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Siklofosfamid Kaynaklı Toksisite Üzerine Salvia virgata Jacq'ın Hematoprotektif ve Antioksidan Etkilerinin Araştırılması

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ÖZET: Bu çalışma, Salvia virgata'nın (SV) Siklofosfamid (CP) kaynaklı toksisite üzerindeki hematoprotektif ve antioksidan etkilerini araştırmayı amaçlamaktadır. Sıçanlar, her biri 7 üyeden oluşan 6 gruba ayrıldı. Bunlar Kontrol grubu, CP Grubu (150 mg kg^{-1}), 100 ve 200 mg kg^{-1} SV Grupları ve CP+100 ve CP+200 mg kg^{-1} SV Gruplarıdır. Tüm sıçanlar, son enjeksiyonlardan sonraki gün kan ve kemik iliği numunelerini toplamak için kurban edildi. Kan örneklerinin bir kısmı lökosit ve trombosit sayımı için kullanılırken, diğerleri toplam antioksidan kapasite (TAC), malondialdehit (MDA), toplam oksidan durumu (TOS), glutatyon (GSH) ve oksidatif stres düzeylerini belirlemek için kullanıldı. indeks (OSI). CP verilen grupta lökosit, trombosit ve kemik iliği çekirdekli hücre sayılarında azalma görülürken, bu gruptaki ratların MDA ve TOC düzeylerinde GSH ve TAC düzeylerinde düşme dışında artış görüldü. Tersine, CP ile indüklenen oksidatif stres ve miyelosupresyon, kombine SV Gruplarında (CP+100 ve 200 mg kg^{-1} SV Gruplarında) tersine döndü, ancak ikinci gruptaki tersine dönüş daha önemliydi. Deneysel sonuçlarımız, SV'nin CP ile ilişkili periferik kan ve kemik iliği toksisiteleri üzerinde antioksidan ve sitoprotektif etkiler gösterebileceğini göstermiştir.

Anahtar Kelimeler: Siklofosfamid**,** hematotoksisite**,** salvia virgata**,** antioksidan**,** sitoprotektif

An Investigation into Hematoprotective and Antioxidant Effects of Salvia virgata Jacq. Upon Cyclophosphamide-Induced Toxicity

ABSTRACT: The present study aims to investigate hematoprotective and antioxidant effects of Salvia virgata (SV) on Cyclophosphamide (CP)-induced toxicity. The rats were divided into 6 groups of 7 members each. These were the Control group, CP Group (150 mg kg^{-1}), 100 and 200 mg kg^{-1} SV Groups, and CP+100 and CP+200 mg kg^{-1} SV Groups. All the rats were sacrificed to harvest their blood and bone marrow samples the day after final injections. While some of the blood samples were used for leukocyte and platelet count, the others were used to determine the levels of total antioxidant capacity (TAC), malondialdehyde (MDA), total oxidant status (TOS), glutathione (GSH), and oxidative stress index (OSI). While a decrease was observed in the leukocyte, platelet and bone-marrow nucleated cell counts of the group given CP, apart from a decline in GSH and TAC levels, the MDA and TOC levels of the rats in this group showed an increase. In contrast, CP-induced oxidative stress and myelosuppression reversed in the combined SV Groups (CP+100 and 200 mg kg^{-1} of SV Groups), although the reversal in the latter group was of more significance. Our experimental results have shown that SV may exert antioxidant and cytoprotective effects upon CP-related peripheral blood and bone marrow toxicities.

Keywords: Cyclophosphamide, hematotoxicity, salvia virgata, antioxidant, cytoprotective

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INTRODUCTION

Cyclophosphamide (CP) is a well and commonly-used potent drug used in treating cancer, particularly in chronic and acute leukemia, breast cancer, multiple myeloma, lymphoma, rheumatoid arthritis, and bone marrow transplants (Kumar and Kuttan, 2005; Cengiz, 2018; Ayhanci et al., 2019). The primary adverse effects of CP are hematopoietic depression, hemorrhagic cystitis, and renal toxicity. This drug is also known to exhibit suggestive adverse effects like hematotoxicity, urotoxicity, teratogenicity, mutagenicity, carcinogenicity, apart from myelosuppression (Buyukokuroglu et al., 2007; Cengiz, 2018).

Phosphoramide mustard (PAM) and acrolein (ACR) are the two active metabolites that CP produces. On the one hand, its anticancer properties are linked to PAM, which is thought to inhibit cell division by binding to DNA, hence facilitating CP's immunosuppressive and antitumor properties (Cengiz et al., 2016; Ayhanci et al., 2008; Taysi et al., 2008; Cengiz, 2018). The toxic action of CP, on the other hand, is due to its active metabolite, ACR, which depletes the tissue antioxidant (AO) defense mechanism, resulting in an overabundance of free oxygen radicals (SOR). As a result, CP causes mutations in mammalian cells (Teksoy et al., 2020; Kawabata et al., 1990; Buyukokuroglu et al., 2007; Cengiz et al., 2018; Ağgül et al., 2021). ACR-induced free radicals disrupt the functions of molecules such as enzymes, receptors, and ion pumps by combining with them. The aforementioned toxic effects should be eliminated via antioxidant agents in order to avoid possible toxic side-effects of ACR during a CP chemotherapy given for neoplastic diseases (Senthilkumar et al., 2006).

It has been demonstrated that the genus Salvia L., known as adaçayı in Turkey, has many traditional uses, both internally and externally, among which are the digestive system (as an appetizer, gas reliever, and stomach smoother), the respiratory system (as a cough suppressant, and bronchitis and asthma reliever), and the immune system (as a protective agent against infections and colds, an antiseptic agent, and a wound-healer) (Cengiz et al., 2016; Cadirci et al., 2012). Previous studies have reported that besides having antioxidant (Ucuncu et al., 2004; Yoruk et al., 2009), anti-tumor, immunomodulatory, anti-inflammatory, and anti-viral activities, SV is used in treating hemorrhoids (Gür et al., 2021; Tepe, 2008; Furtado et al., 2010). The present study aims to investigate possible cytoprotective effects of Salvia virgata on peripheral blood and bone marrow nucleated cells in order to eliminate the dose-limiting side effects of CP, a powerful antitumor agent, thus enabling this drug to be safely used in higher doses.

MATERIALS AND METHODS

Injection of chemical substances

CP (Sigma-Aldrich) and SV extract were obtained commercially. SV was dissolved in saline and two different doses of this extract (100 and 200 mg kg^{-1}) were administered intraperitoneally (i.p.). As for CP, 500 mg was dissolved in 25 ml of distilled water to make it eligible for injection, which was performed i.p. via sterile disposable injectors. The chemical injections were applied shortly after they had been prepared. All the animals were weighed before the first injection and the sacrificing process so as to determine the drug doses to be administered. The experimental study was conducted with the approval of the Local Ethics Committee for Animal Experiments at Eskisehir Osmangazi University (No: 799-1/2020). All the animals in the experimental groups except those in the Control Group were anesthetized 24 hours after CP injection. The animals in the SV+CP Groups were given only SV for five days in a row before they were injected a combination of 150 mg kg^{-1} of CP and SV on the 6th. These

animals were sacrificed to harvest their blood and bone marrow samples the very next day (Cengiz et al., 2019; Buyukokuroglu et al., 2007; Ayhanci et al., 2008; Cengiz, 2018).

Counting blood and bone-marrow nucleated cells via biochemical measurements

All the experimental studies were carried out in a sterile environment via sterile instruments. Intracardiac blood was harvested from the rats anesthetized with 50/10 mg kg^{-1} of Ketamine-Xylazine. One-fifth of these blood samples were placed in citrate tubes and counted by the rat calibration of the Hemavet 850 brand and model blood counting device. Serum and plasma samples were obtained from the remaining part of the blood by centrifugation at 3000 rpm for 10 minutes with an Eppendorf Centrifuge 5804 R brand and model device and then transferred to polyethylene tubes to be preserved in a -80˚C freezer for biochemical analyses.

Determination of Plasma Malondialdehyde (MDA) levels

The Thiobarbituric Acid Reactive Substance (TBARS) method was used to detect the quantity of MDA in plasma samples. The spectrophotometric measurement of the red color formed as a result of the reaction between lipid peroxidation product (MDA) and thiobarbituric acid (TBA). The serum lipids and proteins in the combination were precipitated with the phosphotungstic acid/sulfuric acid method to remove the water-soluble compounds that would react with TBA and yield the same color. After adding 150 mL plasma, 1.2 mL H2SO4, and 150 mL phosphotungstic acid to the mixture, which was centrifuged at 1500 g for 10 minutes, the test tube was properly mixed and kept in it for five minutes. By removing the upper clear section, the absorbances were read at 532 nm wavelength. 1 mmol 1,1,3,3-tetra methoxy propane was incubated for 1 hour at 50 °C in 100 ml 0.01 M HCl with 10, 5, 3, 2, 1, 0.5 nmol MDA solution generated as a result of this compound's hydrolysis. Based on the findings, a standard graphic was created. The amount of plasma MDA was calculated as nmol MDA/ml using this graph (Cengiz et al., 2020).

Determination of glutathione (GSH) levels

GSH levels were measured by a commercial colorimetric kit (Glutathione Assay Kit CS0260-1 KT) (Fraiser et al., 1991).

Determination of Total Oxidant Capacity (TOC)

The TOC value was measured by a commercially available colorimetric assay kit (Erel, 2005).

Determination of Total Anti-Oxidant Capacity (TAC)

The TAC value was measured by a commercially available colorimetric assay kit (Erel, 2005).

Determination of Oxidative Stress Index (OSI)

The OSI value was calculated considering the TOC/TAC ratio. The formula was [(TOS, μmol H2O2 equivalent L^{-1}) / (TAC μmol Trolox equivalent L^{-1}) x100] (Aycicek et al., 2005).

Statistical evaluations

The standard error of the mean (SEM) was used to express the results of this investigation. In a similar vein, one-way ANOVA was used to examine independent and normal distribution data, while Kruskal-Wallis one-way analysis was used to analyse variables with the aberrant distribution. The differences observed in the groups were considered statistically significant as long as the p-value was between 0.001 and 0.05.

RESULTS AND DISCUSSION

Recovery of CP-induced hematotoxicity with SV

Figure 1 shows the impact of these chemicals on the numbers of thrombocytes, leukocytes, and bone marrow cells in the SV (100 and 200 mg kg^{-1}) and CP groups. In the experimental groups given SV at doses of 100 and 200 mg kg^{-1}, there was no statistically significant difference in leukocyte, platelet, or bone marrow cell counts compared to the Control Group ($p>0.05$). When compared to the Control Group, the experimental group receiving 150 mg kg^{-1} CP had a statistically significant drop in leukocyte, thrombocyte, and bone marrow cell counts ($p<0.001$). One of these studies reported that 150 mg kg^{-1} of CP reduced the number of platelets, leukocytes, and bone marrow cells by 54%, 92%, and 94%, respectively (Taysi et al., 2008; Ayhanci et al., 2009). In a similar experimental study, the injection of CP is reported to have reduced the number of leukocytes (Fraiser et al., 1991). In another study, 40 mg kg^{-1} of CP given to baboons is reported to have temporarily reduced leukocyte numbers (Schuurman et al., 2005). Still another study emphasized that 20 and 40 mg kg^{-1} of CP had a mutagenic effect upon the spleen and bone marrow. (Moore et al., 1995). Cengiz et al. have recently shown that 200 mg kg^{-1} of CP significantly decreased leukocyte (96%) and platelet (41%) numbers, along with the number of hemoglobin levels (21%) (Cengiz, 2018). In the same vein, Trasler et al. found that when high doses of CP were given to mice, a dramatic decrease was observed in erythrocyte, leukocyte, and bone marrow cell numbers (Trasler et al., 1987). The results of the present study are consistent with those published in the literature. On the other hand, a statistically significant increase was determined in the leukocyte, thrombocyte and bone marrow counts of the experimental groups given 150 mg kg^{-1} of CP plus 100 and 200 mg kg^{-1} of SV when compared to the CP Group. However, the increase in the number of leukocytes, thrombocytes, and bone marrow nucleated cells was higher in the group treated with 200 mg kg^{-1} of SV compared to the one treated with 100 mg kg^{-1}.

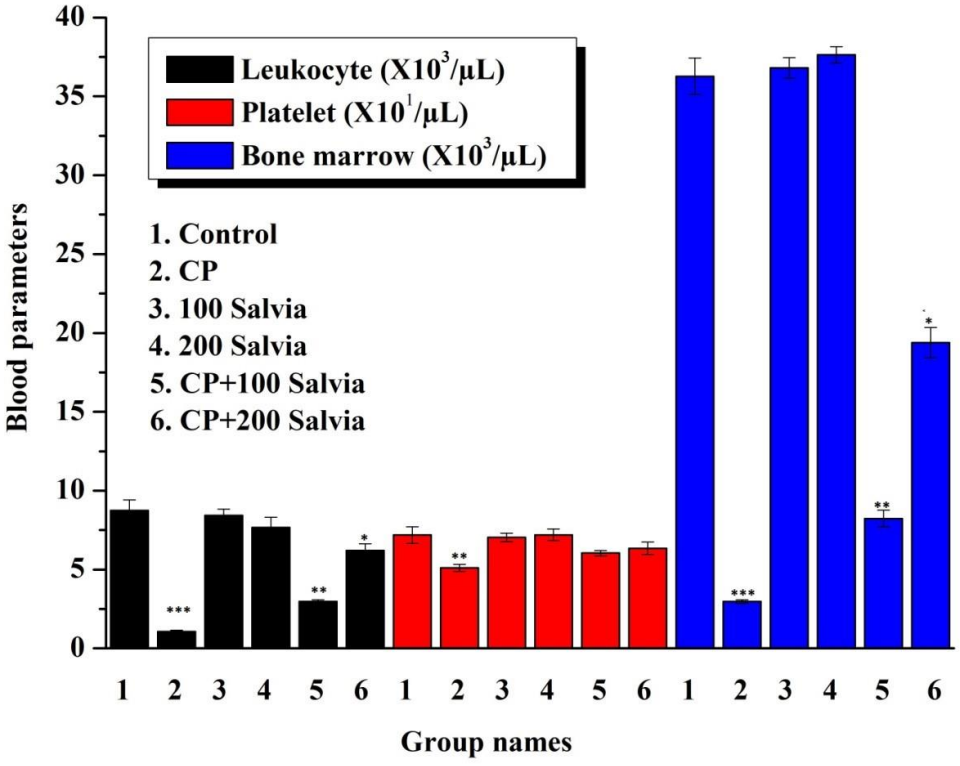

Figure 1. All of the experimental groups' leukocyte, thrombocyte, and bone marrow counts changed. In comparison to the Control Group, *p<0.05, **p<0.01, ***p<0.001 were found.

SV improves CP-induced oxidative stress and lipid peroxidation

Table 1 and Figure 2 show that whereas CP raised MDA, TOS, and OSI levels when compared to the Control Group, it lowered GSH and TAC levels ($p<0.001$). MDA, TOS, and OSI levels, on the other hand, were significantly lower in the CP and SV groups, although GSH and TAC levels were significantly higher ($p<0.001$).

Table 1. The effects of CP and SV upon the levels of MDA and GSH

Groups	MDA	GSH
Control	0.483±0.012	5.79±0.09
CP	0.763±0.055[a]	2.72± 0.19[a]
100 SV	0.440±0.026	5.78± 0.7
200 SV	0.460±0.022	6.22± 0.8
CP+100 SV	0.590±0.013[b]	3.86 ± 0.05[b]
CP+200 SV	0.510±0.02[c]	4.97 ± 0.05[c]

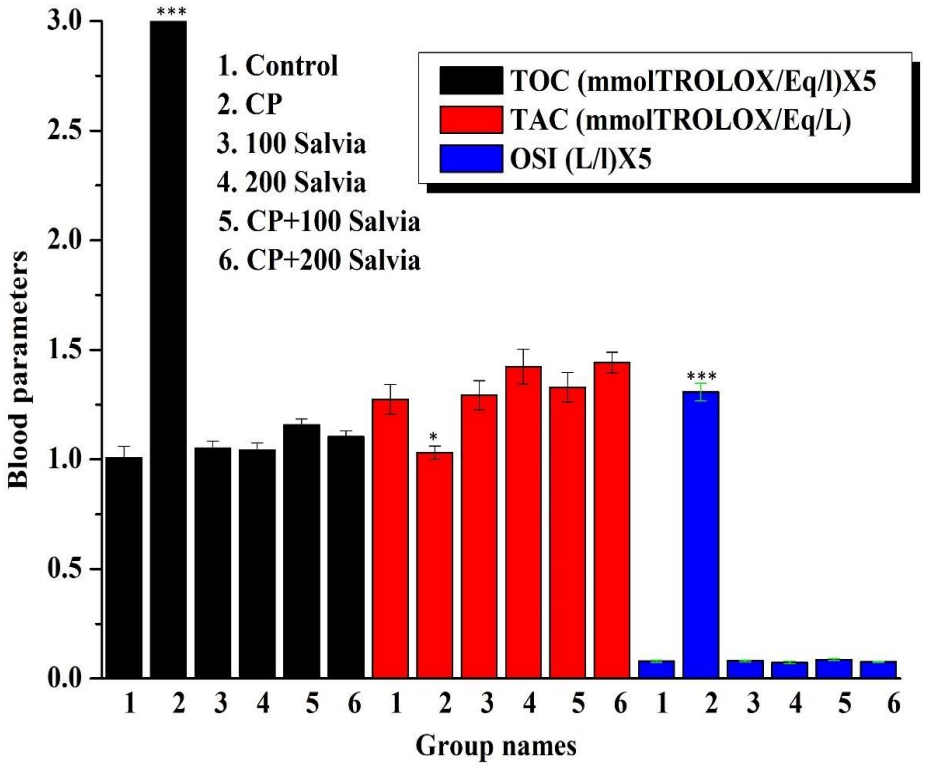

Figure 2. The effects of CP and SV upon the TAC, TOC, and OSI parameters of all the study groups ***$p<0.001$, *$p<0.05$ compared to Control Groups

CP's toxicity is linked to its active metabolite, ACR, which depletes the tissue antioxidant (AO) defense system, resulting in a high rate of ROS production and lipid peroxidation in cells (Kawabata et al., 1990; Buyukokuroglu et al., 2007; Cengiz et al., 2018). By interacting with components like enzymes, receptors, and ion pumps, ACR-induced free radicals disturb their functionality (Senthilkumar et al., 2006). The present study determined that while MDA, TOS, and OSI levels significantly increased in the group given 150 mg kg^{-1} of CP, GSH and TAC levels decreased, which appears to be consistent with the results in the literature.

During CP chemotherapy for neoplastic diseases, the toxic effects should be detoxified thanks to antioxidant agents so that the abovementioned toxic side effects of ACR can be avoided. It has also been

reported that SV has such various biological activities as an antioxidant, antiviral, and anti-inflammatory (Tepe, 2008; Furtado et al., 2010). That both doses of SV significantly prevented myelosuppression, leukopenia, and thrombocytopenia that developed in the experimental groups given CP was demonstrated by the fact that the number of peripheral blood cells and bone marrow nucleated cells had increased and the antioxidant parameters of GSH and TAC had reached those of the control (Figure1 and Table 1). Nonetheless, 200 mg kg^{-1} of SV was more effective in preventing CP-induced toxicity than was 100 mg kg^{-1}.

CONCLUSION

Based on the findings of this research, it was determined that SV can protect against the damaging effects of CP. As a result, we believe that SV could be a promising treatment for CP and other anticancer drug-related side effects. However, more research is needed to fully understand the processes by which SV lowers CP-induced cytotoxicity.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

İlknur Kulcanay Şahin (Ph.D.): Methodology, Investigation Formal analysis, Software, Investigation, Data Curation, Review, Writing-Original Draft Preparation.

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