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# Determination of CTLA-4 levels in spleen tissue of rats treated with ginger oil (Zingiber Officinale) by immunohistochemical methods

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**Abstract**
This study was investigated to determine CTLA-4 levels in the spleen tissue of rats treated with ginger oil (Zingiber officinale) by immunohistochemical methods. For the study, 21 male Sprague-dawley rats of 250-300 g weight were used. The groups were formed as control group, ginger 100 mg/kg group and ginger 500 mg/kg group. Histological and immunohistochemical methods were applied to the spleen tissues taken at the end of the study. Red and white pulp, central artery, trabeculae and vena trabecularis were found to have normal histological structure in the spleen tissue of all groups. Weak CTLA-4 immunoreactivity was detected in the red and white pulp in the spleen tissue of the control and ginger 100 mg/kg groups, and moderate CTLA-4 immunoreactivity was detected in the ginger 500 mg/kg group. In our study, it was determined that ginger oil administration at different doses did not cause histological changes in the spleen tissue and increased CTLA-4 immunoreactivity in the ginger 500 mg/kg group. Based on our results, it is concluded that in order to fully explain the effects of ginger administration on the spleen, it is necessary to determine the efficacy of different doses, forms and administration forms of ginger.
***Keywords:*** *CTLA-4, ginger, immunohistochemistry, spleen*

## *1. Introduction*

The spleen, one of the organs of the immune system, is involved in the differentiation of leukocytes and the destruction of erythrocytes. The part of the spleen called red pulp consists of reticular connective tissue and is rich in blood vessels. Red pulp is the area where erythrocytes are stored. Platelets, granulocytes, plasma cells and macrophages are also found in the red pulp. Within the red pulp there are groups of white lymphocytes, which are called white pulp. Immunoglobulins enter the circulation where the white pulp borders the red pulp. T-lymphocytes are located around the arteries in the white pulp. B-lymphocytes are found in lymphocyte clusters in this region. Therefore, the spleen is an organ with intense cell traffic.[1]

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is a member of the CD28:B7 immunoglobulin superfamily and is involved in modulating the immune system. CTLA-4 is also known as CD152.[2] Under normal conditions, CTLA-4 is present in small amounts on the surface of effector T cells and Treg (regulatory T cell) cells and is involved in regulating the severity of T cell activation in the early period.[3] Inhibition of CTLA-4 increases cytotoxic T cell activation and stops Treg cell-dependent immunosuppression.[4]

Research with natural products that regulate immunity by increasing or decreasing it is gaining importance. Immunomodulatory agents act as immunostimulants in cases of immune deficiency and as immunosuppressants in abnormally increased immunity. In this way, they protect the body against infections. Ginger (Zingiber officinale) has been used since ancient times both as a spice and as a therapeutic agent. The therapeutic properties of ginger can be attributed to the fact that it has about 400 different components, mainly gingerols.[5-8] Ginger (*Zingiber officinale Roscoe*) is a member of the plant family Zingiberaceae.[9] It has been suggested that ginger is the most widely used herbal medicine in many countries. Ginger is known to have many beneficial properties, including antioxidant and anti-inflammatory effects, and ginger has neuroprotective effects.[10,11] Ginger is one of the medicinal plants that are widely used in pharmaceutical products and foods and have beneficial effects on health. The pharmacological effects of crude ginger extract are known. Ginger rhizome is often added to foods as a spice or taken as a dietary supplement. Ginger has been widely used in traditional medicine throughout history.[12,13]

In this study, it was aimed to determine CTLA-4 levels in spleen tissue of rats administered ginger oil (zingiber officinale) by immunohistochemical methods. It is thought that the findings to be obtained will contribute to the determination of the dose-dependent effects of ginger oil, which is known to have a role in immune regulation, and to elucidate the mechanisms of action in the spleen tissue.

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### *2. Material and Method*

For the study, 21 male Sprague-dawley rats weighing 250-300 g were used. Rats were randomly selected and placed in cages with 7 rats in each cage. Rats were housed in laboratory conditions at 50% relative humidity, 20 ± 2°C and 12:12 light/dark cycle. Rats were fed pellet feed (22% HP and 2400 kcal). Feed and water were provided ad libitum to all animals. Groups were formed as follows:

**1. Control Group (n=7):** Rats were administered 1 ml distilled water by oral gavage for 10 days.

**2. Ginger 100 mg/kg Group (n=7):** Rats were administered 100 mg/kg/day ginger oil in 1 ml distilled water by oral gavage for 10 days.

**3. Ginger 500 mg/kg Group (n=7):** Rats were administered 500 mg/kg/day ginger oil in 1 ml distilled water by oral gavage for 10 days.

Rats were fasted for the last 12 hours of the study and sacrificed under anaesthesia (ketamine hydrochloride 75 mg/kg and xylazine 15 mg/kg, IM). Afterwards, spleen tissues were removed and fixed in 10% formol solution.

#### *2. 1. Histological investigations*

The tissues were blocked in paraffin by applying routine tissue monitoring procedure. The 5 µm serial sections taken from the paraffin blocks were stained with Haematoxylin & Eosin to examine the general structure of the spleen tissue.

#### *2. 2. Immunohistochemical investigations*

Streptavidin-biotin peroxidase method was applied to the sections taken on slides coated with chromium alum gelatin. After routine deparaffinisation and rehydration, the sections were shaken in PBS (0.1 M, pH, 7.2) and incubated in 3% $H_2O_2$ prepared in 0.1 M PBS for 15 min. It was then boiled in citrate buffer solution (pH: 6.0) for 10 min in a microwave oven at maximum temperature (800 watt). Then incubated with Large Volume Ultra V Block solution (Thermo Scientific/ LOT: PHLT811) for 10 min. CTLA-4 (Santa Cruz/ sc-376016) (1/500 dilution) primary antibody was added to the sections and kept for 1 hour in a humid environment at room temperature. The sections were washed with PBS and Biotinylated Goat Anti B Polyvalent (Thermo Scientific/ LOT: PHLT811) and Streptavidin Peroxidase solutions (Thermo Scientific/ LOT: PHLT811) were dropped onto the sections and incubated at room temperature for 15 min each. DAB-$H_2O_2$ (Diaminobenzidine hydrogen peroxide) (Thermo Scientific/ LOT: HD53495) Substrate Solution was added for chromogen application and modified Gill III haematoxylin solution (Sigma-Aldrich/ LOT: HX29596474) was used for counterstaining. The preparations were examined under a research microscope and photographed. Immunohistochemical examination was performed by looking at the staining properties of the target cells and the staining intensity of the stained target cells. In the examination, values were given by two independent observers according to the characteristics of no staining (-), weak staining (+), moderate staining (++), and strong staining (+++).[14]

### *3. Results*

#### *3. 1. Histological results*

The histological structure of the spleen tissue was determined in all groups. Red and white pulp, central artery, trabeculae and vena trabecularis were found to have normal histological structure (Figure 1).

#### *3. 2. Immunohistochemical results*

Weak CTLA-4 immunoreactivity was detected in the red and white pulp in the spleen tissue of control and ginger 100 mg/kg group and moderate CTLA-4 immunoreactivity was detected in the ginger 500 mg/kg group (Table 1, Figure 2).

### *4. Discussion*

The functions of the spleen are focussed on the systemic circulation. Therefore, it lacks afferent lymphatic vessels. It consists of two functionally and morphologically different parts, the red pulp and the white pulp. The red pulp is a blood filter that removes foreign substances and damaged erythrocytes. It is also a storage area for iron, erythrocytes and platelets.[15] The spleen is the site of haematopoiesis in rodents, especially in animals in the foetal and neonatal period. It is also the largest secondary lymphoid organ, containing about a quarter of the body's lymphocytes. The spleen is the site of initiation of immune responses to blood-borne antigens.[16,17] This function of the spleen is fulfilled by the white pulp surrounding the central arterioles. The white pulp consists of three subdivisions: the periarteriolar lymphoid sheath (PALS), follicles and the marginal zone.[15] There is growing interest in investigating the phytomedicinal properties of plants and studies investigating the effects of different plant phytochemicals on immunology and spleen cell development are gaining popularity.[18-20] In the spleen, it has been reported that chilli pepper causes an increase in weight and leukocytes, so excessive consumption may cause damage to the spleen.[21] *The effects of Crataegus aronia var. dentata Browicz* extract on spleen were evaluated by using different histochemical methods and it was reported that 1% plant extract application did not cause any change in some carbohydrate molecules and haemosiderin in spleen.[22] In our study, it was determined that there were no histological changes in the spleen tissue of rats treated with different doses of ginger oil.

CTLA-4 maintains the tolerance of peripheral T lymphocytes at some immune system control points such as the programmed death-1 (PD-1) pathway, TIM-3 and LAG-3, prevents autoimmunity and suppresses inflammation during acute/chronic infection.[23,24] The CTLA-4 gene is located in the chromosomal 2q33 region. It maintains T cell homeostasis by encoding a protein that acts as a negative regulator of T cell responses.[25] In the absence of CTLA-4, fatal tissue damage occurs due to increased activation of peripheral T cells.[26] CTLA-4 is an immunological checkpoint and has a central and important role in maintaining peripheral tolerance.[27,28] CTLA-4 is homologous to CD28 but is located in the intracellular compartment and is only minimally expressed on the surface of resting T cells.[29] The gene encoding CTLA-4 has been suggested to be a candidate gene for confer-

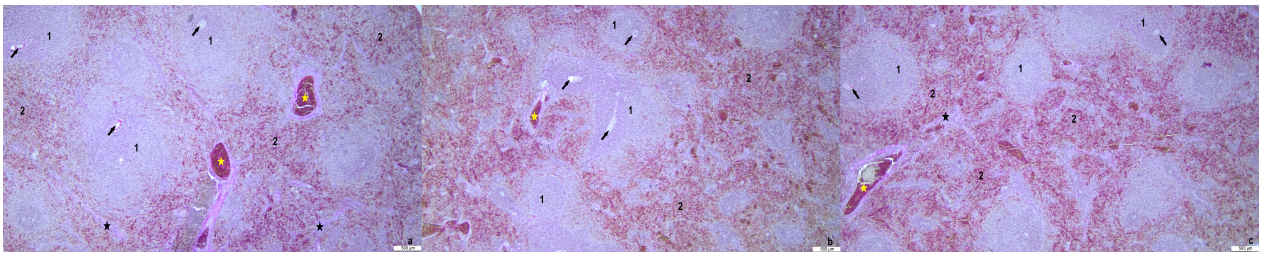

**Figure 1.** Histological structure of rat spleen tissue. Control group (a), Z1 group (b), Z5 group (c). White pulp (1), red pulp (2), arteria centralis (arrows), trabeculae (black stars), vena trabecularis (yellow stars). H-E staining.

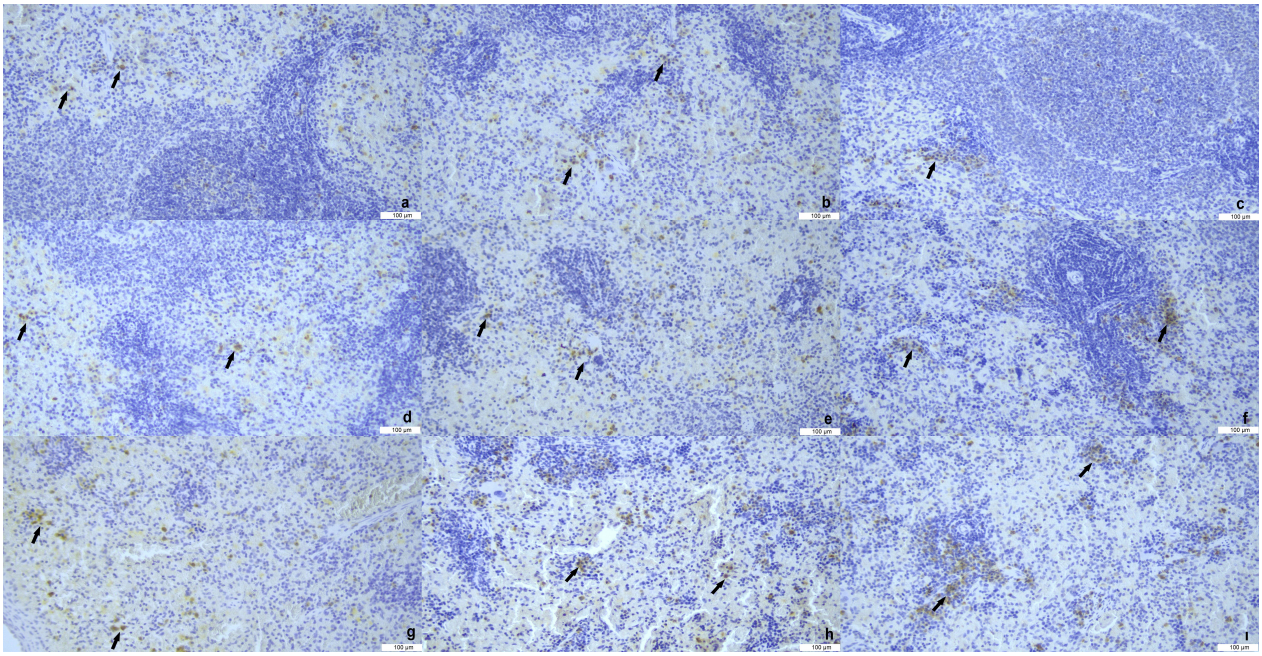

**Figure 2.** CTLA-4 immunoreactivity in rat spleen tissue. Control group (a, b, c), Z1 group (d, e, f), Z5 group (g, h, i). CTLA-4 immunoreactivity positive cells (arrows).

**Table 1.**Semiquantitative scoring of CTLA-4 immunoreactivity in rat spleen tissue.

| Groups | Red pulp | White pulp |
|---|---|---|
| Control | + | + |
| Ginger 100 mg/kg | + | + |
| Ginger 500 mg/kg | ++ | ++ |

No staining (-), weak staining (+), moderate staining (++), strong staining (+++)

ring susceptibility to autoimmunity.[30] Mutations in this gene have been associated with autoimmune diseases such as type 1 diabetes mellitus, celiac disease, systemic lupus erythematosus.[31-33]

Ginger; It is a plant known to have anti-inflammatory, antioxidant and anti-serotonin effects. It has been reported that ginger may have protective functions in arthritis, arthritis and musculoskeletal disorders. In addition, it has been stated that it induces helper T2 cells and anti-inflammatory cytokines such as IL-4, IL-10, increases glutathione level and antioxidant enzyme activity such as superoxide dismutase, inhibits the release of substance P, which is a mediator of inflammation and pain, and decreases TNF-α, IL-1β, IL-6, IL-2 and prostaglandin levels. In a study, it has been suggested that ginger has positive effects in reducing pain associated with inflammation and oxidative stress. It has also been stated that the use of ginger can reduce muscle pain caused by exercise.[34,35] In a study on mice, it has been suggested that ginger can improve inflammation in the airways by increasing the activation of T cells that play a key role in immunity. In addition, it has been stated that by regulating calcium channels, it can relax the smooth muscles of the respiratory tract and improve asthma symptoms.[36] In our study, Weak CTLA-4 immunoreactivity was detected in the red and white pulp in the spleen tissue of control and ginger 100 mg/kg group and moderate CTLA-4 immunoreactivity was detected in the ginger 500 mg/kg group

### *5. Conclusion*

Spleen is an organ that has important functions in the immune system. In studies investigating the effect of various plants on spleen tissue, it was emphasised that plant extracts applied at high doses would cause histological changes in spleen tissue. In our study, it was determined that ginger oil application at different doses did not cause histological changes in the spleen tissue

and increased CTLA-4 immunoreactivity in the ginger 500 mg/kg group. Based on our results, it is concluded that in order to fully explain the effects of ginger administration on the spleen, it is necessary to determine the efficacy of different doses, forms and administration forms of ginger.

### *Ethical approval*

The approval for the study was obtained from the Local Ethics Committee for Experimental Animals of Kafkas University on 06.03.2024 with the ethical committee number 2024/045.

### *Authors contribution*

ŞYA, AG, SEY, MÖ: Research, planning, article scanning, writing-original draft & review.

### *Conflict of interest*

There are no conflicts of interest associated with this re-search publication, according to the authors.

### *Data availability*

Data will be made available on request

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### *References*

1. Karaçalı S. Glikobiyoloji güncel moleküler biyoloji. Turkish Journal of Veterinary & Animal Sciences. 2003;27:489-495.
2. Chowdhury F, Dunn S, Mitchell S, et al. PD-L1 and CD8 + PD1 + lymphocytes exist as targets in the pediatric tumor microenvironment for immunomodulatory therapy. OncoImmunology. 2015;4(10): e1029701.
3. Gibney GT, Weiner PLM, Atkins PMB, et al. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. The Lancet Oncology. 2016;17(12):542-551.
4. Şimşek M, Tekin SB, Bilici M. Immunological agents used in cancer treatment. The Eurasian Journal of Medicine. 2019;51(1):90–94.
5. Said HM, Abdelaziz HO, Abd Elhaliem NG, Mohammed SA. A comparative study between ginger and echinacea possible effect on the albino rat spleen of experimentally ınduced diabetes. Egyptian Journal of Histology. 2020;43:763-76.
6. Widyaningsiht TD, Martati E, Lukitasari DM. Immunomodulatory effects of black cincau (Mesona palustris bl.) Supplement on Escherichia coli Strain O157-Infected Mice. Asian J Pharm Clin Res. 2017;10:326-30.
7. Ezzat MI, Hassan M, Abdelhalim MA, El-Desoky AM, Mohamed SO, Ezzat SM. Immunomodulatory effect of Noni fruit and its isolates: insights into cell-mediated immune response and inhibition of LPS-induced THP-1 macrophage inflammation. Food & Function. 2021;12(7):3170-3179.
8. Jafarzadeh A, Nemati M. Therapeutic potentials of ginger for treatment of Multiple sclerosis: A review with emphasis on its immunomodulatory, antiinflammatory and anti-oxidative properties. Journal of Neuroimmunology. 2018;324:54- 75.
9. Singletary, K. Ginger: An overview of health benefits. Nutrition Today. 2010;45:171–183.
10. Kubra IR, Rao LJM. An impression on current developments in the technology, chemistry, and biological activities of ginger (*Zingiber officinale Roscoe*). Critical Reviews in Food Science and Nutrition. 2012,52:651–688.
11. Arcusa R, Villaño D, Marhuenda J, Cano M, Cerdà B, Zafrilla P. Potential role of ginger (*Zingiber officinale Roscoe*) in the prevention of neurodegenerative diseases. Frontiers in Nutrition. 2022; 9:809621.
12. Fakhri S, Patra JK, Das SK, Das G, Majnooni MB, Farzaei MH. Ginger and heart health: from mechanisms to therapeutics. Current Molecular Pharmacology. 2021;14:943–959.
13. Mohd Yusof YA. Gingerol and ıts role in chronic diseases. Advances in Experimental Medicine and Biology. 2016; 929:177–207.
14. Eliş Yıldız S, Bakır B, Yediel Aras Ş, Dağ S, Karadağ Sarı E. Immunohistochemical distribution of somatostatin in gastric tissue of diabetic rats treated with cinnamon extract. Kafkas Universitesi Veteriner Fakultesi Dergisi. 2019; 25 (3): 427-433.
15. Schauer R. Sialic acids: fascinating sugars in higher animals and man. Zoology. 2004;107:49-64.
16. Al Makdessi S, Sweidan H, Dietz K, Jacob R. Protective effect of crataegus oxyacantha against reperfusion arrhythmias after global no-flow ischemia in the rat heart. Basic Research in Cardiology.1999;94:71-77.
17. Holubarsch CJF, Colucci WS, Meinertz T, Gaus W, Tendera M. The efficacy and safety of Crataegus extract WS® 1442 in patients with heart failure: the SPICE trial. European Journal of Heart Failure. 2008;10:1255-1263.
18. Seo N, Ito T, Wang N, Yao X, Tokura Y, Furukawa F, Takigawa M, Kitanaka S. Anti-allergic Psidium guajava extracts exert an antitumor effect by inhibition of T regulatory cells and resultant augmentation of Th1 cells. Anticancer Research. 2005;25:3763-3770.
19. Sharififar F, Pournourmohammadi S, Arabnejad M. Immunomodulatory activity of aqueous extract of Achillea wilhelmsii C. Koch in mice. Indian Journal of Experimental Biology. 2009;47:668-671.
20. Zandonai RH, Coelho F, Ferreira J, Mendes AKB, Biavatti MW, Niero R, Cechinel V, Bueno EC. Evaluation of the proliferative activity of methanol extracts from six medicinal plants in murine spleen cells. Brazilian Journal of Pharmaceutical Sciences 2010;46:323-333.
21. Al-Dahmesh B, Dkhil MA, Al-Quraishy S. Chili pepper induced injury to splenic tissue of rabbit. Journal of Medicinal Plants Research. 2011;5:2015–2020.
22. Keskin N, Mammadov R, İli P. Crataegus aronia var. dentata Browicz ekstraktının dalak üzerindeki etkilerinin araştırılması: histokimyasal çalışma. Pamukkale Tıp Dergisi. 2012;5(2):68-74.
23. Boussiotis VA. Molecular and Biochemical Aspects of the PD-1 Checkpoint Pathway. The New England Journal of

Medicine. 2016;375:1767-1778.

24. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006;439:682-687.

25. Dariavach P, Mattei MG, Golstein P, Lefranc MP. Human Ig superfamily CTLA-4 gene: chromosomal localization and identity of protein sequence between murine and human CTLA-4 cytoplasmic domains. European Journal of Immunology. 1988;18:1901–1905.

26. Lo B, Zhang K, Lu W, Zheng L, Zhang Q, Kanellopoulou C, Zhang Y, Liu Z, Fritz JM, Rececca M, et al. AUTOIMMUNE DISEASE. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. Science. 2015;349:436-440.

27. Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. Immunological Reviews. 2008; 224: 166–82.

28. Melero I, Hervas-Stubbs S, Glennie M, Pardoll DM, Chen L. Immunostimulatory monoclonal antibodies for cancer therapy. Nature Reviews Cancer. 2007;7:95-106.

29. Linsley PS, Bradshaw J, Greene J, Peach R, Bennett KL, Mittler RS. Intracellular trafficking of CTLA-4 and focal localization towards sites of TCR engagement. Immunity. 1996;4:535-543.

30. Kristiansen OP, Larsen ZM. CTLA-4 in autoimmune diseases--a general susceptibility gene to autoimmunity. Genes & Immunity. 2000;1:170-184.

31. Douroudis K, Prans E, Uibo R. CTLA-4 promoter polymorphisms are associated with latent autoimmune diabetes in adults. Human Immunology. 2009;70:921-924.

32. Hunt KA. A common CTLA-4 haplotype associated with coeliac disease. European Journal of Human Genetics. 2005;13:440-444.

33. Shojaa M. Association between 318C/T polymorphism of the CTLA-4 gene and systemic lupus erythematosus in Iranian patients. International Journal of Rheumatic Diseases. 2017;20:2040-2044.

34. Aryaeian N, Tavakkoli H. Ginger and its effects on inflammatory diseases. Advances in Food Technology and Nutritional Sciences. 2015;1(4):97-101

35. Leach MJ, Kumar S. The clinical effectiveness of ginger (zingiber officinale) in adults with osteoarthritis. International Journal of Evidence-Based Healthcare. 2008;6(3):311-320.

36. Townsend EA, Siviski ME, Zhang Y, Xu C, Hoonjan B, Emala CW. Effects of ginger and its constituents on airway smooth muscle relaxation and calcium regulation. American Journal of Respiratory Cell and Molecular Biology. 2013;48(2):157-163.