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The Protective Effect of Curcumin Used as an Antidiabetic Agent on Hematological Parameters

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ABSTRACT
The study aimed to determine curcumin's effect, an effective antioxidant, and anti-inflammatory. It is used as a spice on some hematological parameters in rats with experimental diabetes with streptozotocin (STZ). Thirty-two male Wistar rats were divided randomly into four groups as follows: Control (K), Diabetes (D), Curcumin (Cu), Diabetes + Curcumin (DCu). RBC, Hb amount, Htc levels, and Plt counts of diabetic rats were significantly lower than the control group (p <0.05). It was observed that while RBC and Htc levels were not greatly affected by curcumin application to diabetic animals, RBC, HTC, and PLT levels tended to increase, and hemoglobin levels increased significantly (p <0.05) compared to the D group. Experimental diabetes caused significant increases in WBC and neutrophil percentage compared to control (p <0.05) and decreased lymphocyte percentage (p <0.05). It is established that there was a significant decrease in the rate of neutrophils and a significant increase in the percentage of lymphocytes in the DC group compared to the D group (p <0.05). These results showed that curcumin application to diabetic rats might positively affect on adverse changes in hematological parameters associated with diabetic damage.
Keywords: *Diabetes Mellitus; Curcumin; Hyperglycemia; hematological parameters, Anemia.*

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic endocrine and metabolic disease that causes carbohydrate, fat, protein and meta,bolism disorders, characterized by chronic hyperglycemia caused by a decrease in insulin secretion or impaired insulin effect [1,2]. DM, which affects approximately 8.5% of the world population, ranks 8th after ischemic heart disease, stroke, lower respiratory tract infections, chronic obstructive pulmonary disease (COPD), diarrhea-related diseases, acquired immune deficiency syndrome (HIV / AIDS), and respiratory route cancer diseases [3].

It is known that oxidative stress and free radicals play a role in developing diabetes and its complications. DM has adverse effects on suppressing antioxidant enzyme activities, an increase of lipid peroxidation, and various hematological parameters [4,5]. Erythrocytes are highly vulnerable to oxidative damage due to their membranes' polyunsaturated fatty acid content. Various Different substances cause damage to the erythrocyte membrane and hemolysis [6]. Adverse effects on erythrocytes and other hematological parameters can lead to various diseases such as anemia, cell cycle anemia, blood platelet disorders, immune system diseases, and inflammation [7]. Besides, T1 and T2 DM have been reported to cause vascular inflammation, autoimmune response activation, and some biochemical changes, including blood cell abnormalities [8].

Pharmacological treatment of diabetes is based on hypoglycemic drugs and insulin. Due to the side effects of these therapeutic agents, interest in herbal and synthetic treatment methods as an alternative therapy has been increasing. It has been put forward that antioxidants can be used to aid in regulating the deteriorated glycemic balance and the fact that diabetes cannot be controlled [9]. Curcumin, the most active ingredient in turmeric, has drawn attention as a potential therapeutic agent in experimental diabetes and the treatment of diabetes-related complications [10,11].

Curcumin has a broad spectrum of biological and pharmacological effects, including anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antidiabetic, antibacterial, antiviral, cardioprotective, nephroprotective, hepatoprotective, immunomodulatory and neuroprotective [10]. Animal studies on this subject have revealed that the effects of curcumin and its analogs are similar to that of the antidiabetic drug, the thiazolidinedione group, through activation of the peroxisome proliferator-activated receptor-(PPAR-γ). Thus, it is thought that curcumin may improve the responsible targets related to glucose and lipid control in the body and play an essential role in the management or treatment of diabetes [12].

This study, it was aimed to determine the effects of oral administration of curcumin, which is known to be highly effective as an antioxidant and also used as a spice, on some hematological parameters in rats with experimental diabetes with streptozotocin (STZ).

MATERIAL AND METHODS

Thirty-two healthy adult male Wistar Albino rats, whose lively weights are similar, were used in the study. Animals were divided into 4 equal groups Control, Curcumin (Cu), Diabetes (D), Diabetes + Curcumin (DCu). The rats were housed in the experimental animal unit, in plastic rat cages, at 23 ± 2 ^{o}C room temperature, 50 ± 10% relative humidity, at 12/12 day/night light periods, with standard rat food *ad libitum*. The rats were kept in front of them with water (~ 50 ml/day/rat) that they could always drink and be refreshed daily.

In groups D and DCu, 60 mg/kg STZ (Sigma S0130-1G) dissolved in 0.1M citrate buffer (pH: 4.5) was administered as a single dose by intraperitoneal injection, and the capillary blood was taken from the tail end 72 hours after STZ administration is checked whether diabetes occurred or not by measuring the fasting blood level with a glucometer (PlusMED) in the fasting blood. Animals with a blood glucose level of 250 mg/dl and above were considered diabetic. 50 mg/kg bw / day of Curcumin (Sigma) was given to Cu and DCu groups throughout the study by gavage. The study was continued ffourr 4 weeks after diabetes occurred.

At the end of the study, enough blood from the heart into anticoagulant (EDTA) wass were taken from the subjects in the groups under general anesthesia (thiopental anesthesia, 40 mg/kg) and with the cardiac puncture. Leukocyte (WBC), erythrocyte (RBC), hemoglobin (HGB), hematocrit (HCT), thrombocyte (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and differential leukocyte counts were determined in the blood samples. Hematological analysis results were obtained using an automatic blood count device (Mindray BC800).

At the end of the study, the statistical analysis of the data and significance of the differences between the groups were performed d by using the SPSS 22.0 package program and the variance analysis by using Duncan's Multiple Range test ($p < 0.05$).

RESULTS

Some hematological parameters and differential leukocyte counts determined in the study groups in the blood samples taken at the end of the trial are given in the tables (Table 1, Table 2).

Table1. Some hematological parameters in experimental groups, X ± SXTable1. Some hematological parameters in experimental groups, X± SX

Parameters	C	Cu	D	DCu
WBC(x10^3/mm^3)	4.48±0.43b	4.40±0.72b	8.61±1.14a	6.51±0.36ab
RBC (x10^6/mm^3)	8.19±0.17a	7.34±0.55ab	5.69±0.91b	6.87±0.31ab
HGB (gr/dl)	14.93±0.27a	13.50±0.97ab	10.42±1.85b	13.91±0.59a
HCT (%)	48.75±1.32a	46.81±2.61a	32.93±6.79b	42.60±4.79ab
PLT (L)	763.16±80.70a	854.50±86.61a	412.0±55.34b	500.66±98.42b
MCV (μ^3)	59.52±1.54	66.86±8.88	55.87±5.01	62.44±7.52
MCH (pg)	18,26±0.33	18.39±0.24	18.03±0.35	20.31±0.69
MCHC (%)	30.66±0.34	29.75±3.49	32.62±5.21	35.46±5.35

a, b; P< 0,05

Table2. Differential leucocyte counts (%) in experimental groups, X± SX

Parameters (%)	C	Cu	D	DCu
Lymphocyte	65.66±3.21^a	62.10±4.0^{5a}	50.01±3.61^b	57.48± 4.75^{ab}
Monocyte	3,85±0,87	3.69 ±1.27	4.82 ± 0.72	3.92 ± 0.95
Neutrophile	28.84 ±4.21^b	32.28 ± 5.09^b	44,02±4,3^a	37.35 ± 2.82^{ab}
Eosinophile	1,33±0,49	1.35 ± 0.28	1.04 ±0.45	1.22 ±0.55
Basophile	0,33±0,42	0.58 ± 0.53	0.68 ± 0.31	0.64 ±0.39

a, b; P< 0,05

DISCUSSION

In recent years, various studies documenting hematological changes in insulin-dependent diabetes mellitus induced by streptozotocin have focused on immunological and hematological modifications [13]. In diabetic patients, it has been reported that the constant high blood glucose level increases the erythrocyte hemolysis by promoting heart damage through hematological changes. As a result, it leads to the development of cardiovascular complications by causing anemia due to a decrease in RBC and HGB levels [14]. It diabetic patients with cardiovascular complications, a decrease in HGB, MCH, and MCHC reduction ratios, which are among the hematological parameters that have a role in erythrocyte function, are observed [15].

The degree of anemia in diabetic patients can be associated with many factors, including glomerular filtration rate, urinary albumin excretion rate, and glucose h (HbA1c) levels. It has been reported that anemia may be due to decreased erythropoietin production due to insufficiency in the kidneys with increased non-enzymatic glycosylation of RBC membrane proteins [16].

According to the results of the research conducted on this subject, it was reported that the WBC count was significantly higher in rats with DM compared to the control, while the red blood cell in. At the same time, RBC, HGB, HCT, MCV, MCH, and MCHC decreased significantly compared to the control group [7,17,18]. Yakchalian *et al.* [13] reported that the platelet count (PLT) in the diabetic group increased significantly compared to the control group.

This study determined that RBC, hemoglobin amount, and hematocrit levels of STZ-induced diabetes rats were significantly lower than the control group ($p < 0.05$). Hemoglobin levels increased significantly ($p < 0.05$) with curcumin administration to diabetic animals compared to the diabetic group. It was also observed that the erythrocyte and hematocrit levels tended to increase when the DC group was compared with the D group. It was determined that the Plt value was significantly decreased in the diabetic group compared to the control and curcumin groups, whil. In contrast, value tended to increase in the DCu group compared to the D group. There was no statistically significant difference between MCV, MCH, and MCHC values (Table 1). In this study, changes in RBC, HGB, and HCT levels of diabetic rats suggest that anemia occurred. The decrease in RBC, hemoglobin, and hematocrit levels appears to be consistent with similar studies compared to the diabetic control group [7,13,17,18].

RBCs are exposed to oxidative damage due to their high cellular HGB content and high fatty acid content in their membranes [19]**.** It is suggested that anemia caused by DM is due to the peroxidation of membrane lipids. The causes of changes such as decreased membrane fluidity, glycosylated oxidation and hemolysis of membrane proteins of RBC, shortening of erythrocyte life, and bone marrow suppression are attributed to oxidative stress [17,20]. It is well known that high glucose causes increased oxidative stress, and oxidative stress plays a vital role in the increase in erythrocyte fragility. It has been reported that oxidative stress caused by diabetes plays a vital role in the rise of membrane damage [21].

In this study, it has been determined that curcumin treatment applied to diabetic animals diminished the decrease in some hematological parameters compared to the diabetic group (Table 1). The positive effect of curcumin on RBC, HB, and Htc of diabetic animals must be due to its effective antioxidant properties. Curcumin is considered a powerful systemic radical cleaner [22]. The beneficial effect of curcumin on hematological parameters may be due to blocking oxidative injuries in DNA and decreasing glycation of membrane proteins, reducing lipid peroxidation, and preventing free radical formation due to the inhibition of some enzymes [22].

Anemia is one of the more common cases in people with diabetes than in people without diabetes. Increased oxidative stress due to diabetes affects all tissues, including blood cells. Therefore, it can be said that curcumin prevents STZ-induced anemia and protects these cells due to these properties. Various proteins such as hemoglobin, albumin, collagen, LDL, or crystalline proteins have been reported to undergo non-enzymatic glycation in diabetes. It is suggested that the decrease in the amount of hemoglobin in diabetic rats is due to the reduction of protein synthesis in all tissues [23].

In this study, diabetes caused significant increases in WBC and neutrophil percentage ($p < 0.05$) and a decrease in lymphocyte percentage ($p < 0.05$) in group D compared to the control. When the data obtained from the DC group with the administration of curcumin to animals with diabetes were examined, it was observed that the percentage of neutrophils was significantly decreased ($p < 0.05$), and the rate of lymphocytes tended to increase compared to the diabetic group. While an increase in the number of neutrophils, one of the leukocyte types, is observed in animals and humans with diabetes, a relative decrease in lymphocyte ratios is mentioned [24,25]. Therefore, Kozlov *et al*. [24] reported moderate neutrophilic leukocytosis and prolonged circulation times of neutrophils and monocytes in diabetic mice and asserted that the number might indicate low-grade inflammation. Supporting the findings of our study, studies are reporting a decrease in lymphocyte ratio among leukocyte types and an increase in neutrophil ratios in animals treated with diabetes [16,26].

It is stated that the increase in total leukocyte count with experimental diabetes may be due to the rise in free radicals, the decrease in antioxidant activity, and the addition of inflammatory cytokines. Some studies have reported a positive relationship between increased inflammatory marker levels (WBC count, CRP, and inflammatory cytokines) in diabetes [27,28]. Tong et al. [29] report that the high WBC count determined in the DM group, associated with macro and microvascular complications in diabetes, was within the normal range. They claim that the high number of WBCs observed in DM reflects inflammation and complications in other tissues [28,29]. In this study, the increase in the percentage of neutrophils determined by diabetes is essential in terms of the immune system's response to the causal effects of diabetes. A significant reduction in both WBC count and neutrophil percentage after curcumin supplementation can be considered a beneficial outcome of curcumin treatment.

A similar study observed that anemia, neutrophilia, and lymphocytosis occurred due to decreased HGB in rats with streptozotocin-induced insulin-dependent Diabetes Mellitus. A significant increase in total leukocyte count (WBC) (leukocytosis) was detected in diabetic rats compared to normal control rats. It has been reported that RBC indices (MCV, MCH, MCHC) also decreased significantly in diabetic rats, but there was no significant difference ($P > 0.05$) in terms of platelet count (PLT) [13].

Diabetes is characterized by chronic inflammation and insulin resistance due to increased pro-inflammatory cytokine production by monocytes infiltrating adipose tissue. This is exacerbated by angiopoietin-like protein 2 (ANGPTL2), which is highly expressed in macrophages and adipose tissue, as its role in T lymphocyte/macrophage activation and accumulation promotes poor insulin response [11]. Leukocytosis, a low-grade inflammation indicator, is a phenomenon that can be observed in obesity and diabetes [25,30]. It has been reported that curcumin treatment insufficiently increases the percentage of WBCs, basophils, monocytes, eosinophils, lymphocytes, neutrophils, and platelets [30,31]. However, in the presented study, no significant change was found in the eosinophil, basophil, and monocyte counts in the diabetic group treated with curcumin.

In animals and human diabetics, a relative decrease in lymphocyte ratios in leukocyte types and an increase in the neutrophil count are among the notifications [30]. Kozlov et al. [24] reported moderate neutrophilic leukocytosis and prolonged circulation of neutrophils and monocytes in diabetic mice, and they assert that the number may also indicate low-grade inflammation. It is also stated that an increase in the number of neutrophils may cause the release of reactive oxygen species (ROS), and in turn, inflammatory responses occur [33,34].

In another study conducted on this subject, while there were significant changes in WBC count, lymphocyte count, platelet count, and granulocyte count among the groups with diabetes, it was reported that RBC, Hb, Htc, MCV, MCH, and MCHC did not differ significantly in all groups. In this study, turmeric was shown to dramatically reduce the level of oxidative stress and improve altered hematological functions to significant levels compared to standard control and insulin-treated diabetic groups. Specifically, curcumin has been proven to have strong antihyperglycemic, anti-anemic, and antioxidant properties [35]. It has been reported in previous studies that turmeric exhibits anticoagulation and antiplatelet activities. Saponins in a very significant amount can prevent platelet aggregation, which could be a possible explanation for the significant decrease in platelet count. This effect of turmeric suggests that it may be helpful in the treatment of patients with vascular thrombosis or as an antithrombotic therapeutic agent after myocardial infarction.

CONCLUSION

An animal study on diabetes emphasized that curcumin and its analogs had a similar mechanism of action to thiazolidinedione, an anti-diabetic drug, through activation of the peroxisome proliferator-activated receptor- (PPAR-). Therefore, curcumin may be effective in the regulation of glycemia and lipidemia.

Therefore, these results showed that curcumin administration to diabetic rats might have beneficial effects on adverse changes in hematological parameters associated with diabetic damage. If these findings can be applied to humans, using these probiotics in diabetic patients points to a promising future in fighting diabetes and related disorders.

AUTHORS CONTRIBUTIONS

ÜS,MÖ and ND designed the research. ÜS and ND performed the experiments. ÜS, MÖ, and ND analyzed the data. MÖ and ND contributed to the production of the manuscript. All authors read and approved the final manuscript.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by Selcuk University Experimental Medicine Research and Application Center Experimental Animals Ethics Committee on 21.03.2017 with a decision no 2017-11.

CONFLICTS OF INTEREST

No conflict of interest is associated with this work.

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