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Modulation of the Immune System and Hemogram Parameters by *Prunus spinosa* in Short-Term Hyperglycemia

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ABSTRACT Diabetes mellitus (DM) is one of the chronic diseases, relationship increased blood glucose level, that requires urgent global attention due to its prevalence and associated complications. DM leads to oxidative stress that plays an important role in the development of various complications in diabetes by suppressing the immune system. *Prunus spinosa* is a plant that has been used in the treatment of many diseases from past to present, thanks to its high antioxidant activity. Therefore, the present study aims to research the effect of *P. spinosa* leaf and flower mixture on immune system during the short-term diabetic condition. In the study, 56 *Wistar albino* male rats divided into 7 groups, one of which control and others six diabetic groups, were used to determine the effects of *P. spinosa* on adenosine deaminase (ADA), (xanthine oxidase) XO and myeloperoxidase (MPO) activities in the liver tissues of diabetic rats as well as on hemogram parameters. Two of these groups were given plant extract in different concentrations (25 and 50 mg/kg bw) and the results were compared with insulin, metformin and acarbose groups. The results showed that both doses administered had a modulating effect on the changing hematological parameters caused by diabetes. Treatment groups significantly decreased ADA, XO, and MPO activities compared to diabetic group. The effects of the PSE50 were found to be more effective than all other treatment. These effects of the plant in diabetesmay be due to its therapeutic immunoregulatory potential. As a result, *P. spinosa* can be a valuable resource as an adjuvant on diabetes.

Keywords: *Adenosine deaminase, Hematology, Immunity, Myeloperoxidase, Prunus spinosa, Xanthine oxidase.*

ÖZ

Kısa Süreli Hiperglisemide *Prunus spinosa* Tarafından Bağışıklık Sistemi ve Hemogram Parametrelerinin Modülasyonu

Diabetes mellitus (DM), prevalansı ve ilişkili komplikasyonları nedeniyle acil küresel dikkat gerektiren, kan glukoz düzeyi ile ilişkili kronik hastalıklardan biridir. DM, bağışıklık sistemini baskılayarak diyabette çeşitli komplikasyonların gelişiminde önemli rol oynayan oksidatif strese yol açar. *Prunus spinosa*, yüksek antioksidan aktivitesi sayesinde geçmişten günümüze birçok hastalığın tedavisinde kullanılan bir bitkidir. Bu nedenle, bu çalışma kısa süreli diyabetik durumda *P. spinosa* yaprak ve çiçek karışımının bağışıklık sistemi üzerindeki etkisini araştırmayı amaçlamaktadır. Çalışmada, diyabetik sıçanların karaciğer dokularında *P. spinosa*'nın adenozin deaminaz (ADA), ksantin oksidaz (XO) ve miyeloperoksidaz (MPO) aktiviteleri ve hemogram parametreleri üzerine etkilerini belirlemek amacıyla biri kontrol, diğerleri altı diyabetik grup olmak üzere 7 gruba ayrılan 56 Wistar albino erkek sıçan kullanıldı. Bu gruplardan ikisine farklı konsantrasyonlarda (25 ve 50 mg/kg canlı ağırlık) bitki ekstraktı verildi ve sonuçlar insülin, metformin ve akarboz grupları ile karşılaştırıldı. Sonuçlar, uygulanan her iki dozun diyabetin neden olduğu değişen hematolojik parametreler üzerinde modüle edici bir etkiye sahip olduğunu gösterdi. Tedavi grupları, diyabetik gruba kıyasla ADA, XO ve MPO aktivitelerini önemli ölçüde azalttı. PSE50'nin etkilerinin diğer tüm tedavi gruplarından daha etkili olduğu bulundu. Bitkinin diyabetteki bu etkileri, terapötik immün düzenleyici potansiyeline bağlı olabilir. Sonuç olarak *P. spinosa*, diyabet üzerinde bir adjuvan olarak değerli bir kaynak olabilir.

Anahtar Kelimeler: *Adenozin deaminaz, Bağışıklık, Hematoloji, Ksantin oksidaz, Miyeloperoksidaz, Prunus spinosa.*

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INTRODUCTION

Diabetes mellitus (DM) is a chronic endocrine disease manifested by increased blood glucose levels resulting from lack of insulin production or inefficient insulin activity (Kaikini et al. 2020). Today, the prevalence of DM is increasing severely around the world. By 2045, the world's diabetic population is expected to reach approximately 800 million, which is expected to pose serious health and economic challenges (IDF 2022). Synthetic drugs (such as acarbose, metformin, and miglitol) are generally used in the treatment of DM. Mechanisms of action of acarbose and miglitol include inhibition of the activities of carbohydrate hydrolyzing enzymes. Metformin also reduces hepatic glucose production; regulates taking and using peripheral glucose; and delays the absorption of intestinal glucose. However, patients using synthetic drugs suffer from side effects such as gastrointestinal discomfort, liver dysfunction, hypoglycemia, icterus and heart failure in humans (Dennis et al. 2019). Therefore, there is a need to investigate non-toxic plants that have strong antihyperglycemic potentials and prevent complications by strengthening the immune system. Epidemiological studies have revealed that dietary preference with plant-based foods can reduce DM complications (Sun and Miao 2020). Phenolic acids, flavonoids, anthocyanins, and proanthocyanidins in plants provide various beneficial health effects. Ascorbic acid, also known as vitamin C, is an organic acid with antioxidant properties that is involved in a series of processes occurring in living cells as one of the essential exogenous vitamins. In addition to polyphenols, ascorbic acid has been reported to reduce the risks of diabetes, atherosclerosis, cardiovascular disease, asthma and various cancer types (breast and colon cancer) thanks to its antioxidant activity (Naidu 2003). Therefore, the use of plant-based products rich in polyphenols and ascorbic acid, which can reduce DM, and its complications and contribute to the treatment, has recently received a lot of attention.

Blackthorn (*Prunus spinosa* L.) is a spiny perennial drupe belonging to the Rosaceae family, mostly grown in Europe and Western Asia. It is also known as wild plum type or sloe.It has a purple-blue-like bloom and yellow-greenish flesh. Blackthorn has a bitter taste when fresh. Therefore, it is harvested after softening by frost. The reason for this bitter taste is the high tannin content, which together with the high anthocyanin content makes these fruits rich in potential antioxidant, antibacterial and anti-inflammatory activities (Pinacho et al. 2015). Previous studies focused on the antioxidant potential of *P. spinosa* and reported that it has antidiabetic, antimicrobial and anticancer properties thanks to its antioxidant effect (Condello et al. 2019; Popovic et al. 2020). The bioactive compounds of the extract are mostly composed of phenolic acids, flavonoids and anthocyanins. Methanolic extract of *P. spinosa* flowers has been shown to be effective against glioblastoma cells by creating an antioxidant effect (Karakas et al. 2019). In addition, the polyphenols found in *P. spinosa* have antioxidant and protective propertiesagainst fibrinogen and other human plasma components (Marchelak et al. 2017). Blackthorn extracts have also been proven to be important for the release of some vital proinflammatory and anti-inflammatory factors in immune cells (Magiera et al. 2022). High levels of reactive oxygen species (ROS) and biomarkers associated with oxidative stress are observed in the blood or inflamed tissues of patients with various chronic diseases. Studies of *P. spinosa* show that it can have beneficial effects on health thanks to its high antioxidant activity (Magiera et al. 2022; Temiz and Okumus 2022). Considering these findings and the fact that *P. spinosa*isa rich source of accessible antioxidants, this study was set up to determine the effect of blackthorn flower&leaf extract on immune system markers in STZ-induced diabetic rat liver tissue.

MATERIAL AND METHODS

This study was conducted with the permission of Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee on 06.02.2020 with the number 2020/01.

Standards and Reagents

Streptozotocin (STZ), adenosine, L-ascorbic standard and sulfuric acid (H_2SO_4) were acquired from Sigma-Aldrich (Seelze).Acarbose, metformin, insulin, ketamine and xylazine were obtained from a local pharmacy. All chemicals used in the study were of analytical grade and were supplied by Merck (Darmstadt, Germany).

Plant Materials and Extraction

The leaves and flowers of *P. spinosa* used in the study were obtained from Tekirdağ city of Turkey in April 2018. The plants were quickly brought to the laboratory where were removed from foreign materials. The extract was prepared as previously described by Temiz and Okumus (2022). Briefly, flowers and leaves were lyophilized (Labconco freeze dryer 117) and then a homogeneous mixturewas formed from 50% leaves and 50%flowers. The resulting mixture was extracted at 50˚C with ethanol-water (3:1 v/v) for 3 hours using magnetic stirrer (Wisd WiseStir MSH-20D). At the end of the period, the supernatant was filtered and lyophilized. The freeze-dried *P. spinosa* extract (PSE) was kept in amber bottles under vacuum at 26°C under nitrogen atmosphere for further analysis.

L-Ascorbic acid Assay

0.1 g of the PSE was homogenized in an ice bath containing 2 mL of 4% metaphosphoric acid with a tissue homogenizer at 30.000 rpm and 30 s. The homogenate was centrifuged at 10.000 x g for 4 minutes at 4°C. Then the supernatant was filtered through a 0.45 µm PTFE filter. Measurements were performed with a HPLC system (Shimadzu LC-20 AD, Kyoto, Japan).A dC18 column (250×4.6 mm, 5 µm, Waters Atlantis) was used to determine L-ascorbic acid. The flow rate was set up as 0.7 mL/min using a mobilephase (water: H_2SO_4, pH 2.54). Detection was made at 25 °C and 244 nm. L-ascorbic acid seen in the chromatograms was defined by comparison with the retention times and standard (Lee and Coates, 1999).

Animals

This study was performed in accordance with the Guidelines for the care and use of laboratory animals issued by Van Yüzüncü Yıl University Animal Researches Local Ethics Committee and was approved by the Committee on the Ethics of Animal Experiments within that institution (decision No. 2020/01). 56 healthy male rats (Wistar albino, 200-300 g and 2-3 months of age) used in the study were obtained from Van Yüzüncü Yıl University (Turkey) Experimental Application and Research Center. Rats were keptat 22±2 °C, 50% humidity, and 12-h dark/light cycle inplastic rat cages.

Experimental Design

The rats were divided into seven groups, each consisting of eight (n=8) animals, and single dose of streptozotocin (STZ) was administered i.p. at 45 mg/kg body weight (bw). Rats with glucose levels ≥ 200 mg/dL 3 days after STZ administration were evaluated diabetic. The experimental groups formed are as follows:

Group 1: Control group (CG), 1 mL of citrate buffer was applied (i.p.).

Group 2: Diabetic group (DG), only STZ injected.

Group 3: Diabetic+PSE-25 (PSE25), 25 mg/kg (bw) PSE per day was administered by gavageto diabetic rats.

Group 4: Diabetic+PSE-50 (PSE50), 50 mg/kg (bw) PSE per day was administered by gavageto diabetic rats.

Group 5: Diabetic+insulin (Insulin) group, diabetic rats were administered 0.5 IU/kg (bw) insulin (Humulin® N Lilly, Turkey) daily (p.o.).

Group 6: Diabetic+metformin (Metformin) group, 100 mg/kg (bw) metformin (Glifor® tablets Bilim, Turkey) daily was administered to diabetic rats.

Group 7: Diabetic+acarbose (Acarbose) group, acarbose (Glucobay® tablets Bayer, Turkey) was administered to diabetic rats at a daily dose of 50 mg/kg (bw).

All groups were given food and water *ad libitum* during the 21-day experiment. Finally, the rats were anesthetized and their blood and tissue samples were collected.

Hematological parameters

Complete blood count was evaluated using an autoanalyzer (Vet-Scan HM2™ Hematology System, Abaxis, Union City, CA).

Biochemical analyses

Rat liver tissues were homogenized for 3 minutes with cold phosphate buffered saline (pH 7.4) using a homogenizer. Then, it was centrifuged at 8.570 x g for 30 min at +4 °C. The supernatants were then placed into Eppendorf tubes, and kept for further analysis at –80 °C.

Measurement of ADA Activity

ADA was measured using the method of Giusti (1974). The method is based on thegeneration of ammonia, which is directly proportional to the extinction of indophenol as a final product. The ammonia reacts with hypochlorite and phenol in an alkaline solution thereby formation of an intense blue color which is measured at 630 nm.

Measurement of XO Activity

XO was determined using the method of Prajda and Weber (1975). The XO method is based on by formation of uric acid from xanthine 37 °C. XO activity was measured at 293 nm and calculated in mmol uric acid produced per min.

Measurement of MPO Activity

MPO was analyzed by the method of Bradley et al. (1982). MPO catalyzes the conversion of hydrogen peroxide (H_2O_2) and chloride (Cl^-) to highly toxichypochlorous acid ($HOCl^-$). The produced oxygen radical (O^-) reacts with o-dianisidine dihydrochloride to form a colored compound which is measured spectrophotometrically at 460 nm.

Statistical Analyses

The findings were presented as Mean±SD. Data showed normal distribution According to result of normality test followed by Shapiro Wilk. Comparisons between of groups were conducted using the one-way ANOVA followed Tukey test. $p<0.05$ was considered to be significant. SPSS 18 statistical software package was used for statistical analyses.

RESULTS

The ascorbic acid content of *P. spinosa* used in the study was determined as 49.33 mg/100 g (not given in the table). The hematological parameters of rat treated on PSE25, PSE50 and all other groups are presented in Table 1.

Table 1: Hemogram parameters of rats.

Blood	Control	Diabetic	PSE25	PSE50	Insulin	Metformin	Acarbose
RBC (10^{12}/L)	8.9±0.5	7.8±0.3[a]	9.1±0.5[b]	9.2±0.3[b]	9.5±1.3[b]	9.1±0.5[b]	9.6±0.7[b]
Hb (g/dL)	15.6±0.4	14.3±0.5[a]	15.9±0.6[b]	16.1±0.5[b]	16.7±0.8[b]	15.6±0.9	16.5±1.2[b]
MCV (fL)	59.9±0.9	54.6±0.8[a]	59.6±1.6[b]	58.9±1.5[b]	59.3±2.0[b]	58.5±0.8[b]	58.6±1.4[b]
HCT (%)	52.7±2.0	44.0±2.9[a]	54.1±2.5[b]	54.6±2.1[b]	56.1±2.6[b]	53.2±2.6[b]	56.1±4.2[b]
MCH (pg)	17.6±0.4	17.9±10.6	17.4±0.3	17.5±0.5	17.5±0.5	17.2±0.4	17.3±0.3
MCHC (g/dL)	29.4±0.4	32.8±1.4[a]	29.0±0.6[b]	29.4±0.6[b]	29.8±0.7[b]	29.5±0.4[b]	29.4±0.6[b]
PLT (10^9/L)	272±87	720±115[a]	272±63[b]	292±53[b]	333±91[b]	301±93[b]	436±108[a,b,c,d]
MPV (fL)	7.19±0.59	8.40±0.46[a]	7.73±0.60	7.61±0.59[b]	7.89±0.16	8.04±0.38[b]	8.21±0.43[b]
WBC (10^9/L)	4.20±0.72	3.02±0.72	3.71±1.02	3.24±0.90	2.87±0.62	3.63±0.93	4.94±1.10[b,d]
LYM (%)	81.9±3.9	63.9±5.4[a]	74.2±5.2[b]	75.9±4.6[b]	65.1±7.3[a,c,d]	67.8±5.3[a]	70.9±6.7[a]
NEU (%)	15.48±2.93	29.09±5.11[a]	18.40±5.11[b]	22.88±4.26	32.68±7.57[a,c,d]	27.46±4.42[a,c]	28.43±6.72[a,c]
MO (%)	0.34±0.15	0.65±0.27	0.40±0.11	0.49±0.16	0.54±0.21	0.71±0.27[a]	0.60±0.24
EOS (%)	0.51±0.17	0.74±0.13	0.51±0.21	0.53±0.15	0.46±0.25	0.64±0.24	0.53±0.23
BAS (%)	0.23±0.09	0.48±0.23[a]	0.31±0.11	0.31±0.11	0.38±0.10	0.28±0.09[b]	0.26±0.07[b]

a: It was significantly different from control group (P<0.05). **b**: It was significantly different from Diabetic group (P<0.05). **c**: It was significantly different from PSE25 group (P<0.05). **d**: It was significantly different from PSE50 group (P<0.05). **RBC**: red blood cells; **Hb**, hemoglobin concentration; **MCV**: mean corpuscular volume; **HCT**: hematocrit; **MCH**: mean corpuscular hemoglobin; **MCHC**: mean corpuscular hemoglobin concentration; **PLT**: platelet; **MPV**: mean platelet volume; **WBC**: white blood cells; **LYM**: lymphocyte count; **NEU**: neutrophile; **MO**: monocytes; **EOS**: eosinophil; **BAS**: basophil.

Although the red blood cells (RBC) (P=0.039), hemoglobin (Hb) (P=0.029), mean corpuscular volume (MCV) (P=0.000), hematocrit (HCT) (P=0.000), and lymphocytes (LYM) (P=0.000) decreased markedly in diabetic group, administration of PSE25 and PSE50 importantly restored the changes in all these variables, similar to the insulin, metformin and acarbose groups. Also, mean corpuscular hemoglobin concentrated (MCHC) (P=0.000), platelet (PLT) (P=0.000), mean platelet volume (MPV) (P=0.000), neutrophile (NEU) (P=0.000) and basophil (BAS) (P=0.003) showed significant increase in diabetic rats (P<0.05).

However, PSE25 and PSE50 administration prevented further increase in these parameters near to the control range. The PLT was considerably higher in the acarbose group compared to the PSE25 (P=0.010) and PSE50 (P=0.034) groups. The LYM value was significantly lower in the insulin group compared to the control (P=0.000), PSE25 (P=0.030), and PSE50 (P=0.006) groups. However, the NEU values were higher in the insulin group compared to the control (P=0.000), PSE25 (P=0.000), and PSE50 groups (P=0.010). High lymphocyte concentration indicates a high level of immunity against pathogens. The decrease in white blood cells (WBC) and LYM may be related to the inhibition of leukocytosis from the bone marrow, which may be due to weak defense mechanism against infection.

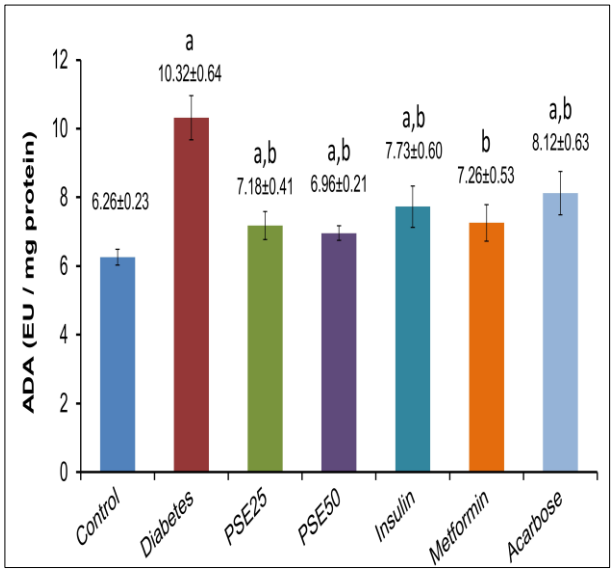

Figure 1: Liver ADA activities of rats.

a: It was significantly different from control group (P<0.05), b: It was significantly different from Diabetic group (P<0.05).

The liver ADA activities of groups are shown in Figure 1. ADA activity was highest in the diabetes group compared to all other groups. The reduction in ADA activity of the treatment groups was found to be significant compared to the control and diabetes groups (P=0.000). The ADA activity detected in the PSE50 group (6.96 EU/mg protein) was lower than the PSE25 group (7.18 EU/mg protein). Although the difference with PSE25 was not significant, the dose of 50 mg/kg PSE had a stronger effect on ADA activity. In addition, both doses (PSE25 and PSE50) showed similar activity to the insulin, metformin and acarbose groups.

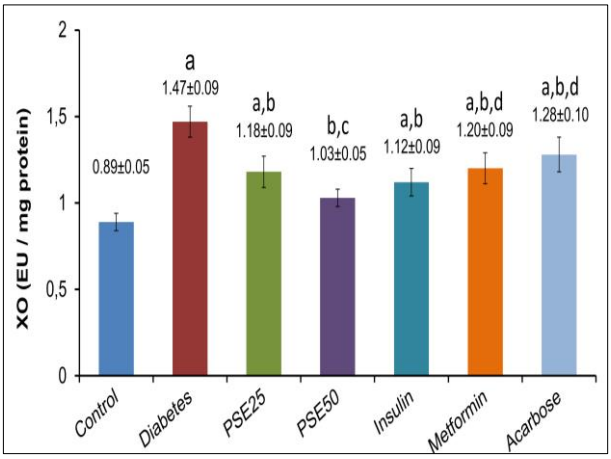

Figure 2: Liver XO activities of rats.

a: It was significantly different from control group (P<0.05), b: It was significantly different from Diabetic group (P<0.05), c: It was significantly different from PSE25 group (P<0.05), d: It was significantly different from PSE50 group (P<0.05).

In Figure 2, the XO activities of the groups are shown. The highest XO activity was found in the diabetes group (1.47 EU/mg/protein), and the lowest in the control group (0.89 EU/mg/protein). This is thought to be due to increased free radical production and oxidative stress in pathological conditions such as diabetes. The decrease in the XO activities of the groups with administered *P. spinosa* (PSE25 and PSE50) was found to be significant (P=0.000). It was determined that the PSE50 showed more XO inhibitory effect compared to the PSE25 (P=0.045). XO activities of metformin (P=0.015) and acarbose groups (P=0.000) were higher than those of the PSE50 group.

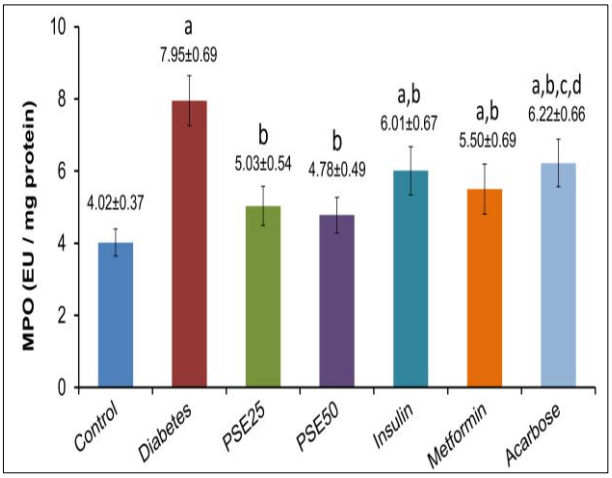

Figure 3: Liver MPO activities of rats.

a: It was significantly different from control group (P<0.05), b: It was significantly different from Diabetic group (P<0.05), c: It was significantly different from PSE25 group (P<0.05), d: It was significantly different from PSE50 group (P<0.05).

Figure 3 shows MPO activities in the liver of experimental rats. The highest MPO activity was determined in the diabetes group and the lowest in the control group. The reduction in PSE50 (P=0.000) and PSE25 (P=0.000)groups was found to be significant compared to diabetes. In the treatment methods applied, PSE50 gave a lower MPO value (4.78 EU/mg protein) compared to PSE25 (5.03 EU/mg protein) (P=0.990). The acarbose group showed higher MPO activity compared to the PSE25 (P=0.025) and PSE50 (P=0.004) groups.

DISCUSSION AND CONCLUSION

Ascorbic acid (vitamin C) is one of the exogenous vitamins and antioxidants that function in the human body. It is involved in the regulation of ROS levels and the effectiveness of other antioxidants. It is effective in stimulating the immune system by increasing the strength and protection of the organism (Bozonet and Carr 2019).In this study,the ascorbic acid result is higher than the amount determined by Kucharska and Sokol-Lętowska (2008) (20-30 mg/100 g) and by Jablonska-Rys et al. (2009) (21.94 mg/100 g). It is thought that this difference is due to the analysis method applied, the soil and climate characteristics of the plant, and the use of the fruit part in the analysis. However, Sikora et al. (2013) determined the amount of vitamin C in *P. spinosa*fruit to be 131.64 mg/100 g dw. As a result, it was determined that the vitamin C content of *P. spinosa* leaves and flowers was close or higher values than the fruit.

Blood is the main transport medium with basic physiological functions in the transport of gases (O_2 and CO_2), nutrient components, and many metabolites. It also includes various immune cells that defend in pathogenic conditions. Evaluation of hematological parameters is a very common routine in clinical trials to determine a person's health status. In this way, it allows the interpretation of the blood-related functions of the consumed substances (Yakubu et al. 2007). In diabetic rats, the RBC count was found to be significantly reduced due to non-enzymatic glycosylation of the RBC membrane protein, which is directly related to the hyperglycemic state ($P<0.05$). Because high glucose level causes to the formation of toxic products, which leads to reduced bone marrow production, low hemoglobin production by affecting the shape of erythrocytes (Singh and Shin 2009). Similarly, pancreatic damage is linked to the occurrence of diabetes. Demolition of pancreatic islet cells results in decreased insulin secretion and increased blood glucose. This increased blood glucose causes oxidative stress and can alter hematological parameters such as HCT, MCV, WBC and LYM (Baltzis et al. 2018). In this study, a decrease in RBC, HCT, MCV, WBC and LYM occurred in the diabetes group. The values found in the PSE25 and PSE50 groups were close to the control group. Similarly, *Prunus spinosa* administration significantly increased Hb, HCT, MCV, and MCH levels against tartrazine toxicity (Balta et al. 2019). Also, it can be concluded that the applied plant extract may have provoked the production of erythropoietin and increased the stem cells in the bone marrow to produce RBC. These results can be featured to the antidiabetic effect of the plant extract (Temiz et al. 2021), and its protection against damage to blood cells of diabetic rats.

In addition, vitamin C in the structure of the plant suppresses the formation of free radicals by preventing oxidative stress. In this way, the cellular antioxidant system is significantly stimulated and the destructive effect on cells is reduced (Gegotek and Skrzydlewska 2022).

Polymorphs include neutrophils, eosinophils, and basophils, and these structures play an important role in immune defense processes. Neutrophils are the main leukocytes and act as the first line of defense. The decrease of neutrophils causes a decline in functional activity, resulting in impaired immunity. In diabetic rats, a decrease of polymorphs is usually observed (Konsue et al. 2017). However, in this study, it was determined that there was an increase in the diabetes group compared to the control group. A similar situation has been found in previous studies where there was a marked increase in the percentage of neutrophils in STZ-induced diabetic rats compared to animals treated with buffer citrate (Mahmoud 2013). Contrary to the current study, Balta et al. (2019) found that *Prunus spinosa* increased PLT and LYM%, as well as decreased MO% and NEU% against tartrazine administration. This contradiction may be due to the fact that diabetes includes many complex conditions.

ADA is an enzyme of the purine degradation pathway and catalyzes the hydrolytic conversion of adenosine and 2'-deoxyadenosine to inosine and 2'-deoxyinosine, respectively. ADA activity is increased in some infectious diseases affecting the immune system. It has been observed that the activity of this enzyme decreases after the treatment of diseases affecting immunity (Bauerle et al. 2011). Therefore, measurement of ADA enzyme activity may be clinically useful in the treatment and follow-up of some immune diseases. It was reported in many studies that ADA activity increased in diabetes (Dayani et al. 2022; Temiz 2023). It probably has a strong relationship between ADA and fasting plasma glucose, i.e., elevated ADA activity is related to reduced glucose uptake in diabetes. However, plant phenolics and flavonoids are compounds that strongly inhibit ADA activity (Egba et al. 2022). Recently, it was reported that *Prunus cerasus* may be beneficial to adenine and oteracil potassium induced hyperuricemia through reduction of ADA activity (Li et al. 2020). Durak et al. (2004) showed that some plant extracts resulted in a significant inhibition of ADA activity in diseased tissues thanks to its high phenolic components. Similar to these results, it can be said that the decrease in the ADA activity of the applied plant extract is due to the high phenolic component content of *P. spinosa* (Temiz and Okumus 2022).

XO is a complex iron-sulfur flavoprotein that catalyzes the hydroxylation of hypoxanthine to xanthine and finally to uric acid. XO is an important precursor for superoxide anion in different tissues in many disease states. It is expressed mainly in the liver and intestine. Itis also important in the pathogenesis of diabetes-associated vascular dysfunctions (Desco et al. 2002). Its inhibition is of great importance for reducing free radicals and ROS.The XO inhibitory potential of plant products used in the treatment of many diseases has been proven by many studies (Mandal et al. 2018; Bhat et al. 2019). This feature is related to the phenolic and flavonoid contents of plants, which are characterized by their antioxidant capacity (Peter and Gandhi 2017). In addition, it has been reported that ascorbate(the form of vitamin C) is effective in reducing XO activity in preventing or reducing reperfusion injuries in stimulated neutrophils (Dwenger et al. 1992). It was found that *Prunus amygdalus* treatmentsignificantly down-regulated XO activity in Fe-nitrilotriacetate (Fe-NTA) toxicity (Pandey et al. 2018). Besides, Yi et al. (2012) showed that *Prunus mume* fruitcould mediate the hypouricemic effect by inhibiting XO activity in the liver.Similar to our results, it was determined in the literature that XO activity was the highest in diabetic rats, and this activity decreased after positive developments in the treatment method (Olugbuyi et al. 2022).

MPO is a hemoprotein associated with many inflammatory events and cardiovascular diseases. The MPO is secreted from leukocytes in response to oxidative stress and plays a crucialfunction in the immune system. In blood, MPO concentrations are measured as a marker of neutrophil initiation and degranulation (Soehnlein 2009). The current results are in line with previousstudies (Aseer et al. 2015;

Olugbuyi et al. 2022) in which diabetes led to elevation of inflammatory status. Phenolic acids and flavonoids are responsible forquenching and/or scavenging the ROS produced in increasing amounts under stress, as well as giving the plants a bitter and sour taste.In this way, it plays a role in regulating the activity of many enzymes. In a previous study by Tabart et al. (2012) on anti-inflammatory capacity, it was stated that the plant extract used could scavenge the ROS produced by neutrophils and inhibit the activity of MPO. Pandey et al. (2018) has stated that *Prunus amygdalus* treatment inhibited the level of MPOas a measure of antioxidant and anti-inflammatory potential during Fe-NTA toxicity.The results obtained from our study were found to be compatible with similar studies.It is thought that the MPO inhibitory activity of *P. spinosa* is due to its phenolic content and high ascorbic acid content, as indicated in its ADA and XO activities.

As a result, *P. spinosa* extract supported to regulate hematological parameters of STZ-induced diabetic rats. In addition, PSE25 and PSE50 groups were found to be a source of ADA, XO,and MPO inhibitors close to insulin, metformin and acarbose in the liver tissues of diabetic rats. Results indicate that *P. spinosa* extract may have an effective potential in protecting the immune system in diabetes.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea / Concept: MAT, EO
Supervision / Consultancy: MAT
Data Collection and / or Processing: MAT, EO
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Writing the Article: MAT, EO
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