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Araştırma Makalesi - Research Article

Siklofosfamid Nedenli Kan Hücreleri ve Kemik İliği Toksisitesi Üzerine Escinin Koruyucu Etkilerinin Sıçanlarda Araştırılması

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ÖZ

Siklofosfamid (CPM), metabolitleri nedeniyle kanserli hücrelerin yanı sıra sağlıklı dokularda da toksisiteye neden olan yaygın olarak kullanılan bir antineoplastik ilaçtır. Bu ilacın ana dezavantajı miyelosupresyondur. Escin (ES), antioksidan, antienflamasyon ve anti-ödem gibi biyolojik özelliklere sahip doğal bir triterpenoid saponin karışımıdır. Bu çalışma, CPM'nin kan hücreleri ve kemik iliği üzerindeki toksik etkileri üzerine ES'nin olası etkilerini araştırmayı amaçlamaktadır. Spraque Dawley erkek sıçanlar, kontrol grubu, 200 mg / kg CPM, 10 mg / kg ES ve CPM+ES olmak üzere 4 farklı gruba ayrıldı. Tüm gruplardaki sıçanlar enjeksiyonlardan 1 gün sonra kesildi. Hayvanlar anestezi altında kesildikten sonra kemik iliği ve kan örnekleri alındı. CPM grubunda eritrosit, hemoglobin, hematokrit, lökosit (% 97), trombositler (% 45) ve kemik iliği hücreleri (% 93.6) sayısında kontrol grubuna göre bir azalma vardı. Tersine, ES + CPM' grubundaki kan hücreleri (eritrosit, hemoglobin, hematokrit, lökosit ve trombositler) ve kemik iliği hücreleri CPM verilen grup ile karşılaştırıldığında artmıştır. Sonuçlarımız, ES ile sıçanların tedavi edilmesinin, CPM'nin kan hücreleri ve kemik iliği üzerindeki toksik etkilerini azaltmaya yardımcı olabileceğini göstermektedir.

Anahtar Kelimeler- Escin, Sitoprotektif, Siklofosfamid, Hematotoksisite, Sıçan

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Investigation into the Protective Effects of Escin on Blood Cells and Cyclophosphamide-Induced Bone Marrow Toxicity in Rats

ABSTRACT

Cyclophosphamide (CPM) is an extensively used antineoplastic drug that induces toxicity in the health cell because of its metabolites. The main disadvantage of this drug is myelosuppression. Escin (ES) is a natural mixture of triterpenoid saponins that has biological properties such as antioxidant, anti-inflammation, and anti-edematous. The present study aims to investigate the possible effects of ES upon the toxic effects of CPM on blood cells and bone marrow. Spraque Dawley male rats were chosen and categorized into 4 different groups, including the control group, 200 mg/kg CPM, 10 mg/kg ES and ES+CPM. Rats in all groups were discontinued 1 day after injections. After the animals were cut under anesthesia, bone marrow and blood samples were collected. A fall in the number of erythrocyte, hemoglobin, hematocrit, leukocyte (97%), platelets (45%), and bone marrow cells (93.6%) was seen in the CPM group. Conversely, blood cells (erythrocyte, hemoglobin, hematocrit, leukocyte, and platelets), as well as bone marrow cells in ES + CPM had increased by comparison with CPM. Our results show that treating rats with a selected dose range of ES may help decrease the toxic effects of CPM upon blood cells and bone marrow.

Keywords- ***Escin, Cytoprotective, Cyclophosphamide, Hematotoxicity, Rats***

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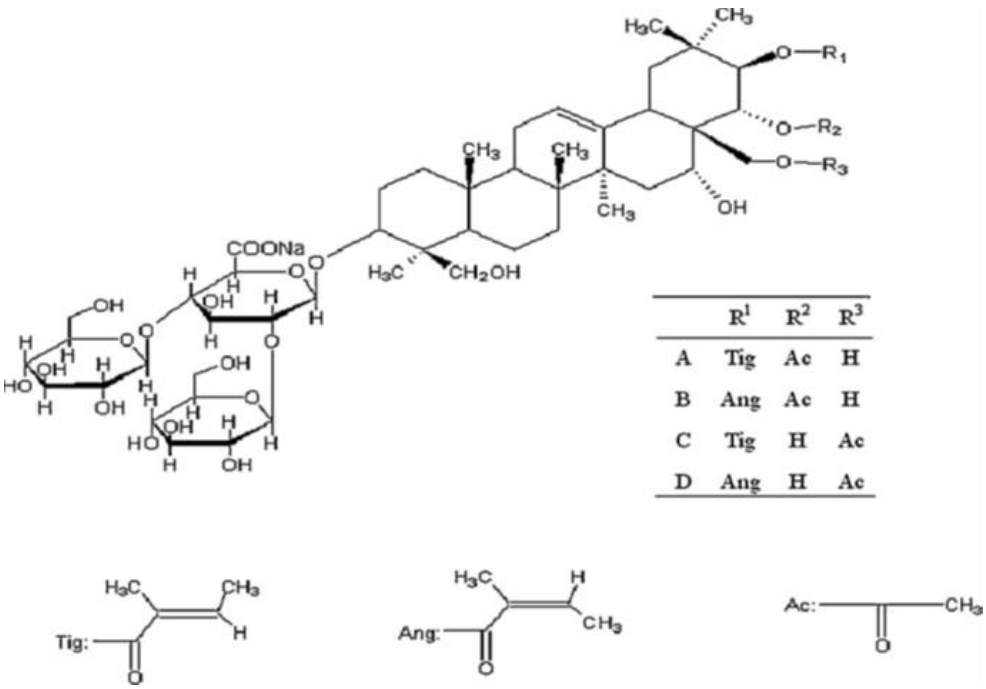
I. INTRODUCTION

Cyclophosphamide (CPM), renowned as a cytotoxic agent, is not just an anti-neoplastic but also an immunosuppressive agent widely used in treating many cancers. However, it a prodrug that has got to be metabolized in the living system to form reactive components like acrolein and phosphoramide mustard [1]. Previous studies have shown these toxic metabolites to decrease lung microsomal enzyme activities, in addition to preventing mammals' antioxidant defense system from functioning properly [2]. The fact is that long-standing CPM treatment may result in side-effects like marked myelosuppression [3], known as a potentially toxic and a dose-limiting effect of CPM. By acting not only on cyclic but also on intermitotic cells, CPM contributes to the cross-linking of DNA, thus inhibiting the synthesis of DNA and causing an overall reduction in immune component cells [4]. Apart from its carcinogenic and teratogenic potentials, CPM also holds recognized toxic effects upon the heart, bladder, and hematopoietic system. It may also cause leucopenia and reduce the platelet number [5]. According to many studies, CPM exposure increases intracellular reactive oxygen species (ROS) production, suggesting that biochemical plus physiological disorders may be caused by oxidative stress [6]. Studies into mammals have revealed that CPM may also give severe harm to the blood-forming tissues of bone marrow. Also, it may temporarily cause a fall in circulating PMNs (polymorphonuclear neutrophils) and so affect our innate immune system. Furthermore, CPM can result in a decrease in microsomal enzyme activity and weaken our antioxidant defense system. This, in turn, weakens our specific immunity by directly depleting lymphoid tissues and keeping the host from giving an acceptable specific immune response [7].

Escin (ES) is known as a regular mixture of triterpene saponins, primarily comprising A, B, C, and D escin (Figure 1) [8]. We know of no less than 3 kinds of pharmacodynamic actions that are closely associated with ES, antioxidant and venotonic properties, as well as anti-edematous properties and anti-inflammatory activities [9, 10]. ES is usually preferred for patients with severe trauma injury as an effective anti-inflammatory and anti-edema agent. As for our study, the rats had been injected ES prior to CPM treatment to investigate the effects of ES on CPM-related hematotoxicity.



	R^1	R^2	R^3
A	Tig	Ac	H
B	Ang	Ac	H
C	Tig	H	Ac
D	Ang	H	Ac

Figure 1. The structure of ES

II. MATERIAL AND METHODS

A. Materials

Not only ES but also CPM was cultivated from Sigma. 500 mg of SF had been dissolved in 25 mL saline in preparation for injection. As for the chemical injections, they were given to the rats intraperitoneally (i.p.) with sterile, disposable syringes subsequent to freshly-prepared solutions. In preparing the orally-given solution, 10 mg/kg dose of ES was dissolved in 0.5 mL of distilled water.

B. Treatment

Our study was launched only after ethical approval had been obtained from the Experimental Animals Ethics Committee of Eskişehir Osmangazi University. The rats to be studied were obtained from the Public Health Center. Care was taken to feed the animals in a standard environment, with drinking water and standard food pellets prepared carefully. The rats were diligently kept under standard humidity (45–50%), temperature (22 ± 2 °C), and light (12 h light/12 h dark) conditions. Their weight was measured not only during the injection process but also before they were slaughtered in the determination of the dose to be applied. Injection of the second group that was given solely CPM was achieved on the very first day. The very next day, they were anesthetized. As to the group given both CPM and ES, the injection was achieved on the first day. Under ether anesthesia, we obtained the blood samples with aid of a cardiac puncture. The study animals were slaughtered on the second day. We flushed their bone marrow with saline into a test tube after both femurs had been dissected. A cell counter (Coulter) was used in counting the homogenized bone marrow, blood cells, and nucleated cells.

C. Statistical Analyses

The expression of the data of the animal experiments was achieved as the standard error of the mean (±SEM). As for the analysis of the independent measurements and continuous data showing a normal distribution, One Way Anova was used. Furthermore, the Kruskal-Wallis test was used in scoring the variants showing an abnormal distribution. The differences in the experimental groups were regarded as significant if the p-value was <0.001 and <0.05.

III. RESULTS

The rise in the number of leukocytes, erythrocyte, hemoglobin, hematocrit, and platelet in the group given 10 mg/kg of ES was not of a statistical significance ($p>0.05$). On the other hand, the rise in the number of bone marrow–nucleated cells was of statistical significance ($p<0.01$). However, 200 mg/kg of CPM resulted in a 97% reduction in leukocyte cells when no other chemicals were added to the dose ($p<0.001$). Injected along with CPM, 10 mg/kg of ES lowered leukocytes as much as 18% ($p<0.01$) (Figure 2). Interestingly, platelets of rats given 200 mg/kg of CPM alone decreased by 45% ($p<0.001$). As for the results of a combination of both CPM and ES, the number of thrombocytes decreased as much as 31% ($p<0.05$) (Figure 3). Bone marrow–nucleated cells reduced as much as 93,6% once 200 mg/kg of CPM was injected ($p<0.001$). In comparison with the respective three doses of CPM, 10 mg/kg of ES induced as much as 49% recovery in bone marrow–nucleated cell numbers (Figure 4). When used alone, CPM decreased the number of erythrocytes as much as 27% ($p<0.01$) (Figure 5). When given with a respective dose of CPM, 10 mg/kg of ES lowered leukocytes as much as 17% ($p<0.01$) (Figure 2). The number of hemoglobin and hematocrit in the animals given 200 mg/kg of CPM alone fallen as much as19 and 23 %, respectively ($p<0.05$). Given along with a respective dose of CPM, 10 mg/kg of ES enlarged the number of hemoglobin and hematocrit by 21 and % 20 ($p>0.05$) (Figures 6 and 7).

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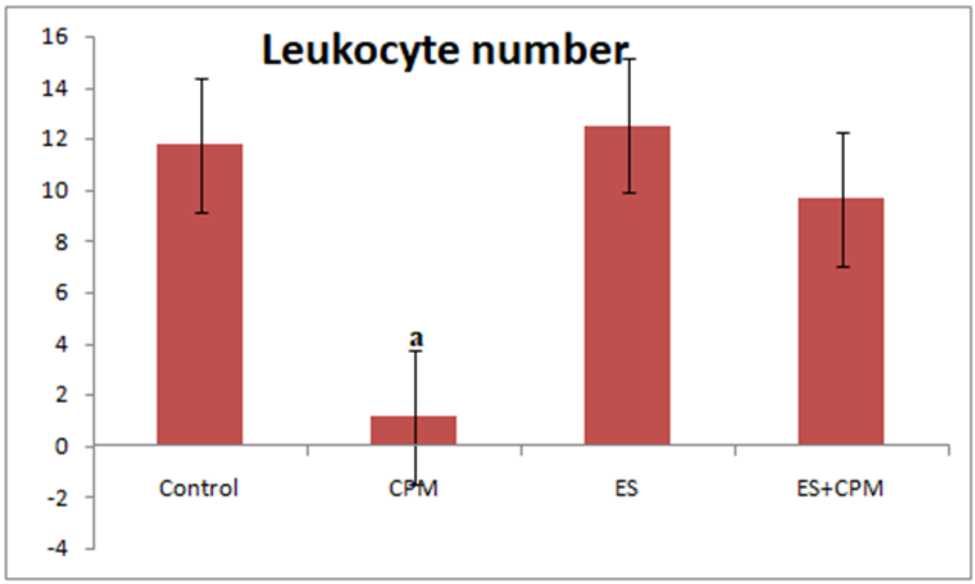

Figure 2. The number of leukocytes in the existence of all groups ($X10^3/\mu L$). [a]*p<0.001* significant difference compared to control

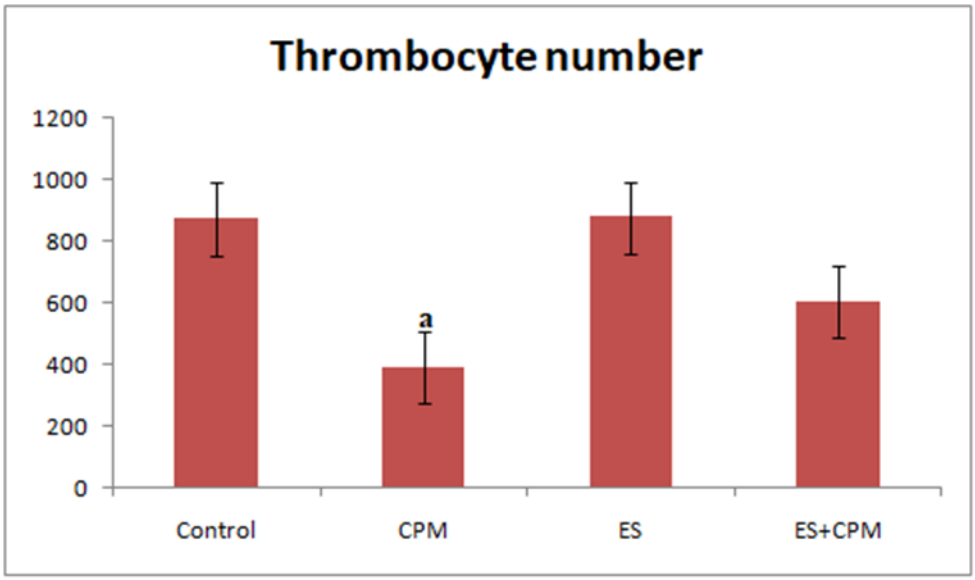

Figure 3. The number of peripheral thrombocytes in the existence of all groups ($X10^3/\mu L$). [a]p<0.001 significant difference compared to control

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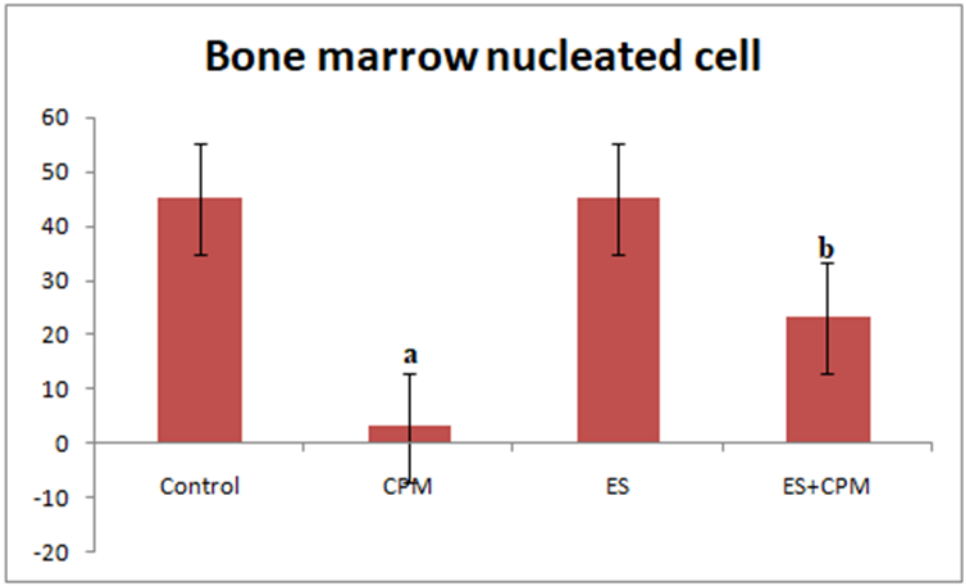

Figure 4. The number of bone- marrow-nucleated cells in the existence of all groups ($X10^3/\mu L$). [a]*p<0.001* significant difference compared to control; [b]*p<0.05* significant difference compared to control

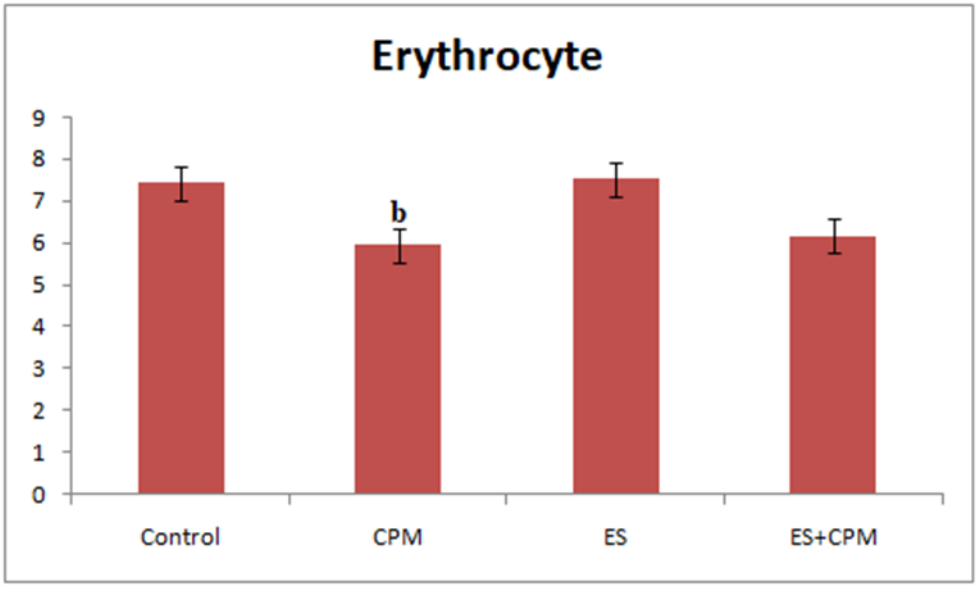

Figure 5. The number of erythrocytes cells in the existence of all groups ($X10^6/mm^3$). [b]*p<0.05* significant difference compared to control

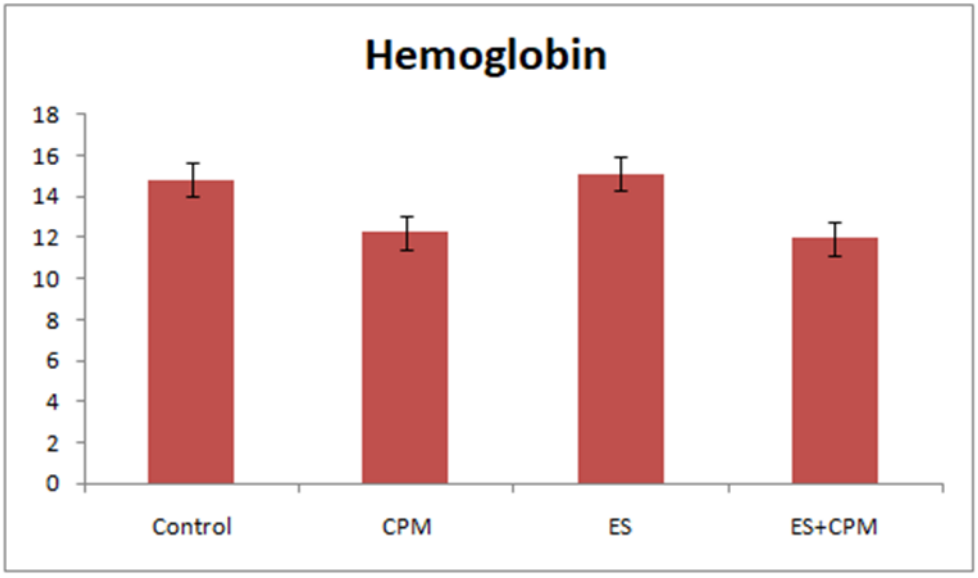

Figure 6. The number of hemoglobin the existence of all groups (g/dL)

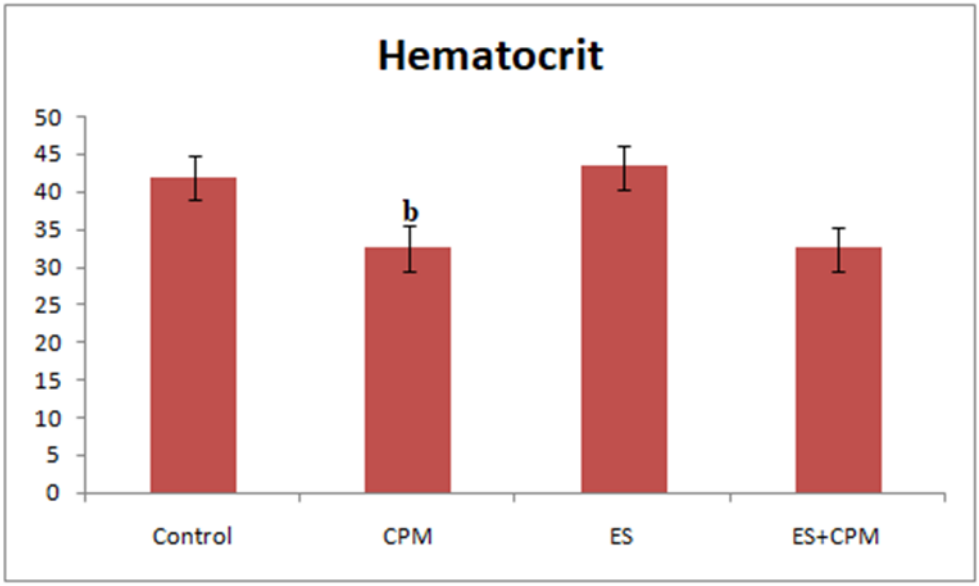

Figure 7. The number of hematocrit the existence of all groups (%).$^{b}p<0.05$ significant difference compared to control

IV. RESULTS

The chemotherapeutic practicality of alkylating agents is closely related to their capability to type a diversity of DNA adducts which can reasonably change DNA structure or function or both at the same time as far as their cytotoxic effect on the cells are concerned. A large number of them are known to go through a rather intricate activation process before they are able to make reactive intermediates happen. The preliminary activation reaction as regards CPM achieved through the microsomal oxidation system in the liver generates 4-hydroxy CPM, a cytotoxic metabolite, diffusing from hepatocytes into plasma before they could be travel in the whole body. Then, 4-hydroxy CPM is transformed into cytotoxic metabolites like PAM and ACR. This situation results in myelosuppression [11, 12], which we know to be a chief potential toxic and dose-limiting adverse effect of CPM.

By acting upon not just cyclic but also inter-mitotic cells, CPM leads to cross-linking of DNA and inhibition of DNA synthesis, which in turn, usually depletes immune component cells [13]. One study reports that 150 mg/kg CPM lowered the leukocyte numbers by 92%, the platelet numbers by 54%, and the bone marrow cell numbers by 94% [14]. In the same vein, a single dose of CPM injection caused leukocyte counts to go down [15]. Moreover, CP (40 mg/kg) given to baboons has caused a temporary fall in White Blood Cell count [16]. Another experimental study emphasizes that CP (20 and 40 mg/kg) may have a mutagenic effect upon the spleen and the bone marrow [17]. In a previous study, we demonstrated that 200 mg/kg CP administration reduced significantly the numbers of erythrocyte (20%), leukocyte (96%), platelet (41%), and hemoglobin (21%), and hematocrit [18]. Trasler et al. [19] referred to the effects of erythrocyte, leukocyte, and bone marrow cell count in that they decreased dramatically when the CPM was given in high doses to the mice. In our study, we witnessed a noteworthy reduction in the number of erythrocytes, leukocytes (97%), platelet (45%), hemoglobin, hematocrit, and bone marrow nucleated cells (93.6%) in the 200 mg/kg CPM group when compared to the control one. Our study results are remarkably consistent with those reported in the literature.

As for ES, it is a natural combination of triterpene saponins that is obtained from the seeds of *Aesculus Chinensis Bge*. or *Aesculus wilsonii Rehd*, largely consisting of A, B, C, and D escin (Figure 1). Mounting evidence suggests that administering ES intravenously results in anti- anti-oxidant [10, 20], inflammatory [8], and anti-edematous effects [21]. Our study is an original one in that while there are many studies into oral and intravenous effects of ES upon the liver, inflammation, and intestinal damage, no studies have been made as regards the protective effects of ES upon bone marrow, blood cell toxicities. Our study is an original one in that while there are many studies into the effects of ES upon the liver [10], inflammation [8], and intestinal damage (whether orally or intravenously), we do not come across any studies into the protective effects of ES upon bone marrow and blood tissue toxicities. In our study, in the group given CPM alone, we observed a reduction in the number of leukocytes, thrombocytes, erythrocytes, hemoglobin, and bone marrow cell counts as 97%, 45%, 20%, 21%, and 93%, respectively when compared to the control group. These rates were of high statistical significance. On the other hand, in the group given both CPM and 1m mg/kg ES, the reduction in the number of leukocytes, thrombocytes, erythrocytes, hemoglobin, and bone marrow cell counts was 18%, 31%, 19%, 20%, and 49%, respectively when compared to the group given CPM alone. These rates were also of high statistical significance. Therefore, ES appears to be toxic neither for bone marrow nor blood cells on its own while CPM is already known to be toxic for bone marrow, leukocytes, and platelets. Based on our study results we conclude that ES may provide protection against the toxic effects of CPM. We, therefore, believe that ES is a potentially effective drug to be used in treating CPM-related damage, which can help prevent damage or treat CPM toxicity. Finally, we suggest that further studies should be conducted in order to discover the underlying mechanism of how ES protects against CPM toxicity.

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