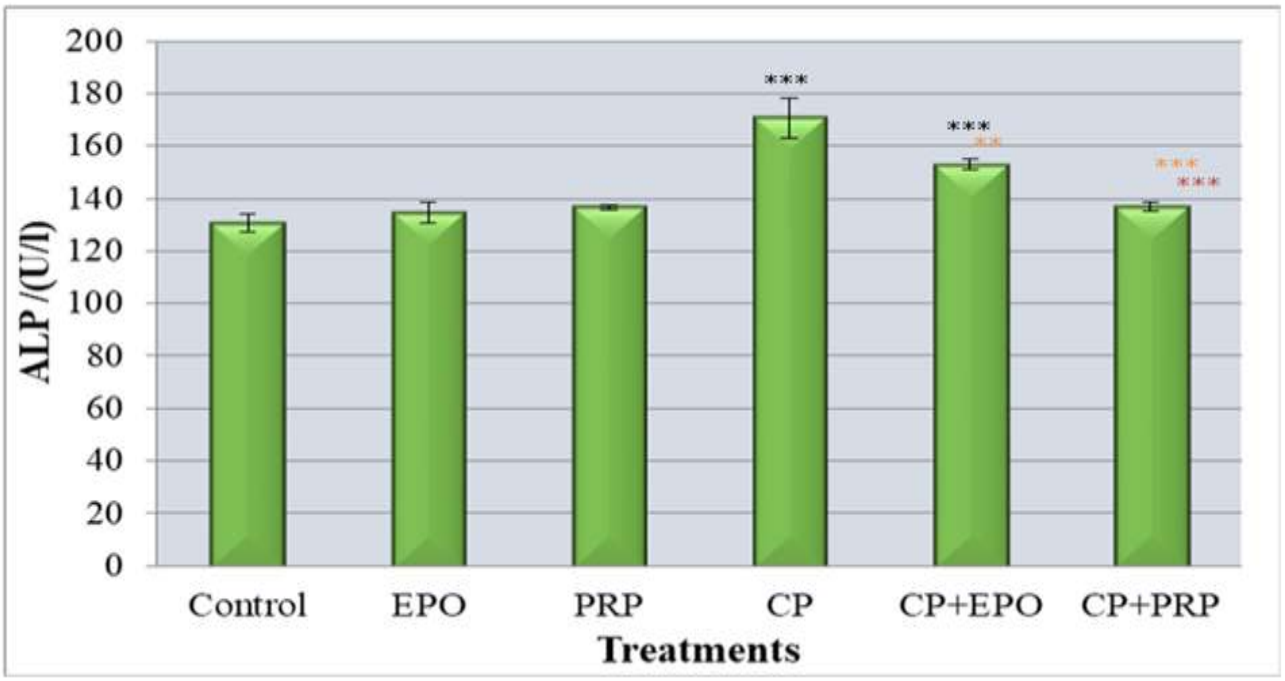

Figure 4: Effect of Erythropoietin (EPO) and Platelete-Rich Plasma (PRP) on the level of ALP of Cyclophosphamide-Treated rats. Values are expressed as mean ± SEM

- **Referring to the significant differences between control group and each treated group separately.**
- **Referring to the significant differences between CP treated group and the groups**
- **that treated with the combination of CP +EPO /or CP+PRP.**
- **Referring to the significant differences between EPO treated group and the group that treated with the combination of CP +EPO.**

Protein Levels

Administration of single intraperitoneal dose of CP (150 mg/kg) resulted in a significant ($p<0.05$) reduction in the levels of total protein, albumin and globulin in the serum as compared to control. Treatment with EPO or PRP significantly ($p<0.05$) elevated serum total protein, albumin and globulin compared to the CP group (Fig.5, 6 and 7).

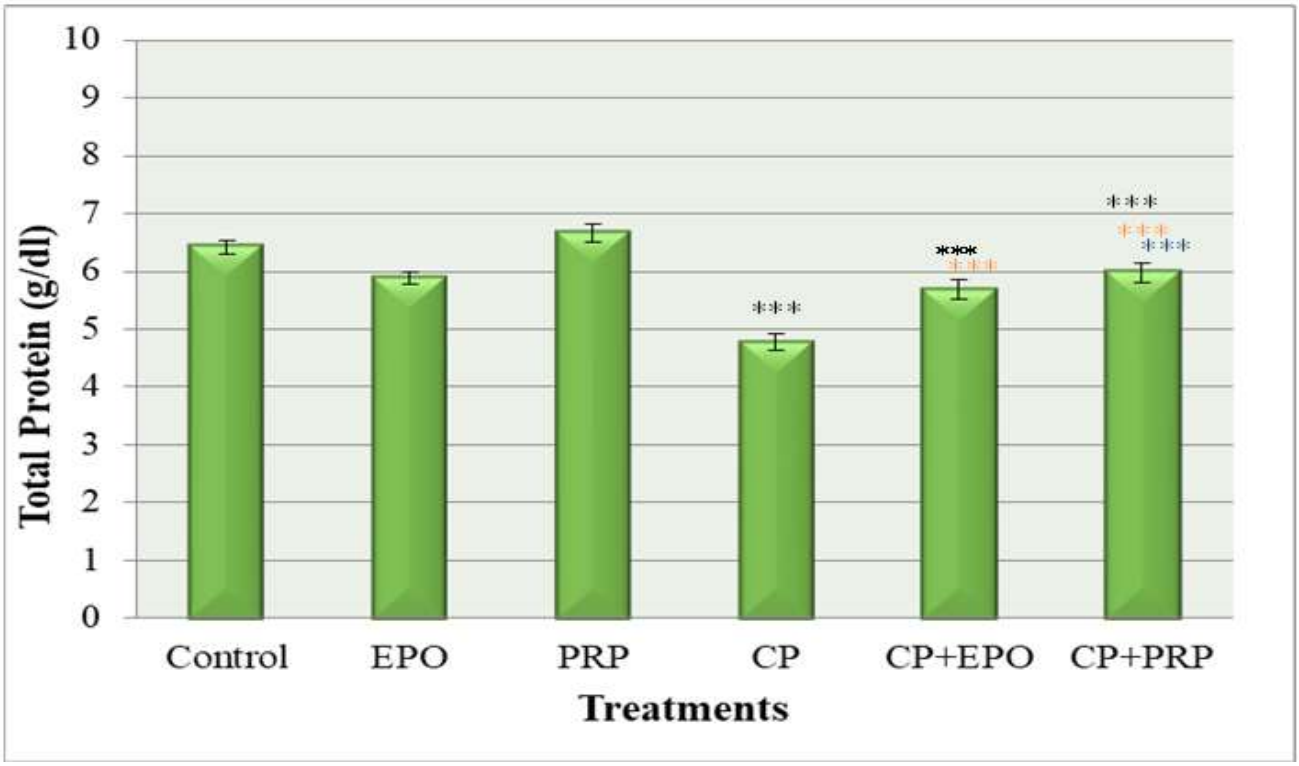

Figure 5: Effect of Erythropoietin (EPO) and Platelete-Rich Plasma (PRP) on Total Protein of Cyclophosphamide-Treated rats. Values are expressed as mean ± SEM

- **Referring to the significant differences between control group and each treated group separately.**
- **Referring to the significant differences between CP treated group and the groups**
- **that treated with the combination of CP +EPO /or CP+PRP.**
- **Referring to the significant differences between PRP treated group and the group that treated with the combination of CP+PRP.**

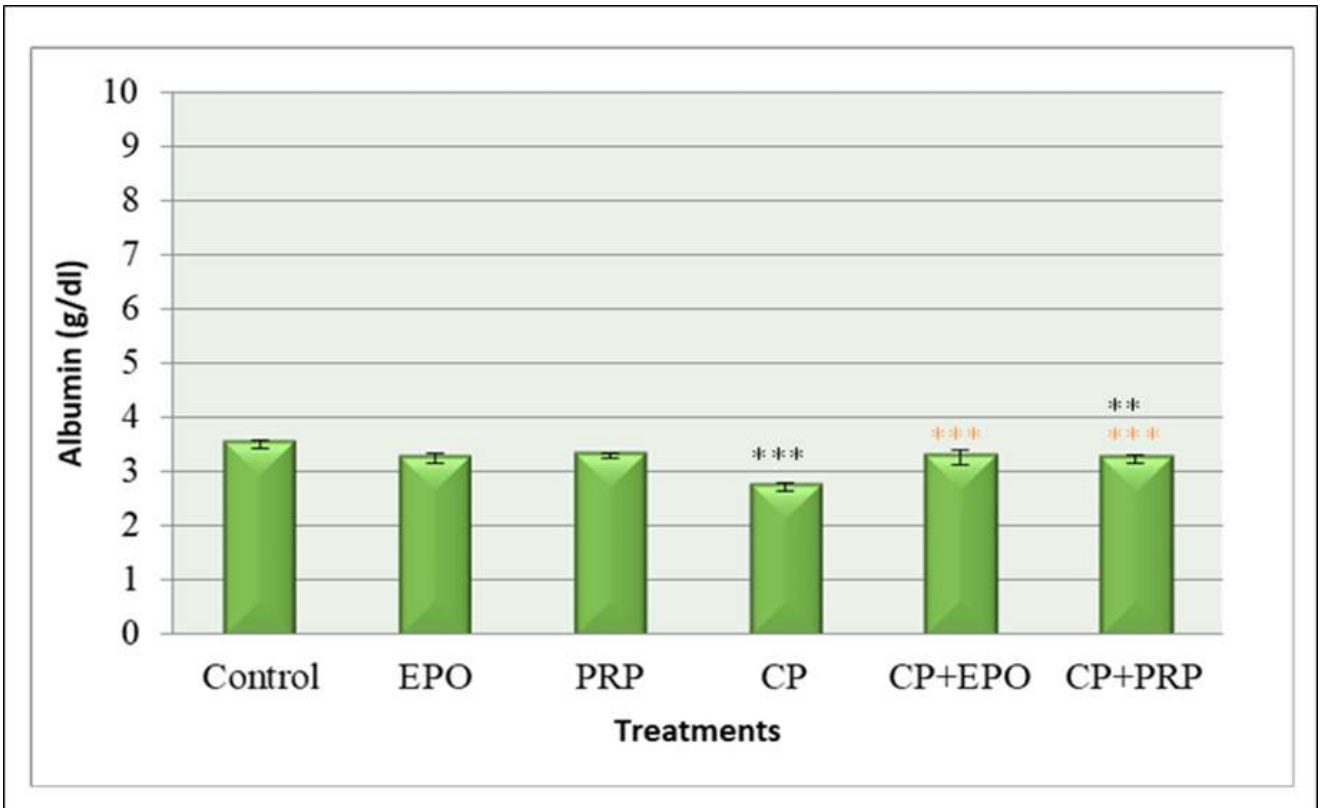

Figure 6: Effect of Erythropoietin (EPO) and Platelete-Rich Plasma (PRP) on Albumin of Cyclophosphamide-Treated rats. Values are expressed as mean ± SEM

- **Referring to the significant differences between control group and each treated group separately.**
- **Referring to the significant differences between CP treated group and the groups that treated with the combination of CP +EPO /or CP+PRP.**

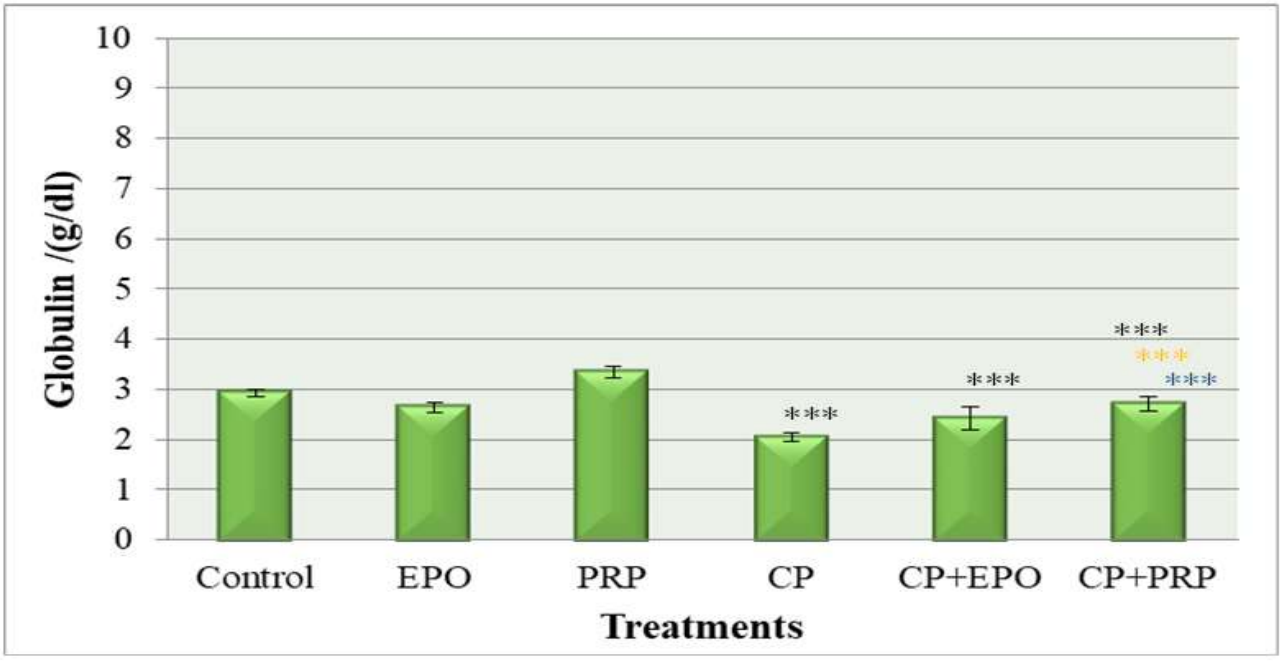

Figure 7: Effect of Erythropoietin (EPO) and Platelete-Rich Plasma (PRP) on Globulin of Cyclophosphamide-Treated rats. Values are expressed as mean ± SEM

- **Referring to the significant differences between control group and each treated group separately.**
- **Referring to the significant differences between CP treated group and the groups that treated with the combination of CP +EPO /or CP+PRP.**
- **Referring to the significant differences between PRP treated group and the group that treated with the combination of CP+PRP.**

Discussion

The present study investigated the ameliorative effect of EPO and PRP on cyclophosphamid-induced hepatotoxicity in male rats. Hepatotoxicity due to administration of CP was confirmed by increased levels of liver function enzymes AST, ALT and ALP with significant reduction in the levels of total protein, albumin and globulin, and these results were consistent with previous studies [14, 15, and 16]. The toxic effect of CP leads to increase the production of ROS, that causes damage to hepatocytes, and increase the level of liver function enzymes in the serum due to tissue destruction, and releasing these enzymes into the circulation [17].

The liver plays a crucial role in the synthesis of proteins; therefore the reduction of total protein, albumin and globulin levels is an indicator of CP-induced liver damage. CP administration obviously reduced appetite and food intake and resulted in body weight loss. This is in accordance with previous studies [18, 19]. The present study demonstrated that administration of EPO and PRP exert hepatoprotective effects against CP-induced hepatotoxicity. Liver toxicity is a major side effect of CP [20]. It is converted by hepatic cytochrome P450 mixed functional oxidase system to produce two metabolites, phosphoramide mustard and acrolein. Phosphoramide mustard has an anti-tumer activity, while acrolein is responsible to CP-induced hepatoxicity [20].

It can stimulate oxidative stress and depress the antioxidant defense mechanism via the production of reactive oxygen species (ROS)[22]. Previous studies suggested that EPO may protect cells by reducing cellular oxidative stress, as one of the most important causes of cellular damage.

Many investigations have detected the direct and indirect anti-oxidative impacts of EPO [23]. In direct anti-oxidative pathway, EPO can increase anti-oxidative enzymes such as glutathione peroxidase, catalase and superoxide dismutase and enhance cellular antioxidant capacity [24]. In indirect anti-oxidative pathway, EPO can inhibit iron-dependent oxidative damage through indirectly depleting body iron.

Furthermore, EPO indirectly reduces cellular oxidative stress by increasing young red blood cells, which can help to ameliorate iron-dependent oxidative damage [25]. Administration of PRP could reduce the tissue damage and accelerate recovery by regulating antioxidant enzymes and

improving the antioxidant defense systems [26]. Previous studies showed that PRP induces the regeneration and repair of damaged cells and tissues by the activation of stem cells via their growth factors and cytokines [27]. The growth factors also promote liver regeneration by stimulating hepatocyte mitosis [28].

Conclusions

To our knowledge, this is the first study to investigate the amelioration of CP-induced Hepatotoxicity by EPO and PRP. The results of the present study demonstrated that EPO and PRP had a significant hepatoprotection against CP-induced hepatotoxicity in male albino rats. PRP has shown higher protective effect than EPO.

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