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Biochemical, Hematological, Peripheral Smear and Weight Evaluation of Low Dose Epigallocatechin Gallate in Diethylnitrosamine-Administered Rats

Dietilnitrozamin Uygulanan Sıçanlarda Düşük Doz Epigallocatechin Gallate'nin Biyokimyasal, Hematolojik, Periferik Yayma ve Ağırlık Değerlendirilmesi

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Abstract

Introduction: Currently, with the development of technology, the use of many chemicals especially Diethylnitrosamine (DEN) in agriculture and industry has increased. The polyphenolic compounds of Epigallocatechin gallate (EGCG) is the active ingredient of green tea. It has been reported that green tea has antioxidant effects. In this preliminary study, effects of low dose EGCG against exposure of DEN administered rats.

Material and Method: As a group, groups were divided into five groups of ten rats for the application as Control, Sham, DEN, EGCG and DEN+EGCG. The parameters analyzed are hemogram, biochemical, peripheral smear and weight.

Results: DEN injection has significantly increased Lactate dehydrogenase (LDH), Aspartate amino transferase (AST), Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP) values, Which are signs of hepatocyte injuries. The number of White Blood Cell Count (WBC)s increased in the EGCG group. In terms of High-density lipoprotein (HDL) and total cholesterol (Tchol) levels, the group in which DEN+EGCG were applied together was found to be the highest and Trigliseride (TG) and Low-density lipoprotein (LDL) levels were found to be lowest. The current study will be a comprehensive study demonstrating the effects of low-dose EGCG against DEN-administred rats.

Conclusion: These results indicate that consumption of low-dose EGCG polyphenolic compound in green tea may be effective against DEN administered rats.

Keywords: Biochemical, diethylnitrosamine, epigallocatechin gallate, hematological, peripheral smear, rat

Öz

Giriş: Günümüzde teknolojinin gelişmesiyle birlikte başta Dietilnitrozamin (DEN) olmak üzere birçok kimyasalın tarım ve sanayide kullanımı artmıştır. Epigallocatechin gallate'nin (EGCG) polifenolik bileşikleri, yeşil çayın aktif bileşenidir. Yeşil çayın antioksidan etkileri olduğu bildirilmiştir. Bu ön çalışmada, düşük doz EGCG'nin DEN uygulanan sıçanlara maruz kalmaya karşı etkileri incelenmiştir.

Gereç ve Yöntem: Gruplar uygulama için Kontrol, Sham, DEN, EGCG ve DEN+EGCG olmak üzere onar (n=10) rattan oluşan beş gruba ayrıldı. Parametreler olarak hemogram, biyokimyasal, periferik yayma ve ağırlık analiz edildi

Bulgular: DEN enjeksiyonu, hepatosit hasarlanmalarının belirtileri olan Laktat dehidrogenaz (LDH), Aspartat amino transferaz (AST), Alanin amino transferaz (ALT) ve Alkaline Fosftaz (ALP) değerlerini önemli ölçüde artırdı. EGCG grubunda beyaz kan hücre (WBC) sayısı arttı. Yüksek dansite lipoprotein (HDL) ve Total kolesterol (TChol) düzeyleri açısından DEN+EGCG'nin birlikte uygulandığı grup en yüksek, trigliserit (TG) ve düşük dansite lipoprotein (LDL) düzeyleri ise en düşük bulundu.

Sonuç: Bu sonuçlar, yeşil çayda düşük dozlu EGCG polifenolik bileşiğinin tüketilmesinin, DEN uygulanan sıçanlara karşı etkili olabileceğini göstermektedir.

Anahtar Kelimeler: Biyokimyasal, diethylnitrosamine, epigallocatechin gallate, hematolojik, periferik kan smear, sıçan

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INTRODUCTION

Diethylnitrosamine (DEN) is an environmental carcinogen found in many products such as smoked, cured foods, nitrite-processed meats such as salami, dairy products, alcoholic beverages, and tobacco smoke.[1] [DEN is frequently used in animal studies and is a liver-damaging agent.[2,3] The intermediates and end products of DEN mediate the binding of tumor initiation sites by covalently binding to DNA with one or two oxidizing electrons.[4-6] As a result, it causes the proliferation of silent hepatocyte cells that carry DEN-induced mutations.[7] DEN has been shown to be a potent compound for the treatment of cancer.

The polyphenolic compounds of epigallocatechin gallate (EGCG) in green tea are consumed and produced in about 30 countries around the world. About two-thirds of the world's population, mainly in India and China, consume green tea. [8,9] Some studies have found that green tea has protective effects against cardiovascular diseases, hypertension, gastrointestinal diseases, some cancers, liver diseases, and arthritis, and apoptosis properties, especially antioxidant properties, have been evaluated.[8-12] The use of EGCG in green tea has been reported in many studies.

Since there are not enough studies on DEN and EGCG, this study aimed to investigate the hematological, biochemical, peripheral smear, and weight effects of the protective property of low-dose EGCG, which has been shown to have many health benefits and is the active ingredient of green tea, in rats treated with DEN.

MATERIAL AND METHOD

In this study, 50 Wistar albino rat, 3 months old,190-225g were used, which were obtained from the Van Yüzüncü Yıl Üniversity Experimental Application and Research Center of Ethics Committee (Project number: 2017/01 TYL 6343). The rats were housed in rooms that had 12 h dark/light for a period of 10 days and the temperature of which had been set as 22±2 °C. Feeding was given with the standard normal feed ad libitum and free access to water. The animals were weighed and their weights were recorded every day before and during the study. As a group, they were divided into five groups as ten in each group for application: The Control, Sham, DEN (Sigma, St. Louis, MO, USA) EGCG (Sigma, St. Louis, MO, USA) and DEN+EGCG. The experimental groups were organized as follows.

Group I (Control): Randomly selected ten rats were separated and was no additional application performed. Animals were allowed for their routine life.

Group II (Sham): On the first day, saline 0.5 ml/kg/i.p. single dose was administered.

Group III (DEN): On the first day, a single dose of 150 mg/kg/i.p. DEN was injected.[13]

Group IV (EGCG): EGCG 10 mg/kg/day were given orally by gavage every day for 10 days from the first day.[14]

Group V (DEN+EGCG): On the first day, a single dose of 150 mg/kg/day DEN i.p. was administered. EGCG 10 mg/kg/day was given by each gavage for 10 days from the first day.

At the end of the 10 days, blood was taken from the hearts of the animals with an injector for blood samples. A drop of blood taken from the rats was spread directly on the slide at an angle of 15°. It was left to dry for 30 minutes at room temperature. Then, it was determined and stained for 5 minutes with May-Grunwald Giemsa Staining. It was then passed through distilled water and allowed to dry for 10 minutes. After drying, Giemsa's pH 7.0 diluted with 10% distilled water was dyed with azur-eosin methylene blue dye for 10-15 minutes.

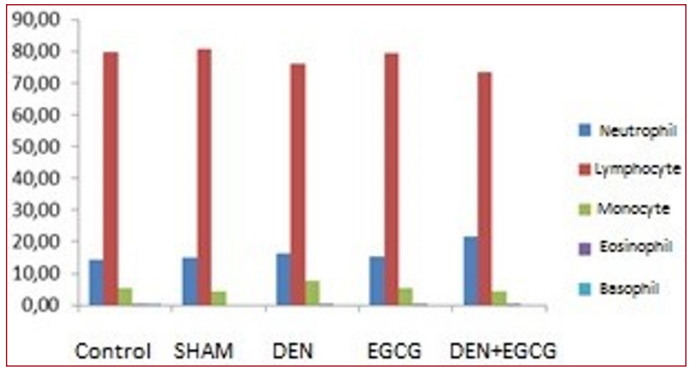
K2-Ethylene Diamine Tetra Acetic Acid (K2-EDTA) Nihon Kohden Celltac G Automatic Hematology Analyzer in blood tubes was studied with Hemolynac-310 and Hemolynac-510 Lysing reagent commercial kit in MEK-9100 device.

Biochemical parameters were determined on Abbott ARCHITECT C 16200, adapted to Abott device, and were studied by spectrophotomeric method with commercial kits. The erythropoietin (EPO) test was studied by the immunoassay method with a commercial EPN kit adapted to the Immulite 2000 device.

All of data were expressed with mean, standard errors, minimum and maximum values. Statistically, the values of the groups were compared using Kruskal-Wallis and Mann Whitney -U tests.

RESULTS AND DISCUSSION

The peripheral smear results of chemicals given in our study show that no statistically significant difference occurred in the control and other groups (**Graphic 1**, **Figure 1-3**). In this case, it can be thought that DEN and EGCG do not have significant effects on platelets, which are the most important cellular element in the coagulation process, at the specified dose and time.[15]



Graph 1. Peripheral smear results belonging to the all of the groups.
DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate,
DEN+EGCG: Diethylnitrosamine+Epigallocatechin gallate.

The fact that haemotological values were observed in all groups (**Table 1**). The lowest Hemoglobin (HGB) and Hematocrit (HTC) values observed in the control group can be explained similarly.

[16] The highest value in the WBC parameter is seen in the EGCG group, and it is seen that EGCG, the active ingredient of green tea, has an increasing effect on the number of WBCs (**Table 2**). It supports that EGCG may have an effect on the immune system. However, the application of EGCG together with DEN reduces this value to a value close to the control. Ramesh et al. (2010) examined the effects of EGCG in rabbits on atherosclerotic diet and observed that EGCG significantly increased WBC compared to the control group.[17] In terms of neutrophil value, EGCG, DEN and DEN+EGCG application caused a significant increase in the Neutrophil parameter compared to the control (**Table 2**). This suggests that neutrophils, one of the indicators of acute inflammation, are increased by these chemicals. The mechanism of the changes in DEN hemogram parameters is not clear. It may be related to immune-mediated mechanisms, non-immunological mechanisms, and bone marrow suppression.[18] The highest monocyte value was observed in the Sham group and the lowest in the DEN+EGCG group (**Table 2**). Similarly, Sham application was observed as the group with the highest increase in Basophil value. The fact that no significant difference was observed in the Control, Sham, DEN, EGCG and DEN+EGCG groups in terms of the platelet parameters examined suggests that the chemicals in question did not affect the platelet values (**Table 3**). However, the specific application of DEN+EGCG application for 10 days increased Red Blood Cell (RBC), HGB and HCT. Hematological parameters can be used to predict DEN damage.

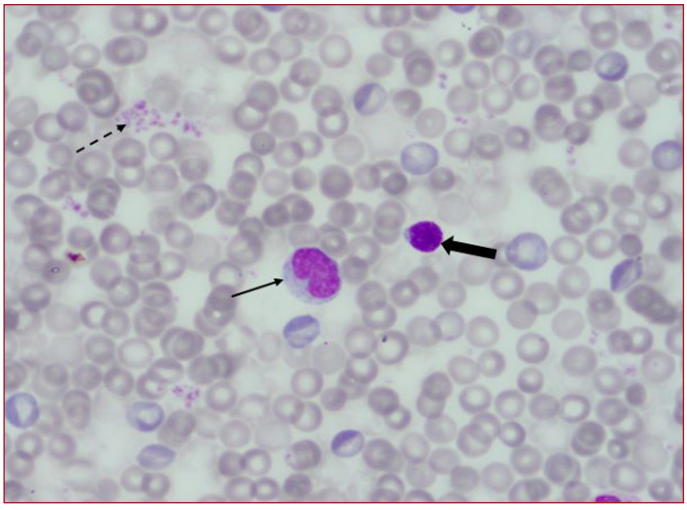


Figure 1. General view of thick arrow sign with lymphocyte, thin arrow sign with monocyte and dashed arrow sign with platelets from peripheral blood cells (May Grunwald-Giemsa, 100x).

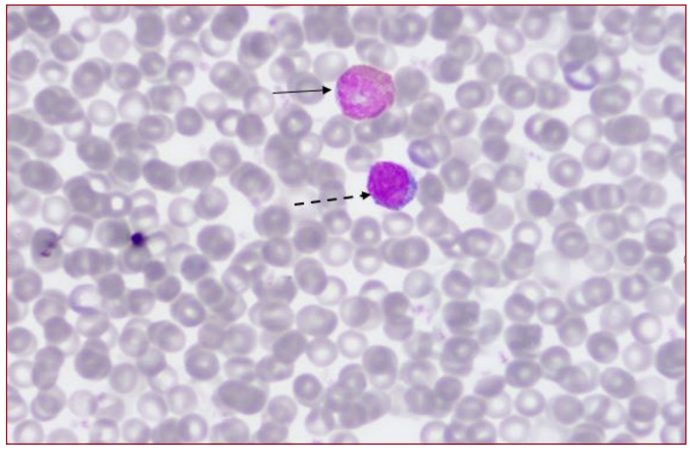


Figure 2. Smooth arrow sign with eosinophil and dashed arrow sign with lymphocyte in peripheral blood (May Grunwald-Giemsa, 100 x).

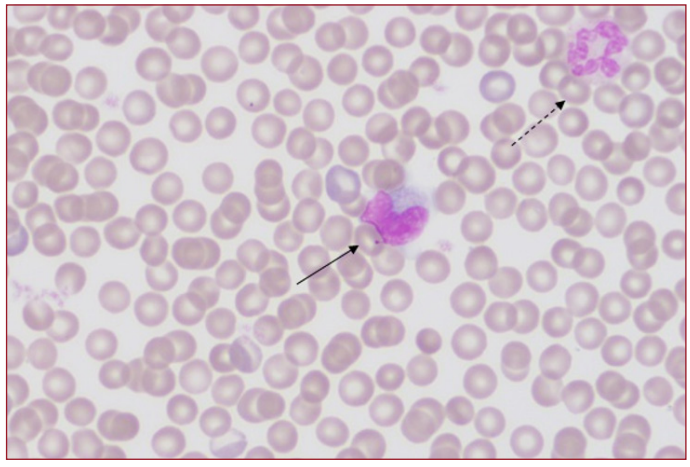


Figure 3. Dashed arrow sign with neutrophil and fine arrow sign with monocyte in peripheral blood (May Grunwald-Giemsa, 100x).

Table 2. Leukocyte numbers of the groups.

	WBC	NE	LY	MO	EO	BA
Control	8,65^{bc}	2,57^{b}	5,35^{a}	0,24^{ab}	0,03^{b}	0,45^{b}
Sham	10,23^{ab}	3,48^{ab}	5,69^{a}	0,33^{a}	0,06^{a}	0,67^{a}
DEN	10,78^{ab}	4,24^{a}	5,73^{a}	0,26^{ab}	0,04^{b}	0,51^{ab}
EGCG	11,21^{a}	3,87^{a}	6,51^{a}	0,27^{ab}	0,06^{a}	0,52^{ab}
DEN+EGCG	7,74^{c}	4,10^{a}	3,14^{b}	0,14^{b}	0,02^{b}	0,34^{b}

DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate, DEN+EGCG: Diethylnitrosamine+ Epigallocatechin gallate. Different letters indicate significant difference between groups p<0.05. WBC: White Blood Cell Count, NE: Neutrophils, LY: Lymphocytes, MO: Monocytes, EO: Eosinophils, BA: Basophils.

Table 1. Hematological parameters of erythrocyte in groups.

	RBC	HGB	HCT	MCV	MCH	MCHC	RDW-CV	RDW-SD
Control	7,33^{b}	14,33^{b}	47,08^{b}	64,59^{a}	19,63^{a}	30,47^{a}	17,97^{a}	46,34^{a}
Sham	8,24^{a}	15,94^{a}	51,50^{a}	62,63^{a}	19,39^{a}	30,94^{a}	18,66^{a}	46,72^{a}
DEN	7,94^{a}	15,80^{a}	50,19^{a}	63,19^{a}	19,65^{a}	31,07^{a}	18,39^{a}	46,47^{a}
EGCG	8,27^{a}	15,72^{a}	51,37^{a}	62,10^{a}	19,00^{a}	30,60^{a}	18,91^{a}	46,99^{a}
DEN+EGCG	8,40^{a}	15,85^{a}	51,06^{a}	60,82^{a}	18,90^{a}	31,05^{a}	18,96^{a}	46,10^{a}

DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate, DEN+EGCG: Diethylnitrosamine+Epigallocatechin gallate. Different letters indicate significant difference between groups p<0.05. RBC: Red blood cell, HGB: Hemoglobin, HCT; Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW-CV: Red blood cell distribution width-coefficient of variation, RDW-SD: Red blood cell distribution width- standard deviation.

Table 3. Platelets belonging to the groups.

	PLT	PCT	MPV	PDW	P-LCR
Control	797,77[a]	0,51[a]	6,37[a]	15,33[a]	18,35[a]
Sham	737,68[a]	0,48[a]	6,44[a]	15,51[a]	18,99[a]
DEN	774,15[a]	0,51[a]	6,36[a]	15,29[a]	17,92[a]
EGCG	793,89[a]	0,51[a]	6,47[a]	15,50[a]	19,42[a]
DEN+EGCG	795,79[a]	0,50[a]	6,23[a]	15,36[a]	17,20[a]

DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate, DEN+EGCG: Diethylnitrosamine+ Epigallocatechin gallate. Different letters indicate significant difference between groups p<0.05. PLT: Platellet, PCT: Platelet crit, MPV: Mean platelet volume, P-LCR: Platelet large cell ratio.

In the study, transferrin, iron and iron binding values were examined in order to evaluate the effect of DEN, EGCG and these two chemicals on iron metabolism of the hematopoietic system (**Table 4**). The fact that there is no statistical difference in these parameters examined suggests that DEN and EGCG do not have a significant effect on these parameters. Although there is no statistical significance, the highest values in Transferrin, Iron binding and Iron values compared to the control were observed in the DEN+EGCG group, although it was not observed in a short time or, suggesting that significant differences may occur with the use of these chemicals in the long term.[19] In research studies, it was reported that after drinking 3-4 cups of tea a day, anemia problem associated with iron deficiency did not occur. In a study conducted by Suliburska et al. (2012), it was found that the glucose and iron levels of the individuals in the group consuming green tea were lower than the control group.[20]

Table 4. Transferrin, iron and iron binding values of the groups.

	Transferrin	Iron binding	Iron
Control	0,95[a]	186,70[a]	112,90[a]
Sham	0,89[a]	198,10[a]	89,20[a]
DEN	0,93[a]	200,40[a]	100,30[a]
EGCG	0,90[a]	182,89[a]	101,67[a]
DEN+EGCG	1,02[a]	216,50[a]	119,00[a]

DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate, DEN+EGCG: Diethylnitrosamine+ Epigallocatechin gallate. Different letters indicate significant difference between groups p<0.05.

The fact that no significant difference was observed between the groups in terms of glucose value was interpreted as that the applied chemicals had no direct effect on glucose metabolism (**Table 5**). In the study of Gubur (2015), glucose levels in rats fed with fructose and in the control group were higher than in the green tea group (p <0.05).[21] DEN+EGCG group High-density lipoprotein (HDL) value increased significantly (p <0.05). In the DEN+EGCG group Trigliseride (TG) value was low compared to the other groups but statistical unsignificance (**Table 5**). In Gubur's study have demonstrated no difference in HDL, Low-density lipoprotein (LDL) and total cholesterol (Tchol) levels, but TG levels in the fructose group were significantly higher than in the control and green tea groups (p<0.05).[20] EGCG, which we tried to protect against DEN, increased HDL levels. Suliburska et al. (2012) found that individuals in the group consuming green tea had high HDL Tchol levels consistent with our study.[21,22] The protective effects of EGCG may be related to its phenolic content.[23]

Hepatocellular damage causes release of Aspartate amino transferase (AST) and Alanine aminotransferase (ALT). Increased levels of AST and ALT are an indicator of cellular infiltration and functional disturbance of liver cell membranes. In our study, hepatocellular function enzymes of ALT and AST values were found to be significantly higher in DEN and DEN+EGCG groups compared to EGCG, Sham and Control groups. In EGCG group administered with DEN, it did not decrease ALT value (**Table 5**). This situation reveals that DEN has a negative effect on liver functions and induced hepatic injury. In many studies, in accordance with our study, it was determined that DEN application caused an increase in liver enzyme activity.[24-29]

The erythropoietin (EPO) hormone increases in conditions where oxygen intake is reduced, such as hypoxia, chronic obstructive pulmonary disease (COPD) and climbing to high altitudes.[30] In our study, the amount of EPO was tried to be measured, but could not be measured because it was outside the limits that can be measured by the device.

After the applications, rats gained weight in all groups (**Table 6**). However, there was no significant difference between the groups in terms of weight gain. This situation suggested that the animals were well cared for in the short study period. But previous studies have been shown that a period of EGCG treatment helps to lose the body weight as well as waist circumference and to improve fecal lipid concentration in most rat experiments.[31]

Table 6. Weight records of the groups.

Weight	Control	Sham	DEN	EGCG	DEN+EGCG
Initially	199,60	194,80	193,20	200,40	200,60
SD	21,04	30,31	21,95	31,96	30,75
Before op	220,00	235,40	225,00	224,40	225,00
SD	19,11	26,25	15,78	19,93	19,89

DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate, DEN+EGCG: Diethylnitrosamine+ Epigallocatechin gallate. Different letters indicate significant difference between groups p<0.05. SD=Standard Deviation.

Table 5: Biochemical parameters of the groups.

	TG	Tchol	LDL	HDL	Glucose	AST	ALT	LDH	ALP
Control	73,50[a]	31,80[b]	15,11[a]	18,74[b]	132,00[a]	54,50[a]	19,30[c]	1237,10[a]	267,80[bc]
Sham	66,40[a]	27,60[b]	15,45[a]	16,29[b]	119,60[a]	49,60[a]	21,00[bc]	1158,50[a]	289,10[abc]
DEN	70,10[a]	30,70[b]	14,43[a]	19,60[b]	106,60[a]	61,70[a]	25,10[ab]	1475,00[a]	327,10[ab]
EGCG	80,78[a]	35,00[ab]	17,80[a]	20,37[b]	115,38[a]	58,56[a]	21,56[bc]	1267,38[a]	226,38[c]
DEN+EGCG	56,50[a]	42,00[a]	16,98[a]	25,91[a]	136,30[a]	77,30[a]	29,20[a]	986,40[a]	339,40[a]

DEN: Diethylnitrosamine, EGCG: Epigallocatechin gallate, DEN+EGCG: Diethylnitrosamine+Epigallocatechin gallate. Different letters indicate significant difference between groups p<0.05. TG: Trigliseride, Tchol; Totol Cholesterol, LDL: Low-density lipoprotein, HDL: High-density lipoprotein. AST: Aspartate amino transferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, ALP: Alkaline Phosphatase. Different letters indicate significant difference between groups p<0.05.

CONCLUSION

DEN is an environmental carcinogen that produces toxic effects primarily in the blood and liver.[3,4] In different studies, EGCG has anti-inflammatory, antimicrobial, antiviral and antioxidant effects, important protective role in a lot of diseases.[32-33] In this study, consumption of the polyphenolic compound of low-dose EGCG in green tea may be protective against DEN administered rats. There are also some limitations in this study. For example, we did not comprehensively evaluate parameters such as biochemical antioxidant parameters. Therefore, future studies are still needed to investigate such parameters. However, it is thought that studies with different doses and different durations are required for clinical use.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Van Yüzüncü Yıl University Ethics Committee (Date: 26.12.2023, Decision No: 6343).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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